INHIBITORY ACTION OF RIBONUCLEASE ON SECRETION OF HYDROCHLORIC ACID BY SURVIVING FROG'S GASTRIC MUCOSA

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The role of nucleic acids, and in particular of ribonucleic acid (RNA), in cell metabolism has been elucidated by the work of M. Brachet [1], Casperson [17, 18], B. Kedrovsky [6, 8], and others. M. Guberniev, I. Kovyrev, and L. Ilina [4, 5] have shown the connection between RNA and the processes of synthesis of digestive enzymes. The important part played by RNA in the functioning of the glands of the alimentary tract has been established by Casperson [17, 18], M. Deken-Grenson [19], and others. The discovery by us of the inhibitory effect of basic dyes on hydrochloric acid secretion [12] led us to the conclusion that, since these dyes combine with nucleic acids, their effect is due to direct blocking of the cyclophorase system, the basic component of which is RNA. D. Yakhina [14] has shown that the RNA activity of the gastric mucosa rises considerably during heightened secretion of hydrochloric acid. Thus the participation of RNA has been demonstrated not only in the synthesis of digestive enzymes, but also in the process of secretion of hydrochloric acid. The active participation of RNA in the secretion of amylase by the pancreas, although not in the process of synthesis of this enzyme, has been shown by L. Hokin [20-22]. A number of recently published papers have shown that protein synthesis and RNA activity do not always vary parallel [19, 23].

Thus, there is every reason to believe that RNA takes part in the accumulation of high-energy phosphate bonds [2, 3, 27], and thus provides for the normal vital activities of cells.

As far back as 1951 we performed experiments on the action of ribonuclease on secreting surviving gastric mucosa of frogs. These experiments were based on Brachet's finding that this specific enzyme is capable of penetration into fixed tissue sections, so serving for the histochemical identification of RNA in tissues. At that time we had no reason to believe that ribonuclease, which has a molecular weight of the order of 12,000-15,000, is able to penetrate into living tissues, although it had a pronounced inhibitory effect on secretion of HCl by surviving gastric mucosa.

It has been shown in the recent papers of J. Brachet [15, 16] and L. Ledoux [24, 25] that ribonuclease is able to penetrate freely into living tissues. This has led us to publish our findings on the inhibitory action of ribonuclease on secretion of HCl by surviving gastric mucosa of frogs.

The opinion has been expressed by some authors working on ribionuclease that it may exert a proteolytic action, owing to its admixture with proteases. This possibility is eliminated if the ribonuclease is prepared according to M. McDonald [26]. We cite below the findings of Yakhina on the action of salivary ribonuclease on secreting surviving frog's gastric mucosa, which exclude the possibility of any such side action of the enzymatic preparation, since it is known that proteolytic enzymes are absent from the saliva. Thermal treatment of saliva has been shown by G. I. Roskin [11] to assure the action of ribonuclease on histological tissue sections, invariably, and beyond any doubt. Ledoux [24, 25] has demonstrated that direct introduction of some proteolytic enzymes into living cells has no effect on cell division.

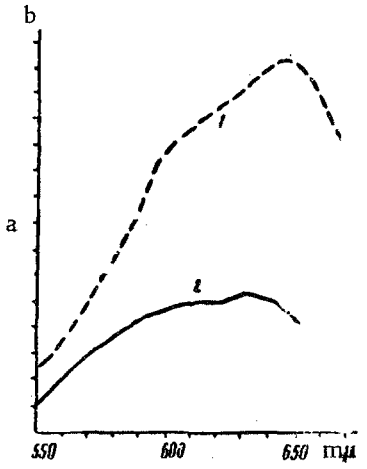
EXPERIMENTAL METHODS

Surviving frog's gastric mucosa was prepared and used for experiment in the way described in a previous paper [12]. To the nutrient medium we added active crystalline ribonuclease, provided by M. V. Pavlova, or else human saliva (1 ml) which had been heated at 70° for 30 minutes; 25 ml of nutrient medium, prepared according to Yakhina, was taken. In order to exclude any possible effects due to ammonium salts used to precipitate ribonuclease, we first removed ammonium ions by passing the enzyme solution through a phenosulfonic cationite in the hydrogen phase.

The amount of hydrochloric acid produced by the surviving tissue during 4 hours was determined by conductometric titration [12]. In some of the experiments we added 1 ml of 0.05% toluidine blue to the medium, in order to check the vitality of the cells, and in others we stained pieces of tissue with the same solution, after completion of the experiment. Living tissues are able to decolorize toluidine blue, changing it into the leuco-form. Apart from this, RNA is able to shift the absorption maximum of toluidine blue, from 630 mμ in its absence to 650 mμ in its presence (see Figure). As a result of this shift, the dye changes color from blue to green. This bathochromic effect is not given by RNA after depolymerization with ribonuclease.

EXPERIMENTAL RESULTS

Our experiments showed that surviving mucosa becomes decolorized if the access to air is restricted (under a cover slip), and that it acquires a violet coloration as soon as the cover slip is lifted. Tissue treated with cyanides or with alcohol immediately acquires a permanent blue coloration. Microscopic examination showed that the toluidine blue stained parts had a greenish tinge in places, whereas after the action of ribonuclease the color changed to blue, approaching a violet tinge.



Absorption spectrum of toluidine blue in the presence of ribonucleic acid (1), and in its absence (2).

The results of experiments on the secretion of hydrochloric acid by frog's surviving gastric mucosa are presented in the Table.

As appears from the Table, ribonuclease powerfully, and in some cases totally, inhibits secretion of HCl by surviving gastric mucosa. This effect can only be explained if we assume that ribonuclease penetrates into the cells, and blocks the biochemical processes responsible for the normal secretion of HCl. Thus, our supposition that RNA plays an essential part in the process of secretion or formation of HCl in the gastric mucosa [13, 14] is confirmed by the direct action of active ribonuclease on RNA.

Enhanced secretion of HCl by surviving gastric mucosa, caused by addition of histamine, is inhibited by addition of ribonuclease to the nutrient medium. This effect of ribonuclease on the activity of surviving gastric mucosa confirms our previously expressed assumptions.

Amount of HCl, in μM per sq. cm of Surviving Gastric Mucosa Produced in 4 Hours in an Aerated Nutrient Solution, in the Absence and the Presence of Active Ribonuclease

Control HCl, μM/sq. cm	Crystalline ribonuclease + + HCl, μM/sq. cm	Saliva* + HCl, μM/sq. cm
Mean of 23 experiments 4.47 ± 0.30	Mean of 7 experiments 2.4 ± 0.40	In 5 experiments 0.00 In one experiment 0.47 In one experiment 1.57 In one experiment 2.97 In one experiment 3.14

* The data of this column are taken from Yakhina.

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EFFECT OF INTRAVENOUS INJECTION OF HETEROLOGOUS SERUM ON GASTRIC SECRETION

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It has been shown by I. P. Razenkov [5] and his co-workers that proteins and other nitrogenous substances are secreted with the gastric juice.

* In Russian.