Histochemistry (1992) 98:311–315

Histochemistry

The intramucosal distribution of gastric alcohol dehydrogenase and aldehyde dehydrogenase activity in rats

I.P. Maly[1], M. Arnold[1], K. Krieger[1], M. Zalewska[2], D. Sasse[1]

[1] Anatomisches Institut der Universität Basel, Pestalozzistrasse 20, CH-4056 Basel, Switzerland
[2] Clinic of Infectious Disease, Medical University of Wroclaw, PL-53–416 Wroclaw, Poland

Accepted: 28 August 1992

Abstract. Using qualitative and microquantitative histochemical techniques, alcohol dehydrogenase and aldehyde dehydrogenase activity was studied in the gastric mucosa of male and female rats. Alcohol dehydrogenase was demonstrated by staining reactions with maximum activity in surface and neck cells and with clearly weaker activity also in parietal cells. Aldehyde dehydrogenase could be detected in surface and neck cells, and also to a comparable degree in the parietal cells. Quantitative analyses of microdissected samples yielded high values for alcohol dehydrogenase activity exclusively in the superficial part of the gastric mucosa, whereas low-K_m aldehyde dehydrogenase activity showed a decreasing gradient from the surface to the deeper parts of the mucosa. Sex differences could not be confirmed.

Introduction

In earlier studies alcohol dehydrogenase and aldehyde dehydrogenase activity was demonstrated to be heterotopically distributed in the liver parenchyma. These enzymes, which are essentially involved in ethanol degradation possess not only a typical intra-acinar distribution pattern but also exhibit activity that varies according to sex in the perivenous zones in the human and rat liver (Maly and Sasse 1985, 1987a, b, 1988, 1991). These results gave support to the hypothesis that sex-specific enzymatic heterogeneity in the liver may be the cause of the earlier and more severe development of alcoholic damage in women than in men. Recent publications, however, emphasize a further important role of ethanol degrading enzymes in the wall of the digestive tract, mainly of the stomach (Lamboeuf et al. 1981; Julkunen et al. 1985). These enzymes probably play the decisive role in the so-called first-pass metabolism of ethanol. It has been claimed that alcohol dehydrogenase in the gastric mucosa shows sex-specific activity, which might, perhaps additionally, contribute to the different susceptibility of the liver to alcoholic beverages (Frezza et al. 1990).

The studies cited above on gastric enzyme activity were carried out in homogenates of bioptic material so that exact localization of the enzymes was not possible. Earlier studies on the topochemistry of alcohol dehydrogenase were either carried out with as yet insufficient techniques (Ferguson 1965), or modern immunohistochemical methods were used without taking into consideration possible sex differences (Pestalozzi et al. 1983). Aldehyde dehydrogenase activity has never before been demonstrated.

In previous studies the technique for the demonstration of alcohol dehydrogenase activity has been improved by using viscous medium and by application of 300 m*M Tris* as an aldehyde trapping agent (Maly and Sasse 1985, 1987b). Furthermore, a histochemical method for the demonstration of aldehyde dehydrogenase activity is described in the present study for the first time. The microquantitative methods in the nanogram range make the interpretation of the staining reactions fully objective. This must contribute to the resolution of the question whether or not the alcohol degrading enzymes show sex specific differences in the gastric mucosa as they do in the liver acinus.

Materials and methods

Ten adult Wistar rats (five males weighing 250.0 ± 3.0 g; five females weighing 189.0 ± 5.0 g) were obtained from Madörin, Füllinsdorf, Switzerland. The animals were kept in separate cages at a constant room temperature of 21° C with a 12 h light/dark cycle (7.00 a.m.–7.00 p.m.). They had free access to water and Kliba standard diet. The rats were stunned and sacrificed by decapitation between 9.00 and 11.00 a.m. and their stomachs were immediately removed. In order to obtain a flat mucosal surface, the stomach was pinned onto a cork plate covered with a thin polyester foil.

