External metabolites in the sea

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WITH THE exception of a hint or two before the turn of the century, by such prophets as BRANDT and NATHANSOHN, it was not until the inter-war period that marine workers began to turn their minds to the possible existence of more subtle ecological relationships than those imposed on organisms by their inanimate environment and by predators. It is relevant that KNÖRRICH and PÜTTER had postulated the direct food value of dissolved organic food substances in the sea, although this view appeared to be finally rejected by KROGH (1931); however, such possibilities are not the main subject of this paper, although they may once again demand investigation. But those who drew our attention once more to the other possible significance of dissolved organic matter deserve our gratitude. Quite the most senior of those now living was HENRY BIGELOW who said in his famous book *Oceanography*, among many other stimulating things, " as yet we know little of the inter-relationships of different species or groups of animals in the sea beyond the obvious fact that some prey on others, but we may be certain that in many cases inter-relationships of less obvious sorts are vital links in the animal economy " (BIGELOW, 1931, p. 131, quoted by RUSSELL, 1936). Before many years elapsed, it seemed clear that he was right and we now know that he was! For the volume in his honour, it is fitting to pay tribute to his foresight.

It would not be proper on this occasion, however, to ignore the others: from JOHNSTONE, SCOTT and CHADWICK (1924)* to ALLEE (1931)† and HARDY (1935)‡. All were feeling towards the certainty of a new type of relationship in ecology, and particularly marine ecology, for which there was then all too little evidence (although it may now seem striking that it was around this very time, in 1929, that the late Sir ALEXANDER FLEMING was making his first observations on the antibiotic influence of Penicillium). It was the stimulus of such hints, and particularly that of working with HARDY when he was evolving his idea of " animal exclusion ", that led Lucas to gather an odd collection of references in support of a speculative theory of " non-predatory " relationships—based on the release and biological influence of metabolites ranging from " toxins to vitamins and hormones " (LUCAS, 1938).

This evidence ranged from the effects of external metabolites in the field of bacteriology, through some of the " mass physiology " experiments of ALLEE's school, to the

* " Also, we are pretty sure that the plankton communities influence each other—that there are what we may call group symbioses on the great scale so that the kind of plankton which we may expect to be present in a certain sea-area must depend, to some extent, on the kind of plankton which was previously present."

† " Once formed, aggregations of aquatic organisms condition the medium surrounding them by the addition of secretions and excretions, the nature and biological effect of which form one of the important problems of mass physiology."

‡ Hardy postulated a " presumably chemical " basis for his theory of " animal exclusion ", and also speculated upon the " biological history " of waters, e.g. the changes which may occur in water passing from regions of predominantly " free " phyto-plankton to regions of " imprisoned " phyto-plankton.

speculations of AKEHURST (1931) about the temporal alternation of oil-producing and starch-producing algae in ponds, and some tentative experiments (Lucas, 1936) regarding " animal exclusion ". With these were coupled ALLEN and NELSON's pioneer demonstrations (1910) of the need for some accessory substances in diatom culture; the use of " soil solution " for more effective growth; and GRAN's observations (1931) on the relatively intensive growth of phytoplankton at the junction of two bodies of water. But more evidence was needed.

More evidence did in fact exist, scattered widely through the field of biological research, and work during the war brought even more, particularly that associated with antibiotics. It thus became possible to review a wider field and to formulate the concept more precisely (Lucas, 1947, and from a more general ecological viewpoint, 1949). In the aquatic field reference was made, among others, to the very different growth-rates of diatoms cultured in different natural sea waters (MATSUDIARA, 1939; HARVEY, 1939), and HARVEY's associated investigations of the effects of various accessory substances on growth; to other examples of the influence of waters previously containing living organisms (or their by-products) upon different organisms in culture (e.g. LEVRING, 1945); and to Fox's demonstrations of the occurrence of carotenoids free in natural waters and their deposits (e.g. 1944). When supported by the rapidly increasing knowledge from the various fields of microbiology, together with a wide range of other biological references, it seemed not unreasonable to come to conclusions along the following lines:

" (1) It is characteristic of cells to liberate certain metabolites, and these are known in a variety of instances to have great influence as endocrines.

