[PERCY SLADEN TRUST RESEARCH]

THE NUTRITION AND METABOLISM OF MARINE ANIMALS
IN RELATIONSHIP TO (a) DISSOLVED ORGANIC
MATTER AND (b) PARTICULATE ORGANIC MATTER OF
SEA-WATER

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A fundamental problem in the economics of life in the ocean is that of the manner in which different types of marine organisms take up their food from the sea. There is no question that bacteria in the sea-water are nourished by food taken up in solution. The distribution of such bacteria is not uniform, they flourish in abundance near any decomposing organic matter of either vegetable or animal origin, and, although invariably present everywhere, they occur in much smaller numbers in purer water some distance from shore, where decomposing organic matter in quantity does not exist. Just as in the case of terrestrial bacteria, it is obvious that bacteria in the sea, growing on or near dead matter from plants or animals, throw out secretions which aid in the solution of such organic matter, and increase in this way the amount of their available pabulum in dissolved form. Such dissolved organic matter is carried away in part by the motion of the sea-water, and along with similar organic matter, such as sewage and humus, from rivers and smaller streams, must give a certain amount of dissolved organic matter to the general bulk of the ocean water.

The amount of such organic matter undoubtedly remains balanced or constant, at a certain value, on account of its being broken down into inorganic matter as it is oxidised by bacteria and other agencies.

It is hence a very probable view, *prima facie*, that many forms of protozoa and other minute organisms possessing no special alimentary system, or free movement of their cytoplasm for enveloping and digesting such particulate food as bacteria, algae or minute débris, may exist and grow and maintain an oxidizing metabolism on the dissolved

organic material so added to the sea, either from the land or from the débris and excreta of larger and higher denizens of the sea.

From this point of view it is of the highest importance that our knowledge of the amount of dissolved organic matter in the sea should be enriched by more quantitative determinations than have hitherto been carried out, so that some idea may be obtained of the value of the sea as a nutrient medium apart from the particulate organic matter in living condition and otherwise which it contains.

Such experimentation upon the dissolved organic matter is made still more essential for two reasons: first, there exist at present most marked divergencies in the experimental findings of observers as to the amount of dissolved organic matter in the sea-water, the figures obtained ranging from quantitatively indeterminate traces of less than 1 mgm. per litre to over 90 mgms. per litre; and, secondly, it has been prominently urged by one investigator in recent years that not only bacteria and protozoa, but many classes of higher invertebrates, and even fishes, obtain the greater part of their nutrition from the dissolved organic matter, rather than, as has hitherto been held, from organisms forming the floating life or plankton of the sea, or from other organisms accumulated along the shore, which form their prey.

From the bio-chemical or physiological point of view, it is quite well known that organic matter destined to form an intrinsic part of the body of an animal must, first of all, be broken down into smaller molecules and dissolved. These soluble molecules are then, in the specific metabolism of the particular animal, built up into the definite colloidal molecules or aggregates which exist in the cell substance of that type of animal.

Even in the simplest and most rudimentary type of digestion, such as that shown by an amoeboid protozoon or a leucocyte, the food particle has not become part of the animal when it is engulfed and surrounded by the cytoplasm. It is afterwards gradually dissolved in so far as the digestive cell-fluids can act upon it, and it is the soluble molecules only which are then aggregated and built up in the single-celled organism to give those specific colloidal structures which lie at the basis of the differentiation of species from one another, and yield those delicate differences of chemical structure which, in turn, give the morphological substratum for functional differences.

The same is true for the metazoa, only the special types of cell fabricate the secretions which act upon the colloidal aggregates of the

food and break them down into soluble molecules to form the building stones, for constructing on different lines the new colloidal aggregates necessary for the animal's own cells with their accompanying peculiar functions. Accordingly, all food of protozoa and metazoa alike must pass through a stage of hydrolysis and disintegration and solution, and there is nothing inherently erroneous in the suggestion as a working hypothesis that the dissolved organic matter of water in which aquatic animals are living may play a large or even a predominating part as a food supply.

On the other hand, in all such preliminary considerations of a general theoretical nature, the fact that a large number of species of protozoa possess means of taking up particulate matter such as organic débris, diatoms and bacteria, must not be lost sight of, as also that with the exception of certain parasitic forms, all of the higher metazoa possess a specially developed system of cells with both mechanical and chemical devices (e.g., intestinal canal and digestive glands) for taking up solid food and rendering it soluble afterwards. In addition, as the scale of life is ascended, there arise special systems for absorption of the material prepared for the nutrition by the solvent action of the digestive secretions, and other systems again for the intermediate metabolism which renders the absorbed material suitable by specialized chemical syntheses for entry into the circulating nutrient fluid which bathes or irrigates the living cells.

This process has proceeded so far in the case of most terrestrial animals that there remains no possibility of their nutrition in any other way than by organic matter taken in by the alimentary canal and passed through the processes outlined above. A great part of the existence of such animals consists either in the pursuit of nutriment or in its ingestion and assimilation, and it is a general law, even up to and including the human species, that the distribution and migration of life follows the distribution in comparative abundance or scarcity of food.

Now, in nearly all the aquatic invertebrata and in the fishes, similar developments for the capture and elaboration of particulate food are to be found, and the same variations in distribution in relationship to abundance or scarcity of other plant and animal life occur, as also the same migrations of motile animals after food. Therefore a purposeful relationship of these factors becomes obvious, and it would appear probable that with the more extensive development of organs designed for the capture, digestion and metabolism of solid food, the direct

absorption of soluble organic food in unchanged condition by organs evidently intended for other physiological purposes, such as the gills of molluscs or fishes, would lessen in degree and become comparatively unimportant as a source of energy, if, indeed, such an intake persisted at all.

Organs specially developed for the performance of such an important function as the intake of nutrition, it can safely be said, would never have evolved to such a high degree of perfection and elaboration if they were only used occasionally as a luxury or an accessory to a main mode of nutrition, in which the food was ready prepared and soluble, and capable of entering the main stream of nutrition at once without injury to the animal. If an abundance of dissolved nutrient matter already existed in the sea, the saving in work by direct utilization of this supply of energy would be so great that digestive and metabolic organs would not develop, and if already developed would atrophy from disuse.

Dissolved organic matter means practically no struggle for existence, and no reason for the development of many organs, such as the sense organs, which have developed in connection with such a struggle.

This is clearly seen in the case of many parasites such as the cestodes, which utilize dissolved nutriment from their hosts.

If the sea-water were a nutrient medium of so considerable concentration in dissolved organic matter as to play any important part in the nutrition of such an animal as a fish, it must not be forgotten that the sea would undoubtedly be swarming with creatures so designed as to be capable of flourishing without solid aliment, and, in addition, there would be a host of other creatures making their prey of these. Life in the ocean away from land would be far more abundant than it is at present. If the sea-water, for example, contained 90 mgms. per litre of organic matter of a type suitable for supporting life, instead of being, as at present, comparatively free from bacteria and containing only 0.83 mgm. per 1,000 litres,¹ it would be a most excellent culture medium, swarming with bacteria and infusoria like the stagnant pools containing decomposing sea-weed found at higher levels along the shore.

Finally, it may be pointed out that as the size of an organism

1. This is the moist weight of bacteria found by Lohmann working at Syracuse in the Mediterranean, as computed by Pütter (*Zeitsch. f. allgem. Physiol.*, Bd. VII, p. 290, 1908) in so large a quantity as 1,000 litres of sea-water. In the 1,000 litres there were only by volume 53.63 cubic millimetres of Plankton made up as follows:—Protophytes No. 2,082,860, vol. 17 c.mm.; Protozoa 325,510, vol. 1.13 c.mm.; Metazoa 17,325, vol. 34.7 c.mm.; Bacteria 785,000,000, vol. 0.80 c.mm. Allowing a specific gravity of 1.080 this gives a total moist weight of Plankton of 55.6 milligrams.

increases, the surface decreases relatively to the weight, and hence a concentration of dissolved organic matter which would be ample for the support of a bacterium or protozoan, would be altogether useless for the support of a fish. When solid organic food is swallowed and digested by a fish, it is taken up in higher concentration from the intestinal tract, and a smaller area of absorptive tract is capable of carrying on the work. But if such a creature were compelled to derive its nutrition from a solution of only one or two parts in a million, the necessary uptake could only be obtained by providing a large surface, such as the lung of an animal, or the green leaf of a plant, or a fish gill, over which the water was very rapidly moved. No such mechanism designed for absorption of soluble food and connected up with a metabolic gland, such as a liver, has ever developed.

The gill of the fish has been supposed by Pütter to carry on such a function in addition to its more obvious function of respiration, but all experimental proof of this supposed function of the gill is lacking. The gill epithelium is not such as to warrant any belief that it can modify any organic matter possibly absorbed, and there is no provision anywhere for a metabolic change of any material absorbed by the gill before it is thrown into the general circulation and carried as nutriment to the tissue cells. All analogy teaches us that promiscuous organic matters so absorbed, and distributed without appropriate intermediate changes, instead of acting as nutriment would produce the effects of a violent poison. The products of digestion removed from the intestine and injected into the blood stream, so that they may reach the tissue cells without passing through the liver, act in man as exceedingly toxic agents and cause death when administered in this way, even in small quantities.

Again, for an adequate uptake of dissolved matter from a very dilute solution, it becomes necessary to have some substance in the circulating fluid with a specific affinity for it. The haemocyanin of crustacean blood and the haemoglobin of fishes is an example of such a body, related to uptake of oxygen in dissolved form by the gills. Yet sea-water contains 8 mgms. of oxygen in solution per litre, it is hence exceedingly difficult to believe that, without any such chemical absorbent, the blood of a fish in process of circulation through the gill could take up anything approaching the amount required for a nutritional balance. Especially is this the case in view of the low limit of organic matter shown by the present experiments as the maximum which can possibly be present in sea-water.

The figures which have been obtained in the present research for the rate of oxidation of organic matter by the species experimented upon, taken in relationship with the low values for dissolved organic matter, demonstrates clearly that the views put forward by Pütter,¹ that marine animals obtain a large amount of their food in soluble form from the sea-water must be regarded as erroneous.

On the other hand, from our own observations, as well as from a consideration of the results of plankton studies of other observers, it becomes evident that many marine animals could not possibly maintain themselves in nutritive equilibrium by any process of consuming plankton as uniformly distributed in the sea without some special local concentration, for the organic material contained in the plankton is also far too small for such a purpose. On the basis of the higher animal acting even as a complete filter to the quantity of water passing through its body, the volumes of water which must necessarily be filtered to yield the daily quota of food are far too large to be credible. There is no mechanism in most species at all comparable to a Chamberland filter for such purposes, since this filter refuses passage even to bacteria, but we find that the combined amounts of particulate organic matter held back by (a) a fine silk netting of 20 gauge, which is the finest used for tow-netting, and (b) that afterwards retained by the Chamberland filter amount together to not more than 1 mgm. per litre, that is to say, to one part in one million of organic matter oxidizable by potassium permanganate. If a calculation is made of the volume of water necessary, as shown in Section B of this paper, to yield the amounts of organic matter experimentally shown to be utilized by those animals with an active metabolism, it becomes at once obvious that the uniformly distributed plankton in this sense cannot play any considerable part in the nutrition of such animals.