Dedicated to Professor Dr. K.S. Ludwig on the occasion of his 70th birthday

Correspondence to: D. Sasse

The pins were inserted along the greater and the lesser curvature and along the borderline between the proventricular and glandular regions. Whereas all other parts were discarded, the area of fundic glands attached to the cork plate was preserved and rinsed for a short time with 60 m*M* potassium phosphate, pH 7.4 at room temperature. In order to protect the epithelial cells on the surface from drying and mechanical destruction, the mucosa was covered with a 3 to 4-mm-thick layer of a homogenized meat paste, analogous to the brain paste (Lowry and Passonneau, 1972).

After these procedures, which lasted no longer than 3 min, the tissue on the cork plate was frozen in liquid nitrogen. The pins and the cork plate with the gel bond foil were then removed in a cryostat at −20° C. The frozen tissue was cut in half with a razor blade perpendicularly to the long axis of the stomach. The tissue was stored in air-tight tubes at −80° C until required.

Fifteen cross-sections (16 µm) of the area of fundic glands from each rat were cut at a cryostat temperature of −20° C. Special care was taken to ensure that the protective meat layer remained attached to the mucosal surface. Five sections were used for the histochemical demonstration of enzyme activity. The other sections of unfixed tissue were lyophilized in a vacuum (666 mPa) at −40° C for about 24 h.

For qualitative histochemistry, cryostat sections thawed on coverslips were dried for 5 min at 37° C. Alcohol dehydrogenase activity was demonstrated in accordance with a method earlier described (Maly and Sasse 1987b). Aldehyde dehydrogenase activity was demonstrated after incubation in the following medium: 60 m*M* sodium phosphate buffer, pH 7.4; 30% polyvinylalcohol, 1 m*M* EDTA, 1 m*M* amytal, 0.5 m*M* methylpyrazole, 1 m*M* nicotinamide adenine dinucleotide (NAD), 5 m*M* nitro blue tetrazolium (NBT), 5 m*M* acetaldehyde and 0.33 m*M* phenazine methosulphate (PMS). Controls were incubated in media without substrates. After incubation all sections were postfixed in a solution of 4% formaldehyde, 136 m*M* $CaCl_2$ and 10% polyvinylpyrrolidone and mounted in glycerin jelly.

Using the stained sections as guides, an area was chosen from the lyophilized tissue where the fundic glands were oriented longitudinally. From each stomach, 6–7 strips of the mucosa were microdissected. Each strip contained 3–4 fundic glands. The protective meat layer and the mucus covering the gastric epithelium, as well as the submucosal tissue at the other end of the strip, were removed. The remaining strip was then subdivided into 5 samples of 50–150 ng, as weighed on a quartz fibre balance.

Microquantitative determination of the alcohol dehydrogenase activity was carried out as described by Maly and Sasse (1985), but with a substrate concentration of 1 *M* ethanol, low K_m aldehyde dehydrogenase activity with 80 µm acetaldehyde as substrate was determined using the method described by Maly and Sasse (1987a). The amount of specifically produced NADH was measured luminometrically.

For the demonstration of the distribution pattern of enzyme activity in the gastric mucosa, the weight of the subsamples was related to the weight of the whole strip. According to its weight, each subsample represented a certain part of the total length of the strip of mucosa. Thus it was possible to plot the enzyme activity (*y*-values) against the relative size and location of the corresponding tissue samples (*x*-values) of one microdissected strip, or of all the strips from one animal. Mean values and standard deviations were calculated for 200 points on the abscissa, which together represented the total height of the mucosa.

Results

Histochemistry

Alcohol dehydrogenase (ADH). After incubation for the demonstration of ADH activity, a deeply stained band marked the luminal part of the mucosa. At higher magnification the reaction product was localized in the basal cytoplasmic regions of the surface cells and the neck cells. In the deeper parts of the glands a moderate reaction was visible in the parietal cells, but the staining intensity sometimes hardly differed from the background reaction which was also present in controls. The chief cells showed a negative staining reaction. Sex specific differences were not discernible (Fig. 1).

Aldehyde dehydrogenase (ALDH). The reaction product occurred maximally in the upper part of the mucosa, but was also localized in the deeper parts of the gastric glands, where the parietal cells were specifically stained. Higher magnification revealed that surface and neck cells reacted with their basal cytoplasmic regions, whereas in parietal cells the total cytoplasm was stained. Chief cells were negative. This heterotopic distribution pattern was similar in both sexes (Fig. 2).

