The Journal of the Soil Association Vol. 16 (1970) p. 21 -25

Increase in Phosphorus and Copper in the Lobster after Moulting

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WHEN an element increases in an animal's body, it is assumed that it has been absorbed, or preferentially incorporated, from its environment. Those who hold this view do not discuss it or carry out any experiments to find out whether the additional quantity of the element could be supplied solely by the environment. In most cases it is true, but it does not necessarily follow that it is so in every instance.

Some scientists have realised that the normal classic concepts fail to fit all their observations. Professor J. M. Pérès, head of the Oceanographic Laboratory at Endoume, in the Faculty of Science of Marseilles, writes on page 24 of his book *La Vie dans l'Océan*: 'The Diatomaceae . . . have need of silica to build the case which encloses their cytoplasm, but insufficiency of environmental SiO_2 never appears to be a limiting factor'. The author does not quote any quantitative details, but he admits that these algae, lacking silica in their ambient medium, are nevertheless still able to make their siliceous casing. This observation on diatoms is not isolated, and in a work *Continuous Creation* (London, 1950) Branfield has given further examples. Various French specialists in marine biology have also confirmed this observation, but to our knowledge no report has been published on systematic research into this endogenous production of silica or on the build-up of other elements. (An investigation is under way in France into the production of calcium by other Protistae (Coccolithae) since the amount of calcium 'fixed' by these microalgae is several times higher than the total calcium contained in the sea water in which they live; the origin of this calcium is not yet known.)

We decided to conduct an experiment to determine whether an animal must necessarily draw on the environment alone in order to increase an element within itself.

Report on an experiment

Lobsters (*Homarus vulgaria or H. gammarus*) weighing about 200 grammes and in the inter-moult period were placed in tanks of normal sea water until they finished their moult. Lobsters of this size moult about twice a year. After moulting, one of them was removed from this water as soon as possible, dried, weighed and then incinerated in an electric furnace at 500°C. The ashes were treated with HCl (17.5 ml. pure HCl in 500 ml.) and the analysis, for different proportions, carried out in this solution.

Another lobster, as soon as its moult was finished, was weighed and then placed in a fresh tank of sea water containing the normal natural content of phosphorus and copper. This water originally contained 1.27 g/l magnesium.

The lobster was left for 17 days in this water without food because we wished to avoid having to take into account the effect of the mineral intake of food. The tank used contained 75 litres of water. This water was in closed circuit; a siphon causes it to circulate permanently over an activated charcoal filter coated with glass silk. The filter is designed to hold back the fragments and dissolved gases derived from the animal's metabolism. The water is aerated by a rotary pump which blows air under pressure to the bottom of the tank, so as to reoxygenate the water. Seventeen days after the moult, this lobster was removed from the water, weighed, incinerated and analysed. There was no loss of P or Cu by reason of absorption on to the filter or the feed pipes.

Results of the analysis

A. PHOSPHORUS.

1. **Balance**

The sea water used, taken near the coast of South Brittany, contained 0.002 ± 0.0001 mg/l P (a relatively low figure). This content gives a total weight of P in the 75 litres of the tank of 0.15 mg.

	At the time of the moult	17 *days later*
In the lobster	380	430
In the water (75 l)	0.15	0.15
	380.15 mg	430.15 mg

In aliquots, the phosphorus was determined by three methods; ceruleomolybdic colorimetry; ascorbic acid + sulfomolybdic acid; and by phosphosmolybdic acid + benzidin acetate. The analysis of water, or of the lobsters, were all carried out again threefold by these different methods, and figures quoted are averages.

The weight of P at the time of moult was checked by two groups of weighings, i.e. weight of the animals in the inter-moult period and at the moment when the experiment started. Males of fairly identical weight were selected; the relation between this weight and the weight of the inter-moult time for the same size, weight and age was found to be almost constant ($\pm 1\%$). The individual weights of the test lobsters were equated to a constant weight after the moult in order

to have valid comparisons over the total time of the experiment.

2. Comments

In 17 days the test lobsters gained 13%. In the tank water there was only 0.15 mg of P. Therefore, in 17 days, there was an increase of phosphorus of more than three hundred times above that present in the environment, while in the water used there was no change in P. An endogenous formation of phosphorus by some unknown enzymatic process seems the only possible explanation. It should be mentioned that we observed similar results in mice which had been given an excess of magnesium in their diets (see: Comptes Rendus de l'Academic d'Agriculture de France du 13.12.67).

There is also another possible origin of P since a link has been noted between N and P—at least in certain micro-organisms. In the light of our work, Professor Komaki, head of the applied microbiology laboratory of Mukagawa University, using several groups of micro-organisms, obtained an increase in the weight of phosphorus built up intercellularly exceeding that contained in the whole culture solution. Certain species of urea-degrading bacteria (*Uro-bacillus, Urococcus*) are capable of building up internally twice as much phosphorus as that contained in the culture medium after 72 hours' incubation at 30°C. Certain species of moulds were found to build up P. under the same conditions of time and temperature, while the culture medium used for growth remained quite free of P. The most active strains were tested by one of Komaki's pupils, Miss Fujimoto, in connection with a thesis. Large scale industrial applications are now in progress for agricultural uses by a leading industrial company, the transition from the applied microbiology laboratory in the university to the industrial stage having been carried out by two agricultural microbiology laboratory workers of the Biology Division of the firm.

We must also refer to the results of analysis presented at the International Congress on 'Lisier', Lyon, at the end of 1967. In the 'lisier' (urine + excreta mixed) of sheep i.e. under anaerobic conditions, P rises by 165% in 48 hours; N (originally in excess of P) decreases by 14% in relation to the total found on the fresh components (urine + excreta). The nitrogen is furnished by the urine and the excreta, the excreta micro-organisms being derived from the flora of the sheep's digestive system. The phenomenon is less marked in the droppings of cattle: N—10%, P+27.7%. Several experiments (including at least one thesis in France) have reported a decrease in phosphorus in the germination of Leguminaceae seeds. There is thus a relationship between different experiments showing that the variation in phosphorus is a clearly observed phenomenon.

