Metabolism of Dog Gastric Mucosa

I. NUCLEOTIDE LEVELS IN PARIELTAL CELLS*

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Adenine and pyridine nucleotide levels as well as those of phosphate, phosphocreatine, lactate, pyruvate, β-hydroxybutyrate, acetoacetate, glucose, and glycogen were measured in histologically defined parietal and mucous cell sections of biopsies of dog gastric mucosa at rest, and in various secretory states. As a result of stimulation of secretion, there appeared to be no change in adenine nucleotide levels, or phosphocreatine, but there was a rise in inorganic phosphate and a fall in phosphorylation potential. However, there was a marked increase in NADH, but no change in NADPH with onset of acid secretion. The increase in the lactate to pyruvate ratio showed that the increased NADH level occurred in the cytoplasm and these data are discussed with reference to change in cell pH.

Proton transport is an almost universal phenomenon occurring in subcellular organelles such as mitochondria (1) and chromatophores (2), as well as in unicellular organisms such as Escherichia coli (3) or yeast (4), and in plants (5) or animal tissues such as kidney (6), pancreas (7), or stomach (8). The latter tissue is probably the most specialized in this function, the mammalian parietal cells being capable of developing a 10^6.6-fold concentration gradient of H^+. Since it is unlikely that a totally novel mechanism evolved in gastric mucosa, it seems that an understanding of the transport process in this organ would be generally applicable to the problem of H^+ transport in most situations.

Measurement of metabolite levels in gastric mucosa therefore has as its main purpose the determination of the primary energy source for H^+ secretion. Thus, measurement of admeine nucleotide and phosphocreatine levels may be able to answer the question as to whether ATP is the primary energy source, and extension of these measurements to other metabolites should be able to provide information as to the pathway or pathways stimulated to provide the primary energy for transport.

Logically, the tissue metabolites should be studied before and after stimulation, and a comparison of the steady state levels should indicate physiologically significant changes. Two types of measurements have been made. Nondestructive techniques such as dual or split beam spectroscopy (9, 10) have provided information on the steady state changes in the oxidation-reduction components of the mitochondrial respiratory chain, and destructive techniques such as described here have given data on adenine nucleotide levels (11, 12). However, these past experiments have been confined almost exclusively to in vitro studies of frog gastric mucosa where the change in H^+ rate is limited, and moreover no distinction has been made between the multiple cell types present in the tissues. The work reported in this and succeeding papers deals with measurements in biopsy samples from an intact mammalian mucosa, in what were determined histologically to be parietal cell regions weighing about 1 µg. The fluorimetric cycling procedures developed by Lowry and his collaborators (13) were used to obtain the necessary sensitivity. This paper reports data obtained for adenine and pyridine nucleotides and metabolites related to the "free" pyridine nucleotide levels in cytosol and mitochondria under resting (i.e. nonsecreting), secretory onset, and steady state secretion in dog gastric mucosa.

METHODS

Gastric Preparation—Adult beagle dogs were anesthetized with Nembutal. The abdomen was incised and the gastric mucosa exposed by an incision along the greater curvature. Resting tissue samples were taken at 15 and 30 min after surgery, at which time histamine infusion was started at a rate of 100 µg/kg/hour to give maximal secretion. In some experiments, 100 µg of urecholine were used in addition to histamine to increase secretion further. After 8 to 10 min of infusion, the gastric mucosa reddened, which occurred 1 to 2 min prior to onset of acid secretion. With histamine and urecholine, a faster onset was seen. A sample was taken from the reddened nonsecreting mucosa during this period and labeled "blood flow" sample. Onset of acid secretion was monitored by measuring the fall of pH in 1 ml of saline (0.9% NaCl solution) held on the mucosa by means of a Lashley suction cup (2.27 cm^2) for 3 min. When pH started to fall, tissue samples were taken at that time (HIH) and another when the pH stabilized at about 2.0 in the approximately 10-fold diluted sample (HII). H^+ rate was obtained by titration to pH 7.0 (Fig. 1). The acid rate for histamine alone was one-third that found with histamine and urecholine in combination, as known previously.

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Precautions were taken to control bleeding, and to keep the mucosa moist and warm at all times. Dogs which showed cyanotic mucosae were rejected as bad experiments.

**Sampling Procedure**—Samples were taken by precooled rectal biopsy forceps, with scalpel excision of the piece of tissue (~100 mg wet weight). The tissue sample was frozen immediately (<3 s) in liquid nitrogen, and stored at -80° until use. Holding the tissue at room temperature for a period of 30 s did not change the adenine or pyridine nucleotide levels, hence the rapidity of the sampling procedure was deemed adequate. Phosphocreatine decreased and lactate increased in this 30-s procedure, but since no change was found in phosphocreatine in the 3-s sample, this was further evidence for adequately rapid sampling.