Microchemistry

ADH. The microquantitative distribution pattern exhibited highest ADH activitiy in the superficial tenth of the mucosa with values of 14.18 ± 2.28 µmol/min per g in males and of 13.91 ± 3.06 µmol/min per g in females. This maximum activity decreased drastically in the direction of the deeper tissue regions, so that in the second tenth there was only one-half of the initial activity. After the first third of the tunica mucosa the values were less than 1, and in the deepest third of the mucosa, ADH activity was no longer detectable. The total values of ADH activity (males, 2.66 ± 0.75; females, 2.60 ± 0.74 µmol/min per g) and the distribution pattern did not indicate any sex difference (Figs. 3, 4).

ALDH (low-K_m). ALDH activity showed maximum values in the superficial part of the mucosa with values of 5.84 ± 0.51 µmol/min per g in males and 5.65 ± 3.17 µmol/min per g in females. A gradual decrease of activity could be observed in the direction of the deeper parts, where in the last quarter the lowest values of 0.79 ± 0.17 µmol/min per g in males and 0.84 ± 0.17 µmol/min per g in females were attained. Neither the total activity (males, 2.55 ± 0.38; females, 2.54 ± 0.41 µmol/min per g) nor the distribution pattern indicated any sex difference (Figs. 5, 6).

Discussion

Microquantitative determinations of enzyme activities in microdissected tissue samples not only improve the objectivity of histochemical staining, but also serve as a specificity control of the qualitative histochemical reaction. On the other hand, the histochemical demonstration of enzyme activity by staining techniques allows the quantified enzyme activity to be localized accurately, which is indispensable because measurements of tissue samples even of only about 100 ng comprise different cell types.

