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Abstract

It has been revealed that ribonuclease (RNase) can penetrate into living cells and inhibits amino acid incorporation into proteins resulting in the suppreion of protein synthesis and growth of living cells. BHIDE, BRACHET[1], KAUFMAN and DAs have proven that the RNase penetrates into onion root-tip cells and induces a number of mitotic abnormalities. KIMOTO and others[2] also have revealed that RNase injection into mice results in the reduction of cytoplasmic basophilia with the morphologic change of endoplasmic reticulum and the disturbances in DNA synthesis as demonstrated histochemically on pancreatic exocrine cells and liver cells. But there is little information so far on the mechanisms of penetration of RNase into living cells. PILLERI[3] and SCHUMAKER4 in Brachet's laboratory have demonstrated the uptake of RNase by pinocytosis in amoebae and cancer cells. This may suggest the penetration of RNase through the membrane of the endoplasmic reticulum in the cells whose RNase contents are low5, however it is reasonablly supposed that some phosphatase may be concerned with the permeability of RNase.

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HISTOCHEMICAL STUDIES OF ADENOSINE TRIPHOSPHATASE ACTIVITY OF LIVER CELLS EXPOSED TO RIBONUCLEASE

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It has been revealed that ribonuclease (RNase) can penetrate into living cells and inhibits amino acid incorporation into proteins resulting in the suppression of protein synthesis and growth of living cells. BHIDE, BRACHET[1], KAUFMAN and DAS have proven that the RNase penetrates into onion root-tip cells and induces a number of mitotic abnormalities. KIMOTO and others[2] also have revealed that RNase injection into mice results in the reduction of cytoplasmic basophilia with the morphologic change of endoplasmic reticulum and the disturbances in DNA synthesis as demonstrated histochemically on pancreatic exocrine cells and liver cells. But there is little information so far on the mechanisms of penetration of RNase into living cells. PILLERI[3] and SCHUMAKER[4] in Brachet's laboratory have demonstrated the uptake of RNase by pinocytosis in amoebae and cancer cells. This may suggest the penetration of RNase through the membrane of the endoplasmic reticulum in the cells whose RNase contents are low[5], however it is reasonablly supposed that some phosphatase may be concerned with the permeability of RNase.

In the present work, an attempt has been made to study the histochemically demonstrable adenosine triphosphatase activity (ATPase) of mouse liver cells exposed to RNase *in vitro*, which may be correlated with permeability of RNase.

The method used for the demonstration of ATPase was the one devised by WACHSTEIN and MEISEL[6] but slightly modified by SENO and TOGAWA[7]. But it has been recently claimed by FREIMAN[8] that the reaction may be mainly of polyphosphatase.

Small pieces of fresh liver tissue of C58 mice, about 3 mm. in size, were put in the phosphate buffer, pH 7.4, containing RNase, 10 mg/dl, and incubated at 37 °C for 60 minutes. After incubation tissues were fixed in 10 per cent chilled formol, 0°～5 °C, and frozen-sectioned. The sections were further incubated with the substrate having the following constituents at 37 °C for 20 minutes.

SUBSTRATE MIXTURE	
ATP Na-salt	25 mg
Distilled water	22 cc
6.2 *M* Tris malate buffer (pH 7.2)*	20 cc
Lead acetate (2 per cent)	3 cc
0.1 *M* magnesium chloride	5 cc

Washing with distilled water the sections were treated with 1 per cent ammonium polysulfate solution, washed with water and observed mounting glycerin. As the controls the tests for ATPase activity were done on the liver slices fixed with chilled formol without pretreatment and on those treated with the buffer solution but containing no RNase.

In the rat liver received no pretreatmentor incubated with phosphate buffer solution the strong activity of "ATPase" is demonstrated on the intra-acinal bile canaliculi selectively and regularly (Figs. 1 and 2). On the other hand, ATPase activity of the liver received the pretreatment with RNase-containing phosphate buffer demonstrates much reduction and irregularity in the ATPase activity of bile canaliculi (Figs. 3 and 4).

Data demonstrate that the liver cells exposed to RNase lose its activities of ATPase or polyphosphatase, which is demonstrable on the cell surface histochemically and supposed to be colsely correlated with the cell permeability or active transport by using the energy of ATP or polyphosphate. The relation between active transport and ATPase has been proved on mitochondria or red cell[9] but only for inorganic ions and glucose, and not for protein. As RNase proves to penetrate into cell through cell surface or endoplasmic reticulum but acts to reduce or obliterate the ATPase activity, it is supposed that RNase will act as to disintegrate molecular structure of the membrane on the cell surface or endoplasmic reticulum and penetrate the cell by passive transport, but not by the aid of ATP and ATPase, though the mechanism should be settled with further study.

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* 6.2 *M* Tris-malate buffer is mixed with 0.2 *M* Tris malate stock solution 200 cc, 1 *N* NaOH 100 cc and distilled water 200 cc, and then this mixtured solution was buffered pH. 7.2 by 1 *N* NaOH.

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EXPLANATION FOR PHOTOS

Fig. 1. ATPase activity of normal mouse liver.

Fig. 2. ATPase activity of mouse liver pretreated with phosphate buffer solution (pH 7.4) containing no RNase.

Fig. 3. ATPase activity of mouse liver pretreated with RNase (10 mg RNase/cc phosphate buffer solution).

Fig. 4. ATPase activity of mouse liver pretreated with RNase. High power view of a part of Fig. 3.


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