**Treatment of Samples**—The samples were placed in a Harris cryostat at -20° and serially sectioned (20 μm thick) until parietal cell areas were seen, as evidenced by the darker appearance of the tissue. The initial sections consisted exclusively of surface or glandular neck cells. The frozen sections were placed in drilled aluminum blocks at -20° and dried under vacuum at -40°. Every fourth frozen section was transferred to a glass slide and stained for succinic dehydrogenase activity using succinate and nitro blue tetrazolium (14); in this manner the parietal cell area was clearly identifiable (Fig. 2). Adjacent lyophilized sections were trimmed using the stained sections as a guide until only defined parietal cell regions remained. Careful dissection of tissue samples from these areas of the sections were used for the extraction procedures. To ensure that the sections would not absorb atmospheric moisture, freeze-dried sections were maintained at -80° under vacuum for storage and dissected quickly in a specially constructed room maintained at <50% humidity and 18°. In separate experiments, direct extraction of frozen sections gave quantitatively similar data, hence the dissection procedure did not result in metabolite destruction. In some cases, antral tissue was sectioned or the mucous cell sections of fundus were used, and the low level of succinic dehydrogenase staining was used to assess freedom from parietal cells.

**Extraction Procedures**—Since we are dealing with extremely small samples of tissue (1 μg), the oil well technique (15) was required. A small volume (1.0 to 2.0 μl) of HCl or NaOH is introduced into a specially constructed Teflon rack 10 wells at a time. The sample tissue is then pushed into the droplet of acid or alkali and oil added quickly to avoid evaporation problems. The procedure is performed under a stereo dissecting microscope. The Teflon rack was heated where necessary (generally 20 min, 80°) to destroy endogenous pyridine nucleotide and enzyme activities. Standards dissolved in acid or alkali are treated the same way as the tissue samples following addition to the oil well.

**Metabolite Levels**—Since several metabolites were measured, tables are presented giving the details of the procedure used for measurement of each metabolite. The sequence of steps is (a) extraction of the tissue sample (Table I) and enzymatic assay utilizing the conversion to pyridine nucleotide, oxidized or reduced; (b) treatment of sample for cycling (Table II); (c) cycling (Tables II and III); (d) indicator step (Table IV). Table II also contains the range of substrate standards used and controls are inserted at each stage. References to the methods are on the tables, and a detailed description is given in Ref. 13.

**Cycling Techniques**—This varies for NAD+, NADH, NADP+, and NADPH, and the procedures are detailed in Table III. This is the method used for determination of levels of the pyridine nucleotides, and also for the pyridine nucleotide produced in the other substrate assay methods with, therefore, the omission of the initial step destroying the oxidized or reduced form of the pyridine nucleotide, which step is performed slightly differently for each substrate as detailed in Table I. Also, the amount of cycling enzyme varies for each substrate, as a function of the number of cycles required and this is detailed in Table II. The other reagent levels remain the same.