It is only in the case of fixed animals with a slow metabolism, such as sponges and ascidians, that the demand for organic nutrition can be satisfied by the minute forms present in plankton by such a reasonable volume as may be supposed to be passed through the animals. (See Section B, p. 281.)

To this extent, therefore, we confirm the view of Pütter, but we disagree with him in that we do not find an available source of supply of adequate value in the soluble organic matter, and believe his results are

1. Die Ernährung der Wassertiere, *Zeitsch. f. allg. Physiol.*, Bd. VII, S. 283-320; Der Stoffhaushalt des Meeres. *Ibid.* S. 321-368; Studien z. vergleichenden Physiologie des Stoffwechsels, *Abhand. d. kgl. Gesellsch. d. Wissensch. zu Göttingen, Math.-Physiol. Klasse*, Neue Folge, Bd. VI, S. 1-79.

due to lack of appreciation of the defects of the ordinary methods of analysis of fresh water when applied to sea-water.

All the wealth of argument so ably applied by Pütter to prove that the organic energy supply of the plankton is inadequate for the support of life, applies with equal force to the dissolved organic matter, for neither source yields more than one part per million.

But if the plankton figures, on the basis of uniform distribution, fail to furnish an explanation, and the organic matter in solution also is inadequate, what, it may be asked, is the food-supply? In our opinion the answer lies in the unequal distribution of the plankton, similar in nature and cause to the unequal distribution in space of the higher marine animals and of nearly all terrestrial animals.

The terrestrial animals have to seek their food, and, as a rule, congregate in flocks or herds for that purpose where food is most abundant and follow the food. Other purposes of a biological nature are also served and fostered by the communities so formed for the primal necessity of obtaining food. Fish in the sea live together in shoals for the same reason, and anyone who has walked along a sea-cliff in summer must have been struck by the fact that birds, such as sea-gulls, follow the movements of the young fish, and do not spread themselves uniformly over the sea-surface regardless of the concentration of the food.

Where there is a good supply of pasturage and water, under natural conditions there will be a greater wealth of herbivorous animals, and where these are found in abundance there will be more of the carnivorous animals which prey upon them. Thus some factor in the environment of a permanent physical nature in soil or water, or of seasonal variation, such as light or heat or moisture, produces abundance or scarcity of one species of animal or plant, and this precipitates a whole train of events, the causal relationship of which can only be unravelled by patient study and experimentation.

There is no prevalence of monotony and uniformity in Nature, all is cyclical and undergoing constant variation, and so also there exists no uniform distribution of plankton in the sea.

The postulate of a uniform distribution of plankton would appear to be assumed by many workers on the floating life of the sea and its variations in season and in space. This postulate is taken as a basis by Pütter when he deduces the inefficiency of the plankton as a food-supply. It underlies all his arguments that the fish, or large invertebrate, is engaged all the time in exhausting a certain volume of water either

(a) of plankton on the views of the orthodox school of plankton workers, or (b) of dissolved organic matter on his own views.

This is the fundamentally wrong conception in the whole argument, because it leaves out of account anything in the nature of a taxis towards the food in the lower species or of what we term instinct in the higher species, and in the case of non-motile organisms any selective action of the organism in choosing a site for its operations more favourable for the capture of the particular kind of food upon which it lives. Yet every observer of distribution of marine animals knows that this is precisely what happens, and that at a given time of year he will find particular species by dredging or fishing in particular spots.

The knowledge of the floating life of the sea and its variations in season, and in locality, is most important, and although the experimental methods available may be imperfect and may not show all variations in the content of the water in living organisms, yet much as to sequence and interdependence of life may be found out by such study.

It is equally important that the limitations of such study should be appreciated, and that figures so obtained should not be applied with a direct mathematical rigour for which there is no warrant.

Tow-nettings taken with care throughout a season may show quite clearly the relative quantities of a certain organism available as food for another, but such figures taken and multiplied into volumes of water to show how many litres of water the latter organism must filter through its body before it can make a sufficient meal from the former constitutes a drifting into ridiculous absurdity, such as usually overtakes the biologist when he applies mathematics in a blindfold fashion without thinking of the many natural limitations unrepresented in his simple formula.

A herring, which swam about aimlessly with its mouth open in a sea containing 0.5 parts in one million of organic matter as plankton, would have to filter a good deal of water before it obtained an adequate supply of food, and might grow very lean in the process; another herring in the same sea making proper use of its sensory and nervous systems might be able to detect enormous variations in the distribution of food and grow fat where the other was starving.

It is, accordingly, the factor of the animal's one action which appears to us to have been disregarded too much, and this explains how a balance of nutrition can be obtained, in spite of the low figures for dissolved organic matter and for plankton on the supposition of a uniform distribution.

These theoretical considerations illustrate the importance of determining such questions as the organic content in solution and suspension of the sea-water, and of the amounts of organic matter required by different types of animal.

In regard to the first part, viz., the organic content of the sea-water, we believe that we have obtained definite results which place a maximum at such a low level as to show that the organic content in solution is almost negligible and lies within the limits of error of determination, and also that the plankton, whether as ordinarily fished with a fine silk netting or as separated by the Chamberland filter, is represented by a very low figure. But in respect to the amounts of organic matter oxidized by the species of animal with which we have worked, we are only able to put preliminary figures forward as yet, and intend to supplement these later by fuller study of each species. This latter portion of the work has opened up certain new problems which will be mentioned in describing the experiments.

The work was carried out at the Marine Biological Station, Port Erin, Isle of Man, during April, 1912, and our thanks are due to Professor Herdman, for the invitation to work at the problem, for the use of the steam yacht 'Runa' in collecting water and specimens, as also for many most valuable suggestions in the course of the work. The expenses of the research have been defrayed by a grant from the Percy Sladen Memorial Trust, and we desire to take this opportunity of expressing our indebtedness to the Trustees.

The water used was collected purposely in different situations, sometimes four miles out at sea, sometimes between one and two hundred yards, sometimes close to shore from beside Port Erin Breakwater, and sometimes in the Aquarium at the Biological Station, so as to follow all variations which might be possible, but all water used was clean fresh sea-water.

The plankton was completely separated from the water in a measured volume passed through the Chamberland pump, in two portions, viz., (*a*) that separated by a No. 20 silk-netting, which is the finest silk gauze made and used for plankton work, and (*b*) that separated by the material of the Chamberland filter candle after passing through the silk.

Determinations were then made (*a*) of the organic matter oxidizable by potassium permanganate, (1) in acid, (2) in alkaline solution; and (*b*) of (1) free and saline ammonia, (2) organic or albuminoid ammonia, in the following portions: (i) The unfiltered sea-water, (ii) The sea-water

filtered through the silk-netting only, (iii) the sea-water filtered through both silk and filter, (iv) the residue caught on the silk, (v) the residue left on the candle of the Chamberland filter.

As the total number of litres filtered for each experiment was known, it follows that such a method of procedure gave (*a*) total amounts of organic matter in unfiltered, partially filtered, and completely filtered water, and (*b*) amount of organic matter removed by ordinary silk tow-netting and by complete filtration of all organic matter, including bacteria.

It may at once be indicated that the organic matter as determined by the direct method was exceedingly small, and fell well within the limits of experimental error. The residues gave reliable titrations, as the suspended organic matter from several litres could be taken for analysis and so experimental errors could be discovered, but when worked out to amount per litre the figures give very small amounts and amply confirm those obtained directly in the analyses in the water, since they fall within one part per million. The fraction stopped by the Chamberland is much greater than that stopped by the silk-netting.

In the second portion of our enquiries, estimations were made of the rate at which organic matter was oxidized by various species of marine animals, by making determinations of the rate of disappearance of dissolved oxygen from a known large volume of *filtered* sea-water in wide-mouthed stoppered bottles in which the animals were enclosed without the presence of air for a known period of some hours' duration. In addition, the carbon dioxide produced in the same period by the respiration of the animals was estimated by simple titration of a known volume of the water with centinormal acid at the end of the experiment, and using phenol-phthalein as indicator. This simple method was found to give results of considerable value. Also the amount of oxidizable matter in the water was determined at the beginning and end of each set of experiments by the permanganate methods, alongside controls in which no animals were present in the water, and the amounts similarly of free and saline, and of albuminoid or organic ammonia. So far from showing any diminution in dissolved organic matter, as might have been expected if dissolved organic matter formed the main source of nutrition, as supposed by Pütter, it was always found that both organic carbon and organic nitrogen were largely increased, and this also even when the animals had been kept without food for twenty-four hours previously, and no obvious solid dejecta were present.

In such experiments as these the animals are, of course, living on the reserve food of their tissues and metabolic organs. The total wastage from the animals is rightly represented by the sum of the oxygen disappearing in oxidizing organic matter (and so yielding energy to the animal for its life-processes) and of the unoxidized organic matter added as waste of excretions and secretions to the water. Both these quantities would be increased in an animal which was fed during an experiment, so that the sum of these two quantities of organic matter must be regarded as the minimal demand. The amounts are given in Section B, and it will there be shown that the amounts of organic matter in solution, or as separated by a Chamberland pump, sufficient to satisfy such demands, would be distributed in too inordinately large volumes of water for the animals to obtain them by fishing out or filtering average sea-water.

It would appear from these experiments as if the higher animals, instead of living upon dissolved organic matter, or bacteria or protozoa, on the contrary contributed energy in the form of organic compounds to sea-water for the use of these lowlier organisms. The source of this organic matter, in addition to that required for their own use, being grosser food-supply, or plankton occurring in shoals.

The results of the investigations will be described in two separate sections, the first dealing with the organic matter of the sea-water and the second with the metabolism of the marine animals.

Section A. THE ORGANIC MATTER OF THE SEA IN SOLUTION AND SUSPENSION

Any accurate determination of the amount of organic matter in sea-water is rendered almost impossible for two reasons: first, the amount of organic matter is excessively small; secondly, the amount of inorganic matter as chlorides and carbonates is relatively enormous, and the chlorides especially interfere with all known methods of oxidation by yielding free hydrochloric acid, and, in presence of oxidising agents, free chlorine which interferes with the titrations. For these reasons all that can at present be given is a very low figure as a maximum, which it can safely be said that the organic matter does not exceed.

Strange as it may seem, in view of the immense superstructures which have been built upon the supposed presence of appreciable amounts of organic matter, we have only been able to find one observer, E Raben,¹

1. Wissensch. Meeresuntersuch. herausg. v.d. Kommission z. Wissens. Untersuch. d. deut. Meere in Kiel u. d. biol. Anstalt auf Heligoland, N.F., Bd. XI, Leipzig, Lipsius u. Fischer, 1910.

who has carried out a series of analyses in filtered sea-water, and the greater part of these are vitiated by being made on polluted water from Kiel Ford, the great naval base of the German Navy.