By combining the qualitative and quantitative histo-

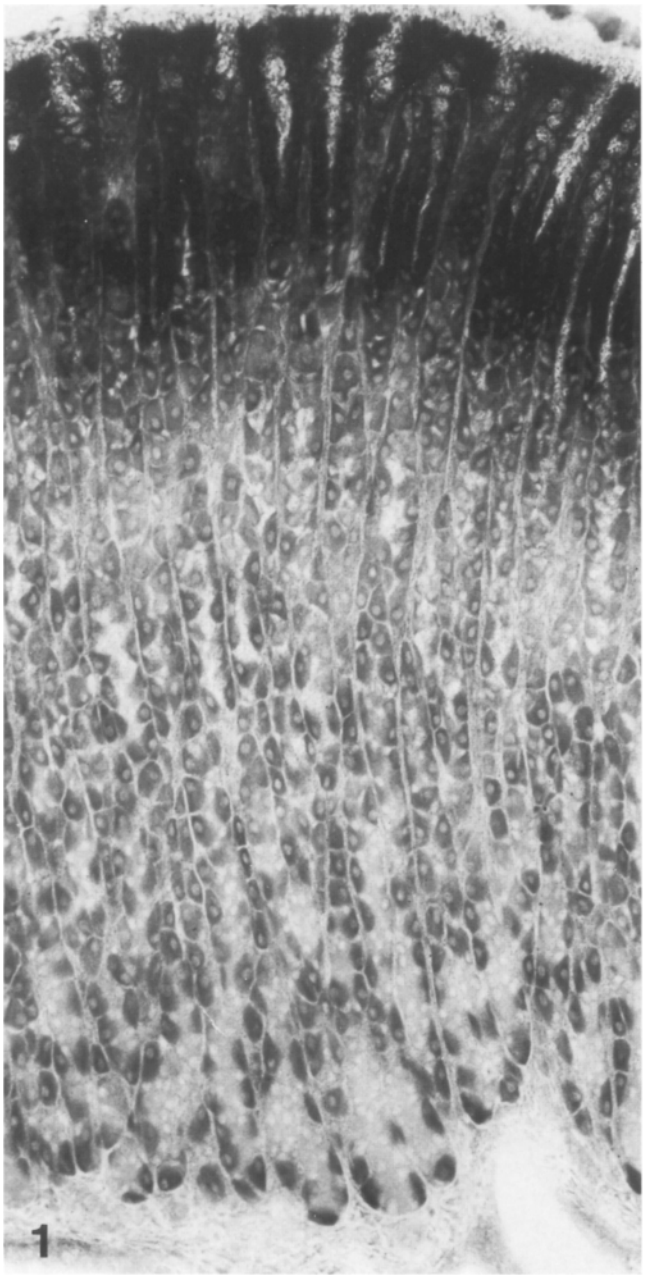

Fig. 1. Histochemical demonstration of alcohol dehydrogenase activity. Reaction product occurs mainly in the surface and neck cells of the gastric mucosa, with a moderate reaction of the parietal cells. × 190

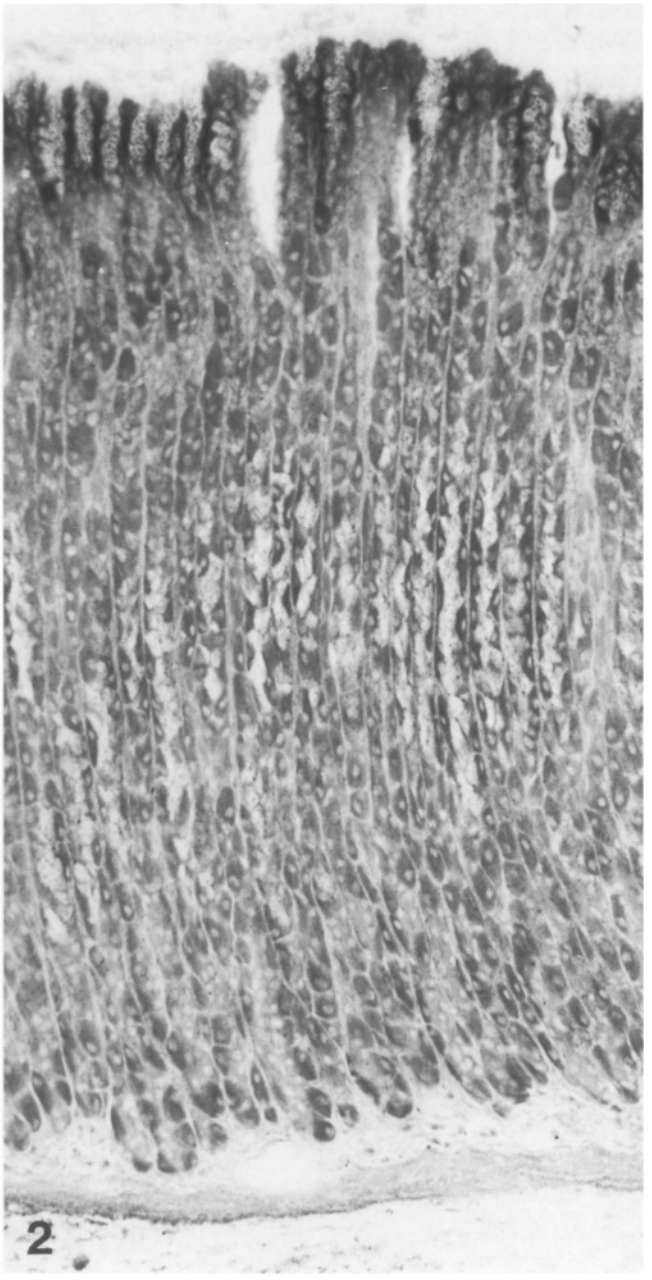

Fig. 2. Histochemical demonstration of aldehyde dehydrogenase activity. The reaction product occurs in the surface and neck cells; in the deeper parts of the mucosa the parietal cells are also markedly stained. × 190

chemical results it becomes evident that ADH has a clear-cut maximum activity in the surface and neck cells of the gastric mucosa. Parietal cells contribute to only a minor part of this total activity, and chief cells appear to be practically negative. The distribution pattern of ADH activity in the stomach of the rat described here parallels the immunohistochemical results obtained from ADH protein in the gastric mucosa of man (Pestalozzi et al. 1983).