**Indicator Step**—In NAD+ cycle, malate levels were measured as the indicator step by direct fluorimetry using malic dehydrogenase, and for
| Substance  | Preparation of sample | Volume | Buffer | Enzymes | Other additions | Preparation for cycling |
|------------|-----------------------|--------|--------|---------|-----------------|------------------------|
| ATP (16)   | 3 µl of 0.1 N NaOH, 15 min, 75° | To 1 µl add 2 µl reagent, 15 min, R.T. | 300 mM Tris, pH 8.0 | 2 µg/ml yeast hexokinase; 0.25 µg/ml glucose 6-P dehydrogenase | 0.05% BSA, 0.5 mM DTT, 5 mM MgCl₂, 1 mM glucose, 60 µM TPN | 1 µl of 1.2 N NaOH, 20 min, 75° |
| ADP (13)   | 3 µl of 0.05 N NaOH + 6 mM H₂O₂, 20 min, R.T. | To 1 µl add 3.3 µl reagent, 20 min, R.T. | 100 mM imidazole, pH 7.0 | Pyruvate kinase, 5 µg/ml; lactic dehydrogenase, 3 µg/ml | 0.02% BSA, 2 mM MgCl₂, 75 mM KCl, 25 µM PEP, 4 µM NADH | 2 µl of 0.5 N HCl, 45 min, R.T. |
| AMP (13)   | 3 µl of 0.05 N NaOH + 6 mM H₂O₂, 20 min, 80° | To 1 µl add 3.3 µl reagent, 20 min, R.T. | 100 mM imidazole, pH 7.0 | Pyruvate kinase, 5 µg/ml; lactic dehydrogenase (BHLDH), 8 µg/ml; myokinase, 2 µg/ml | 0.02% BSA, 2 mM MgCl₂, 75 mM KCl, 25 µM PEP, 7.5 µM NADH, 5 µM ATP | 1 µl of 0.5 N HCl, 45 min, R.T. |
| PCr (16)   | 3 µl of 0.01 N NaOH, 15 min, 75° | To 1 µl add 2 µl reagent, 30 min, R.T. | 300 mM Tris, pH 8.0 | Creatine kinase, 2 mg/ml; hexokinase, 2 µg/ml; glucose-6-P dehydrogenase, 0.25 µg/ml | 0.16% BSA, 5 mM MgCl₂, 1 mM glucose, 60 µM NADP⁺, 0.02% ADP, 0.5 mM DTT | 1 µl of 1.2 N NaOH, 30 min, 75° |
| Glucose    | 1 µl of 0.02 N HCl, 20 min, 60° | 2 µl reagent, 30 min, R.T. | 100 mM Tris, pH 8.0 | Yeast hexokinase, 2 µg/ml; yeast glucose-6-P dehydrogenase, 0.25 µg/ml | 0.15 µM NADP⁺, 100 µM ATP, 5 mM MgCl₂, 0.02% BSA | 1 µl of 1.2 N NaOH, 30 min, 80° |
| Glycogen (17) | 7.6 µl of 0.02 N NaOH, 20 min, 60° | To 1 µl add 1 µl 100 mM acetate buffer, pH 4.0, with 2 mg/ml amyloglucosidase, 20 min, 55°, add 1 µl reagent react 30 min, R.T. | 300 mM Tris, pH 8.9 | Yeast hexokinase, 5 µg/ml; yeast glucose-6-P dehydrogenase | 100 µM NaDP⁺, 200 µM ATP, 10 mM MgCl₂, 0.04% BSA | 1 µl of 1.2 N NaOH, 30 min, 80° |
| P₃ (44)    | 1 µl of 0.1 N NaOH, 15 min, 65° | 0.5 µl imidazole, pH 7.0 | Phosphofructokinase 30 µg/ml; phosphoglucomutase 3 µg/ml; glucose-6-P dehydrogenase 1.5 µg/ml | 0.027 BSA, 2 mM EDTA, 20 µM 5-AMP, 60 µM NADP⁺, 5 mM glycogen | 1 µl of 1.2 N NaOH, 15 min, 75° |
| Lactate (13) | 1 µl of 0.02 N NaOH, 20 min, 80° | 5 µl reagent, 60 min, R.T. | 100 µM AMP, pH 9.75 | Beef heart lactic dehydrogenase, 400 µg/ml; glutamate pyruvate transaminase, 50 µg/ml | 2 µl of 0.25 N NaOH, 30 min, 80° |
| Pyruvate (13) | 1 µl of 0.02 N NaOH, 20 min, 80° | 2 µl reagent, 20 min, R.T. | 50 µM imidazole, pH 7.0 | Beef heart lactic dehydrogenase, 0.2 µg/ml | 2 µl of 0.025 N NaOH, 20 min, 80° |
| β-Hydroxybutyrate (18) | 1 µl of 0.025 N HCl, 20 min, 80° | 2 µl reagent, 30 min, R.T. | 300 µM Tris hydroxylamine, pH 8.5 | β-Hydroxybutyrate dehydrogenase, 10 µg/ml | 0.02% BSA, 0.5 N HCl, 45 min, R.T. |
| Acetoacetate (18) | 1 µl of 0.02 N NaOH, 20 min, 80° | 2 µl reagent, 90 min, R.T. | 200 µM imidazole, pH 7.0 | β-Hydroxybutyrate dehydrogenase, 40 µg/ml | 1 µl of 0.5 N HCl, 30 min, R.T. |

References are given in parentheses.

R.T., room temperature; BSA, bovine serum albumin; DTT, dithiothreitol; NADP⁺, triphosphopyridine nucleotide; PEP, P-enolpyruvate; BHLDH, beef heart lactic dehydrogenase; PCr, phosphocreatine; NAD⁺, diphosphopyridine nucleotide; AMP, 2-amino-2-methylpropanol.

Corrected for glucose blank.

the NADP⁺ cycle, 6-phosphogluconate was measured directly using 6-phosphogluconate dehydrogenase (Table IV). The sequence of steps is summarized in Fig. 3 for the entire procedure.

Materials—All enzymes were obtained from Boehringer-Mannheim except beef heart lactic dehydrogenase which was purchased from Worthington and further purified with activated charcoal. Substrates were purchased from Sigma Chemical Co., and the Trizma (Tris(hydroxymethyl)amino methane) buffer from Sigma is treated with Norit before use.

Results

Acid Secretion

As shown in Fig. 1, the dog gastric mucosa initially secretes no measurable acid, zero time on the graph representing 30 min after surgery. Following infusion of histamine there is a 10-min lag, and with histamine and urecholine a 3-min lag. Then reddening of the mucosa is observed consonant with increased...
Table II

