Histochemical Findings in the Rat Gastric Mucosa during Starvation

M. Zaviačič and M. Brozman

Institute of Pathology, Medical School, Komenský University Bratislava (Head: Prof. Dr. M. Brozman, DrSc) Sasinkova 4 CS-80100 Bratislava, Czechoslovakia

Summary. The influence of starving on the activity of enzymes of the rat gastric mucosa was investigated by selected histochemical methods. Besides the conventional methods of enzymatic histochemistry the technique of semipermeable membranes was used in the proof of lysosomal enzymes. Dehydrogenases were proved in aqueous and also in gel media with PMS.

During the starvation in the parietal cells a marked increase took place in the activity of acid phosphatase, E-600 resistant esterase, less in β-glucuronidase. High activity of the lysosomal enzymes in macrophages did not change during starvation. Nor did any changes took place in the activity of alkaline phosphatase in the endothelium of the capillaries. The chief cells in the control and starving animals, in contrast to the human gastric mucosa, did not contain any non-specific esterase. Concerning dehydrogenases, parietal cells with a different activity of these enzymes were observed both in starved and control animals.

In the rat gastric mucosa starving induced changes in the activity of the enzymes which mark important organelles of the cells. Thus it is possible to consider the observed histochemical changes as a functional manifestation of morphological damage of cellular structures which are affected during starvation.

Introduction

The influence of starvation on the structure of tissues and organs was examined in experiments by electron microscopy. Attention was particularly devoted to the liver (David, 1960; Herdson et al., 1964; Scarpelli et al., 1968; Cardell, 1971; Bednář et al., 1973) and to the pancreas (Paradisi and Cavazzuti, 1965; Komáromy et al., 1967; Tigić et al., 1967; Gasbarrini et al., 1968; Kapeller et al., 1971; Webster et al., 1972; Nevalainen and Janigan, 1974).
Up to the present, on the whole, only little attention has been focused to the gastric mucosa. The influence of starving on the ultrastructure of fundal glands of the mouse was described (Helander, 1962; Komnick, 1963). We investigated the ultrastructure and histochemical parameters of the human gastric mucosa in healthy volunteers during 240h of starvation (Zaviačič, 1974a; Zaviačič et al., 1975). In our further studies, we analysed the influence of starving on the ultrastructure of the rat gastric mucosa (Zaviačič et al., 1976b) and other papers were devoted to the ultrastructure and cytochemical picture of the rat gastric endocrine cells and human gastric G cells during starvation (Zaviačič et al., 1976a, c).

In the present paper the influence of starving on the histoenzymatic character of the rat gastric mucosa during 168 h of starvation is described.

**Material and Methods**

In the present experiments 90 rats of the Wistar strain (*Rattus norvegicus var. alba*) 3–4 months old were used, weighing about 230 g. Thirty rats were control animals. Sixty rats were divided into 5 groups, fasting 24, 48, 72, 144 and 168 h respectively. The starving animals were placed singly into large glass vessels provided with a steel wire-net 10 cm over the bottom of the vessel (prevention of coprophagia). A half of the animals in the individual groups (6 animals) starved and had rest access to water, remaining rats (6 animals) could drink ad libitum. At the end of fasting, the animals were decapitated (always between 8–9 a.m. in order to exclude the possible variation, as a consequence of circadian rhythm). After opening the stomach, excisions from the corporal and antral mucosa were taken out.

For the majority of the histochemical examinations, the excisions were frozen by a mixture of propane-butane-dry ice (−70 °C) and cryostat sections were prepared at −20 °C, using a Pearse-Slee cryostat, or an American Optical Corp. Cryo-Cut microtome. Another portion of samples was fixed in a cooled Baker solution (formol and CaCl₂) at 4 °C 20–24 h for preparation of frozen sections (Lojda, 1965a), or only a short fixation was used by cooled Baker’s solution (2 h) followed by a shortened embedding in paraffin (Lojda, 1970). The third portion of samples was fixed in 10% neutral formol and embedded in paraffin.

Acid phosphatase was demonstrated by the azocoupling method with naphthol-ASBi or α-naphthyl phosphate (Lachema, Brno, ČSSR) and hexazonium-p-rosaniline (HPR) after Lojda et al. (1964), or with naphthol-ASBi-phosphate and Fast Blue BB (Lachema) after Burstone (1962), modif. Lojda (1966).

β-glucuronidase was detected with naphthol-ASBi-β-D-glucuronide (Koch-Light, Colnbrook, England) and HPR (Hayashi et al., 1964, modif. Lojda, 1968).

Acetyl-β-D-glucosaminidase was demonstrated with naphthol-ASBi-acetyl-β-D-glucosaminide (Lachema) and HPR (Hayashi, 1965, modif. Lojda, 1968). Sections prepared by different means were used for the proof of lysosomal enzymes. The best results were obtained with cryostat sections in the method of semipermeable membranes (Lojda, 1972).

For the demonstration of nonspecific esterase were used α-naphthyl acetate (Lachema) and HPR (Davis and Ornstein, 1959, modif. Lojda, 1966), naphthol-AS-acetate (Lachema) and HPR (Lojda, 1966), α-naphthyl acetate and Fast Blue BB (Lachema) after Lojda, 1966; 5-bromindoxyl acetate (Lachema) after Holt and Withers (1958), modif. Lojda (1966), naphthol-AS-acetate and Fast Blue BB (Burstone, 1962, modif. Lojda, 1966). Lysosomal E-600 resist. esterase was detected with α-naphthyl acetate and HPR following the inhibition by Mintacol (Bayer, Leverkusen, BRD) 10⁻² M (Lojda, 1966). We used frozen sections, cryostat sections shortly fixed by cooled Baker’s solution (10') and paraffin sections following a shortened embedding (Lojda, 1970).

Alkaline phosphatase was proved with naphthol-ASMX or α-naphthyl phosphate and Fast Blue BB (Lachema) after Burstone (1962), modif. Lojda (1966). Frozen sections as well as paraffin sections following a shortened embedding (Lojda, 1970) were used.