

## BIOCHEMISTRY AND BIOPHYSICS

### CHANGES IN THE SPEED OF INCORPORATION OF METHIONINE S<sup>35</sup> INTO THE PROTEIN OF THE MUCOSA DURING EXPERIMENTAL GASTRIC ULCER

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In seeking a model which could be used to test the effectiveness of pharmaceuticals in preventing the development of gastric ulcers, we worked out a method of reproducing gastric ulcers by causing "excessive" irritation in the duodenal area.

It was found (by means of microscopy) that destructive changes in the gastric walls appear as hemorrhages, erosions, and ulcers 3-6 hours after trauma is caused in the duodenal area.

Pharmacological and physiological analysis of the mechanisms producing the experimental gastric ulcer showed that they are not the direct results of tissue injury, but are produced by the reflexes which arise as a consequence. This is substantiated both by the location of the ulcers in the mucosa of the stomach at a distance from the traumatized area and by the fact that the ulcers can be averted by cutting the vagus nerves below the diaphragm or by the administration of drugs which interrupt the reflex arc (barbiturates, ganglion-blocking agents). In a series of experiments, carried out on a dog with a stomach isolated by I. P. Pavlov's method and with a fistula of the duodenum, it was established that prolonged electric stimulation of the duodenum produces changes by reflex action in the functional state of the gastric glands. A decrease in the amount and a change in the quality of the secretions produced in response to stimulation by food is observed. The amount of enzymes was reduced considerably, and the digestive power of the gastric juice was diminished [1].

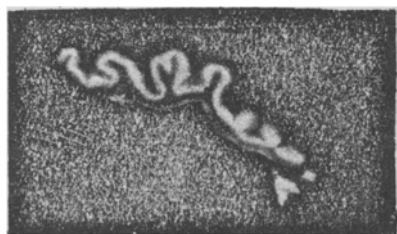
According to data in the literature, lowered enzyme production by the gastric glands serves as an indicator of a disturbance of the tissue metabolism (trophe) of the gastric mucosa [2-5].

As a result of such prolonged and regular irritation of the duodenal mucosa by electric current (for a month), the dog suffered lesions of the gastric mucosa and died of gastric hemorrhage. The development of severe dystrophy of the gastric mucosa proved incontrovertibly that the change in the amount of enzymatic proteins in the gastric juice was actually the first sign of a disturbance in the trophe of the mucosa. However, the recording of the enzymatic proteins in the gastric juice makes it possible to judge only regarding the synthesis of those proteins which form a part of the secretions, while it is much more important to know the rate at which the proteins which are a basic part of the gastric cell structure are synthesized in order to judge the nutritional levels.

The purpose of this work was to determine the changes in the cellular metabolism of the gastric walls under the influence of reflexes arising as a result of duodenal irritation. Tagged atoms were used for this purpose.

## EXPERIMENTAL METHOD

We judged the rate of exchange of the proteins in the gastric wall by the speed with which radioactive amino acid — sulfur-tagged methionine — was absorbed. A solution of methionine  $S^{35}$  was administered subcutaneously to the guinea pigs, using an amount which would give 4000 pulses per gram of body weight. Before administration, the number of pulses emitted by 1 ml of the solution per minute was determined on an end counter (type "B" apparatus). The volume of the solution administered per guinea pig varied between 0.3 and 2 ml, depending on the radioactivity of the compound.



Autoradiograph of a section of a guinea pig's gastric wall one hour after administration of methionine  $S^{35}$

removal of the organs to the precipitation of the proteins, were carried out as quickly as possible, with ice cooling. The precipitated protein was washed with a 5% solution of trichloroacetic acid and centrifuged several times, until the wash liquid was no longer radioactive. The washed protein was separated from the lipoids by extraction with 10 times its volume of alcohol for 12-20 hours, followed by washing with alcohol and centrifuging. Then the protein precipitate was dissolved in 1 N solution of KOH. 0.2 ml of this solution was used for the determination of radioactivity by means of an end-counter, and 0.2 ml for colorimetric determination of the amount of protein by the biuret method. In all cases, the incorporation of methionine  $S^{35}$  was expressed in impulses per minute of 10 mg of protein.