| Substance | Aliquot from oil well for cycling | Volume & condition for cycling | Enzymes utilized | Substrate standard utilized (x 10^{-16} mol) |
|-----------|----------------------------------|--------------------------------|------------------|-------------------------------------------|
| ATP       | 1 µl                             | 100 µl, 60 min, 37°            | Beef liver glutamic dehydrogenase, 100 µg/ml; glucose-6-P dehydrogenase, 50 µg/ml | 4.34-8.68 in 0.1 N NaOH |
| ADP       | 1 µl                             | 100 µl, 60 min, 27°            | Alcohol dehydrogenase, 120 µg/ml | 4.82-9.64 in 0.05 N NaOH |
| AMP       | 1 µl                             | 100 µl, 60 min, 27°            | ADH, 100 µg/ml; MDH, 15 µg/ml | 1.67-3.33 in 0.05 N NaOH |
| PCR       | 1 µl                             | 100 µl, 60 min, 27°            | Glucose-6-P dehydrogenase, 100 µg/ml; MDH, 15 µg/ml | 2.3-5 in 0.02 N HCl |
| Glucose   | 1 µl                             | 100 µl, 60 min, 27°            | Glucose-6-P dehydrogenase, 100 µg/ml; MDH, 15 µg/ml | 7.5-30 in 0.02 N NaOH |
| Lactate   | 1 µl                             | 100 µl, 60 min, 27°            | Glucose-6-P dehydrogenase, 100 µg/ml; MDH, 15 µg/ml | 5-20 in 0.02 N NaOH |
| Pyruvate  | 1 µl                             | 100 µl, 60 min, 27°            | Glucose-6-P dehydrogenase, 100 µg/ml; MDH, 15 µg/ml | 0.96-1.92 in 0.02 N NaOH |
| β-Hydroxybutyrate | 1.3 µl       | 100 µl, 60 min, 27°           | Glucose-6-P dehydrogenase, 100 µg/ml; MDH, 15 µg/ml | 0.404-0.808 in 0.025 N NaOH |
| Acetoacetate | 3 µl                            | 100 µl, 60 min, 27°          | Glucose-6-P dehydrogenase, 100 µg/ml; MDH, 15 µg/ml | 1.25-5 in 0.02 N NaOH |
| P,        | 0.535 µl                         | 100 µl, 60 min, 37°           | Glucose-6-P dehydrogenase, 100 µg/ml; MDH, 15 µg/ml | 9-16 in 0.02 N NaOH |

ADH, alcohol dehydrogenase; MDH, malate dehydrogenase; PCr, phosphocreatine; GDH, beef liver glutamic dehydrogenase; G6PDH, glucose-6-P dehydrogenase.

Table III

| Substance | Cycling reagent | Procedure | Standard range (10^{-16} mol) |
|-----------|-----------------|-----------|-------------------------------|
| NADP     | 100 mM Tris-HCl buffer, pH 8.0 | 1 µl of 0.025 N HCl with 300 mM ascorbate, 75°, 20 min, cool on ice, 12 µl cycling reagent, incubate 37°, 90 min, stop, 100°, 4 min, cool, 10 µl to 1 ml indicator reagent | 0.144-0.228 |
| NADPH    | 100 mM Tris-HCl buffer, pH 8.0 | 1 µl of 0.02 N NaOH, 75°, 15 min, cool 12 µl cycling reagent, incubate 37°, 60 min, 100°, 4 min, stop, cool, transfer 10 µl to 1 ml indicator reagent | 0.144-0.228 |
| NAD      | 100 mM Tris-HCl buffer, pH 8.0 | 1 µl of 0.02 N HCl, 80°, 20 min, cool on ice, 9 µl cycling reagent, 27°, 60 min, stop, 100° for 10 min, 7 µl to 1 ml indicator reagent | 1.25-5 |
| NADH     | 100 mM Tris-HCl buffer, pH 8.0 | As above, but double enzyme concentration | 1 µl of 0.02 N NaOH with 1 mM cysteine, 80°, 20 min, cool on ice, 9 µl cycling reagent, 38°, 1 hr, stop, 100°, 10 min, transfer 7 µl to 1 ml indicator reagent | 0.25-1 |

ADP—The levels found in the parietal cells are given in Table V and in the mucous cells in Table VI. The levels found are quite similar to those of other tissues, liver for example (22). The levels found in mucous cells are somewhat lower (65%) than those of parietal cells. It is striking, also, that no change is observed with increase of blood flow or acid secretion in the level of this nucleotide.

Adenine Nucleotides

AMP—The level of AMP increased by about 20% with onset of mucosal perfusion until acid secretion started and stabilized thereafter (Table V). No measurements of this nucleotide were made in the mucous cells. The ATP to ADP ratio suggests an active adenylate kinase.

Total Nucleotides—No significant change was noted in the total adenine nucleotide level found in the parietal cell region as a function of acid secretion (Table V).
blood flow, and secreting samples. It should also be pointed out, that in some dogs, although not reflected in the means of the totals, there is a distinct, significant rise in phosphocreatine with onset of acid secretion (Table V). The level in mucous cells is similar to that found in parietal cells (Table VI).

**ATP/ADP Ratio**—From the data for the individual values, there is no change in the ATP/ADP ratio. Mucous cells show a ATP/ADP ratio of about 1.0 (Table VI), much less than for parietal cell regions, which is about 2.3.

**NAD⁺**—Table VII shows that there is no significant change in the levels of NAD⁺ between rest and secretion. The level in mucous cells is lower by about 30% than those of parietal cells (Table VI).

**NADH**—In contrast to the constant values obtained for NAD⁺ there is a significant (80%) increase in NADH with onset of acid secretion which is maintained during steady state secretion (Table VII). The level in mucous cells is extremely low (Table VI), being only 20% of the parietal cell value.

**NAD⁺/NADH Ratio**—As can be predicted from the above, there is a significant fall in this ratio, with H⁺ secretion in the parietal cells, but even at peak secretion, there is about 3 times as much NAD⁺ as NADH. Also it should be noted that with onset of acid there is a slight (8%) increase in the total nicotinamide adenine nucleotide level. The ratio NAD⁺/NADH in the mucous cells is about 30, and increases with secretion to 48.