" (2) It is now well known that a number of these potent metabolites are eliminated as secretions or excretions by the organisms themselves, and many other chemicals are eliminated which are not yet known to have any specific effects within the body.

" (3) Particularly insofar as any of these metabolites are . . . parts of the environment of other organisms, they may be expected to have immediate potency for many of them. . . . The term ' ectocrines ' has been suggested for such metabolites.

" (4) More generally, however, . . . the capacity for adaptation of most organisms suggests that further differentiation between beneficial and antagonistic relationships would be likely to have developed between the producers and those affected. In the extreme instances escape, exclusion, or death must be expected on the one hand, and obligatory association (parasitism, symbiosis) on the other.

" (5) Such processes are believed to be important in evolution, and they are considered to mediate communal relationships in ecology, which is the contemporary aspect of evolution. They should be seen as part of the nexus which also includes physical and chemical relationships as well as those of prey and predator " (Lucas, 1949, pp. 353–354).

If these seemed then to be rather premature speculations, they now have much more specific support. It is not possible to review the whole field here and this note is simply intended to bring together a few references indicating some of the lines along which progress is now being made. However, the suggestions could properly be regarded as stemming from, and partly supported by, the theory of " animal exclusion ", and evidence (BAINBRIDGE, 1952) has recently been brought to bear against the only laboratory experiments (Lucas, 1936) specifically made to investigate that theory. It seems necessary, therefore, to mention BAINBRIDGE's experiments first,

and it is particularly appropriate that they were made by one who has worked with both HARDY and LUCAS. LUCAS concluded that his very preliminary experiments were not inconsistent with HARDY's theory and, indeed, appeared to offer support for it. The work had to cease and they went no further than that. BAINBRIDGE's work was not only more detailed but much more precisely executed, and it represents a real contribution to our knowledge of the prey-predator relationship. His criticism of LUCAS's " light and dark " experiments is that they simply reflected the tropism of the animals—a possibility discussed by LUCAS. It is and always was relevant. For all that, the very weaknesses in LUCAS's work and the greatly improved techniques in BAINBRIDGE's are very relevant, and they may still mean that BAINBRIDGE's results are not necessarily so critical of the theory of animal exclusion as they seemed at first sight.

BAINBRIDGE was careful to use only algal cultures aged not more than " a week or so " (his p. 391) after inoculation, whereas LUCAS probably seldom used such fresh cultures in his work. Indeed, LUCAS's cultures may, in the light of modern ideas (many of his experiments were made in 1934), have been approaching senescence at times and, whatever may be their potency during the early phase of a culture, we can now see that metabolites released during the later stages (e.g. PRATT, 1943) may well have been harmful and have acted more as deterrents than as attractions. This is, of course, far from certain and exploratory experiments on these lines would be useful. In any event, it is also relevant that, whilst BAINBRIDGE found that most of his plankton animals were attracted by, or at least did not appear to avoid, the majority of the denser phytoplankton cultures, several were either neutral or were markedly avoided or even lethal. Two of the more " harmful " were flagellates. It is also significant that not only did most of his cultures influence the animals but, in several experiments, so did the culture fluids in the absence of the plant cells. The main conclusion is that his experiments demonstrated the release by plant cells of substances which were frequently attractive and occasionally repellent to many of his animals (BAINBRIDGE, 1952, p. 429). Like LUCAS, also, he found evidence of an optimum density of plant cells below and above which the animals were presumably either starved or poisoned.

However, other evidence was accumulating. In the first place, there was the work of LWOFF's school (1943), which demonstrated convincingly the release of " vitamins " into their environment by some micro-organisms, and the vital need for such vitamins by some other forms which are unable to synthesize them. LWOFF saw this as evidence of a progressive loss of physiological function during evolution, but it provided also a mass of evidence of mainly beneficial inter-relationships, mediated by the release of metabolites potentially of communal significance.