In regard to unfiltered water the amount of quantitative work is also most meagre. Not only has Pütter failed to make any determinations of the amount of organic matter in water completely cleared of suspended matter by Chamberland filtration, there is no note in his paper that the water was filtered from plankton by silk-netting or filter paper, and one is left from the description to understand that the water analysed was entirely unfiltered water from the Gulf of Naples. Twelve analyses are given, and, as Henze points out, although these were taken on days close together, the values obtained show enormous variations from 68 mgms. of organic carbon per litre to 134 mgms. Such variations might have caused Pütter to suspect the accuracy of the method used, but he draws no attention to them. In discussing the nature of the supposed organic carbon, he concludes from the simple process of distillation in acid, and catching the distillate in decinormal alkali, that no less than 36 mgms. per litre are present as volatile organic acids. One would have thought that this large quantity of volatile organic acids would have afforded an opportunity of finding out the nature of such acids, but Pütter replaces this experimental work by a theoretical speculation, as a result of which he places the average molecular weight of the volatile fatty acids at 128. Inspired by this result, he proceeds to fix on a theoretical basis, the composition and oxygen value of the remaining part of the supposed organic matter amounting to two-thirds of the whole.

There is little doubt that the supposed volatile organic acids of Pütter consist of hydrochloric acid distilled off from his mixture of sea-water and sulphuric acid, and the same type of explanation holds for his supposed 68 to 134 mgms. of organic carbon per litre of sea-water, upon which the whole superstructure of his solution-nutrition theory is based. This organic carbon in Pütter's experiments probably arose, as Raben¹ points out, from acetic acid set free from lead acetate, which Pütter interposed to take up the evolved hydrochloric acid. The volatile acetic acid was carried over to the glowing tube containing lead chromate and copper oxide, and there combusted. So that Pütter's figures for organic carbon represent a variable amount of organic matter arising from one of the reagents used in the analyses.

The method used was that known as Messinger's wet combustion

1. *Loc. cit.*

method, in which the organic matter is oxidised by a mixture of potassium bichromate and strong sulphuric acid, or by chromic acid. This method was originally designed for use in potable waters where the amount of chlorides is low. When transferred to use in the case of sea-water, the utmost care must be taken that hydrochloric acid or chlorine bodies are not allowed to pass over and become absorbed in the weighed potash bulbs intended for absorption of the carbon dioxide.

Shortly afterwards Henze¹ repeated Pütter's work, taking care by the interposition of a tube of metallic antimony and of a glowing tube of lead chromate and copper oxide to prevent any chlorine compounds or acid reaching the carbon-dioxide absorption apparatus. With such precautions, Henze found that while organic matter added to sea-water was readily oxidizable and re-obtained with ease, the amount of organic matter in sea-water under natural conditions was such as to fall within the region of experimental error. Henze concludes that so far as Pütter's views are based on presence of appreciable amounts of organic matter in the sea-water they are devoid of all experimental proof.

In his later monograph,² Pütter refers to this work of Henze's, and appears to accept it as proving that his former work was based on much too high figures, and without any further experimentation discards his earlier average amount of 90 mgms. of organic carbon, and replaces it by a new figure of 4 to 5 mgms.

In this later monograph on the subject, Pütter admits that the Messinger method of determining organic carbon cannot be utilized, and suggests its replacement by determinations of the amount of oxygen necessary for oxidation of the organic matter as completely as may be done by potassium permanganate in acid or alkaline solution.

This method is used by Pütter in his respiratory experiments, and he appears to be unaware of the fact that it also is upset by the action of the hydrochloric acid and chlorine set free by the interaction of the acid and permanganate upon the chlorides present in such preponderating quantity.

As will be shown later, the method can only be used with the greatest caution, *in the cold*, and using as a zero a blank, in which no organic matter but an equal amount of chlorides is present, under comparable conditions throughout.

When this is done, the difference in readings between sea-water and

1. Pflüger's *Archiv. f. d. ges. Physiol.*, Bd. CXXIII, S. 487, 1908.

2. Die Ernährung der Wasserthiere, und der Stoffhaushalt der Gewässer, Fischer, Jena, 1909, pp. 49 and 99 to 101.

the inorganic control fall practically to zero, showing that organic matter is scarcely detectable in the sea-water.

But Pütter recommends that the mixture of sea-water, acid, and permanganate shall be boiled for ten minutes.

In an experiment carried out by us to test the effects of such a procedure, six grams of pure fused sodium chloride were taken and dissolved in 250 c.c. of distilled water, so as to represent approximately the concentration in chlorides of sea-water, and the usual amount taken in actual experiments. To this, 10 c.c. of 25 per cent. sulphuric acid and 10 c.c. of N/20 KMnO_4 solution were added, the same quantities as might be used in an experiment.¹ The whole was then boiled for only five minutes, instead of the ten recommended by Pütter, cooled and titrated with N/10 $\text{Na}_2\text{S}_2\text{O}_3$, after adding excess of potassium iodide to release iodine, when only 5.8 c.c. were required instead of 10 c.c. A parallel experiment with 250 c.c. of distilled water and no sodium chloride, treated otherwise similarly, gave no loss of chlorine, requiring at the end 10 c.c. of decinormal thiosulphate for neutralization.

This shows clearly that any kind of result as to content of oxidizable organic matter may be obtained by following the method described by Pütter in his monograph. For example, the above experiment would fallaciously show 13.4 mgms. per litre of oxygen required for oxidation of organic matter, this in an artificial sea-water containing not a trace of organic matter but only pure sodium chloride fused before use.

The experiments recorded below with 'Falk' salt,² and the salts of sea-water after evaporation and ignition over a bunsen-burner, show the same effects, but in lessened degree, because the experiments were carried out in the cold and in stoppered bottles. These figures form the blanks to the sea-water. Where organic matter is actually present as in the residues on silk-netting and Chamberland, the change in the figures shows that such true organic matter is readily oxidizable by the permanganate, and results can be obtained when proper precautions are taken.

For these reasons, the results obtained by Pütter by the permanganate method resemble those with the Messenger method in being vitiated by experimental errors, and all proof is lacking of any appreciable amount of oxidizable organic matter in normal filtered sea-water.

1. See below.

2. This is a pure table salt manufactured by the Salt Union, Ltd., Liverpool. It contains no appreciable amount of organic matter, but in any case it was always heated before use.

More recently, an important contribution to the subject of the amount of organic matter in sea-water has been made by Raben, who has, like Henze, demonstrated the illusory character of Pütter's analyses, and expresses his complete concurrence with Henze's concluding statement quoted above.

Four out of the six complete and careful analyses made by Raben were carried out upon water from the 'Kieler Förde,' and two only with water from the open sea. The results show a wide difference, the four from Kiel yield 13.9, 12.8, 11.4, and 10.9 mgms. per litre respectively, while those from the open sea yield only 3.0 and 3.2 mgms. respectively. Raben ascribes this difference to three causes, viz., nearness to land, presence of the German fleet in the harbour, and the access of sewage (Schmutzwasser).

An amount of 3 mgms. per litre is equivalent to 0.3 mgm. in the quantity of water taken for analysis, viz., 100 c.c., which would yield 1.1 mgm. of carbon dioxide or approximately 0.5 c.c. This is a quantity, as Henze states, which is well within the limits of experimental error with such a complicated apparatus, working for a period of two hours in a stream of air.

The exacter experiments of Henze and of Raben have accordingly shown that the organic carbon dissolved in sea-water is an exceedingly minute quantity lying at the limits of detectability by the best known methods.

There are one or two figures given by earlier observers indicating a higher value, but the methods of analysis are so questionable in the light of more recent knowledge as to rob them of all value. In connection with the Challenger Expedition, J. Y. Buchanan made a few estimates of organic carbon by the rather drastic procedure of distilling to dryness with potassium permanganate, and found in different samples from 2.3 to 7.7 mgms. of organic carbon per litre. But in summarizing, he himself states: 'The few determinations of "organic carbon" by means of permanganate of potash were experimental, and were entered in my journal in course. I was, however, so dissatisfied with the experiments that I soon gave them up, and I attach no value to the results.'

The early observations of Natterer in 1892 need only be mentioned because Pütter seems to attach some importance to them at the present day. They were made long before the difficulties of the problem were appreciated, and gave results varying in different samples from 10 to 50 mgms. per litre. But when it is explained that they were obtained

by heating an alcoholic filtrate, still containing a large residue of inorganic salts, in a test-tube until the test-tube began to soften, the difference in weight of the tube before and after being taken as the amount of organic matter, chemists of to-day will know how much credence to give them.

Pütter in his monograph¹ also lays considerable stress on an organic substance obtained from sea-water resembling stearin or palmitin. This substance was obtained at the end distillation of 200 litres of water, thrown together from all sources at the end of the expedition, as Natterer himself states it came over 'in ganz geringer Menge' only a few centigrams from the whole 200 litres, of a miscellaneous collection of water samples at the end of the trip, and the only evidence of palmitin or stearin is that in heating in an open tube it gave a smell resembling overheated palmitic or stearic acid, and that it melted at approximately the same temperature as stearic acid.

It is certainly remarkable to find such an isolated experiment of such a type as this quoted fifteen years later, without any further experimental work by the person quoting it, as evidence of the presence of higher fatty acids in sea-water.

The most reliable method for obtaining the organic carbon in well and river water consists in evaporating down to dryness in presence of sulphurous acid to drive off the inorganic carbonate, and then subjecting the residue to combustion by either the ordinary dry method or a wet method, and estimating the carbon dioxide produced either volumetrically by gas analysis, or by absorption in potash balls and weighing.

This method is rendered useless in the case of sea-water by the enormous amount of chlorides present compared to the small amount of organic matter. When sea-water is evaporated to dryness in a porcelain basin and then heated over a Bunsen burner, there is just a faint yellowing of the mass and then it turns white. To obtain enough carbon to give any appreciable reading in a combustion, would require enough sea-salt to fill a combustion tube.

The method used throughout was wet oxidation with potassium permanganate in the cold for a number of hours stated in each case. As soon as we appreciated the precautions required we employed a control, carried out under like conditions, of ignited sea-salt or Falk table salt, also ignited before use.

For the greater number of cases oxidation in acid solution was

1. *Loc. cit.*, p. 102.

employed, but as we thought part of the difficulty might be avoided by oxidizing in alkaline solution, in later experiments we also used alkali. It would appear, however, that the greater part of the difficulty with the chlorides is attached to the actual titration at the end, which must be done in both cases in acid solution, and when allowance is made for the fact that in alkaline reduction only 3, in acid solution 5 atoms of oxygen are set free, there is practically no difference in the two sets of results.