**NADP⁺**—Table VII shows that with blood flow and onset of acid secretion there is no change in the level of this nucleotide,

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**TABLE IV**

**Indicator steps**

| Substance          | Value       |
|--------------------|-------------|
| NADP⁺/NADPH (19)²  | 20 mM Tris buffer, pH 8.0 |
|                    | 0.02 mM NADP⁺ |
|                    | 0.01 mM EDTA |
|                    | 5 µg/ml 6-phosphogluconate dehydrogenase |
|                    | 1 ml reagent to each tube, react 30 min, 22°C, measure NADPH fluorometrically |
| NAD⁺/NADH (21)     | 6-PG⁺ + NADP⁺ R-5-P + NADPH CO₂ |
|                    | 50 mM 2-amino-2-methyl propanol buffer (AMP), pH 9.9 |
|                    | 0.02 mM NAD⁺ |
|                    | 10 µM glutamate |
|                    | 2 µg/ml GOT |
|                    | 1 ml reagent to each tube, react 22°C for 10 min, measure NADH fluorometrically |
|                    | Malate + NAD⁺ OAA + NADH + H⁺ |
|                    | OAA, glutamate → aspartate + α-KG |

²References are given in parentheses.

*6-PG, 6-phosphogluconate; R-5-P, ribulose-5-P; MDH, malate dehydrogenase; GOT, glutamate-oxalacetate transaminase; OAA, oxalacetate; α-KG, α-ketoglutarate.

**TABLE V**

**Metabolite levels in parietal cells**

The values are the means ± S.E. of n dogs, at least four measurements having been made on each sample in each dog. Blood flow refers to the state of reddening of the mucosa, HI the sample when acid secretion was detected in that area, and HII a sample at steady state secretion. Values are given, when appropriate, in millimoles kg⁻¹ dry weight.

| Substance          | Resting | Blood flow | III |
|--------------------|---------|------------|-----|
| ATP                | 12.438 ± 0.410 (n = 17) | 12.414 ± 0.627 (n = 13) | 12.12 ± 0.627 (n = 11) |
| ADP                | 5.691 ± 0.192 (n = 17) | 5.741 ± 0.175 (n = 13) | 5.779 ± 0.269 (n = 11) |
| ATP/ADP            | 2.267 ± 0.102 (n = 17) | 2.218 ± 0.133 (n = 13) | 2.372 ± 0.207 (n = 11) |
| AMP                | 1.175 ± 0.121 (n = 15) | 1.360 ± 0.130 (n = 10) | 1.463 ± 0.122 (n = 11) |
| Total nucleotide   | 19.30 ± 0.18 (n = 15) | 19.49 ± 0.23 (n = 10) | 19.36 ± 0.31 (n = 11) |
| Phosphocreatine    | 4.149 ± 0.318 (n = 17) | 4.788 ± 0.434 (n = 13) | 4.724 ± 0.270 (n = 11) |
| [ATP][AMP]/[ADP]²  | 0.45     | 0.52       | 0.53 |

FIG. 3. A schematic representation of the stages used for analysis of cellular levels of metabolites, NBT, nitro blue tetrazolium.

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**References**

References are given in parentheses.

6-PG, 6-phosphogluconate; R-5-P, ribulose-5-P; MDH, malate dehydrogenase; GOT, glutamate-oxalacetate transaminase; OAA, oxalacetate; α-KG, α-ketoglutarate.
but at steady state secretion there is apparently a decrease in the level of oxidized nucleotide. The level of this nucleotide is lower than in the mucous cells.

**NADPH**—Corresponding to the values of NADP⁺ found in the parietal cell region of the mucosa there is no significant change in NADPH levels, during the stages of acid secretion (Table VII). Mucous cells have a slightly lower value of NADPH than parietal cells (Table VII). No change occurs in the mucous cells with secretion.

**NADPH/NADP⁺ Ratio**—The value for this ratio does not change during onset of acid secretion, but at the steady state level there is a slight rise in the value, with no change in mucous cells.

**Pyruvate**—Table VIII shows that there is a progressive rise in pyruvate from the resting sample to blood flow and to secretion in the parietal cells; the levels in the mucous cells are about the same as in the parietal cells (Table VI), but do not change with the onset of acid secretion.

### Table VI

**Metabolite levels in mucous cells**

| Substance | Resting | Histamine |
|-----------|---------|-----------|
| ATP       | 8.13 ± 0.93 | 7.66 ± 0.38 |
| ADP       | 7.82 ± 0.68 | 6.66 ± 0.44 |
| Phosphocreatine | 5.53 ± 0.106 | 5.19 ± 0.110 |
| NAD⁺      | 1.29 ± 0.029 | 1.34 ± 0.040 |
| NADH      | 0.046 ± 0.008 | 0.028 ± 0.004 |
| NADP⁺     | 0.088 ± 0.012 | 0.067 ± 0.036 |
| NADPH     | 0.161 ± 0.016 | 0.178 ± 0.014 |
| Lactate   | 4.33 ± 0.254 | 4.61 ± 0.252 |
| Pyruvate  | 0.766 ± 0.060 | 0.768 ± 0.020 |

The values given are in terms of n dogs ± S.E. at rest and during acid secretion in millimoles kg⁻¹ dry weight.

