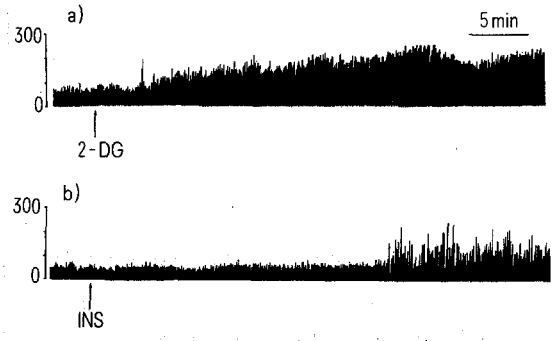

Fig. 1. Effect of intracarotid injection of 2-deoxy-D-glucose and insulin on the efferent activity of the gastric vagus nerve. *a*) Nerve activity of gastric vagus in response to an injection of 2-DG (50 mg/kg, 2-DG). *b*) Insulin (5 units/kg, INS) similarly increased the activity of the gastric vagus nerve. Ordinates indicate nerve activity (spikes/5 sec).

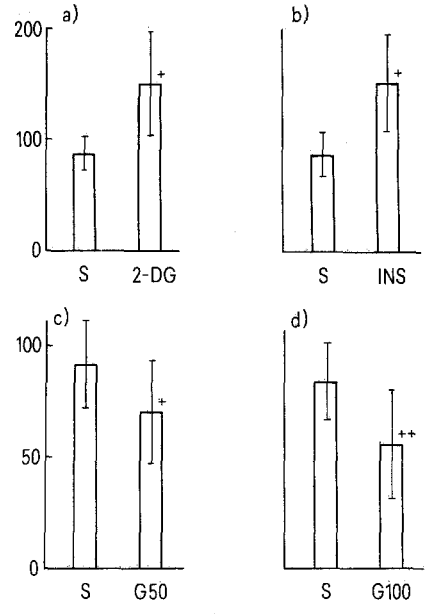

Fig. 2. Graphic presentations of the effect of 2-DG (a), insulin (b) and glucose (c) (d) on the efferent activity of the gastric vagus nerve. *S*, spontaneous activity; 2-DG, maximum activity within 60 min after the injection of 2-DG (50 mg/kg); INS, highest activity within 60 min after insulin injection (5 units/kg); G50, lowest activity within 5 min after the injection of glucose (50 mg/kg); G100, nerve activity after glucose (100 mg/kg) injection. Ordinates indicate nerve activity (spikes/5 sec). Vertical bars, SD. +, significantly different from the spontaneous activity ($p < 0.01$). ++, $p < 0.001$.

peak activity within 60 min after the injection and that to glucose by the lowest activity within 5 min. Injections were made through a catheter inserted into the cephalic end of the left carotid artery (i.c.a.). Data were collected from the 1st response to a certain drug. 2-DG and insulin were not tested in the same animal. Values were expressed as mean ± SD. Difference was evaluated by Student's t-test. 2-DG and glucose were prepared as 5% solutions is distilled water.

Results and discussion. A marked increase in the activity of the gastric vagus nerve was found after the injection of 2-DG (50 mg/kg, i.c.a.) (fig. 1,a). Spontaneous activity increased from 88.6 ± 14.7 spikes/5 sec to 150.7 ± 47.2 spikes/5 sec within 60 min after the injection ($p < 0.01$, paired t-test, $n = 10$) (figure 2,a). An injection of insulin (5 units/kg, i.c.a.) caused a gradual increase in the vagus activity (figure 1,b). Spontaneous activity increased from 88.2 ± 19.6 spikes/5 sec to 154.5 ± 44.6 spikes/5 sec ($p < 0.01$, paired t-test, $n = 7$) (figure 2,b). 25 rats were used to examine the effect of glucose on efferent activity of the gastric vagus nerve. Intracarotid injections of glucose (50 mg/kg) immediately suppressed the efferent activity of the vagus nerve by 24% ($p < 0.01$, $n = 11$) (figure 2,c). A larger dose (100 mg/kg) caused a 33% increase in suppression ($p < 0.001$, $n = 14$) (figure 2,d).