It has been suggested by Dupré¹ that the method can be used in presence of chlorides, and even in sea-water, if glacial phosphoric acid be used instead of sulphuric acid. Accordingly, we experimented with this modification, but without change in the result. In presence of sodium chloride the titration figure showing remaining excess of permanganate at the end is always lower than with distilled water, so falsely indicating a small amount of organic matter.

The nearest we were able to approach the problem, accordingly, consisted in carrying out a control under as like conditions as possible to give a zero, and when this is done the organic matter found in the sea-water is negligibly low.

The method is too well known to require detailed description.² The following is an outline:—250 c.c. of the water were taken, alongside other samples and controls, in wide mouthed glass-stoppered bottles of about 400 c.c. capacity, 10 c.c. of one in four sulphuric (or phosphoric acid) was added, and the appropriate amount of N/20 or N/80 KMnO_4 solution, such as 10 or 20 c.c. of N/80, or 5, 10, or 15 c.c. of N/20, as detailed below. The bottles were left at laboratory temperature, except where otherwise stated, for a number of hours, but all for some time. A few drops of a 5 per cent. solution of potassium iodide are then added, so as to give excess and set free iodine corresponding to the unused excess of potassium permanganate, and the iodine was then titrated in the usual way with a N/40 solution of sodium thiosulphate, using starch indicator.

EXPERIMENT I.—The water used for this experiment was taken on the steam yacht 'Runa,' about four miles west of Port Erin, in the open sea. It was filtered on board the yacht, and the potassium permanganate and sulphuric acid were at once added on board, and it was then taken to the laboratory for analysis. In addition, about five litres of the completely filtered water were retained for determinations of the free and saline

1. *Analyst*, Vol. X, 1885.

2. See Sutton, *Volumetric Analysis*, tenth edition, 1911, pp. 484-488.

ammonia and of the organic or albuminoid ammonia. The removal of the non-dissolved constituents took place in two stages. The water coming up from the sea in a rubber hose pipe of about one inch bore (2.5 centimetres), which was sunk about 4 metres in the sea, before passing to the Chamberland pump was attached to a piece of wide glass tubing about two feet long. This glass tube passed through a cork about four inches from one end. The cork fitted into the neck of an ordinary filter flask, such as is used with a suction pump in ordinary laboratory work. A piece of the finest silk netting (20 mesh gauze) was used to make a tube about five inches long, somewhat narrower at the closed end, and fitting by its wider open end around the cork, so that it was enclosed airtight between the cork and the neck of the filter flask, and all the water filtered had to pass through it. The side outlet of the filter flask was connected by a second short piece of hose with the Chamberland pump. The silk-netting accordingly retained the amount of plankton separated from the quantity of water filtered in any such experiment, the filter flask contained water which had been silk filtered only, the deposit on the Chamberland candle at the end gave the non-soluble matter in the volume of water filtered, which was too small to be removed by the silk netting, and the completely filtered water was obtained and measured as it came from the pump. At the time of the experiment a sample of unfiltered water was taken by the closing water bottle from the same depth as the open end of the inlet hose. In this way five things, viz., two deposits and three waters, were taken at one time from the same spot for analysis.

The quantity of water filtered in this particular experiment was forty litres. Two samples of the Chamberland filtered water, and one sample each of the unfiltered and silk-filtered waters, were analysed, 10 c.c. of $N/80 \text{ KMnO}_4$ (1 c.c. = 0.0001 gram of oxygen) being added to each, and all being then kept in the incubator at 25°C . for four hours. As the first amounts added were decolourized, 10 c.c. of $N/20 \text{ KMnO}_4$ were added later in each case. The results obtained are shown in the following table in milligrams of oxygen required for oxidation of organic matter per litre, but as explained above these results really show that no organic matter is present, for the amounts are no greater than those shown in Experiment V, D, where no organic matter is present, and merely represent a small amount of hydrochloric acid decomposed with liberation of chlorine and its partial escape. This loss is higher than in subsequent experiments, because the samples were heated in an incubator to 25°C . instead of being kept at air temperature.

No. 1.	Chamberland filtered water	=3.16	mgms.	per	litre.
No. 1a.	„ „ „	=3.16	„ „		
No. 2.	Silk filtered water	=2.48	„ „		
No. 3.	Unfiltered water	=3.84	„ „		

The fact that the completely filtered samples appear to contain more organic matter than that only filtered through silk, is confirmatory evidence that it is not really organic matter which is determined. But even making no allowance for experimental errors, it is seen that the amount of organic matter possibly present taken at a maximum is very small.

As at this period the disturbing influence of the chlorides was not fully appreciated, the two deposits on silk and Chamberland candle, respectively, were taken up in sea-water in this experiment and the next one. In later experiments the deposits were taken up in distilled water. Allowances based on the above data were made as deductions for the amounts of filtered sea-water so used in taking up the residue. The residue on the silk-netting, which was very small in amount, required permanganate representing 11.6 mgms, and as this came from 40 litres, the amount of plankton, separable from the sea-water by such silk, corresponded to 0.29 mgms. of oxygen¹ per litre. Similarly, the Chamberland deposit, which was more copious, was taken, mixed thoroughly with 400 c.c. of filtered sea-water, and 50 c.c. were taken for the analysis. This portion was equivalent to 4.55 mgms., or 36.4 in the whole amount of 40 litres, which works out at 0.91 mgms. of oxygen per litre. The total amount of matter in suspension is therefore equivalent in oxygen to:—silk=0.29, Chamberland 0.91=1.2 mgms. of total plankton. This figure is free from the chloride error, and as the titration figures are quite large, it probably fairly accurately represents the plankton on that day, when fairly heavy takes were being recorded by the ordinary tow-nettings of the Biologists on board. It is to be noted that the Chamberland here retains about three times the quantity removed by the silk.

The free and saline, and albuminoid ammonia as determined by the usual water analysis method, and testing by Nessler's reagent against standard dilutions of ammonium chloride solution, gave 0.04 parts per million of free and saline ammonia, i.e., 0.04 mgms. per litre, and of albuminoid ammonia 0.2 parts per million or 0.2 mgm. per litre. If all

1. One milligram of oxygen may be roughly taken as equivalent to 2.5 milligrams of organic matter, and this factor must be used throughout, see Table at end of this section of the paper.

this albuminoid ammonia be taken as protein it must be multiplied by the factor 5 to get protein, which would give 1 mgm. per litre of protein.

EXPERIMENT II. In this experiment the oxidation with permanganate and sulphuric acid was carried out at laboratory temperature for a period of about fifteen hours. The volume of water filtered was 43 litres. It was taken from three to four metres below the surface, on the yacht 'Runa,' on the afternoon of the same day as that of the preceding experiment, the situation from which it was taken was about three miles north-west of Port Erin in the open sea.

The analyses concern one sample of water completely filtered through Chamberland, two samples filtered through silk only, one of unfiltered water, one the deposit retained by the silk, and one of the deposit on the Chamberland candle.

The water analyses gave the following figures, to which the same remarks apply as in the preceding experiment:—

No. 1.	Chamberland filtered water	= 2.24	mgms.	per litre
No. 2.	Silk filtered water	= 1.89	„	„
No. 2a.	„ „	= 2.24	„	„
No. 3.	Unfiltered water	= 1.73	„	„

The deposit on the silk-netting, taken up in 240 c.c. of filtered sea-water, required 6.35 mgms. of oxygen for oxidation. On making allowance for the sea-water, this gave 5.79 mgms. in 43 litres, or 0.13 mgms. of oxygen per litre.

The deposit on the Chamberland candle was taken up in 600 c.c. of filtered sea-water, and 50 c.c. of this, or $\frac{1}{12}$ th, with 50 c.c. additional of filtered sea-water were taken for analysis. Its organic matter required 2.75 mgms. of oxygen, which amounts to 2.64 mgms. for the fraction of the residue only, multiplied by 12, this gives 31.68 mgms. from the 43 litres, or 0.74 mgm. per litre. The total suspended matter per litre is accordingly represented by 0.87 mgm. of oxygen, of which about $\frac{1}{6}$ is removed by the silk and $\frac{5}{6}$ by the Chamberland.

The ammonia determinations were made in this case on the unfiltered water, and gave:—Free and saline ammonia=0.1 mgm. per litre; albuminoid ammonia=0.16 mgm. per litre. These do not differ greatly from the results of the previous experiment with filtered water, and illustrate the excessively low organic content of pure sea-water whether filtered or unfiltered.

EXPERIMENT III. The oxidation in this experiment was also carried out in the cold for a period of fifteen hours, and phosphoric acid (orthophosphoric prepared by acid combustion and oxidation of phosphorus in the laboratory) was substituted for sulphuric acid to attempt to minimize loss of chlorine from the chlorides as recommended by Dupré (*loc cit.*). No difference, however, was found, as will be seen by comparing the results with sea-salt and 'Falk' salt after ignition as shown in Experiment V, D.

The water was filtered and treated on the yacht, taken on Saturday morning, April 13th.

The volume of water filtered was 53·2 litres. The silk netting deposit was so meagre that it was not regarded as worth doing, and it may be remarked that the ordinary tow-nettings taken on that occasion for biological purposes were very meagre. There was no visible deposit on the silk-netting in this experiment at the end of filtration of the 53·2 litres.

One sample each of the three conditions of the water were analysed for oxidizable organic matter, using in all 25 c.c. of N/20 KMnO_4 and 10 c.c. of 25 per cent. phosphoric acid to 250 c.c. of sea-water, the amounts used up of the standard KMnO_4 were only 0·7, 2·2, and 2·3 c.c. respectively in the three samples, and the results per litre work out as follows:—

1. Chamberland filtered water	= 1·12 mgms. per litre.
2. Silk filtered water	= 3·52 „ „
3. Unfiltered water	= 3·68 „ „

The residue in the Chamberland candle showed 9·38 mgms. of oxygen required for 53·2 litres, corresponding to 0·18 mgms. per litre.

In this experiment, both filtered and unfiltered water were analysed for ammonia with the following results:—

Unfiltered water:—Free and saline ammonia = 0·06 per million or 0·06 mgm. per litre; albuminoid ammonia = 0·17 per million or 0·17 mgm. per litre.

Chamberland filtered water:—Free and saline ammonia = 0·02 per million, or 0·02 mgm. per litre; albuminoid ammonia = 0·1 per million or 0·1 mgm. per litre.

Such a water would be put down as excellent amongst potable waters as regards organic matter. The figures again show what an infinitesimal trace of organic matter is present, especially in the filtered water, which contains less than the unfiltered, but all figures lie at the limit.

EXPERIMENT IV. The water used in this experiment was taken, on

the yacht, about 200 yards off Sugar Loaf Rock, outside Calf Island Sound in deep water, about two metres from surface, on Monday afternoon, April 15th. The volume of water filtered was 25·2 litres. The oxidation was carried out in the cold for fifteen hours, using 20 c.c. of $N/80 \text{ KMnO}_4$ and 10 c.c. of 25 per cent. glacial phosphoric acid to the 250 c.c. sample of water. The reagents, as usual, were added at once on the yacht.