**Lactate**—Lactate levels double with the onset of acid secretion, and are maintained during steady state secretion (Table VII). The lactate level in the mucous cells is similar to that of resting parietal cells and shows no change (Table VI).

**Lactate/Pyruvate Ratio**—The lactate/pyruvate ratio rises with onset of acid secretion to nearly twice its previous value, but does not change in mucous cells.

**Acetoacetate**—Table VIII shows that there are no changes observed with this keto acid over the period of observation. The value obtained for gastric mucosa is higher than that observed for control fed rat liver, for example (22), but lower than others have found for fasted liver (23) and higher than in rat brain (24).

**β-Hydroxybutyrate**—The level of the reduced product of acetoacetate, as was found for lactate, shows an increase with onset of acid secretion, which is maintained with continued secretion (Table VIII). The level found, however, is low compared to levels obtained for fasted liver.

**β-Hydroxybutyrate/Acetoacetate Ratio**—There is a progressive rise in this value from onset of acid secretion into the steady state H⁺ secretion sample, the final result being an approximate doubling of the ratio found. It should be noted that this ratio is the inverse of what is found in rat liver (22), under either fed or fasted conditions.

**Glucose**—The level of glucose remained quite constant over the period of observation, showing an insignificant 10% increase (Table IX).

**Glycogen**—There is a gradual continuing decline in glycogen levels with onset and steady state acid secretion (Table IX), with a net 15% fall.

**Inorganic Phosphate**—There is a significant rise in levels of inorganic phosphate found in parietal cells with acid secretion, but no change with blood flow (Table IX), the maximal change being about 30%. The rise in this metabolite is unexpected in view of the lack of change of the adenine nucleotides and phosphocreatine, and may be due to entry of phosphate from the blood.

### DISCUSSION

Hypotheses as to the energy source for acid secretion have had a major effect on the concepts prevalent in the field. The earliest suggestion postulated that protons could be derived

### Table VII

**Metabolite levels in parietal cells**

| Substance | Resting | Blood flow | HI | HII |
|-----------|---------|------------|----|-----|
| NAD⁺      | 1.970 ± 0.074 | 1.962 ± 0.074 | 1.882 ± 0.070 | 1.933 ± 0.074 |
|           | (n = 17) | (n = 13)   | (n = 11) | (n = 13) |
| NADH      | 0.295 ± 0.090 | 0.315 ± 0.097 | 0.594 ± 0.074 | 0.507 ± 0.070 |
|           | (n = 17) | (n = 13)   | (n = 11) | (n = 13) |
| NAD⁺/NADH | 6.68 ± 0.04  | 5.91 ± 0.04  | 3.60 ± 0.07  | 3.81 ± 0.07  |
|           | (n = 17) | (n = 13)   | (n = 11) | (n = 13) |
| NADP⁺     | 0.069 ± 0.008 | 0.069 ± 0.012 | 0.064 ± 0.008 | 0.046 ± 0.010 |
|           | (n = 16) | (n = 7)    | (n = 14) | (n = 11) |
| NADPH     | 0.219 ± 0.012 | 0.223 ± 0.014 | 0.230 ± 0.013 | 0.230 ± 0.072 |
|           | (n = 20) | (n = 14)   | (n = 12) | (n = 14) |
| NADPH/NADP⁺ | 3.17 ± 0.31 | 3.23 ± 0.31 | 3.59 ± 0.33 | 4.76 ± 0.23 |
|           | (n = 16) | (n = 7)    | (n = 12) | (n = 17) |

* p < 0.001.
* p < 0.05.
of these components were measured; the hypothesis was that, because of the large change in energy consumption by the
ments of adenine and pyridine nucleotides, steady state levels
a sense blurred the distinction between ATP- and oxidation-
generation or dissipation of an H+ or potential gradient, has in
the problems in the field of oxidative phosphorylation. Here
els in frog mucosa during onset or increase of acid rate has also

Values are given, when appropriate, in millimoles kg$^{-1}$ dry weight.

| Substance                   | Resting  | Blood flow | HI        | HI1       |
|-----------------------------|----------|------------|-----------|-----------|
| Lactate                     | 3.909 ± 0.251 | 4.679 ± 0.588 | 7.822 ± 1.191$^a$ | 7.494 ± 1.090$^a$ |
| (n = 17)                    | (n = 12) | (n = 11)   | (n = 13)   |           |
| Pyruvate                    | 0.678 ± 0.032 | 0.747 ± 0.046 | 0.760 ± 0.074 | 0.817 ± 0.080 |
| (n = 10)                    | (n = 12) | (n = 11)   | (n = 13)   |           |
| Lactate/pyruvate            | 5.77 ± 0.32 | 6.26 ± 0.53 | 10.29 ± 1.16$^a$ | 8.87 ± 1.90$^a$ |
| (n = 15)                    | (n = 12) | (n = 11)   | (n = 13)   |           |
| Acetoacetate                | 1.379 ± 0.131 | 1.309 ± 0.084 | 1.377 ± 0.148 | 1.25 ± 0.115 |
| (n = 7)                     | (n = 7)   | (n = 7)    | (n = 7)    |           |
| $\beta$-Hydroxybutyrate     | 0.101 ± 0.013 | 0.157 ± 0.021 | 0.257 ± 0.024$^a$ | 0.333 ± 0.008$^a$ |
| (n = 7)                     | (n = 7)   | (n = 7)    | (n = 7)    |           |
| $\beta$-Hydroxybutyrate/    | 0.140 ± 0.019 | 0.135 ± 0.028 | 0.211 ± 0.040$^c$ | 0.327 ± 0.113$^c$ |
| acetoacetate                | (n = 7)   | (n = 6)    | (n = 7)    |           |

