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Effects of Ribonuclease on the Metabolism of Living Root-Tip Cells

USING ribonuclease as an analytical tool, Gale and Folkes[1] have recently brought forward new evidence for the hypothesis that ribonucleic acid plays an important part in protein synthesis[2,3]. A recent report, by Kaufmann and Das[4], that ribonuclease induces mitotic abnormalities in root-tip cells, shows that the enzyme actually can penetrate into living root-tips. These findings led to the present study on the effects of ribonuclease on the metabolism of nucleic acids, protein and respiration in onion root-tips. The experiments have been performed with the technical help of J. Brygier, E. Baltus and F. Vanderhaeghe.

When intact onion root-tips are treated with crystalline ribonuclease (1 mgm./ml.) for a few hours prior to immersion in water containing a labelled amino-acid (radioactive glycine or phenylalanine), there is considerable inhibition of the incorporation of amino-acid into the proteins: a 50 per cent inhibition is observed after pre-treatment for 1 hr. with ribonuclease, and it reaches almost 90 per cent after treatment for 3 hr. with the enzyme. These biochemical results have been confirmed by using an autoradiographic method: as seen in Figs. 1 and 2, treatment for 3 hr. with ribonuclease considerably reduces the uptake of labelled phenylalanine into the cells. Inactivated crystalline ribonuclease showed no effect on the incorporation of the labelled amino-acids into the proteins of the root-tips.

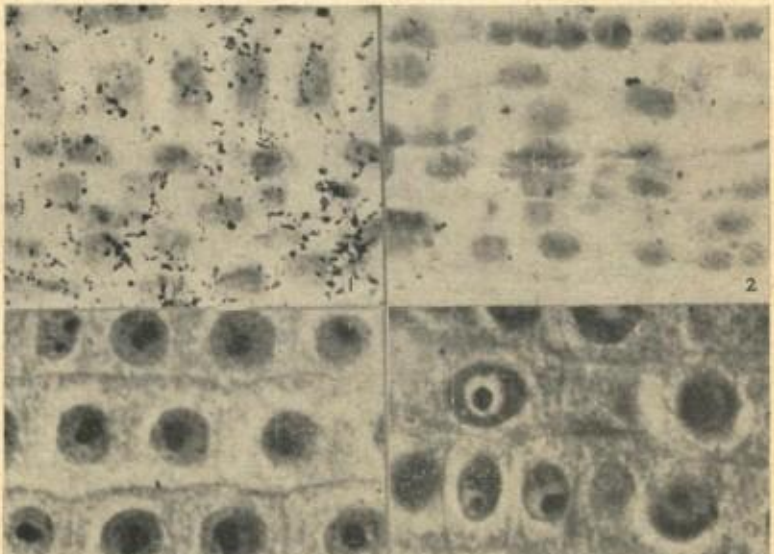
Very different results are obtained when the incorporation of labelled adenine in the nucleic acids is studied: treatment with ribonuclease first increases the incorporation of adenine into ribonucleic acid, the increase reaching 60 per cent for a 3-hr. period. Incorporation then steadily decreases, so that in root-tips treated for 20 hr. with ribonuclease, 80 per cent inhibition is found. As regards deoxyribonucleic acid, the incorporation of labelled adenine is immediately reduced: the inhibition reaches 30 per cent during a period of 1–3 hr. and it becomes practically complete after 20 hr. These observations suggest that ribonuclease immediately slows down deoxyribonucleic acid synthesis, leading to the cytological abnormalities described by Kaufmann and Das[4], while it first stimulates, then inhibits ribonucleic acid metabolism.

In contrast to these striking changes in protein

and nucleic acids metabolisms, the oxygen consumption of the root-tip meristematic tissue remains unaffected by ribonuclease, even after treatment for 15 hr.

While the oxidative activities of the mitochondria thus apparently remain normal, phosphorylations are effected by the ribonuclease treatment: the inorganic phosphate content drops by 20 per cent during the first 3-hr. period, without any further changes. At the same time, acid-labile organic phosphate (presumably adenosine triphosphate) shows a 30 per cent increase during the first 3 hr. of the ribonuclease treatment and remains constant afterwards. These results probably mean that adenosine triphosphate is not utilized and thus accumulates, when protein and deoxyribonucleic acid metabolisms are inhibited by ribonuclease.

Ribonucleic acid, when estimated spectrophotometrically according to the method of Ogur and Rosen[5], shows a 20 per cent drop after the root-tips have been treated for 3 hr. with ribonuclease; there is no further decrease afterwards, even after 18 hr. Soluble nucleotides seem to drop slightly at the



Track-radioautograph of onion root-tip cells incubated in radioactive phenylalanine. Control (1) and after treatment by ribonuclease (2)
Sections of onion root-tip cells stained by Mazia's method. Control (3) and after treatment by ribonuclease (4)

beginning (1–3 hr.), then steadily increase, finally reaching a value 40–50 per cent above controls after 18 hr. It should be pointed out, however, that classical methods of estimation of ribonucleic acid might be misleading if the nucleic acid of the cells is partly degraded by ribonuclease. It seems, nevertheless, that the strong inhibition of protein metabolism is not linked to any extensive degradation of ribonucleic acid.

This conclusion is confirmed by cytochemical observations on cells treated with ribonuclease : after Unna staining, the cells of the outer layers are more basophilic than normally after 3 hr. in ribonuclease. The increased basophilia is due to ribonucleic acid, as shown by the ribonuclease test[3] ; it is thus likely that the enzyme first induces a synthesis of ribonucleic acid in the external cells. Later on, after 6 hr., for example, this outer layer of cells loses its basophilia almost completely.

An interesting point is that when sections of roots previously treated *in vivo* for 3 hr. with ribonuclease are stained with Mazia's[4] method for proteins, an empty space becomes visible around most of the nucleoli (Figs. 3 and 4). This finding might mean that, as proposed by Caspersson[2], the nucleolus plays a very important part in protein synthesis, in agreement with the experimental data obtained in this laboratory by A. Ficq[5].

These results, in showing that ribonuclease produces a very strong inhibition of amino-acid incorporation into the proteins of living cells, bring fresh evidence for the view that ribonucleic acid is intimately concerned with protein synthesis. The accumulation of adenosine triphosphate in the treated cells as well as the fact that ribonucleic acid only partly disappears after treatment by ribonuclease agree well with Dounce's hypothesis[6] that a phosphorylated form of ribonucleic acid is involved in a template mechanism of protein synthesis. It should finally be pointed out that ribonuclease, when acting on living cells, exerts effects comparable to those of the removal of the nucleus in unicellular organisms[3] ; for in such cases, ribonucleic acid decreases together with protein anabolism, while adenosine triphosphate increases and oxygen consumption remains unaffected.

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