The results were as follows:—

No. 1. Chamberland filtered water	= 0·82	mgms.	per litre.
No. 2. Silk filtered water	= 0·82	,,	,,
No. 3. Unfiltered water	= 0·89	,,	,,

In regard to the silk-netting and Chamberland candle deposits, these, instead of being taken up in sea-water, were washed off by distilled water, so as to get rid of adverse influence of chlorides. The entire residue was taken in each case, in 250 c.c. of distilled water, and 10 c.c. of $N/20 \text{ KMnO}_4$ and 10 c.c. of 25 per cent. glacial phosphoric acid were used for oxidation.

The silk-netting deposit was oxidised by 0·664 mgm. of oxygen, which represents 0·03 mgm. of oxygen per litre.

The Chamberland candle deposit required 2·33 mgms., which gives 0·09 mgm. of oxygen per litre.

The total plankton is accordingly represented by 0·12 mgm. of oxygen per litre.

The unfiltered water gave the following low figures in the ammonia determinations:—Free and saline ammonia = 0·035 per million, and albuminoid ammonia = 0·08 per million.

EXPERIMENT V. The sea-water used in this experiment was pumped from the beach at the shore end of Port Erin Breakwater, when the water was about eight or nine feet deep, and the inlet hose was about five or six feet below the surface. A volume of 140 litres was pumped, and in addition to the analyses this water was utilized for Experiment VII of Section B for the oxygen exchanges of the animals.

Twelve analyses of oxidizable organic matter with permanganate were made in all in this experiment. Six of these were with the three conditions of the water, viz., Chamberland, and silk filtered, and unfiltered (*a*) in acid solution, and (*b*) in alkaline solution. Four were made on the two deposits, on silk and on Chamberland, (*a*) in acid and (*b*) in alkaline solution.

Two analyses were made upon 8 grams of 'Falk' salt made up, after

ignition to destroy any possible organic matter, to 250 c.c. with distilled water, so as to give an artificial sea-water free from any organic matter to serve as a control. All were carried out alongside of one another in similar bottles of equal size, and all conditions made as closely identical as possible.

The results of these two controls may also be used for the previous experiments, and show that the apparent small amounts of organic matter can all be accounted for on the basis of chloride decomposition.

The results were as follows:—

(A) WATERS IN ACID SOLUTION

No. 1. *Unfiltered water*

250 c.c. + 20 c.c. N/80 KMnO_4 + 10 c.c. of 25 per cent. glacial phosphoric acid, used up 1.4 c.c. = 0.56 mgm. per litre.

No. 2. *Silk-filtered water*

250 c.c. + 20 c.c. N/80 KMnO_4 + 10 c.c. of 25 per cent. glacial phosphoric acid, used up 1.16 c.c. = 0.46 mgm. per litre.

No. 3. *Chamberland filtered water*

250 c.c. + 20 c.c. N/80 KMnO_4 + 10 c.c. of 25 per cent. glacial phosphoric acid, used up 0.96 c.c. = 0.38 mgm. per litre.

(B) WATERS IN ALKALINE SOLUTION

No. 4. *Unfiltered water*

250 c.c. + 20 c.c. N/80 KMnO_4 + 5 c.c. N NaOH, at the end of 20 hours made acid (with glacial phosphoric acid 25 per cent., 11 c.c.) and titrated used up 2 c.c. = 0.48 mgm. per litre.

No. 5. *Silk-filtered water*

250 c.c. + 20 c.c. N/80 KMnO_4 + 5 c.c. N NaOH, titrated as above in acid, used up 1.16 c.c. = 0.28 mgm. per litre.

No. 6. *Chamberland filtered water*

250 c.c. + 20 c.c. N/80 KMnO_4 + 5 c.c. N NaOH, titrated in acid, used up 1.16 c.c. = 0.28 mgm. per litre.

(C) DEPOSITS IN ACID AND ALKALINE OXIDATION

No. 7. *Silk-netting Deposit oxidized in acid solution*

One half of the deposit + 250 c.c. distilled water + 10 c.c. N/20 KMnO_4 + 10 c.c. of 25 per cent. glacial phosphoric acid, after 20 hours at air temperature, used up 1.66 c.c. of N/20 KMnO_4 = 0.664 mgm. = 1.33 mgm. in whole deposit, this in 140 litres = 0.009 mgm. of oxygen per litre.

No. 8. *Silk-netting Deposit oxidized in alkaline solution*

One half of the deposit + 250 c.c. distilled water + 10 c.c. N/20 KMnO_4 + 5 c.c. N NaOH, after 20 hours at air temperature, made acid with 11 c.c. of 25 per cent. glacial phosphoric acid, used up 3.16 of N/20 KMnO_4 = 0.758 mgm., so in whole deposit = 1.516 mgm., as this is in 140 litres, the amount is 0.011 mgm. of oxygen per litre.

No. 9. *Chamberland deposit in acid solution*

One-tenth of Chamberland deposit + 250 c.c. of distilled water + 10 c.c. N/20 KMnO_4 + 10 c.c. of 25 per cent. glacial phosphoric acid, left 20 hours at air temperature, used up 2.4 c.c. of N/20 KMnO_4 = 0.96 mgm., therefore whole deposit = 9.6 mgms., this in 140 litres = 0.069 mgm. of oxygen per litre.

No. 10. *Chamberland deposit in alkaline solution*

One-tenth of Chamberland deposit + 250 c.c. of distilled water + 10 c.c. of N/20 KMnO_4 + 5 c.c. N NaOH., at end of 20 hours at air temperature made acid with 11 c.c. of 25 per cent. glacial phosphoric acid, used up 3.8 c.c. of N/20 KMnO_4 = 0.92 mgm., so in whole deposit there are 9.2 mgm., as this is in 140 litres the amount per litre is 0.066 mgm. of oxygen.

The total suspended matter removable both by silk and Chamberland is when oxidized in acid solution = 0.078 mgm., in alkaline solution 0.077 mgm. In working out the calculation it is to be remembered that the oxidizing power of the permanganate in alkaline solution is only three-fifths of its power in acid solution. Also the end titration in both cases is in acid solution. These concordant results demonstrate clearly that the oxidizing value of the organic matter in suspension, or total plankton in this water, is less than 0.1 mgm. per litre. The factor for conversion of oxygen used into total organic matter, as shown by combustion, ranges between two and six for different waters, taking the highest value, the maximum amount of organic matter in the total plankton lies at 0.4 mgm. per litre.¹ The amount of material available in the deposits from 140 litres was quite sufficient to give good figures, there were practically no chlorides present, and there is, therefore, little question as to the accuracy of these figures.

1. The remaining eight-tenths of the Chamberland deposit was dried and weighed and found to be 0.195 gram, this gives 0.247 gram for the entire deposit. This deposit was found by careful incineration to contain 77.5 per cent. of ash and 22.5 per cent. of organic matter. The amount of organic matter in the total 140 litres stopped by the Chamberland filter was accordingly 56 milligrams or 0.4 milligram per litre. On comparison with the result by the permanganate method in the text, this yields a multiplier of 6.

(D) 'FALK' SALT AFTER INCINERATION

No. 11. '*Falk*' salt (ignited), oxidized in acid solution

Eight grams 'Falk' salt + 250 cc. distilled water + 20 c.c. N/80 KMnO_4 + 10 c.c. of 25 per cent. glacial phosphoric acid left for 20 hours at air temperature, used up 3.52 c.c. of N/80 KMnO_4 = 1.40 mgms. per litre.

No. 12. '*Falk*' salt (ignited), oxidized in alkaline solution

Eight grams of 'Falk' salt + 250 c.c. of distilled water + 20 c.c. of N/80 KMnO_4 + 5 c.c. N NaOH, left at air temperature for 20 hours, then made acid with 11 c.c. of 25 per cent. glacial phosphoric acid and titrated, used up 4.72 c.c. of N/80 KMnO_4 , equivalent to 1.13 mgms. per litre.

These two samples of artificial sea-water, quite free from organic matter, hence show more organic matter per litre than the natural water, and prove that the slight amounts of organic matter there indicated lie within the limits of experimental error, and are probably due to slight chlorine escape.

The ammonia figures were also obtained for this filtered sea-water and confirm the above results in showing the usual amounts of organic matter present. The results are as follows:—Free and saline ammonia = 0.04 per million; albuminoid ammonia = 0.21 per million.

EXPERIMENT VI. This experiment was carried out to provide more controls than those mentioned in the previous experiment.

No. 1. *Sea-water titrated at once without allowing time for oxidation.*

A volume of 250 c.c. of Chamberland filtered sea-water was taken, 10 c.c. of N/20 KMnO_4 , and 10 c.c. of 25 per cent. sulphuric acid, then excess of potassium iodide solution was added at once without waiting and it was at once titrated. It appeared to have used up 0.8 c.c. of N/40 thiosulphate = 1.6 c.c. of N/80 KMnO_4 = 0.16 mgm. of oxygen or 0.64 mgm. per litre.

No. 2. A volume of 250 c.c. of sea-water was taken to dryness in a porcelain evaporating dish, and incinerated over a Bunsen burner. The salt turned slightly yellowish and then white. The residue in which organic matter had been thus destroyed was taken up in 250 c.c. of distilled water + 15 c.c. of N/20 KMnO_4 + 10 c.c. of 25 per cent. sulphuric

acid, after standing 15 hours this had used up 2.1 c.c. of N/20 KMnO_4 = 0.84 mgm. of oxygen or 3.36 mgm. per litre.

No. 3. A similar experiment but kept for 15 hours in the oven at 25° C., used up 4.4 c.c. of N/20 KMnO_4 = 1.76 mgm. or 7.04 mgms. per litre.

No. 4. Eight grams 'Falk' salt (unincinerated) + 250 c.c. distilled water + 20 c.c. N/80 KMnO_4 + 10 c.c. of 25 per cent. glacial phosphoric, for 24 hours at air temperature, uses up 1.8 c.c. = 0.18 mgm. of oxygen or 0.72 mgm. per litre.

No. 5. Same as No. 4, but 'Falk' salt, incinerated beforehand, uses up 2.1 c.c. = 0.21 mgm. oxygen or 0.84 mgm. per litre.

No. 6. Same amounts as Nos. 4 and 5, but in alkali instead of acid and unincinerated, uses up 4.6 c.c., which with factor of 3 instead of 5 oxygen, amounts to 0.27 mgm or 1.08 mgm. per litre.

No. 7. Same as No. 6, also in alkali, but the 'Falk' salt incinerated, used up 4.3 c.c. = 0.26 mgm., or 1.04 mgm. per litre.

No. 8. Sea-water, 250 c.c. evaporated down and incinerated, gave 8.2 grams of salt, this made up again to 250 c.c. with distilled water, 20 c.c. of N/80 KMnO_4 and 10 c.c. of 25 per cent. glacial phosphoric acid added, and left 24 hours at room temperature alongside Nos. 4 to 7 of this series, used up 2.7 c.c. = 0.27 mgm., or 1.04 mgm. in one litre.