Quantitatively, ADH activity of the apical tenth of the gastric mucosa shows a ratio to the second and third tenths of 4:2:1. This means that the first step of enzymatic ethanol degradation is mainly restricted to the upper third of the mucous membrane, and that the deeper parts must be considered to be of minor functional importance. These results further support earlier reports that the enzymatic degradation of ethanol is not restricted to the liver, significant fractions of oral doses of alcohol being oxidized in the gastrointestinal tract, particularly in the stomach (Julkunen et al. 1985; Caballeria et al. 1987).

As a consequence, acetaldehyde and acetate appear

in the veins draining the stomach following the intragastric administration of ethanol. Julia et al. (1987) reported that, in the rat, ADH-1 is the only isoenzyme that is responsible for this initial step of gastric ethanol metabolism. The ADH-1 isoenzyme shows a high K_m for ethanol in the molar range, and is therefore clearly different from the liver ADH-3 isoenzyme. A similar result was recently described by Yin et al. (1990) for human gastric ADH. Our microchemical results on ADH activity were achieved by using a substrate concentration of 1 *M* ethanol.

The concentration of ADH activity in the upper part of the mucosa could be relevant for the interpretation of alterations in the first-pass ethanol metabolism under the conditions of alcohol consumption. Kvietys et al. (1990) found that after 40 min of perfusion with 20% ethanol, nearly all of the surface cells are lucent or even detached from the mucosa. Thus, the intragastric administration of ethanol leads to injuries of exactly those cells which, according to the results presented here, are mainly involved in ethanol oxidation. Consequently, the observations of Chey (1972) that in rats chronically fed