Next there is the work of LEFÈVRE and his school (1952) which, while collectively reviewed under the title of " Auto. et hétéroantagonisme chez les algues d'eau douce ", demonstrates also quite clearly the very real influence, both favourable as well as unfavourable, which one micro-organism may exert upon others through the mediation of its secretions or excretions. In brief, these workers concluded that the three groups of bacteria, algae and fungi, all have " Faculté d'élaborer des substances actives autoantagonistes, hétéroantagonistes ou favorisantes. Spécificité des substances actives produites. . . . Décharge rapide des substances accumulées par les cellules quand on les replace dans un milieu neuf. . . . Solubilité des substances actives dans

l'eau ou dans certains solvants organiques. . . . D'autre part, il n'est pas impossible que les substances actives produites par des Algues se developpant massivement dans une collection d'eau aient une influence directe sur la multiplication et le développement des animaux aquatiques: Entomostracés, Insectes, Mollusques, Vermes et peut-être même Poissons " (LEFÈVRE, JAKOB and NISBET, 1952).

The limitations implied by the title of their paper and the wider relevance of the text (summarized on their pages 173–181) are not without interest, for there seems to have been a strong tendency on the part of various workers to anticipate unfavourable reactions rather than favourable ones in given circumstances; e.g. the review by MCCOMBIE, 1953, mentions only the possibility of harmful effects of free metabolites,* while one or two workers, in referring to LUCAS' papers, have noticed only his references to harmful effects. The fact is that one organism's " meat " may be another's " poison " in the ecological nexus, and terms such as " harmful " and " beneficial " can only be used in an immediate and limited sense. In this sense, much of the evidence demonstrates the development of " favourable " relationships, although admittedly some of the most striking are unfavourable. One of the latter is instanced by the phenomenon of " red tide ", with its harmful effects on marine animals and unfortunate repercussions on man. LUCAS instanced such effects of the secretions of plants or animals, and much more evidence has been accumulated since (e.g. BRONGERSMA-SAUNDERS, 1948). There is now no doubt that, even though in their more striking forms such phenomena can be regarded as abnormal, they are, in fact, far from unusual in a lesser degree and, further, they are mediated by the release of " toxic " substances, frequently by flagellates. Their nature and the more precise conditions which lead to their production in a mild or extreme form, are being intensively investigated in several laboratories.

The major task now is to determine the nature and effects of some of the more significant metabolites in aquatic ecology, to trace their probably variable distribution in some natural waters, and to determine the conditions leading to their production. Several lines of work have recently been developed. In the United States, PRATT (1943) has produced clear evidence that *Chlorella* cells in culture release a growth inhibiting substance, whilst RICE (1954) has grown *Chlorella vulgaris* and *Nitzschia frustulum* (both fresh water algae) together, and demonstrated clearly that neither grows so satisfactorily in the company of the other as it does in pure culture (depending upon the size of the populations used). Each was similarly inhibited when grown in the culture medium of the other, after its cells had been removed by filtration, and both were also inhibited when grown in a culture medium prepared with pond water which had supported a dense growth of *Pandorina* before filtration. Again, the metabolites in solution could be absorbed in charcoal and removed by autoclaving, suggesting volatile substances.

HARVEY, at Plymouth, following on his pioneer culture experiments with growth substances, is now attempting to review all the evidence available so as to define more

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* LUCAS drew attention to this tendency (1944 and later) in respect of the term " antibiotic ". He pointed out that not only were antibiotics necessarily favourable to those organisms, such as man, which are preyed upon by the object of antibiosis, but that the antibiotic might well prove beneficial to those organisms succeeding its producer in the ecological chain (just as AKEHURST, 1931, had suggested the autotoxic secretions of one algae may be beneficial for its successors). Indeed, the ecological successors of the producers of antibiotics can only succeed by virtue of being adapted to the presence of the antibiotics or their degradation products, and there is already some evidence that this may be true even for a potent substance such as penicillin. Here is a possible theoretical basis for ecological succession.