The present results show that injections of 2-DG as well as of insulin increased the efferent activity in the gastric vagus nerve while intracarotid injection of glucose caused a transient depression of vagus activity. Colin-Jones and Himsworth[7] reported that only the direct injection of 2-DG into the lateral hypothalamic area increased acid secretion in the stomach and the authors concluded that a chemoreceptor which is responsive to a lack of metabolized glucose can initiate the vagally mediated acid secretion. The present results are in keeping with the view that 2-DG and insulin induce gastric acid secretion by increasing the efferent activity of the gastric vagus nerve while glucose inhibits acid secretion by reducing the vagus activity.

1 This work was supported by research grant No. 212105 from The Ministry of Education, Science and Culture of Japan.

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Stimulation of pyruvate carboxylation by gastric secretagogues[1]

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Summary. Pyruvate carboxylation was stimulated by 2 gastric secretagogues, histamine and dibutyryl cyclic AMP, and by butyrate. Thiocyanate, an inhibitor of acid secretion, produced a slight decrease. Avidin significantly reduced acid secretion and this effect was overcome by biotin and oxalacetate. The results suggest that carboxylation of pyruvate is one of the reactions controlling oxidative metabolism and acid secretion in toad gastric mucosa.

The exact mechanism by which gastric secretagogues stimulate acid secretion (qH^+) is not known, but current evidence indicates that the activation of the oxidative metabolism may play an important role[3-9]. Therefore, a prerequisite for a better understanding of the mechanism of qH^+ is to characterize the metabolic responses to gastric secreta-

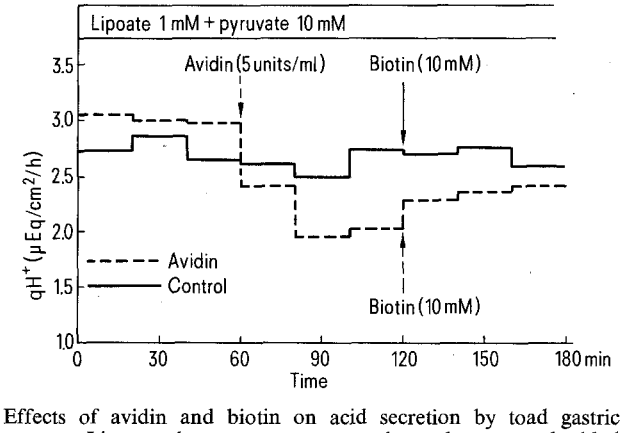
gogues. Stimulation of qH^+ is associated with an increased oxidation of fatty acids[6,8] and carbohydrates[3,4,10], which in turn leads to an elevation of acetylcoenzyme A (CoA-S-Ac) concentration[10]. We have previously reported that oxalacetate (OAA) concentration is regulated by pyruvate carboxylase activity and is one of the factors controlling oxygen uptake and qH^+ in the amphibian gastric mucosa[11]. In the present paper we report the effects of gastric secretagogues on the rate of pyruvate carboxylation and the effect of avidin, and inhibitor of the pyruvate carboxylase enzyme, on the rate of qH^+.

Materials and methods. All experiments were performed in vitro on gastric mucosa from fasted Venezuelan toads *(Bufo marinus)*. The rate of pyruvate carboxylation was estimated by measuring the net fixation of label from $H^{14}CO_3^-$ as previously described[4,11]. This method has proved to be valid for estimating the rate of the pyruvate carboxylase reaction in the intact tissue. Briefly, after the toad was pithed, the stomach was removed and the muscularis layer was stripped from the gastric mucosa and discarded. Paired sections of gastric mucosa were incubated 2 h at 30 °C in 5 ml of a Ringer solution containing 17 mM N-tris-(hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES) at pH 7.4 as a buffer, 10 mM $NaHCO_3$ containing 1 µCi $H^{14}CO_3^-$, 10 mM pyruvate, and the testing compounds. At the end of incubation, the tissue was quickly frozen in liquid nitrogen and immediately pulverized with a stainless steel percussion mortar previously cooled in dry ice. In the next steps, the intermediates of the Krebs cycle were extracted by perchloric acid according to the method of Williamson and Corky[12]. The radioactivity of 0.5-ml aliquots of each extract in 10 ml of Aquasol (New England Nuclear) was counted in duplicate in a liquid scintillation counter (Nuclear Chicago, model Mark III). For measurements of qH^+, the mucosa was divided longitudinally into paired halves and qH^+ was measured as previously reported[4,11,13], using the pH-stat method with the stat set at 5.6. TES at 17 mM, pH 7.4, was used as a buffer in the nutrient (submucosal) side, and both the secretory and the nutrient solutions were gassed with 100% oxygen. The statistical significance of the differences was calculated using the Student's t-test.

