LETTERS TO THE EDITORS

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Protein Synthesis in Nucleated and Non-nucleated Halves of *Acetabularia mediterranea* studied with Carbon-14 Dioxide

It is generally assumed, after Caspersson[1], that the nucleus is the main centre of protein synthesis in the cell. However, this opinion is based on cytochemical and biological observations only (for example, the incapacity for sustained regeneration in many unicellular organisms), and it appeared desirable to test this assumption on biochemical grounds also.

An especially well-suited material has been found in the giant unicellular alga *Acetabularia mediterranea*, which has been extensively studied by Hämmerling[2]. The algæ were collected at the marine laboratory of Villefranche-sur-Mer, thanks to the kindness of Prof. Tregouboff, and they were grown according to Hämmerling's technique. Our biological observations concerning the extensive capacity for regeneration of nucleated fragments entirely confirm Hämmerling's; non-nucleated fragments of this alga are also able to initiate regeneration and even the formation of an umbrella. However, as Hämmerling has rightly noted, it is not easy to decide whether this corresponds to a real synthesis or to a mere elongation, due, for example, to a change in hydration.

In order to clear up this point, we carried out the following experiment: 50–100 nucleated and as many non-nucleated fragments, carefully washed and cleaned by means of a small paint-brush, were put into 12 c.c. of sterile medium (Hämmerling's) in a 50 c.c. Erlenmeyer flask; 2–4 mgm. radioactive barium carbonate (containing 1–2 μcuries of carbon-14) were placed in a small vial hanging from the stopper; radioactive carbon dioxide was liberated by adding a few drops of perchloric acid to the barium carbonate and the flask was immediately closed. The fragments thus kept in the presence of carbon-14 dioxide were exposed to the light of two 40-watt fluorescent tubes at a distance of 50 cm. After 24–38 hr., the fragments were rapidly washed free of radioactive medium, and sorted out (nucleated and non-nucleated fragments are readily distinguished, for the nucleus is located in the rhizoids of the alga).

The two groups of fragments were ground in 10 per cent trichloracetic acid in a small homogenizer, the insoluble substances were washed once with trichloracetic acid, and then hydrolysed for 18 hr. at 145° in 6 *N* hydrochloric acid, in sealed tubes. The amino-acids resulting from the hydrolysis of the proteins were decarboxylated with ninhydrin according to Van Slyke[3]; the carbon dioxide liberated was trapped in sodium hydroxide, then precipitated as barium carbonate, the specific radioactivity of which was determined by means of a thin-window counter.

The results of such experiments, performed with different batches of algæ that have been studied at different times after sectioning into two fragments, are given in the accompanying table.

Time since sectioning	Time of CO_2 incorporation	Specific activity of CO_2 groups of proteins / Specific activity of CO_2 in the medium $\times 100$		Specific activity of non-nucleated fragments / Specific activity of nucleated fragments
		Nucleated	Non-nucleated	
9 days	38 hr.	8·6	8·7	1·01
11 ,,	26 ,,	6·4	6·5	1·02
16 ,,	24 ,,	8·3	7·6	0·92
23 ,,	24 ,,	7·2	4·4	0·61
33 ,,	24 ,,	4·1	3·2	0·78
38 ,,	27 ,,	4·5	3·1	
		4·8	2·9	0·70

Preliminary experiments had shown that the incorporation of carbon-14 dioxide into the carboxyl groups of the amino-acids is linear for at least 48 hr.; it seems thus that we are very probably dealing with a real protein synthesis. It might be added that control experiments have conclusively shown that the whole of the carbon dioxide liberated by ninhydrin really came from protein amino-acids and that the contribution in this process of contaminating organisms is negligible.

In conclusion, it can be advanced that, contrary to the generally accepted views, but in harmony with Hämmerling's biological observations, the presence of the nucleus is not necessary for protein synthesis to continue for a considerable time. It is not until two weeks after enucleation that incorporation of carbon dioxide in the protein begins to drop; but even after more than five weeks this incorporation is very far from negligible. The fact that according to London, Shemin and Rittenberg[4] hæmin synthesis takes place in reticulocytes (that is, basophilic non-nucleated erythrocytes) and that the same is probably true for globin and other proteins (Borsook *et al.*[5]) suggests that our conclusions are probably also valid for animal cells without photosynthetic activity. Our results agree also with those of Hultin[6], Keller[7], Siekewitz and Zamecnik[8], according to which cytoplasmic microsomes rich in ribonucleic acid show the highest rate of incorporation of labelled amino-acids into proteins of all cellular fractions obtainable by differential centrifugation, including nuclei.

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A Technique for taking Successive Shadowgraphs of Oat Seedlings

Owing to the great variability in growth-rate and tropic curvature of the coleoptile shown by oat (*Avena*) seedlings similarly treated, it is for many experimental purposes desirable to be able to take successive measurements of growth or curvature on the same individuals rather than on a fresh sample each time. Thus in experiments involving curvatures, in view of the difficulty in obtaining really straight seedlings to start with, doubtless partly due to nutation[1], it is most desirable to have initial records before treatment; it may also be useful to record the degree and kind of curvature at successive intervals after