rigorously than has previously been possible the precise requirements of phytoplankton organisms in nature and in culture. Meanwhile, PROVASOLI, HUTNER, and their colleagues at the Haskins Laboratories, have also very rightly undertaken fundamental and precise experiments on the basic requirements for the growth of marine and fresh water micro-organisms. PROVASOLI and PINTNER (1953), in their review of the ecological implications of the nutritional requirements of algal flagellates, have brought together a large number of references to the need of various micro-organisms for growth factors and trace elements. To these they have added the striking results obtained in their own laboratory. The evidence ranges from the early demonstration by HUTCHINSON (1943) of the actual existence of thiamin in natural waters, to the significant requirement of various forms for cobalamin (vitamin B_{12}) in pure culture within chemically defined media. Of particular importance is the rigorous technique, necessary and adopted, in their work. All the results to date demonstrate aspects of " non-predatory " relationships. As they say:

" It is a reasonable assumption that if an organism requires a growth factor *in vitro*, then this metabolite or its physiological equivalent should be found in significant amount in the environment " (p. 845).

" The water environment is the one in which metabolites are interchanged most efficiently. It is to be expected that the interdependent growth of the different groups of water organisms should sensitively reflect the excretion and consumption of metabolites. Undaunted by new intricacies, we should envisage all the possibilities in these relationships, and not hesitate to follow LUCAS's lead in constructing theoretical frameworks upon which to hang data. In the present paper, only a few aspects of the nutrition of phytosynthetic forms are considered. It is possible, nevertheless, to state more definitely some of the interdependencies based upon ' external metabolites ': (1) the interchange of growth factors; (2) the lowering of inhibitory concentrations of several major mineral nutrients, especially PO_4; and (3) the preferential utilization of minerals, including trace metals, may condition waters, bringing their concentrations into the optimal zones for succeeding forms. The practical aim—to predict algal successions and blooms—may be achieved through a comprehensive knowledge of vitamin cycles as well as mineral cycles. An immediate problem is to trace the thiamine and cobalamin cycles " (p. 849).

DROOP (for example, 1954) is pursuing a similar course at Millport, in Scotland, and has shown the need of several marine flagellates* for vitamin B_{12}. LEWIN, in Canada, has also found B_{12} essential for the growth of the alga *Stichococcus* in sea water (1954). The presence of free B_{12} in natural sea water has been demonstrated, and its distribution is being examined in more than one laboratory. DROOP is now developing an assay for it in sea water. Along a rather different line FOGG (1952) has suggested that some at least of the external metabolites (for instance, the polypeptides of blue-green algae) may further communal growth, via chemical linkage, by making relatively insoluble nutrients available in a form more suitable for assimilation.

Turning now to the animal field, several workers have followed ALLEE in linking aggregations with the release of metabolites (for example, COLE and KNIGHT-JONES, 1949, and KNIGHT-JONES, 1950). Again, there was the demonstration by ALLISON and COLE (1935) that the feeding movements of barnacles can be correlated with the

*Droop has now demonstrated this need in the diatom *Skeletonema costatum* (DROOP 1955).

abundance of dinoflagellates in the water (with the postulate that this is mediated by a by-product) and the subsequent hint (MIAZAKI, 1938) of the effect of an alga upon the spawning of the male oyster. Now, COLLIER and his colleagues, at Galveston, Texas, have moved into this field from a rather different angle. Whilst studying the effects of industrial wastes on oysters, they deduced a generalized influence upon their pumping rates, which was found in due course to be correlated with the presence or absence in the water of a carbohydrate-like substance; this is either truly soluble or colloidal, and may attain densities in neritic waters of up to 25 mg/l (COLLIER, RAY, MAGNITSKI and BELL, 1953). During further work (COLLIER, 1953) they found that, along with tyrosine-tryptophane, these carbohydrates have a marked diurnal variation in abundance, and their production is associated with light and aeration—so that they are probably the by-products of plant growth. In addition, a rhamnoside (WANGERSKY, 1952) has been isolated from oceanic water, and particularly from " red tide " water up to quantities of 50 mg/l, whilst minute quantities of ascorbic acid and some other carbohydrates of very low molecular weight are being isolated for identification. The significance of such substances to the oyster may be two-fold, but it is at least clear that one of the substances in question is both widespread and very variable in quantity, and that it acts as a remarkably effective (and almost instantaneous) pumping stimulus. It would appear that the substance is also absorbed by the oysters (up to 50 mg per hour), although it remains to be seen whether such substances are of positive and substantial food value (see, for example, KORRINGA, 1949, and JØRGENSON, 1952)—a possibility which would greatly have interested both PÜTTER and KROGH—or whether their role is limited to providing sensory stimuli and perhaps growth factors. At the moment, the chief point is that they " found a biologically active compound (or group of compounds) to which an organism would respond quantitatively ", and drew attention to the link between this work and the probable significance of external metabolites or " ectocrines ".