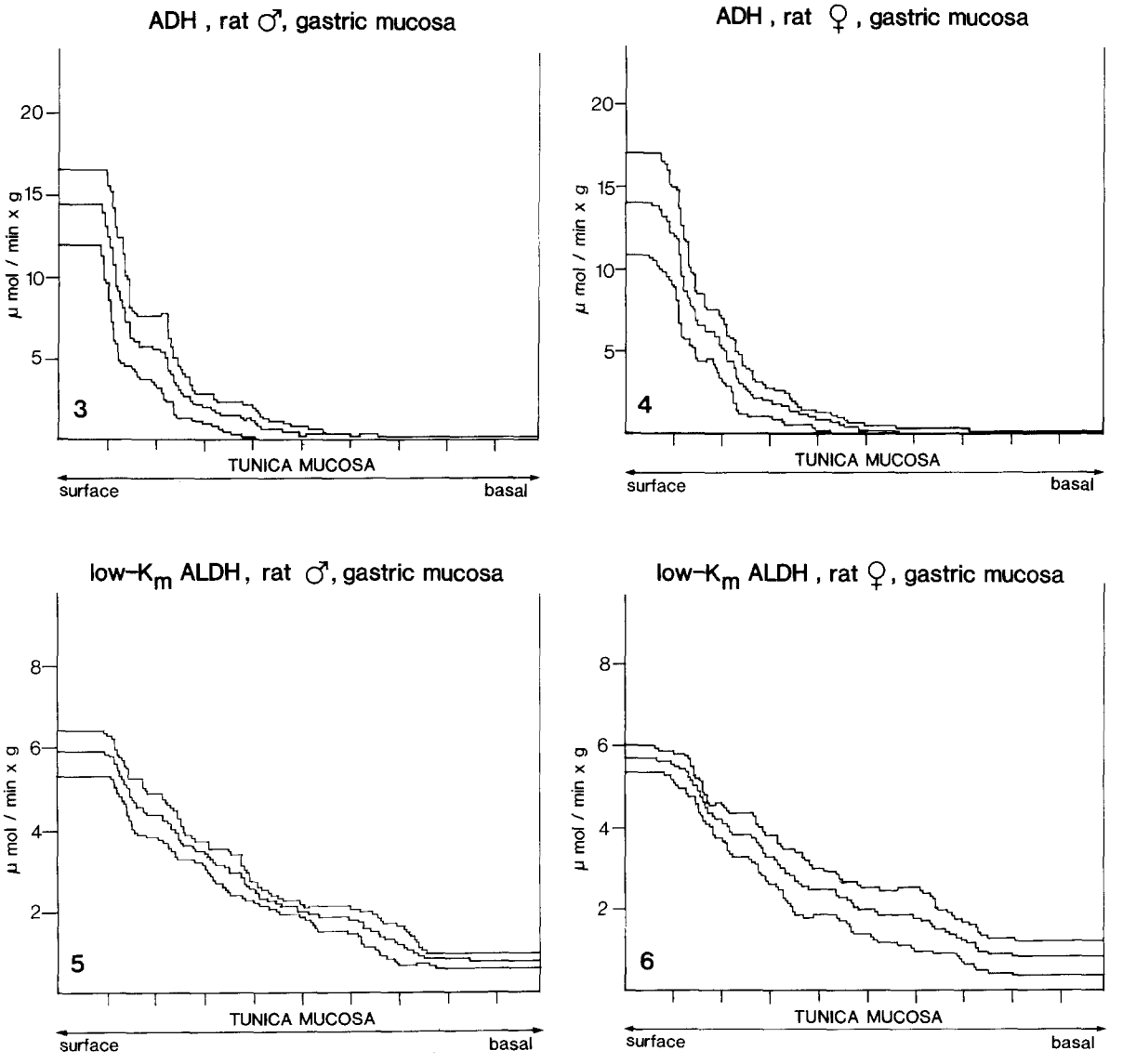

Fig. 3. Microquantitative distribution of alcohol dehydrogenase activity in the gastric mucosa of male rats. Activity was calculated from 160 microdissected samples of 32 surface/basal strips of tissue. Data are of mean values (median line) and standard deviations

Fig. 4. Microquantitative distribution of alcohol dehydrogenase activity in the gastric mucosa of female rats. Activity was calculated from 170 microdissected samples of 34 surface/basal strips of tissue. Data are of mean values (median line) and standard deviations

Fig. 5. Microquantitative determination of low-K_m aldehyde dehydrogenase activity in the gastric mucosa of male rats. Activity was calculated from 150 microdissected samples of 30 surface/basal strips of tissue. Data are of mean values (median line) and standard deviations

Fig. 6. Microquantitative determination of low-K_m aldehyde dehydrogenase activity in the gastric mucosa of female rats. Activity was calculated from 165 microdissected samples of 33 surface/basal strips of tissue. Data are of mean values (median line) and standard deviations

on an alcoholic diet there is a decrease in gastric ADH activity, can be interpreted as the result of mucosal injury. Seitz et al. (1983) found that chronic alcohol ingestion results in a significant increase in DNA synthesis in the gastric mucosa. This was interpreted as the consequence of the cellular hyper-regenerative response, which is accompanied by an increase in the parietal cell mass (Lillibridge et al. 1973), as seen also in dogs. It is feasible that, following injury to the mucosal surface, the parietal cells on the more protected sites of the gastric glands try to compensate for the loss of ADH activity.

The appearance of acetate in the effluent venous blood of the stomach (Julkunen et al. 1985) suggests that there is further degradation of ethanol in the gastric mucosa. The ALDH distribution profiles described here indicate that the surface and neck cells also play a major role in acetaldehyde oxidation. As a consequence, the upper part of the gastric mucosa is mainly involved in acetaldehyde oxidation. However, the ALDH distribution profiles show that the deeper parts of the mucosa also are capable of the degradation of acetaldehyde. The decreasing gradient of mucosal ALDH activity corresponds to the decrease in the number of parietal cells towards the deeper parts of the gastric glands. Therefore acetaldehyde produced mainly in the upper third of the mucous membrane, can at least be partly oxidized.

Frezza et al. (1990) reported sex dependent differences of ADH activity in men and women, with a male/female ratio of 1.68:1. The authors concluded that because of the lower gastric first-pass metabolism in the female, the bioavailability of ethanol is much greater in women than in men, a phenomenon which should contribute to the higher susceptibility to liver injury found in women compared with men. The results presented here give no hint of sex differences in the gastric mucosa of rats, although sex dependent distribution patterns of ADH and low-K_m ALDH activity are clearly evident in the liver (Maly and Sasse 1985, 1987a). These different enzymatic localizations are modified by sex hormones (Maly and Sasse 1987b). In a recent study (Maly and Sasse 1991), the sex differences observed in rats could also be confirmed for the enzymes ADH and ALDH in the liver parenchyma of men and women. Further studies using our methodology should elucidate the discrepancy between our observations in rat stomach and the reports by others on sex-specific differences of ADH in human gastric mucosa.

