Remarks on preparations of ribonuclease from different manufacturing sources

The action on cancerous cells of different preparations of ribonuclease (RNase; Armour, Worthington or General Biochemical Incorp. (G.B.I.)) has been studied[1,2]. The experimental material was obtained by chromatographic separation of these batches following the procedure described by HIRS, MOORE AND STEIN[3].

The fractions used were apparently the same, as discussed below (fraction C[4]) but the observed biological effects differed considerably.

When treated with the RNase from G.B.I. or Worthington, the cells of the Ehrlich carcinoma show cytochemically rapid changes in their basophily; this change is quite slow in the case of Armour RNase. Moreover, cellular malformations appear in the first two cases but not with the Armour product.

From a biochemical point of view, one observes pronounced modifications of the metabolism of the ribonucleic acids (RNA). With G.B.I. or Worthington material, an immediate synthesis of RNA in the cell takes place, under certain conditions, followed by a slow disappearance of the intracellular RNA. With Armour RNase, one observes a very slow disappearance from the first moment of interaction[3].

This is not due to a difference in enzymic activity only. Whilst the Armour product has half the *in vitro* activity of that from G.B.I., equal activities still produce the same different biological results.

Similar results have also been obtained with *Amoeba*: when submitted to RNase, the basophily of the cytoplasm and the nucleoli rapidly decreases with the G.B.I. enzyme; the Armour RNase is about 5 times less active and the experiments are not so easily reproducible. However, G.B.I. and Armour products give the expected results in the case of the incorporation of labelled amino acids in living root tips (BRACHET[5]): under similar experimental conditions, G.B.I. RNase is about twice as effective in inhibiting the incorporation as the Armour product.

These differences in biological behaviour can not so far be explained on the basis of corresponding changes in physico-chemical properties.

The patterns of chromatographic and sedimentation diagrams (sedimentation experiments by Dr. K. V. SHOOTER) are on the whole very similar. None of the fractions seems to be contaminated by proteinous material carrying other enzymic activities. On the other hand, the RNase material, as purchased, contains low molecular weight impurities which account for *ca.* 25% of the dry weight in each case. Also the U.V. spectrum which corresponds to that described by SHUGAR[7] shows with all the preparations, no gross variations. The observed values agree with those obtained by sedimentation experiments and chemical analysis.

In consequence, these preparations appear to possess similar protein content but different enzymic or biological activities. It is perhaps too early to attempt an interpretation of these facts but it is important to draw attention to them at this juncture.

As a suggestion, these products could represent either slightly altered or oxidised forms of the same protein or, as an extreme, even really different structures[8,9,10].

It was previously reported[4,6,11] that two sluggish –SH groups, probably linked with other groups of the protein, were present in native ribonuclease and played an important part in enzyme activity. In connection with what was said above, it should be emphasised here that these results were obtained with G.B.I. and Worthington material only and may not reflect fully the properties of the Armour product.

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