# HUMAN SALIVARY RIBONUCLEASE ACTIVITY IN NORMAL HEALTH, GASTRIC CARCINOMA, FOOD POISONING, AND BACILLARY DYSENTERY

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UDC 616.33-006.6 + 616.9-022.38 + 616.935-07: 616.316-008.831 : 577.155.2-074

The salivary ribonuclease activity was investigated in healthy human subjects and patients with various gastro-intestinal diseases (carcinoma of the stomach, bacillary dysentery, food poisoning). Both acid and alkaline ribonucleases were detected in the saliva. The latter differs in its properties from pancreatic ribonuclease. The salivary ribonucleases activity is clearly reduced in patients with gastric carcinoma.

* * *

Because of the important biological role of ribonuclease in cell activity, the properties of this enzyme have been intensively studied in extracts and homogenates of many tissues and organs [2, 5, 7, 8, 11]. However, little information is available on the ribonuclease activity of human saliva [3, 6].

In the present investigation certain properties of human salivary ribonucleases were investigated and the salivary ribonuclease activity under normal conditions and in certain pathological states was compared.

## EXPERIMENTAL METHOD

Ribonuclease activity of the saliva was determined in 24 healthy persons, 15 patients with carcinoma of the stomach, 10 patients with food poisoning, and 21 with bacillary dysentery. The saliva was collected in the morning before breakfast, without the use of sialogogues. The samples of saliva were cooled and centrifuged at 2-4°.

High-polymer cytoplasmic RNA isolated from rat liver by the Kirby – Georgiev method [1] was used as substrate. The solution of high-polymer RNA was used for 7-10 days.

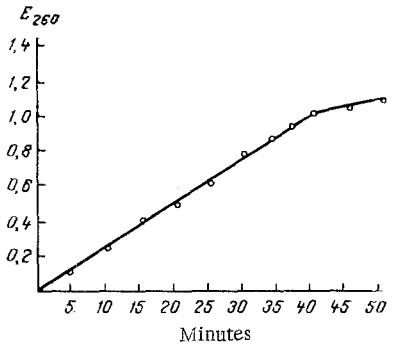


Fig. 1. Salivary ribonuclease activity as a function of time. Composition of incubation mixture given in text. Incubation at 37°.

The incubation mixture contained (in 3 ml): 150 μmoles Tris-HCl buffer (pH 7.8), 330 μmoles NaCl, 1.5 mg RNA, and 0.1 ml saliva. Incubation continued for 15 min at 37°. The precipitating agent was perchloric acid (final concentration 0.5 M). The optical density was determined in a type SF-4 spectrophotometer at 260 mμ. The unit of ribonuclease activity which was adopted was the quantity of acid-soluble fraction producing a change in optical density of 0.1 under standard conditions. Protein was determined by Lowry's method [9]. The specific activity was calculated per milligram of protein.

The effect of pH, temperature (with determination of the temperature optimum), and $mg^{2+}$ ions on ribonuclease activity and its resistance to heat were all studied in an incubation mixture of the composition described above.

## EXPERIMENTAL RESULTS

Salivary ribonuclease activity as a function of time is illustrated in Fig. 1. The linear relationship persists for 40 min.

Department of Medical Biochemistry, Central Postgraduate Medical Institute, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR F. G. Krotkov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 12, pp. 49-53, December, 1968. Original article submitted June 28, 1967.

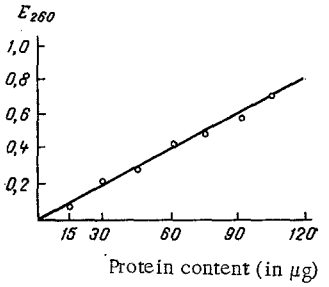


Fig. 2

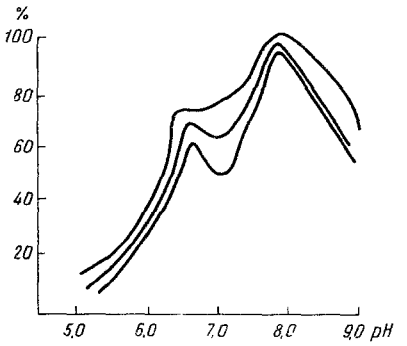


Fig. 3

Fig. 2. Salivary ribonuclease activity as a function of protein concentration. A volume of saliva containing from 15 to 120 μg protein was added to the incubation mixture. Incubation for 15 min at 37°.

Fig. 3. Effect of pH on salivary ribonuclease activity. Buffer solutions (0.1 M): Tris-HCl buffer, pH 7.6-9.0; phosphate buffer, pH 6.2-7.5; acetate buffer pH 5.0-6.1. Maximal enzyme activity taken as 100%.

During this period ribonuclease activity was proportional to enzyme concentration (Fig. 2). The results of experiments to determine the effect of pH on ribonuclease activity of saliva obtained from 3 adult males aged from 25 to 40 years are shown in Fig. 3. High ribonuclease activity was maintained over a wide range of pH values (from 6.3 to 8.5). The principal optimum occurred in an alkaline medium (pH 7.8-8.0), and a subsidiary optimum was found in an acid medium (pH 6.3-6.5).