Acknowledgements. The stay of M.Z. was financially supported by the Schweizerischer Nationalfonds (Soforthilfemassnahmen Osteuropäische Staaten). We thank D. Müller for typing the manuscript and Dr. F. Steel for his help with the English text.

References

Caballeria J, Baraona E, Lieber CS (1987) The contribution of the stomach to ethanol oxidation in the rat. Life Sci 41:1021–1027

Chey WY (1972) Alcohol and gastric mucosa. Digestion 7:239–251

Ferguson MM (1965) Observations on the histochemical distribution of alcohol dehydrogenase. Q J Micr Sci 106:289–297

Frezza M, di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS (1990) High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. N Engl J Med 322:95–99

Julia P, Boleda MD, Parés X (1987) Kinetic properties and physiological significance of the ADH-1 isoenzyme of rat stomach alcohol dehydrogenase. In: Flynn TG, Weiner H (eds) Enzymology and molecular biology of carbonyl metabolism. Liss, New York, pp 189–201

Julkunen RJ, Tannenbaum L, Baraona E, Lieber CS (1985) First pass metabolism of ethanol: an important determinant of blood levels after alcohol consumption. Alcohol 2:437–441

Kvietys PR, Twohig B, Danzell J, Specian RD (1990) Ethanol-induced injury to the rat gastric mucosa. Role of neutrophils and xanthine oxidase-derived radicals. Gastroenterology 98:909–920

Lamboeuf Y, de Saint Blanquat G, Derache R (1981) Mucosal alcohol dehydrogenase- and aldehyde dehydrogenase-mediated ethanol oxidation in the digestive tract of the rat. Biochem Pharmacol 30:542–545

Lillibridge CB, Yoshimori M, Chey WY (1973) Observations on the ultrastructure of oxyntic cells in alcohol-fed dogs. Am J Dig Dis 18:443–454

Lowry OH, Passonneau JV (1972) A flexible system of enzymatic analysis. Academic Press, New York San Francisco London

Maly IP, Sasse D (1985) Microquantitative determination of the distribution patterns of alcohol dehydrogenase activity in the livers of rat, guinea pig and horse. Histochemistry 83:431–436

Maly IP, Sasse D (1987a) Microquantitative determination of intra-acinar distribution profiles of low-K_M and high-K_M aldehyde dehydrogenase activity in rat liver. Eur J Biochem 170:173–178

Maly IP, Sasse D (1987b) The intra-acinar distribution patterns of alcohol dehydrogenase activity in the liver of juvenile, castrated and testosterone-treated rats. Biol Chem Hoppe-Seyler 368:315–321

Maly IP, Sasse D (1988) Nutritional and gonadal effects on the intra-acinar profiles of low-K_M and high-K_M aldehyde dehydrogenase activity in rat liver. Histochemistry 88:387–393

Maly IP, Sasse D (1991) Intraacinar profiles of alcohol dehydrogenase and aldehyde dehydrogenase activities in human liver. Gastroenterology 101:1716–1723

Pestalozzi DM, Bühler R, von Wartburg JP, Hess M (1983) Immunohistochemical localization of alcohol dehydrogenase in the human gastrointestinal tract. Gastroenterology 85:1011–1016

Seitz HK, Czygan P, Kienapfel H, Veith S, Schmidt-Gayk H, Kommerell B (1983) Changes in gastrointestinal DNA synthesis produced by acute and chronic ethanol consumption in the rat: a biochemical study. Z Gastroenterol 21:79–84

Yin SJ, Wang MF, Liao CS, Chen CM, Wu CW (1990) Identification of a human stomach alcohol dehydrogenase with distinctive kinetic properties. Biochem Int 22:829–835