Fixation of label from $H^{14}CO_3^-$ by toad gastric mucosa

Condition (n)	Label fixation (µmoles/g wet wt/2 h)	%Δ
Control (14)	2.07±0.11	
0.1 mM histamine (6)	3.12±0.41	+51%*
10 mM db-cAMP (4)	3.46±0.34	+67%*
10 mM butyrate (11)	2.91±0.22	+41%*
10 mM thiocyanate (4)	1.84±0.13	−11% NS

10 mM pyruvate was used as the substrate in all experiments. %Δ is the change expressed as percentage of the control values. Values are means±SE. The numbers in parentheses indicate the number of experiments. * $p < 0.025$ vs control; NS, not significant.



Effects of avidin and biotin on acid secretion by toad gastric mucosa. Lipoate plus pyruvate were used as substrates and added at time 0 to both paired halves of a gastric mucosa. At 60 min, avidin was added to one half (– – –) and buffer was added to the other half (——), which served as control. At 120 min, biotin was added to both halves.

Results and discussion. The effect of different agents on the rate of pyruvate carboxylation by toad gastric mucosa are shown in the table. Histamine and dibutyryl cyclic AMP (db-cAMP), 2 gastric secretagogues, produced a significant increase of 51 and 67%, respectively, above the control value. Butyrate, a substrate which is oxidized to Ac-S-CoA units, also produced a significant increase of 41%. Thiocyanate, an inhibitor of qH^+, produced a decrease of 11% but this was not statistically significant. Pyruvate carboxylase is a biotin-containing enzyme which can be inhibited by avidin[11]. If the rate of pyruvate carboxylation were one of the factors controlling oxidative metabolism and qH^+ in toad gastric mucosa, a reduction of qH^+ should be expected by inhibiting the pyruvate carboxylase enzyme. Therefore, the effect of avidin on qH^+ was investigated. Avidin at 2.5 units/ml significantly inhibited qH^+ by 20% (9 experiments, $p < 0.05$). Biotin alone had no significant effect on qH^+ but it stimulated in the presence of avidin. This latter effect is shown in the figure. In this illustrative experiment, avidin at 5 units/ml reduced qH^+ to about 65% of the control value, and subsequent addition of biotin restored qH^+ to about 85% of the control value. OAA at 10 mM had a similar effect (not shown).

The present results permit the suggestion that the pyruvate carboxylase reaction is one of the reactions controlling oxidative metabolism and acid secretion in toad gastric mucosa, and that its stimulation is one of the metabolic responses to gastric secretagogues. It may be possible that gastric secretagogues stimulate the rate of substrate oxidations (fatty acids[6,8] and carbohydrates[3,4,10]) producing an increase in Ac-S-CoA concentration[10] which in turn would activate the pyruvate carboxylase enzyme, stimulating the rate of conversion of pyruvate to OAA. This latter intermediate is necessary for the oxidation of Ac-S-CoA in the Krebs cycle and for the continuing oxidation of both pyruvate and fatty acids. Ac-S-CoA is a well known positive modulator of pyruvate carboxylase in animal tissues[11].

1 This investigation was supported by Consejo de Desarrollo Científico y Humanístico de la Universidad del Zulia, and by CONICIT Grant S1-0455.
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