Work being undertaken by WILSON, at Plymouth, is also relevant. For many years he has been concerned with the problems of breeding and growing planktonic larvae, particularly polychaetes. On the one hand he noticed that sands from some areas were more suitable for the settlement of polychaete larvae than others (e.g. WILSON, 1948 and 1953) whilst, on the other hand, he found that, with the passage of years, his success with rearing these and other larvae was tending to decrease (WILSON, 1951). Continued and painstaking investigations in various directions now seem to make it clear, however, that the suitability of sands for larval settlement must be determined by the existence on them of other forms of life, probably micro-organisms—" Organic material, living or dead, on the sand grains plays an important role in rendering a sand attractive or repellent to the larvae " (WILSON, 1954). Whilst we may not be so obviously concerned here with a free metabolite (although that is quite possible), we have to deal once again with the significance for living larvae of organic remains, and *with their detection* by these larvae.

The other instance is of even more direct interest. WILSON associated his decreasing success with the now familiar change known to have taken place whereby the waters of the English Channel have been much less productive since 1930 than in the 1920s. Superficially, this was due to reduced phosphate content, but the possibility of more subtle factors remained, and comparative experiments were made by rearing poly-chaete and echinoderm larvae in (a) " local " Channel water collected in the Plymouth

neighbourhood (as had been usual) and (b) water collected from the Celtic Sea, in which the typical plankton community is normally similar to that of the " local " water before 1931. The results were striking: good growth of polychaete and echinoderm larvae was obtained in the latter and only poor or deformed growth in the former. Appropriate combinations of experiments suggested the presence of a " beneficial " substance in the Celtic water, and its lack in the local water, rather than the existence of a harmful substance in the latter. More recent work has shown that Clyde water also tends to be more suitable than " local " Channel water and, although there are variations, the inferences seem to be unambiguous. In association, WILSON and ARMSTRONG (1954) have shown that heating the waters for shorter or longer periods generally had an adverse effect on the larvae bred in them, thus suggesting the existence of a beneficial substance of a more or less volatile nature. Their analyses have not yet been carried to the stage of demonstrating any particular component or fraction which is responsible for the biological difference.

Recently, at Aberdeen, JOHNSTON has extracted various fractions of the organic matter in natural sea waters, and made some preliminary biochemical analyses. Certain of these were tested in bio-assay on a number of phytoplankton diatoms and flagellates (with results summarized by JOHNSTON in his Table I, 1955, from a paper read to the International Council for the Exploration of the Sea in 1954). It was found that the growth of many was favoured and of some others hindered relative to controls, although, " since the tests were limited to one concentration, further (and perhaps different) instances of these effects would probably have been observed by testing a range of concentrations. One particular fraction was found to promote greater growth in 9 of the 11 species tested." The initial information so gained is providing a basis for the bio-assay of sea water samples from different areas, with a view to a preliminary labelling of such waters according to their physiological effects. Then, further attention will be given as far as possible to identifying some of the substances thus found to be of biological significance. JOHNSTON tentatively discusses the possible value of such information to the fisheries worker.

In all such experimental work, tribute should be paid to the surveys made by RUSSELL (e.g., 1939) and FRASER (e.g., 1952). By distinguishing on biological evidence between water masses, which have often been indistinguishable on familiar hydro-graphical criteria, they are providing the experimenter and biochemist with invaluable clues.