It appears, accordingly, from the results of these controls that an amount of 1 to 3 mgms. per litre must be deducted from the results obtained above with the sea-water and the various filtrates, in order to show any organic matter, and when this is done all organic matter disappears; in other words, dissolved organic matter in pure sea-water is so small in amount as to fall within experimental error, and in the average certainly can be safely said not to exceed one mgm. per litre.

In other words, the titration figure of the permanganate solution in presence of so much chlorides is slightly lower than in distilled water, so falsely showing a trace of organic matter if compared with the titration in distilled water; when allowance is made for this, no organic matter is shown by this method, which would certainly detect it if any appreciable amount were present.

For this reason, the organic ammonia in the case of sea-water gives a more satisfactory guide. Even when all the organic ammonia is taken as protein, there is less than 1 mgm. per litre present, and it is probable that only a fraction is present as protein.

In all cases analysed, the amount of organic matter as plankton is surprisingly low. Although the Chamberland filtration removes about three times as much as silk-net filtration, the two together only form a fraction of a milligram per litre.

It may be concluded that at the season and under the conditions of our experiments, the combined dissolved and suspended organic matter does not on the average much exceed 1 to 2 mgms. per litre.

The various results of Experiments I to V on amounts of plankton are shown in the accompanying Table, in which the last column gives organic matter obtained by multiplying the oxygen consumed by 2.5, the average factor¹:—

TOTAL PLANKTON IN MILLIGRAMS PER LITRE						
		Silk Netting.	Chamberland.	Total.	Organic Matter.	
Expt. I	...	0.29	0.91	1.2	3.00	
Expt. II	...	0.13	0.74	0.87	2.17	
Expt. III	...	Nil.	0.18	0.18	0.45	
Expt. IV	...	0.03	0.09	0.12	0.30	
Expt. V, in acid	...	0.009	0.069	0.078	0.19	
„ in alkali		0.011	0.066	0.077	0.19	

Section B. THE RATE OF OXIDATION OF ORGANIC MATTER IN CERTAIN MARINE ANIMALS

The amount of oxidizable organic matter in solution and suspension in sea-water, under conditions of average distribution, having been determined as nearly as possible in the preceding experiments, we passed on to obtain some idea of the rate at which certain marine animals consumed organic matter, as measured by the rate at which they consumed oxygen dissolved in the water. Three series of experiments were made, in the first two series the animals were used as soon as taken from the sea, and the considerable amount of excrement in some cases showed that digestion was actively proceeding; in the third, the animals were kept without feeding, in filtered sea-water for 24 hours before commencing the experiment, in order to obtain results in the analyses of the water afterwards as free as might be from the disturbing influence of excretion of solid matter.

In the latter case the period of deprivation of food was not of

1. The actual value of this factor cannot be determined for the sea-water. The estimation of the organic matter in the Chamberland deposit of Expt. V leads as already indicated to a value of 6, but this is probably too high.

sufficient duration so far to reduce the reserve store of metabolized material as to decrease the rate of using up of food by oxidation. The volume of sea-water allowed was in most cases large compared to the volume of the animals, and under such conditions it might have been expected on the hypothesis of Pütter that the amount of dissolved organic matter would have decreased, but, on the contrary, it was always considerably augmented.

Both this increased amount of dissolved and suspended organic matter, and the amount which had disappeared, corresponding to the amount of oxygen used up by the animal in its respiratory exchanges, must be added together to indicate the amount which under natural conditions would have to be replaced by the food of the animal.

These two quantities of organic matter are the index of the demand of the animal for a food supply, the amount of the oxygen used up gives a measure of the energy utilized by the animal, and the organic matter added to the water shows the amount passed through without utilization.

Each type of animal was kept separately for an observed time-interval in a large wide-mouthed bottle, the average volume of the bottle being 2,800 c.c. A control bottle was kept under like conditions throughout the experiment without any animals, so as to give a deduction for any consumption of oxygen by bacteria or small plankton during the experiment, but the loss of oxygen in this way was found to be very small.

In starting the experiment the animal was transferred to the bottle in which it was to be kept, and well washed with filtered sea-water coming directly from the Chamberland pump. The bottle was then filled in a large clean bucket, which was also full of the filtered water, so that the lid or stopper of the bottle could be slipped on under water without including any air. Each experiment was carried out in two stages. Samples were taken out at the expiry of the first period mentioned in each case for the determinations to be indicated presently, and then a sufficient amount of filtered sea-water was added to replace that removed, and the experiment allowed to proceed overnight till the following day, when it was finally interrupted and the determinations made.

The most important determination in this series was (*a*) the amount of dissolved oxygen in the measured volume of water at the beginning and end of the experiment, but in addition (*b*) the output of carbon dioxide was determined by titration, as described below, of the water at beginning and end with centinormal caustic alkali using phenol-phthalein

as indicator, (c) the oxidizable organic matter by the permanganate method as in Section A, (d) the free and saline and organic ammonia, (e) the moist weight of the animals, the total dried weight, the weight of organic matter when dried, the weight of skeleton, and the ash in the skeleton.

The method used for determining the dissolved oxygen was that of Winckler, which has been so often described in connection with similar work and in text-books of water analyses,¹ that it need not be given in detail. In principle it consists in utilizing the free dissolved oxygen to convert manganous to manganic oxide, then by acting upon this with hydrochloric acid in presence of hydriodic acid to set free the corresponding amount of iodine, which is then titrated in the usual fashion by standard sodium thiosulphate in presence of starch indicator.

The work is carried out in an ordinary narrow-mouthed bottle of about 300 c.c. capacity, such as is used for bench reagents in the laboratory. This is completely filled with water, so that the stopper is insertable without any air-bubbles, then the stopper is removed, and the volume it contains is measured and noted. Such a bottle of measured volume is completely filled with the sample of sea-water to be estimated, taking care this is not aerated in the process. By means of a pipette 1 c.c. of a 10 per cent. solution of manganous chloride is introduced to the bottom of the bottle, and followed by 3 c.c. of a mixture of saturated caustic potash containing 10 per cent. of potassium iodide, also passed in at the bottom. The stopper of the bottle is then inserted without introducing any air-bubbles and the contents mixed by rotation. The bottle is left for some time for the manganous hydrate to take up all oxygen and settle to the bottom. In about half an hour, the bottle may be opened and 5 c.c. of concentrated hydrochloric acid passed to the bottom by a pipette, the stopper is again inserted and the contents thoroughly mixed, when the acid re-dissolves the mixed hydrates of manganese, and an amount of iodine is set free corresponding to the amount of oxygen in the water. The contents are transferred to a flask and titrated with thiosulphate.

EXPERIMENT VII. The water used in this experiment was Chamberland filtered, being a portion of the 140 litres pumped from beside the Breakwater as described in Experiment V, Section A. Three large wide-mouthed bottles of 2,800 c.c. capacity were completely filled,

1. See Sutton's *Volumetric Analysis*, tenth edition, 1911.

the lids being fitted on under the sea-water, so as to exclude all air, and contained as follows:—(a) Control containing the filtered sea-water only, (b) a large-sized sponge, (c) a group of ascidians.

The experiment was commenced at 11 a.m., April 16th, and was interrupted in $8\frac{1}{2}$ hours, viz., at 7.30 p.m., to take samples for determination of dissolved oxygen, a small volume of fresh sea-water was then added to replace that removed for analysis (about 300 c.c.), and the experiment was allowed to continue till 11 a.m. on April 18th; that is, for 48 hours in all. The results of the two intervals are shown in Table I, Part 1 and Part 2, as under:—

TABLE I.—*Part 1*Dissolved Oxygen after $8\frac{1}{2}$ hours

		Milligrams per litre.	Total mg. in 2,800 c.c.	Milligrams of Oxygen used by Animals.
(a) Control	...	7.93	22.20	—
(b) Sponge	...	7.42	20.77	1.43
(c) Ascidians	...	7.00	19.60	2.60

TABLE I.—*Part 2*

Determinations at end of 48 hours

1. Dissolved Oxygen used up

		Milligrams per litre.	Milligrams in 2,800 c.c.	Milligrams used by Animals in 48 hours.
(a) Control	...	7.02	19.66	—
(b) Sponge	...	6.46	18.09	1.57
(c) Ascidians	...	6.82	19.10	0.56

2. Oxygen given out in form of Carbon dioxide

		Milligrams per litre.	Milligrams in 2,800 c.c.	Milligrams excreted by Animals in 48 hours.	Respiratory Quotient.
(a) Control	...	1.28	3.58	—	—
(b) Sponge	...	2.40	6.72	3.14	2.1
(c) Ascidians	...	1.12	3.14	0.44	Indeterminate

3. Oxygen required for oxidation of organic matter

		Milligrams per litre.	Milligrams in 2,800 c.c.	Added to water by Animals.
(a) Control	...	1.04	2.91	—
(b) Sponge	...	1.84	4.15	1.24
(c) Ascidians	...	1.16	3.25	0.34

4. Ammonia determinations (a) Free and Saline, (b) Albuminoid

FREE AND SALINE.				ALBUMINOID.		
	Mg. per litre.	Mg. Total.	Mg. excreted by Animals.	Mg. per litre.	Mg. Total.	Mg. excreted by Animals.
(a) Control ...	0·07	0·20	—	0·22	0·62	—
(b) Sponge ...	0·33	0·92	0·72	1·20	3·36	2·74
(c) Ascidians ...	0·03	0·08	0·12	0·42	1·18	0·56

5. Constants for Animals (Weights, etc.)

		Moist Weight in grms.	Total Dry Weight.
(a) Control ...		Nil	—
(b) Sponge ...		43·9	9·345
(c) Ascidians ...		11·3	1·092

REMARKS.—The chief points to be noted about this experiment are:—(1) That there was an ample supply of water, because when allowance is made for specific gravity, the volume of the Sponge would be about 40 c.c., and that of the Ascidians 10 c.c., and as the volume of water was 2,800 c.c., the Sponge had about 70 times its volume of water and the Ascidians about 280 times their own volume of water. Also the supply of oxygen was ample, as shown by the slight fall in the 48 hours. With larger volumes it would have been impossible to estimate the gaseous exchanges. (2) The very small amounts of oxygen required by these two types of animals in the 48 hours, less than two milligrams by the Sponge, and about half a milligram by the Ascidians, and the very much greater output of carbon dioxide by the Sponge, the amount for the Ascidians, although it is negative apparently, probably lies within the limits of experimental error. (3) So far from using up any dissolved organic matter, both types distinctly increase the amount of organic matter in the water, and this is confirmed by two quite independent methods, viz., oxidation by permanganate and determination of albuminoid ammonia. Permanganate oxidation is not complete, and, on an average, multiplication of the weight of oxygen used by 2·5 gives the weight of organic matter present. In the case of the Sponge this gives 3·1 mgms., and for the Ascidians 0·85 mgm. of organic matter excreted and added to the water in the 48 hours. (4) In the complete oxidations of respiration, the weight of oxygen required may approximately be taken as equal to the weight of organic matter combusted. If the higher figure of the oxygen in the carbon dioxide be taken, the Sponge shows about 3 mgms.

combusted. Similarly, taking the higher figure of oxygen consumed for the Ascidians, the amount combusted is about 0·6 mgm. (5) Adding together the organic matter under sources (3) and (4), the probable amounts used in the 48 hours are: Sponge, 6 mgms.; Ascidians, 1·5 mgms. (6) The determinations given under Experiment V, for the total plankton in the water used for the experiment, are very concordant for the acid and alkaline oxidation, giving for oxygen used, 0·078 in acid, and 0·077 mgm. in alkali per litre, taking 0·08 mgm. as a round number and multiplying by the factor 2·5, the amount of organic matter in the plankton becomes 0·2 mgm. per litre. (7) From these data it can be calculated what volume of sea-water would require to pass through each type. The amount works out at 30 litres for the Sponge, and 7·5 litres for the Ascidians, in 48 hours, or approximately 600 c.c. per hour for the Sponge and 150 c.c. per hour for the Ascidians. Taking the volume of organism used as the unit, viz., 40 c.c. and 10 c.c., this may be expressed by saying that in each case the organism requires fifteen times its own volume of sea-water per hour. It may be pointed out that this sample of water was the lowest of the series in plankton, and on the other hand, that in the sea the animals would possibly have been more active and had a higher metabolism.