B. COPPER

1. Balance

The copper content of the raw water used in the lobster tests was 0.026 mg/1 (1.95 mg/75 1). At the end of the experiment (17 days after the moult) it was found to be 0.066 mg/1 (4.95 mg/75 1).

	At the time of the moult	*17 days later*
In the lobster	3.4	4.95
In the water (75 l)	1.95	5.51
	5.35 mg	10.45 mg

Increase (total) 95% (61% in the lobster: 131% in the water). Copper content was determined on aliquots by 2—2¹ diquinolyl colorimetry and by addition of cuprethol in a cuvette at 434 mμ (without extraction). The figures quoted were obtained by assays in a Beckman spectrophotometer with atomic absorption and they were very near those of the average figures obtained by chemical methods. We have performed the assays in a Beckman apparatus in order to verify the chemical analysis by a physical method.

2. **Comments**

Copper is an important element of crustaceans (and other marine creatures) since their 'blood' (haemolymph) contains haemocyanin, an oxygen exchange protein with copper, instead of iron as in haemoglobin. Hence, various investigations have been carried out on copper content of lobsters. Ranzi has discovered about a x5 increase in copper in the embryo of the cuttle-fish egg during incubation; but here again it has been implicitly accepted, without research, that the copper came from the environment. Similarly, the research by Zuckerkandl has shown a variation in the total copper from 1 to 20 according to the inter-moult periods in crabs (sea spiders=*Maia squinado*) such variations being attributed implicitly to exchanges with sea water alone. Our experiments show that the phenomenon is apparently quite different.

At the end of the experiment the lobster contained 2.11 mg more Cu. Since there was only 1.95 mg Cu. in all the tank water this could not account for such an increase in the lobster. At the same time there was also a considerable increase of Cu. in the water, this being the result of the catabolism of the lobster which produced an excess of Cu. which it excreted.

Previous researches had indicated a link between copper and iron. We therefore have analysed the iron in the water at the beginning of the experiment and at the end (17 days afterwards). Analysis was performed by chemical methods and confirmation obtained by the Beckman spectrophotometer. We have found respectively 0.0845 mg/l and 0.069 mg/l (average of several replicates); that is 6.337 mg and 5.5175 mg 75/1, a decrease of 18%. (In this raw water Fe/Cu =3.25).

Conclusion

It seemed to us important to present fully the results of this unique type of experiment in the hope that others might repeat it or reproduce a similar report on a larger number of test animals (we used a total of 8 lobsters) in order to check for reproducibility. There appears to be some rather more general biological phenomena responsible for such demonstrations of the non-zero balances in biology

—which have been widely observed elsewhere.

It must also be pointed out that in oceanographic centres which are not by the seaside, lobsters are left in the same water in closed circuit for two or three years. During this period those lobsters which initially weigh around 200 grammes may have 6 or 7 moults before the water is changed; i.e. they are in constant contact with the original stock of minerals present in the tank water. Fresh water, not sea water, is added from time to time to compensate for evaporation. There is a slight mineral contribution through the food, very light perhaps but not entirely negligible, over 2 or 3 years. However, it may be seen from the first fortnight following the first moult in the tank that the total content of P and Cu in the water is insufficient to explain the increase of these elements found in the lobsters, which could have certainly not taken such quantities of mineral elements from the medium (water) in which they lived.

There is therefore very likely to be an endogenous production of these elements which is apparently inexplicable by the laws of physics and chemistry as we understand and interpret them at present, hence more experiments on these newer lines are advocated under defined biological conditions. An unknown enzymatic action would appear to be responsible. It seems to us that the whole of the foregoing opens up a vast field for research extending through physico-chemistry, linked with enzyme action, extending perhaps to within the atomic nuclei on weakly linked protons. Chemistry does not explain all the life processes and it may be necessary to revise several laws relating to classic agriculture, cattle-breeding, medicine, etc.—laws based upon possible error where biology has previously been equated back to the present laws of chemistry. Life is more complex than that, as the above experiment has shown.

Breeding and observation of the animals at the Centre Oceanographique G. Bollore, Odet, near Quimper (Brittany).

BIBLIOGRAPHY

J. M. Peres—La Vie dans l'Océan—Edit. du Seuil, Paris 1966.

R. Heim—paper read before the French Academy of Sciences, Paris, 29.6.64, on the researches of P. Fusey and G. Hyvert showing the increase of Ca in 'sick' stones (through the reaction of Streptomyces) without outside contribution of Ca, but with decrease of Si.

E. Zuckerkandl—La teneur en cuivre de l'hémolymphe de *Maiasquinado* aux différents stades de l'intermue. Thesis, Paris 1959. Also papers in C.R. Soc. Biol. n°3 1957, etc.

Hisatoki Komaki—Formation de protéines et variations minérales par des microorganismes—Rev. de Pathol. Comparée et de Medec. experim. Mars/avril 1969.

C. L. Kervran—Augmentation de calcium et du phosphore chez l'animal par une surcharge alimentaire en magnésium. Rev. de Pathol. Paris, Juillet/Août 1968 and Académie d'Agriculture de France, Decembre 1967.

C. L. Kervran—Preuves relatives à l'existence de transmutations biologiques—Edit. Maloine, Paris, 1968 (under press: published by Crosby Lockwood, London, the translation of Kervran's book: A la découverte des transmutations biologiques, edit. Le Courrier du Livre, Paris).