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EFFECT OF BILE ON AMYLOLYTIC ACTIVITY OF SOME BIOLOGICAL MEDIA

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KEY WORDS: amylase; bile; changes in enzyme activity.

Changes in enzyme activity are among the principal mechanisms of metabolic adaptation *in vivo*. Many of the factors determining the level of catalytic enzyme activity have been elucidated [3]. As regards amylase, which takes part in intestinal digestion, there is evidence that it is affected by activity of bile acids [2] or of whole bile [4], present in the intestine. Components of bile are contained in the internal medium and tissues of the body [1], where their concentration may rise in biliary stasis.

The aim of the present investigation was to discover whether an increase in concentration of components of bile is reflected in amylase activity in these media.

EXPERIMENTAL METHOD

The effect of bile on the amylolytic activity of human saliva, blood plasma and homogenates of the pancreas and mucosa of different parts of the small intestine of rats was investigated *in vitro*.

Blood and tissues of these organs were obtained from the rats after decapitation. The pancrease and the intestinal mucosa, washed to remove chyme, were homogenized with a Teflon pestle in Ringer's solution, pH 7.0, at the rate of 1 ml of liquid to 100 mg tissue. Plasma was obtained by centrifugation of blood mixed with 5% NaCl solution in the ratio of 1:50; saliva was diluted before analysis with Ringer's solution in the ratio of 1:20,000. All media for study were prepared at 0-4°C.

Amylase activity was determined by Ugolev's method [5] and expressed in milligrams starch hydrolyzed per minute per milliliter (of saliva or blood plasma) or per gram of tissue (pancreas and intestinal mucosa). Bile was added to the test media in doses of 0.1, 0.2, 0.5, and 1 ml. An increase in the concentration of bile acids by 2.5, 5, 12, 5, and 25 mg%, respectively, was created in this way in the medium.

The numerical data were subjected to analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

Addition of bile to a medium in which enzymic hydrolysis of starch takes place was shown to accelerate the course of the reaction. Activation was not associated with the presence of amylase in the bile itself (its activity was accounted for in the calculations).

The presence of bile had a stimulating action on amylase activity, which differed in its origin. An increase in activity of amylase synthesized by cells of the salivary glands, pancreas, and intestinal mucosa was observed. Meanwhile, with the same quantity of bile, the increase in amylase activity differed in different media. On addition of 0.2 ml of bile, amylase activity in the blood plasma increased by 280%, in saliva by 146%, in homogenate of the pancreas by 152%, in the jejunal mucosa by 114%, and in the ileal mucosa by 150%.

The degree of increase in amylase activity depended on the quantity of bile added to the medium. With a gradual increase in the quantity added, the maximum of the effect was observed

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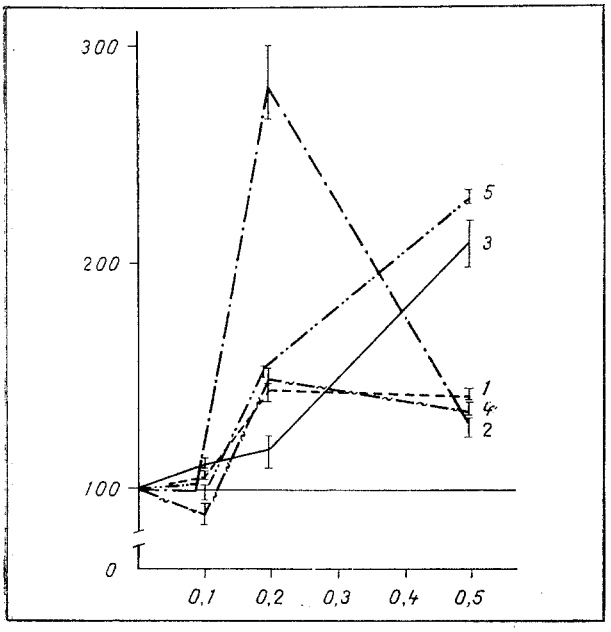

Fig. 1. Effect of bile on amylolytic activity of saliva, blood, and homogenates of the mucosa of the proximal and distal portions of the small intestine and pancreas. Abscissa, quantity of bile (in ml); ordinate, enzyme activity (in % of control, taken as 100). 1) Saliva; 2) blood; 3) proximal portion; 4) distal portion; 5) pancreas.

in the presence of a definite quantity of bile, which differed for amylases of different origin (Fig. 1): For amylase of blood plasma, saliva, and ileal mucosa it was 0.2 ml, for amylase of the pancreas and jejunal mucosa it was 0.5 ml. On addition of a larger quantity of bile, the stimulating effect was reduced. The action of bile on the rate of the enzymic reaction was observed in concentrations which did not change the pH of the medium.