EXPERIMENT VIII. This experiment was also carried out with Chamberland filtered water obtained from the same spot by the Breakwater on April 19th. The details were as described in the previous experiment. The experiment was commenced at 4 p.m., April 19th, was interrupted to take oxygen samples at 8 p.m. (i.e., in 4 hours), a small volume of water was added to replace that removed, and the experiment allowed to go on till next morning at 11 a.m. (i.e., total duration was 19 hours). Volume of filtered sea-water=2,800 c.c. in each case. The results are shown in Table II.

TABLE II.—*Part 1*
Time-interval=4 hours

Dissolved Oxygen used up		Milligrams per litre present at end.	Milligrams in 2,800 c.c.	Milligrams used by Animals.
(a) Control	...	7·42	20·78	—
(b) <i>Aplysia</i> (6 specimens)		7·48	20·94	0·16
(c) <i>Fusus</i>	...	6·63	18·56	2·22
(d) <i>Echinus</i>	...	7·33	20·52	0·26
(e) Cancer	...	1·07	2·99	17·79

TABLE II.—*Part 2*

Determinations at end of 19 hours

1. Dissolved Oxygen

		Milligrams per litre at end.	Milligrams in 2,800 c.c.	Milligrams used by Animals in 19 hours.
(a) Control	...	7.94	22.23	—
(b) Aplysia	...	6.37	17.84	4.39
(c) Fusus	...	2.75	7.70	14.53
(d) Echinus	...	6.76	18.93	3.30
(e) Cancer	...	0.98	2.74	19.49

2. Oxygen given out in form of Carbon dioxide

		Milligrams per litre.	Milligrams in 2,800 c.c.	Amount formed by Animals.	Respiratory Quotient.
(a) Control	...	2.2	6.16	—	—
(b) Aplysia	...	6.4	17.92	11.76	2.68
(c) Fusus	...	12.0	33.60	27.44	1.88
(d) Echinus	...	3.8	8.40	2.24	0.68
(e) Cancer	...	17.6	49.28	43.12	2.21

3. Ammonia excreted (a) Free and Saline, (b) Albuminoid

FREE AND SALINE			ALBUMINOID			
	Mg. per litre.	Mg. Total.	Mg. excreted by Animals.	Mg. per litre.	Mg. Total.	Mg. excreted by Animals.
(a) Control	...	0.07	0.20	—	0.22	0.62
(b) Aplysia	...	0.16	0.45	0.25	0.54	1.51
(c) Fusus	...	0.87	2.44	2.24	1.10	3.08
(d) Echinus	...	0.14	0.39	0.19	0.35	0.98
(e) Cancer	...	0.11	0.31	0.11	0.49	1.32

4. Constants of Animals (weights, etc.) in grams

	Moist Weights.	Total Dry Weights.
(a) Control	...	—
(b) Aplysia	...	47.95
		3.935
		Soft Parts + Skeleton.
(c) Fusus	...	249.80 (115 grams soft parts)
(d) Echinus	...	277.15
(e) Cancer	...	236.20

REMARKS.—(1) The most remarkable feature in this experiment is the great consumption of oxygen by the crustacean, as compared with the other types. Thus in the first four hours nearly 18 mgms. of oxygen are

used up, and the dissolved oxygen has been reduced to a very low level. In the remaining 15 hours, the available oxygen was so little that only 2·30 mgms. was used in this longer period, while the total output of oxygen as carbon dioxide for the nineteen hours was 43·12 mgms., or more than double the oxygen taken in during the same period. Similar results with another species of crab of smaller size are seen in the next experiment, and these findings seem to us of such importance that it is our intention to make a special and more detailed examination of the matter. Both animals, although very quiescent, were quite healthy at the end, and recovered and became quite active when placed in ordinary fresh oxygenated water. On account of the rapid fall in dissolved oxygen, it is probable that the 17·79 grams used up in the first four hours do not represent the full oxidation if the dissolved oxygen had not fallen, but taking them as a basis for calculation, the hourly requirement would be 4·45 mgms., and if the total plankton be again taken at 0·08 mgm. per litre, this would require 55 litres of water completely cleared per hour, or approximately 250 times the animal's own volume per hour. This would appear to demonstrate that such a type of animal can only live by the capture of food in other ways. (2) It is again to be noted that the animals add dissolved organic matter to the water in all cases, and that there is accordingly no evidence of consumption of dissolved organic matter by any of these types. (3) With the exception of *Echinus esculentus*, where the production of carbon dioxide and uptake of oxygen were both so small as to be liable to considerable experimental error, the oxygen given out as carbon dioxide is much higher than the oxygen intake from the water, so leading to such respiratory quotients as 1·88, 2·21, 2·68, the possible meaning of this peculiar respiratory phenomenon is discussed at the end of this section. (4) The greater output of albuminoid nitrogen in the case of *Fusus* is in all probability due to the large amount of mucin excreted by this type. (5) Calculations of the volumes of water containing the amount of organic matter as plankton required, on the basis of 0·2 mgm. per litre, yield the following results:—*Aplysia*, 3·7 litres per hour or 82 volumes per hour; *Fusus*, 10 litres per hour or 90 times volume of soft parts per hour; *Cancer* has already been given at 250 times its volume per hour. These values would appear too great to support purely plankton feeding.

EXPERIMENT IX. This experiment was carried out with Chamberland filtered water pumped from the supply to the Fish Hatchery. The details were as described under Experiments VII and VIII. The

experiment was commenced at 12 noon, April 21st, the first part of the experiment lasted $7\frac{1}{2}$ hours, the second $16\frac{1}{2}$ hours, making 24 hours in all. All the animals were alive at the end, at noon, April 22nd. The results are given in Table III.

TABLE III.—*Part 1*
Time-interval = $7\frac{1}{2}$ hours

Dissolved Oxygen used up				Milligrams per litre.	Milligrams in 2,800 c.c.	Milligrams used by Animals.
(a)	Control	...	...	7.64	21.39	—
(b)	Fish (Blennius)	...	...	7.72	21.62	0.23
(c)	Echinoderm (Asterias)	...	...	5.72	16.02	5.37
(d)	Mollusc (Buccinum)	...	...	7.81	21.67	0.28
(e)	Crustacean (Eupagurus)	...	...	2.75	7.70	13.69

TABLE III.—*Part 2*
Determinations at end of 24 hours

1. Dissolved Oxygen used up				Milligrams per litre.	Milligrams in 2,800 c.c.	Milligrams used by Animals.
(a)	Control	...	...	7.34	20.55	—
(b)	Fish (Blennius)	...	...	3.98	11.14	9.41
(c)	Echinoderm (Asterias)	...	...	1.22	3.42	17.13
(d)	Mollusc (Buccinum)	...	...	5.79	16.21	4.34
(e)	Crustacean (Eupagurus)	...	...	0.97	2.72	17.83

2. Oxygen given out as Carbon-dioxide				Milligrams per litre.	Milligrams in 2,800 c.c.	Milligrams excreted by Animals.	Respiratory Quotient.
(a)	Control	...	...	1.92	5.38	—	
(b)	Fish (Blennius)	...	...	7.04	19.71	14.33	1.52
(c)	Echinoderm (Asterias)	...	...	9.60	26.88	21.50	1.25
(d)	Mollusc (Buccinum)	...	...	3.84	10.75	5.37	1.24
(e)	Crustacean (Eupagurus)	...	...	8.96	25.09	19.71	1.11

3. Oxygen required to oxidize organic matter in water at end				Milligrams per litre.	Milligrams in 2,800 c.c.	Milligrams added by Animals.
(a)	Control	...	...	0.38	1.06	—
(b)	Fish (Blennius)	...	...	1.32	3.70	2.64
(c)	Echinoderm (Asterias)	...	...	1.52	4.26	3.20
(d)	Mollusc (Buccinum)	...	...	2.00	5.60	4.54
(e)	Crustacean (Eupagurus)	...	...	1.64	4.59	3.53

4. Ammonia excreted (a) Free and Saline, (b) Albuminoid

			Milligrams per litre.	FREE AND SALINE Mg. in 2,800 c.c.	Mg. excreted by Animals.
(a) Control	...	...	Nil	Nil	—
(b) Fish (Blennius)	...	...	0·07	0·20	0·20
(c) Echinoderm (Asterias)	...	...	0·20	0·56	0·56
(d) Mollusc (Buccinum)	...	...	0·02	0·06	0·06
(e) Crustacean (Eupagurus)	...	...	0·27	0·76	0·76

			Milligrams per litre.	ALBUMINOID M.g. in 2,800 c.c.	Mg. excreted by Animals.
(a) Control	...	...	0·05	0·14	—
(b) Fish (Blennius)	...	...	0·31	0·87	0·73
(c) Echinoderm (Asterias)	...	...	0·41	1·15	1·01
(d) Mollusc (Buccinum)	...	...	0·35	0·98	0·84
(e) Crustacean (Eupagurus)	...	...	0·37	1·04	0·90

5. Constants of animals (weights, etc.) in grams

			Moist Weights.	Dry Weights of Soft Parts.
(a) Control	...	...	—	—
(b) Fish (Blennius)	...	...	10·57	1·918
(c) Echinoderm (Asterias)	...	...	80·60	5·031
(d) Mollusc (Buccinum)	...	...	49·68*	16·565
(e) Crustacean (Eupagurus)	...	...	34·34*	4·046

			Wt. of Dried Skeleton.	Organic Matter in Skeleton.	Ash in Skeleton.
(a) Control	...	...	—	—	—
(b) Fish (Blennius)	...	...	1·486	1·151	0·335
(c) Echinoderm (Asterias)	...	...	10·129	4·024	6·105
(d) Mollusc (Buccinum)	...	...	37·332		
(e) Crustacean (Eupagurus)	...	...	5·757		

*In each case without shell, the Mollusc shell weighed 56·88 grams, the shell inhabited by the Hermit crab was not weighed.