In conclusion, it can be said that, before the war, a few people were moving towards a conception of ecological inter-relationships which seemed likely to be an important complement to the already familiar relationships existing between the organism and its physical environment, and those between prey and predator. Limited observations in diverse fields suggested that these non-predatory relationships would be mediated by the release of metabolites of varying potency for other members of the community. Further work during the war made it possible to gather much more support for the suggestion, and it is now quite clear that dissolved or colloidal organic matter may be present in natural fresh and marine waters in greater quantities than was originally envisaged. Indeed, it is still uncertain whether these may not in themselves provide, for some forms, nutrients in the ordinary sense of the word*. It is also clear that within

*See, for example, MORRIS, 1955.

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this general heading of organic matter are included, sometimes in very minute quantities, substances whose effects within the community may not unreasonably be compared with those of endocrine metabolites within the body. It now seems necessary to believe that such substances play a considerable part in the growth of aquatic communities of bacteria, algae and fungi (we still have much to learn of the activities of the last in natural waters), so that the success of various organisms, and consequently their ecological succession, may be largely determined in this way. The next point is that we now have a number of instances in which such free metabolites affect not only the lives of protozoans (whose communal life may be expected to be very similar to that of the micro-plants, except in so far as their need for external metabolites can be expected to be much greater), but also those of higher animals such as worms, echinoderms and molluscs and probably crustaceans. We can confidently expect this list to be extended, probably even to include fish.

For example, HASLER and WISBY (1951) have obtained most interesting evidence of the influence of different natural waters upon the movements of fish. They found that minnows were able to discriminate between the waters of two Wisconsin creeks after as little as two months conditioning, while cautery of their olfactory epithelia rendered them unresponsive to conditioning. It appeared, therefore, that olfaction was the principal, if not the sole, means of discrimination. They further demonstrated that the chemical response was not to carbon-dioxide in the creek waters, for example, but that the significant fraction was probably organic, in the usual sense, in that the minnows reacted to the distillate rather than the residue (vacuum distillation at 25° C), so that the existence of a volatile substance in the water can be anticipated. Here then is the essence of one reaction system on which " homing ", and perhaps other aspects of migration, might reasonably be based. Indeed, HASLER's preliminary tests suggest that salmon can detect such " odours " of streams and discriminate between them. In that instance, it would be necessary to imagine the salmon being conditioned during its early fresh water life to the " odours " of the " home " tributary. HASLER has also been able to demonstrate that minnows could respond to such odours after " forgetting periods " which were longer in fishes trained when young than in old ones. It may not therefore be so unreasonable to think of such reactions as even applying to the movements of other and wholly marine fish.

Lastly, when mentioning this particular possibility as one of the possible uses to which such fundamental research might ultimately be put in a fisheries laboratory, JOHNSTON (1955) also referred to the possibility that " fertilization " of natural waters, normally by enrichment with phosphates and nitrates, might come to include the addition of minute amounts of critical metabolites. Ideally, at least, these would be selected as those likely to mediate the succession of " desirable " algae in relation to the favoured crop. For example, it has seemed at times that the risks of fertilization being followed by blooms of " undesirable " algae are considerable, but it does not seem unreasonable to imagine that such a succession could, to some extent, be controlled if the fertilizer included not only normal manures but a specific metabolite antagonistic to these algae and preferably favouring other forms. Indeed, it may not be too speculative to suggest that some natural waters, normally producing little in the way of a desirable crop, might be induced to undergo a complete ecological change by the addition of a critical metabolite alone. The apparent scarcity of nutrients in some of these waters may not be so significant as it may seem. Not only may there

be great reserves of nutrient in the bottom deposits but, as the Haskins Laboratories have demonstrated, many algae can tolerate only quite dilute nutrient solutions, and can thrive on them, so long as the necessary free metabolites are present. Perhaps particularly in some " barren " fresh waters, it may be that certain types of ecological development are blocked, just as in some laboratory experiments, by the natural absence of a specific metabolite essential to the life of an essential organism in fish management.

Such possibilities as these various lines of work indicate may make this field an attractive one to fisheries worker and biologist alike. Certainly, considerable development can be expected during the next few years. A number of those working in the various fields, however, may possibly have missed something of the community of interest they share with many others. With a few striking exceptions, what now seem to be very relevant cross references are frequently missing from bibliographies. Perhaps this paper may serve to emphasize, where necessary, some of the fundamental features thought to be common to the various investigations.

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