The mode of action of ribonuclease on living root-tips

It has been shown earlier (BRACHET[1,2]) that ribonuclease (RNase) produces a strong inhibition of growth and incorporation of labelled amino-acids into the proteins of living onion roots. These results suggest that RNase, when it penetrates into the living cells, inhibits net protein synthesis. New experiments, in which both the growth in length and the protein nitrogen (Kjeldahl) have been simultaneously measured, according to the techniques outlined in a previous note (BRACHET[2]), have now confirmed this viewpoint: in 3 experiments, RNase (Armour, 1 mg/ml) produced, in 20–24 h, an 86% inhibition of growth in length and an 89% inhibition of protein synthesis. It can safely be concluded that, in living onion roots, RNase strongly inhibits net protein synthesis.

The mechanism remains, however, unclear: there is no doubt that RNase interferes with ribonucleic acid (RNA) metabolism (BRACHET[1]); but, as pointed out earlier, the possibility that RNase acts as a basic protein and not as a specific enzyme, thus combining with RNA without breaking it down, cannot be excluded (BRACHET[2]). The following experiments suggest that both mechanisms probably act; after the initial formation of a RNA-RNase complex (which is enough to inhibit protein synthesis), RNA is apparently broken down.

1. Basic proteins (histone, protamine, cytochrome *c*) exert less cytological effects on living root-tip cells than ribonuclease (KAUFMANN AND DAS[3]); however, histone and salmine (1 mg/ml) strongly inhibit the incorporation of labelled phenylalanine into the proteins of the growing roots, presumably because they form a complex with the nucleic acids. Cytochrome *c*, which quickly stains the roots in deep red, exerts only a slight inhibitory (10%) effect on this incorporation. The difference is probably due to the fact that cytochrome *c* has less affinity for RNA than histones and protamines.

2. As shown earlier (BRACHET[1]), RNase treated cells do not lose their basiphily unless they have been acted upon for more than 3 hours; such a 3 h treatment is sufficient to inhibit almost completely growth and incorporation of amino-acids into the proteins. But, if the roots are fixed by freeze-substitution (instead of Zenker acetic, as previously used), cytoplasmic basiphily (Unna's staining) disappears already after 1 h in the outer layers; nucleolar basiphily is abolished in almost all cells. After 3 h RNase treatment, basiphily (except for chromatin) has practically disappeared in the freeze-substitution fixed roots; it remains unimpaired in the Zenker-fixed ones. Obviously, RNase builds, especially in the nucleoli, a complex with RNA: the latter does not stain with pyronine any more, unless the complex is broken down by an energetic fixative like Zenker.

3. On the other hand, experiments by M. MORTREUIL have shown that, while ribonuclease exerts only a slight inhibitory effect on the uptake of ^{32}P (10–30%) in onion roots, it very strongly inhibits its incorporation into RNA (inhibitions: 63% after 1 h, 80% after 2 h, 97% after 3 h). The inhibitions are less for DNA (7% after 1 h, 46% after 2 h, 71% after 3 h). The incorporation of ^{32}P into RNA and the incorporation of amino-acids into proteins are equally sensitive to RNase in the treated cells. Estimations still in progress of the basic composition of the RNA present in the RNase treated cells indicate that, after treatment for 1 h, the ratio of the bases is not significantly altered; after 4 h, however, RNA has lost 80% of its uridylic acid, and 35% of its adenylic and cytidylic acid content.

4. Experiments in which the effects of RNase on amino-acids incorporation have been compared at 2 different temperatures (4° and 20°) failed to give clear-cut results, because both the penetration of RNase (the RNase content of the treated roots increases 2–3 times at 20°) and the incorporation of the amino-acids into the proteins are strongly temperature-dependent.

It can be concluded that RNase first forms a complex with the intracellular RNA in the living roots; this complex formation suffices to inhibit RNA and protein metabolisms. At a later stage, RNA is broken down in the usual way.

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