TABLE IV

TEMPERATURE OF SEA AND AIR DURING EXPERIMENTS OF SECTION B.

	Sea.	Air.		Sea.	Air.
April 16th, 1912—			April 18th, 1912—		
Morning	8·0° C.	8·3° C.	Morning ...	8·0° C.	8·6° C.
Afternoon ...	8·6° C.	11·4° C.	Afternoon ...	8·6° C.	9·4° C.
April 17th, 1912—			April 19th, 1912—		
Morning ...	8·0° C.	8·0° C.	Morning ...	8·3° C.	9·0° C.
Afternoon ...	8·6° C.	10·0° C.	Afternoon ...	8·3° C.	10·8° C.
			Sea.	Air.	
April 20th, 1912—					
Morning ...	8·0° C.	9·4° C.			
Afternoon ...	8·0° C.	11·6° C.			

TABLE V

COMPOSITION OF THE SOFT PARTS OF CERTAIN OF THE ANIMALS
USED IN SECTION B.

After removal of the skeleton the soft parts were dried and the percentage of mineral matter and of organic matter, and in the latter of fat and protein were determined. It was found impossible without a great expenditure of labour to estimate carbohydrates on account of the interference of the proteins. All figures are in percentages of total dried weights, it may be especially emphasized that this is so in the case of fat and protein which are not percentages of the organic matter. Attention may be called to the high percentage of inorganic matter even in the soft parts of these animals. Fat means total ethereal extract, and proteins were obtained by estimating the total nitrogen by Kjeldahl's method and multiplying by the factor 6·25.

			Ash.	Organic Matter.	Fat.	Protein.
Sponge ...	...	...	59·54	40·46	2·75	18·8
Ascidians ...	...	...	46·43	53·57	2·35	28·8
Aplysia ...	...	...	28·58	72·42	2·87	37·0
Fusus ...	...	...	12·49	87·51	8·47	52·6
Echinus ...	...	...	60·75	39·25	1·95	7·8
Cancer ...	...	...	10·06	89·94	14·06	50·7

THE RESPIRATORY QUOTIENT IN INVERTEBRATES

In nearly all the cases shown in the tables above, the respiratory quotient is greater than unity; that is to say, the volume of carbon-dioxide produced is greater than that of the oxygen used up. In many cases the over-production of carbon-dioxide is very marked, and in the case of the two crustaceans in the later stages of the experiment, scarcely any oxygen is taken up, while a considerable evolution of carbon-dioxide continues.

Such a process is also found in the ripening processes of seeds and fruits, and has been observed in animals well fed with carbohydrates which are in process of fattening.

A respiratory quotient greater than unity is impossible if complete oxidation of foodstuffs takes place, and such a quotient is an indication of the linking together of a reducing and an oxidizing agency, whereby a smaller quantity of a substance of higher chemical energy potential is formed, so setting free oxygen which is used in oxidizing more material, as a result of which energy for the use of the organism is set free without any intake of oxygen.

If carbohydrate be completely oxidized, since hydrogen and oxygen are present in the proportions to form water, a volume of carbon-dioxide is set free exactly equal to that of the oxygen used for combustion, so that the respiratory quotient is unity.

If fat be completely oxidized, since there is a large excess of hydrogen, this uses up a great deal of oxygen which appears as the end-product, water, and as a result the carbon-dioxide formed is considerably less than the oxygen used. As a result, the respiratory quotient falls to 0.5, or even less. Proteins lie intermediately, but much nearer to the carbohydrate, as there is very little hydrogen excess.

As the energy yielded weight for weight by hydrogen in undergoing combustion is much higher than that given by carbon, it follows that the heat value of the fats is much greater than that of the carbohydrates or proteins for equal weights.

In the building up of fats, proteins or carbohydrates in the organism, these processes are reversed, and, if, as commonly happens, fat is synthesized from carbohydrate, twice the weight of carbohydrate at least must be used up to yield a given weight of fat. The process is one of reduction, and if the end members of the molecular groupings be neglected, the carbon atom of the carbohydrate may be represented as

$$\begin{array}{c} | \\ \text{H}-\text{C}-\text{OH} \\ | \end{array}$$
 while that of the fat may be written as
$$\begin{array}{c} | \\ \text{H}-\text{C}-\text{H} \\ | \end{array}$$
 there is

hence an atom of oxygen set free from each carbon atom, which may be utilized to oxidize carbon down to carbon-dioxide in another carbohydrate molecule, and so yield energy for the formation of the fat. If this process be aided by a little oxygen from outside, still more carbohydrate may be oxidized, and the net result is that the exo-thermic or carbohydrate oxidizing reaction runs the endo-thermic or fat synthesizing reaction, and a surplus of energy is left over for the uses of the animal.

When such a linkage of reactions has occurred, the respiratory quotient may much exceed unity in value, and there is no doubt that, even in mammalia, when fat is being produced from carbohydrate, such a condition of affairs does arise.

It was observed by Gautier,¹ in 1881, that even warm-blooded animals lived in part in an anaerobic fashion, producing carbon-dioxide without equivalent uptake of oxygen, so obtaining a portion of oxygen not from the air but by fermentative processes from oxygen-containing organic bodies. Gautier also indicated that a portion of such oxygen might arise from fat formation from carbohydrate.

Hanriot,² in 1892, showed that after heavy meals of carbohydrate the respiratory quotient might rise as high as 1.30, and attributed this to conversion of carbohydrate into fat. Hanriot illustrates how such an effect might arise by the equation:—

In this equation the formula $\text{C}_{55}\text{H}_{104}\text{O}_6$ represents a neutral fat containing one molecule each of oleic, palmitic and stearic acid.

Thus Hanriot was the first observer clearly to put forward the view mentioned above in explanation of respiratory quotients greater than unity.

In the course of work somewhat similar to our own, upon the respiration of invertebrates, Vernon³ found respiratory quotients greater than unity, and also, like us, found that certain of these animals could continue living with a very minimal oxygen supply, and during this period produced large amounts of carbon-dioxide, so that the respiratory quotient in one case rose to over four.

Vernon attempted to drive all the carbon-dioxide out of the water by heating in a vacuum, and as completeness here is almost impossible,

1. *Comptes rendus* CXIV, p. 374, 1892.

2. *Ibid.*, p. 371.

3. *Journ. of Physiol.*, XIX, p. 18, 1895.

it is probable that his results ought to be even higher than they are. Vernon ascribes his results to the animals being in a pathological condition and moribund, but since, like our own, most of them recovered when fresh oxygen was admitted we scarcely can agree in this view. Also, in our case we have obtained similar results long before there was any real dearth of oxygen in the water.

Athanasiu,¹ in 1900, found a mean respiratory quotient of 1.24 in frogs, which were not feeding during the experiments, and concluded that frogs stored up oxygen in their tissues for use during such a period of hibernation.

Pembrey,² on the other hand, found an exceedingly low respiratory quotient in the hibernating marmot, but a quotient of well over unity (viz., 1.24, 1.28, and 1.39) during the feeding and fat-storing period in the same animal.

Pembrey agrees with Hanriot as to the explanation being the conversion of carbohydrate into fat.

It is somewhat difficult to believe that fat storage can occur during starvation, except for a short period, and it may be that the real explanation in our own experiments may be as suggested by Athanasiu, some oxygen storage in the tissues in some form at present unknown to us. Certainly the amounts of fat present at the end, as shown by Table VI, are not very high; and this, so far as it goes, indicates that either some reduced substances other than fat were formed, or that there is normally a storage of oxygen in some organic form which was utilized during the period of experiment.

Similar results were obtained by Pütter,³ by keeping leeches in an atmosphere free from oxygen. This observer traces the results to the substitution of hydrolytic cleavages for oxidations, but the amount of energy obtainable in this way is very small.

Granting, however, that such a linkage of reactions can occur, as is premised above, then the effect might be shown by such an equation as the following:—

1. *Journ. de Physiol. et de Path. gen.*, II, p. 243, 1900.

2. *Journ. of Physiol.*, Vol. XXVII, p. 407, 1902.

3. *Zeitsch. f. allgem. Physiol.*, Bd. VII.

The two amounts of carbohydrate on the left-hand side are written separately to indicate that nineteen molecules go to form the two molecules of neutral fat shown on the right-hand side, while the eight additional molecules are completely oxidized in combination with the oxygen set free in the fat formation to form carbon-dioxide and water, so yielding more than enough energy to run the reaction exo-thermically.

The weight of carbohydrate expressed in gramme-molecular weights is 4,860 grammes, the weight of fat formed is 1,776 grammes, carbon-dioxide is 2,112 grammes, and water 972 grammes.

Taking the relative caloric values of fat and carbohydrate as 9 to 4, the 1,776 grammes of fat are equivalent in energy to 3,996 grammes of carbohydrate, and subtracting this from the original weight there is the energy of 864 grammes of carbohydrate to run the reaction exo-thermically, or about 32,000 large calories.

This may be stated otherwise thus:—Without any oxygen from without, carbohydrate can yield about one-third of its weight of fat, and at the same time yield a supply of energy equivalent to about one-sixth of that total amount which it would yield if directly and completely combusted to carbon-dioxide and water.

A parallel case of utilization of carbohydrate energy by partial reduction and partial oxidation, linked together by the agency of a living cell, is the formation of alcohol from glucose by the yeast cell.

All degrees between these two cases (where the respiratory quotient is, of course, infinity) and those exemplified above in invertebrate animals with respiratory quotients lying between 1 and 2, can be obtained by varying intakes of oxygen.

If, for example, in the above equation eight gramme molecules more of carbohydrate were taken in on the left-hand side, and forty-eight gramme molecules of oxygen for their complete oxidation, there would then appear a total of ninety-six gramme molecules of carbon-dioxide on the right-hand side, and the carbohydrate would now yield only about one-fourth of its weight for storage as fat. Under such conditions the respiratory quotient would be 2 exactly, and there would be a large amount of energy for heat and external work.

It is most interesting to note that these considerations indicate fat formation in the tissues under conditions of diminished oxidation, which is in accord with physiological facts.

An animal with a rich reserve of carbohydrate might also actually increase its fat content during inanition and inactivity, for a short period, until carbohydrate became exhausted. Such a process as this actually does occur in the ripening of many fatty seeds.

Our thanks are due to Mr. A. Webster for much valuable assistance in the practical work, and especially for the determinations contained in Table V.