The results thus indicate that components of bile which under normal circumstances are present in certain quantities not only in the intestinal lumen, but also in the internal medium of the body, may perform the role of factor influencing amylase activity in various tissues and biological fluids.

The stimulating effect may be connected with the influence of bile acids [6] or other components present in the bile.

Considering the conditions of the experiments described above, a direct effect of components of the bile on conformation of enzyme protein molecules must be accepted. Components of bile such as bile acids, cholesterol, bilirubin, and phospholipids, are known to be adsorbed on the surface of plasma proteins [1]. Depending on variants of molecular forms of the enzyme (isozymes) conformational changes resulting from exposure to identical factors may be different [3]. This may account for the unequal increase in activity of amylases of different origin under the influence of bile.

Conditions of the experiments *in vitro* demonstrate the effect of bile on the activity of enzymes already synthesized. Changes in amylase activity were observed in the presence of components of bile in concentrations corresponding to the upper limit of normal and its transition to cholemia. This state of affairs must be taken into account when enzyme tests are used for diagnostic purposes.

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BIOCHEMICAL CRITERIA OF SENSITIVITY AND RESISTANCE OF GASTRIC TUMORS TO 5-FLUOROURACIL

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KEY WORDS: 5-fluorouracil; gastric tumors; alkaline phosphatase; uridine kinase; thymidine kinase.

Because of the biochemical differences between experimental tumors sensitive and resistant to 5-fluorouracil (5-FU) [1, 2], it was decided to undertake corresponding investigations on human gastric carcinoma tissue.

EXPERIMENTAL METHOD

Gastric carcinoma tissue was obtained in the cold state after removal from patients admitted to a Regional Cancer Dispensary. The intensity of accumulation of [6-^{3}H]-5-FU (specific radioactivity 104-192 GBq/mmole, Czechoslovakia) in tumor tissue RNA was studied after incubation for 30 and 60 min. The tissue was then washed and homogenized in the cold. The acid-soluble fraction and lipids were extracted from the homogenates and DNA was separated from RNA [6]. Radioactivity of the samples was determined on a Mark III scintillation counter (from "Nuclear Chicago," USA).

Alkaline phosphatase (AlP) activity was determined [7] in tumor homogenates, and uridine kinase [5] and thymidine kinase [3] activity in partially purified homogenates by a radioisotope method. Protein was determined by Lowry's method [4].

EXPERIMENTAL RESULTS

Incorporation of [6-^{3}H]-5-FU into RNA of different tumor cells took place with different intensities, and it resembled very closely incorporation of labeled 5-FU into experimental tumors sensitive and resistant to the compound. In some tumors incorporation of 5-FU into RNA took place in the same intensity as in tumors sensitive to 5-FU, and in others, with the same intensity as in tumors resistant to 5-FU [2]. In agreement with these data, the tumors studied were divided on the basis of incorporation of [6-^{3}H]-5-FU into RNA into those sensitive and resistant to the compound (Table 1).

Activity of AlP, uridine kinase, and thymidine kinase was studied in these same tumors, and in tumors sensitive to 5-FU it was found to be significantly higher than in resistant tumors (Table 2).

The correctness of our views on sensitivity of gastric tumors to 5-FU on the basis of biochemical tests was confirmed by clinical trials.

In the period from 1976 through 1978 we studied 123 tumors removed from patients at the Regional Cancer Dispensary. After the operations these patients received prophylactic chemotherapy with 5-FU. All these tumors were divided on the basis of AlP activity into those sensitive (58) and resistant (65) to the compound. AlP activity in tumors sensitive and resistant to 5-FU was 20.88 ± 0.60 and 11.13 ± 0.67 μmoles p-nitrophenol/mg protein/min, respectively ($P < 0.001$).

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