Increased Incorporation of L-[¹⁴C]Valine into Proteins of the Nuclear Fraction from Guinea-Pig Brain Slices Incubated with Ouabain

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The effect of ouabain on the incorporation of L-[¹⁴C]valine into the protein of brain slices has been investigated as a part of a study of the action of electrical stimulation on cerebral protein synthesis, since ouabain mimics the effect of electrical stimulation, to some extent, by increasing $[Na^+]_{in}$ and decreasing $[K^+]_{in}$.

Ouabain (20 μ M) abolished the 5 min. lag period in the incorporation of L-[¹⁴C]valine into protein that occurred after the addition of the labelled compound to the controls. As a result the rate of incorporation for 15 min. from zero time with ouabain was similar to that exhibited by the controls after the initial lag period. Ouabain was without effect on incorporation when $[Na^+]_{out}$ was decreased from 164 mM to 40 mM.

The initial increase in incorporation in the presence of ouabain was not a consequence of an increased influx of L-[¹⁴C]valine, since the intracellular content of the labelled compound was always lower in the presence of ouabain than in the controls. Nor could the stimulation be ascribed to an increase in the intracellular ATP concentration after inhibition of the sodium pump, since the ATP and creatine phosphate contents of the tissue remained unchanged for 3 min. after the addition of ouabain and thereafter declined.

It seemed possible therefore that ouabain caused a selective and enhanced incorporation of label into some subcellular compartment despite the low overall intracellular concentration of L-[¹⁴C]valine. Fractionation of the slices incubated with L-[¹⁴C]valine showed that 20 μ M-ouabain caused a doubling of the incorporation of label into the proteins of the nuclear fraction over a period of 30 min. This increase in incorporation did not occur in nuclei isolated from slices incubated with ouabain and 40 mM- Na^+ . Incorporation into the microsomal and supernatant fractions was inhibited by ouabain, whereas in the mitochondrial fraction (which also contained nuclei) incorporation was slightly increased during the first 10 min. but was inhibited thereafter. The enhanced incorporation of label into nuclear protein from slices incubated with ouabain may be a consequence of an increased uptake of L-[¹⁴C]valine into the nuclei as a result of a rise in the intracellular concentration of Na^+ (see Allfrey, Meudt, Hopkins & Mirsky, 1961; Navon & Lajtha, 1969) after inhibition of the sodium pump.

Allfrey, V. G., Meudt, R., Hopkins, J. W. & Mirsky, A. E. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 907.
Navon, S. & Lajtha, A. (1969). *Biochim. biophys. Acta*, **173**, 518.

Isolation of Ribosomes from Cell Walls of Barley (*Hordeum vulgare*)

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We have shown that if highly purified cell walls from barley seedlings are incubated with 0.3 M-KOH for 17 hr. at 37° ribonucleotides are released, and the base composition of the ribonucleotide mixture is identical with that of cytoplasmic ribosomal RNA. Extraction of purified cell walls with deoxycholate, with saline or with phenol has no effect on the yield of ribonucleotides, so we conclude that the RNA is not due to the presence of contaminating cytoplasm (see Phethean, Jervis & Hallaway, 1968).

The base composition of the ribonucleotide mixture released from walls by alkali hydrolysis suggests it is derived from ribosomes held within the walls. We have now isolated ribosomes from the walls in the following way. A suspension of purified cell walls in 0.25 M-sucrose buffered at pH 7.8 with 5 mM-tris containing $MgCl_2$ (1 mM) and sodium deoxycholate (0.2%) was passed through a French press at 8000 kg./cm.². The resulting fine suspension was filtered on a sintered-glass funnel (no. 1 porosity) to remove cell-wall fragments. The filtrate was centrifuged at 30000g for 30 min. to remove suspended material. The supernatant was layered on top of 2.0 M-sucrose buffered at pH 7.8 with 5 mM-tris containing $MgCl_2$ (1 mM) (Jackymezyk & Cherry, 1968). This gradient was then centrifuged at 105000g for 2 hr. and the pellet was resuspended in 0.25 M-sucrose buffered at pH 7.8 with 5 mM-tris containing $MgCl_2$ (1 mM). The suspension had a maximum u.v. absorption at 258 nm. and a minimum at 242 nm., the $E_{max.}/E_{min.}$ ratio being 1.20. After a preliminary centrifugation at 10000g for 10 min. to remove aggregated material, the suspended 105000g pellet was resedimented by centrifugation at 105000g for 2 hr. This procedure did not increase the $E_{max.}/E_{min.}$ ratio.

The RNA in the 105000g pellets accounted for approx. 25% of the RNA of the original purified walls, about 65% was recovered from the wall fragments retained on the filter and the remainder was in the pellet sedimented at 30000g.

Jackymezyk, W. J. & Cherry, J. H. (1968). *Biochim. biophys. Acta*, **157**, 368.
Phethean, P. D., Jervis, L. & Hallaway, M. (1968). *Biochem. J.* **108**, 25.