$^a$ p < 0.001.
$^b$ p < 0.01.
$^c$ p < 0.05.

directly from oxidation-reduction of a substrate with secretion of H$^+$ and donation of electrons to an unspecified acceptor (25). This oxidation-reduction hypothesis was amplified further, based on work in yeast (26, 27) and data obtained from dual-split beam spectroscopy (28) have been interpreted as supporting an oxidation-reduction mechanism. On the other hand, measurements showing correlations between ATP levels and acid secretion (29, 30) or results obtained from the use of inhibitors (31, 32) have been interpreted as for (29) or against (30) an ATP-based mechanism. The discovery of ATPase in gastric mucosal membranes, either $\text{HCO}_3^-$- or K$^+$-stimulated (33, 34) has also been used to support an ATP hypothesis. Measurement of adenine nucleotide and phosphocreatine levels in frog mucosa during onset or increase of acid rate has also implicated ATP as the primary energy source (35).

The conflict of interpretation of the data is reminiscent of the problems in the field of oxidative phosphorylation. Here the proposal of chemiosmotic coupling (30) whereby the breakdown or synthesis of ATP is linked intimately to the generation or dissipation of an H$^+$ or potential gradient, has in a sense blurred the distinction between ATP- and oxidation-reduction-linked mechanisms.

Since techniques have not been developed for flux measurements of adenine and pyridine nucleotides, steady state levels of these components were measured; the hypothesis was that, because of the large change in energy consumption by the tissue, at some point in the transition between rest, tissue perfusion, and varying secretory states, critical steady state changes would be observed.

The primary reaction, that of H$^+$ transport, may be the site of stimulation of metabolism. The largest changes in the tissue should be observed in the acid-secreting cells, the parietal cells, and changes in surface epithelial cells should be minimal. The data obtained showed no change in levels of ATP and other adenine nucleotides, as well as no change in phosphocreatine, for either the parietal or the mucous cells. Moreover there was no significant difference in the levels found when the histamine and histamine + urecholine experiments were compared, in spite of the widely differing acid rates.

There is a variety of possible interpretations. The high mitochondrial content of the parietal cells (>32%) suggests that this tissue may well be capable of a rapid response to widely varying energy demands, so that there is little change in ATP or phosphocreatine. In this case, however, since there is no change in total ADP, the primary metabolic stimulus for the increased $O_2$ consumption (37) must be elsewhere in metabolism or due to changes in intramitochondrial ADP. It may be that mitochondrial metabolism is substrate-limited, rather than ADP-limited, i.e. that the mitochondria in gastric mucosa are in State 2 rather than State 4, as has been suggested elsewhere (28).

Compartmentation of the various nucleotides between mito-
show reduction of pyridine nucleotide with stimulation of this area has been confined to spectroscopy where the data the NAD⁺ level was still greater than NADH. Previous work in level. Although the NAD⁺/NADH ratio fell from 6.68 to 3.81, observed consistent and significant increases in the NADH latter consistent with the effects of increased CO₂, adenylate kinase to maintain this equilibrium. It can be concluded that gastric parietal cells contain sufficient reaction, the inverse of the above ratio, is 2.26 at pH 7.4. It can changes in the ratio of these due to a shift in NAD⁺/NADH due involved in calculation of the phosphorylation potential, ATP and a rise in ADP may be due to poorer oxygenation of the matrix. In the stomach, whereas the acetoacetate/butyrate ratio increases ranging from 0.3 to 1 pH unit. A fall in cell [H⁺] will result in activation of phosphofructokinase as well as a decrease in the NAD⁺/NADH, hence [pyruvate]/[lactate] ratios. Since pyruvate is the oxidized form, and glyceraldehyde-3-phosphate is the reduced form of the two oxidation-reduction couples involved in calculation of the phosphorylation potential, changes in the ratio of these due to a shift in NAD⁺/NADH due to a change of pH will cancel, assuming equilibrium operation. Data from frog gastric mucosa (35) which show a slight fall in ATP and a rise in ADP may be due to poorer oxygenation of the in vitro mucosa, with also slower removal of HCO₃⁻, with subsequent hypoxia and alkalization of the parietal cell, the latter consistent with the effects of increased CO₂ in vitro (40). The increase in phosphocreatine observed (35) was explained by the shift in cell pH. Since, however, the equilibrium constant for creatine kinase is 7.2 x 10⁻⁹ at pH 7.4, and 2.98 x 10⁻⁹ at pH 9.8 (41), the predicted change as a function of pH would be a fall in phosphocreatine, rather than a rise, or a fall in ADP with a rise in ATP. Hence, the constancy, or increase of phosphocreatine is difficult to reconcile with ADP driven metabolic transition. It should be pointed out that constancy in ATP, and ATP/ADP ratios is also found in muscle during a work load (42). However, associated with this, there is a fall in phosphocreatine contrary to what was observed here, and in frog mucosa (35).