In order to watch the speed with which the methionine was incorporated into the proteins of the gastric mucosa, i.e., in order to observe the rate of synthesis of these proteins, we killed the experimental animals 1, 3, and 6 hours after administration of the sulfur-tagged amino acids and determined the amount of  $S^{35}$  in the tissues of the gastric wall by the amount of radioactivity. For this purpose, 2-g of gastric wall tissue was carefully crushed and ground in a mortar. Then the protein was precipitated by a 10% solution of trichloroacetic acid. All the operations, from the

## EXPERIMENTAL RESULTS

The experiments showed that only one hour after subcutaneous administration of the methionine, it was found in the proteins of the gastric wall of the control group of guinea pigs, which were not subjected to any manipulations. In order to locate the position of the gastric wall tissue which incorporates the majority of the methionine, autoradiographs of sections of the experimental animals' stomachs were made.

The tagged methionine was administered subcutaneously in the usual amounts. An hour later, the animals were killed; but instead of analyzing it chemically, we fixed the gastric wall with Carnoy's solution, embedded the piece under analysis in paraffin, and prepared sections of it  $9\mu$  thick. Then we laid photographic film over the section and exposed it for several days in the dark. On the developed film could be found an autoradiograph, i.e., a print of those areas of the section which had incorporated the radioactive methionine. The print of one of these autoradiographs on photographic paper is illustrated. The strongest radiation, indicating the greatest incorporation of radioactive methionine, was observed in the mucosa of the gastric wall, which is represented in the illustration as a light strip, sharply differing from the considerably darker reflection of the deep tissues of the gastric wall.

The next series of experiments on guinea pigs was carried out in order to determine the effect of reflexes arising during "excessive" irritation of the duodenal area on the protein synthesis of the gastric wall. For this purpose, with light ether anesthesia and under aseptic conditions, the abdominal cavity of the animals was opened along the white line of the belly, and Pean's forceps were applied to the duodenum for fifteen minutes. After this trauma, the wound in the abdominal wall was sewed. An hour later, the usual dose of methionine was administered to the experimental animals subcutaneously; after another hour, the animal was killed and the amount of marked methionine was determined.

A control group of animals was subjected to the same conditions with the exception of the trauma caused by Pean's forceps in the duodenal area.

Inclusion of Methionine S<sup>35</sup> into the Proteins of the Gastric Wall of Guinea Pigs under Normal Conditions and after the Infliction of Trauma in the Duodenal Area

| Date of experiment | Control animals |                             | Experimental animals |               |                             |
|--------------------|-----------------|-----------------------------|----------------------|---------------|-----------------------------|
|                    | Weight (in g)   | Number of pulses per minute | Date of experiment   | Weight (in g) | Number of pulses per minute |
| 5/22               | 730             | 222                         | 5/6                  | 710           | 68                          |
| 3/25               | 500             | 204                         | 3/25                 | 500           | 37                          |
| 3/31               | 770             | 278                         | 3/31                 | 570           | 84                          |
| 4/2                | 710             | 216                         | 4/2                  | 770           | 86                          |
| 4/5                | 450             | 200                         | 4/5                  | 450           | 234                         |
| 4/7                | 600             | 238                         | 4/7                  | 600           | 182                         |
| 4/9                | 870             | 200                         | 4/9                  | 880           | 104                         |
| 4/23               | 760             | 295                         | 4/23                 | 760           | 120                         |
| 4/27               | 650             | 168                         | 4/27                 | 750           | 120                         |
| 5/3                | 710             | 235                         | 4/3                  | 700           | 131                         |
| 4/21               | 700             | 290                         | 6/4                  | 720           | 96                          |

Average

231

114

The control experiments showed that short-term ether anesthesia and the cutting of the abdominal wall had no effect on the protein synthesis by the gastric wall; and that tagged methionine was incorporated into these proteins at the same rate as it was in guinea pigs which were not subjected to any manipulations.

In the experimental animals, a decrease in the rate of incorporation of methionine S<sup>35</sup> into the proteins of the gastric wall was observed (see table).

As seen from the data in the table, compared with the control group of animals, there was a 55% reduction in incorporation of methionine when there was "excessive" irritation of the duodenum.

Thus, the results of our experiments show that interference with protein synthesis was already apparent 2 hours after trauma, i.e., even before the appearance of destructive changes in the gastric wall, which were found on microscopical analysis.

It can be presumed that delay in protein synthesis is one of the first indicators of a disturbance in the nutrition of the mucosa of the gastrointestinal system. Reflexes which arise on irritation of the duodenum disrupt protein synthesis, thus having a deleterious effect on the nutrition of the gastric mucosa, which is, in turn, the reason for the formation of ulcers.

The results of our work show that the speed with which sulfur-tagged amino acids are incorporated into protein is a sensitive and exact indicator of changes in the synthesis of tissue proteins, i.e., an indicator of the nutritional condition of gastric tissues.

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