Investigation of the resistance of salivary ribonuclease to heat demonstrated its high thermostability. After heating to 95° for 8 min at pH 3.5, no decrease in the ribonuclease activity of the saliva was observed. Heating the saliva for 10 min under the same conditions reduced the ribonuclease activity by 8-10%. Incubation of saliva with substrate under the same conditions but at different temperatures showed maintenance of high ribonuclease activity between temperatures of 50 and 70°. The temperature optimum under these conditions was 65°. Addition of $Mg^{2+}$ ions in different concentrations lowered the ribonuclease activity: in a concentration of 0.003 M by 6-8%, 0.006 M by 9-11%, and 0.012 M by 23-27%.

The next investigations were to study salivary ribonuclease activity under normal and pathological conditions. No definite changes in ribonuclease activity dependent on sex or age could be discovered. In patients with gastric carcinoma a marked decrease in ribonuclease activity was observed, compared both with the control group ($P < 0.001$) and the groups of patients with bacillary dysentery ($P < 0.01$) and with food poisoning ($P < 0.001$; Table 1). The distribution of ribonuclease activity in patients with carcinoma of the stomach compared with healthy persons is interesting. The number of samples with activity between 0 and 19 in the group with gastric carcinoma was 40%, and in the control group it was zero; the number of samples with activity from 0 to 29 was 86.6% in the group of patients with gastric carcinoma and 12.5% in the control group. The decrease in ribonuclease activity in patients with food poisoning and bacillary dysentery compared with the control group was not significant ($P > 0.05$).

Bearing in mind that the mean volume of saliva secreted per diem is 1500 ml, the daily excretion of alkaline salivary ribonuclease calculated as crystalline pancreatic enzyme is 63 μg.

The results indicate that saliva contains both alkaline (pH 7.8-8.0) and acid (pH 6.3-6.5) ribonuclease. The study of the pure secretions of the parotid and submandibular salivary glands under conditions precluding contamination with microorganisms and leukocytes revealed similar pH optima of salivary ribonuclease activity [9]. High ribonuclease activity of the total saliva was found to be due to the ribonuclease activity of the pure secretions of the parotid and submandibular salivary glands. Acid and alkaline ribonucleases have also been found in homogenates of mouse salivary glands [4]. It is unlikely that contamination of the oral cavity by microorganisms and leukocytes has any significant effect on the total salivary ribonuclease activity.

TABLE 1. Salivary Ribonuclease Activity of Patients with Gastric Carcinoma, Food Poisoning, and Bacillary Dysentery

| Source of salivary ribonuclease | Number of determinations | M±m | P |
|---|---|---|---|
| Healthy persons | 24 | 45.4±3.2 | – |
| Patients with gastric carcinoma | 15 | 24.4±2.7 | < 0.001 |
| Patients with food poisoning | 10 | 42.3±3.8 | > 0.05 |
| Patients with bacillary dysentery | 21 | 37.1±3.4 | > 0.05 |

It is an interesting fact that the study of the ribonuclease activity of various human physiological fluids (blood serum, cerebrospinal fluid) has revealed only an alkaline enzyme. The presence of an acid ribonuclease likewise has not been demonstrated on the skin surface. Only in human urine have both acid and alkaline ribonuclease been discovered [2]. Consequently, only human saliva and urine contain acid and alkaline ribonuclease. Since only the alkaline enzyme is found in the blood serum, this cannot be the source of the acid ribonuclease. The most probable source of the enzyme is the salivary glands. Since the salivary ribonucleases were not purified in this investigation, the information obtained regarding the properties of the alkaline ribonuclease must be regarded as preliminary. According to these preliminary data (pH optimum 7.8, temperature optimum 65°, high thermostability), salivary alkaline ribonuclease is indistinguishable from the recently described [11] alkaline ribonuclease of the human pancreas. Similarity between pancreatic ribonuclease and the alkaline ribonuclease of the blood has been demonstrated previously [10]. Besides the salivary glands as a probable source of salivary alkaline ribonuclease, the possibility of a pancreatic origin of this enzyme thus cannot be ruled out.

The possibility of a pancreatic origin of the salivary alkaline ribonuclease is in agreement with results obtained for the activity of this enzyme in various diseases of the gastro-intestinal tract. The hormone secretion, produced mainly in response to stimulation of the duodenal mucous membrane by hydrochloric acid and products of digestion arriving from the stomach (peptones, higher fatty acids and their salts) is known to participate in the stimulation of pancreatic enzyme secretion. In carcinoma of the stomach, with low acidity and disturbance of digestive processes in the stomach, this stimulant action on pancreatic enzyme secretion is weakened.

Since no determination of salivary ribonuclease activity have previously been made in various pathological states, the marked decrease in ribonuclease activity demonstrated by these experiments in patients with carcinoma of the stomach is of considerable interest. A study of ribonuclease activity in patients with carcinoma of the stomach at different stages of the disease could help to establish the diagnostic importance of the demonstration of a lowering of its level.

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