Since the ratio [ATP]·[AMP]/[ADP]² remains fairly constant at about 0.5 and since the equilibrium constant of the reaction, the inverse of the above ratio, is 2.26 at pH 7.4, it can be concluded that gastric parietal cells contain sufficient adenylate kinase to maintain this equilibrium.

In contrast to the constancy of adenine nucleotide level, we observed consistent and significant increases in the NADH level. Although the NAD⁺/NADH ratio fell from 6.68 to 3.81, the NAD⁺ level was still greater than NADH. Previous work in this area has been confined to spectroscopy where the data show reduction of pyridine nucleotide with stimulation of secretion (10, 28) or at least reduction of the rest of the respiratory chain (39), but some disagreement exists for this finding (9). The spectroscopic data naturally do not distinguish di- or triphosphopyridine nucleotides, nor the cell type or cellular location of the change. NADP⁺ and NADPH change significantly with acid rate changes, and the NAD⁺/NADH ratio doubles in the mucous cell, hence the spectroscopic data probably do indeed relate to the parietal cell (oxyntic cell in amphibia).

It is possible to calculate the NAD⁺/NADH ratio in the cytoplasm and mitochondria (43) on the assumption that (a) lactic dehydrogenase and α-glycerophosphate dehydrogenase: β-hydroxybutyrate dehydrogenase and glutamic dehydrogenase are located in the cytoplasm or mitochondria exclusively, (b) their activity is sufficient to maintain equilibrium, (c) there is no pH gradient between mitochondria and cytoplasm.

The equilibrium constants for lactic dehydrogenase is 1.11 x 10⁻¹¹ M, and hence from the equation

\[
\frac{\text{NAD}^+}{\text{NADH}} = \frac{1}{K_{\text{LADH}}} \left(\frac{\text{pyruvate}}{\text{lactate}}\right) \left(\frac{\text{H}^+}{\text{K}_{\text{LADH}}}\right)
\]

The cytoplasmic ratio NAD⁺/NADH at pH 7.4 is calculated to be 620 and to change to 348 with secretion. The ratio changes of α-glycerophosphate and dihydroxyacetone phosphate are similar to the lactate/pyruvate changes (39), hence the assumption that these dehydrogenases are sufficiently active to maintain equilibrium seems justified (Table XI).

This may well not be so for the β-hydroxybutyrate in stomach which has low activity in relation to liver. In the liver, the direction of change of acetoacetate/β-hydroxybutyrate and α-ketoglutarate NH₃/glutamate correspond, showing identity of the NAD⁺/NADH ratio in mitochondrial membrane and matrix. In the stomach, whereas the acetoacetate/butyrate ratio falls, the α-ketoglutarate ratio increases (39, and subsequent papers) by 50%. This implies that the β-hydroxybutyrate dehydrogenase has too low an activity to maintain equilibrium in parietal cells, and assuming at least no fall in NH₃, that the mitochondrial NAD⁺ pool is relatively oxidized with onset of acid secretion. The ratio for cytoplasm is much larger than the measured ratio, presumably due to greater binding of NADH relative to NAD⁺ (43).

The influence of pH on this equilibrium is large. Thus, a

| Sample | Calculated ATP/ADP-P | Measured ATP/ADP-P |
|--------|----------------------|--------------------|
| Resting | 46.23 | 9699 |
| Blood flow | 50.08 | 2080 |
| HI | 3201 | 1553 |

| Sample | Calculated NAD⁺/NADH ratio | Cytosol (pyruvate/lactate) | Total measured NAD⁺/NADH |
|--------|-----------------------------|-----------------------------|--------------------------|
| Resting | 62.20 | 6.88 |
| Blood flow | 570 | 5.91 |
| HI | 348 | 3.60 |
| HII | 404 | 3.81 |

S. J. Hersey, personal communication.
change of pH from 7.4 to 7.7, as has been suggested to occur in
in vitro frog mucosa, would be sufficient to induce a 2-fold
change in the ratio, as observed here. Since, however, there is
no difference between the two maximal secretory rates, where
an additional change in cell pH might be expected, and also
since there is no change in the NADP+/NADPH ratio which
would be equally pH-sensitive, it may be that in vivo, little
change in the ratio, as observed here. Since, however, there is
no difference between the two maximal secretory rates, where
an additional change in cell pH might be expected, and also
since there is no change in the NADP+/NADPH ratio which

In some dogs a sample was taken after prolonged anesthesia
and secretion. In those dogs there was a significant fall in ATP
and phosphocreatine, as well as a large rise in lactate and
NADH. These data could be mimicked by holding the biopsy
sample in the forceps for 3 min prior to freezing. Hence anoxia
could be a significant factor in these samples. Maintaining
anoxia in a biopsy from resting and secreting mucosa did not
give significant differences in ATP fall between rest and
secretion. Data obtained for rat gastric mucosa (total thick-
ness) show similar changes with hemorrhagic shock (45).
The mucous cells showed little change in any of the
metabolites with onset of acid secretion. This shows a lack of
effect of histamine on this cell type in the dog.

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