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Nicotinamide–adenine dinucleotide phosphate in gastric mucosa

The hydrogen ions secreted by gastric mucosa are probably generated by an oxidation–reduction mechanism in the oxyntic cells[1]. This view is supported by studies of the efficiency of acid secretion in relation to oxygen consumption[2,3]. The components of the oxidation–reduction mechanism are not known. KIDDER, CURRAN AND REHM[4] recently demonstrated the existence of extramitochondrial cytochrome *c* in bullfrog gastric mucosa, and suggested its involvement in the mechanism of acid secretion. The present communication reports the finding of a high level of NADPH in frog gastric mucosa, high activity of extramitochondrial NADHP-generating enzymes, and appreciable NADPH:cytochrome *c* oxidoreductase activity.

Gastric mucosae were obtained from fresh frogs of the species *Discoglossus pictus*. The levels of $NADP^+$ and NADPH in the mucosae were determined in neutralized acid and alkaline extracts, respectively, by the method of VILLEE[5]. In the enzyme assays, the mucosae were homogenized with 9 vols. of ice-cold 0.12 M KCl and the homogenate was centrifuged at 0–4° at 20000 × *g* for 30 min. The supernatant was used for the assays. D-Glucose-6-phosphate:$NADP^+$ oxidoreductase (EC 1.1.1.49) was determined by the method of LÖHR AND WALLER[6]; L_S-isocitrate:$NADP^+$ oxidoreductase (EC 1.1.1.42) by the method of OCHOA[7]; NADPH:cytochrome *c* oxidoreductase (EC 1.6.2.3) by the method of HAAS, HORECKER AND HOGNESS[8]. Protein concentrations were determined by a biuret method[9]. The coenzyme and enzyme assays were also carried out on frog liver for the purpose of comparison.

TABLE I

LEVEL OF $NADP^+$ AND NADPH IN FROG GASTRIC MUCOSA AND LIVER

The values are means ± S.E. of determinations on five animals. Units refer to wet wt.

Tissue	*$NADP^+$ (μmoles/g)*	*NADPH (μmoles/g)*	*NADPH/$NADP^+$*
Gastric mucosa	0.015 ± 0.004	0.103 ± 0.018	10.6 ± 3.8
Liver	0.059 ± 0.006	0.163 ± 0.032	2.7 ± 0.4

TABLE II

ACTIVITY OF $NADP^+$-LINKED ENZYMES IN 20000 × *g* SUPERNATANT OF HOMOGENATES OF FROG GASTRIC MUCOSA AND LIVER

Initial activities were measured at 25° in terms of μmoles $NADP^+$ reduced per min in the case of the NADPH-generating enzymes, and in terms of μmoles cytochrome *c* reduced per min in the case of NADPH:cytochrome *c* oxidoreductase. Values are means ± S.E. of determinations on five animals. Units refer to protein content of supernatant.

Enzyme	*Gastric mucosa (initial activity/mg protein)*	*Liver (initial activity/mg protein)*
Glucose-6-phosphate: $NADP^+$ oxidoreductase	0.260 ± 0.015	0.038 ± 0.007
Isocitrate:$NADP^+$ oxidoreductase	0.066 ± 0.017	0.012 ± 0.002
NADPH:cytochrome *c* oxidoreductase	0.009 ± 0.001	0.010 ± 0.001

The levels of $NADP^+$ and NADPH observed in the gastric mucosae and livers are shown in Table I. It is seen that a high level of NADPH is maintained in both tissues. The NADPH/$NADP^+$ ratio is apparently higher in gastric mucosa than in liver (P about 0.10 in the data). This correlates with the finding of higher activities of NADPH-generating enzymes in the mucosae (Table II). The activity of NADPH: cytochrome *c* oxidoreductase was found to be the same in the mucosae and livers (Table II).

Extramitochondrial cytochrome *c* may function as an electron acceptor in the generation of hydrogen ions for secretion in gastric mucosa[4]. The present findings suggest the possibility that NADPH may be the source of the reducing equivalents. Although direct evidence remains to be discovered, it is of interest to suggest that the hydrogen ions produced in the oxidoreduction between the NADPH and the cytochrome *c* are captured for secretion.

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