or 20% of the sera or their non-dialyzable fractions. Usually 1×10^5 cells per 1 ml of medium were incubated in Müller flasks or test tubes, and the percentage of the initial inoculum reached by cultures with experimental and control sera was calculated from 4 vessels (8 hemocytometer counts) in repeated time-independent experiments.

Results. (Table.) The yield of the L-As and LA cells after 3 days of incubation was higher with the sera from cold-exposed rats than with the sera of controls. Dialysis lowered the cell yield, but the difference between the experimental and control sera did not disappear. In the combination L-As cells-sera 2, the effect was practically the same for native and dialyzed sera. In the system LA cells-sera 1, the response to dialyzed experimental serum was lower than to native serum, but still present. Decreased serum concentration in the medium decreased the total cell yield, but did not influence the result. For human diploids LEP 19, native sera were toxic, but with inactivated sera, the cell yield was the same as in mouse L cells, only after a longer incubation. Higher serum concentration improved the cell yield. The difference between the experimental and control sera was in the range of mouse heteroploids. 1st-passage embryonic rat liver cells and 5th-passage newborn rat hepatocytes were not able to multiply with the control sera, but did so with the experimental sera. Their response was lower than that of mouse heteroploids and phase II human diploids. Adult rat kidney primaries unable to overcome rapidly the stress of explantation suffered great cell losses, but even here slight evidence of a positive reaction was found.

Discussion. The mitogenic effect of the serum of cold-exposed rats was neither species nor tissue specific. The serum stimulated multiplication of mouse, human and rat cells, fibroblastic and epithelial, heteroploid and diploid. It differed thus from the tissue specific growth- and metabolism-stimulating sera of rats after hepatectomy[5] and uninephrectomy[6]. The absence of tissue specificity was to be expected in view of the in vivo induction of DNA synthesis and the increase in cell number in many organs of cold-acclimated rats[1,2,7,8]. The high sensitivity of the quickly growing cell types and the low sensitivity of those growing slowly was in agreement with the known positive correlation between the proliferative capacity of cultured cells and their requirements for serum and its growth-stimulating macromolecules[9].

The nature of the mitogenic stimulus in the sera of cold-exposed rats is not known. One might speculate about some hormones which take part in the development of cold acclimation, for instance triiodothyronine, a stimulator of liver thermogenesis in vivo and in cultured hepatocytes[10]. Triiodothyronine also stimulates the production of growth hormone whose mediators are the growth-promoting somatomedins[11]. Another mitogenic peptide, the epidermal growth factor (urogastrone), was recently shown to induce DNA synthesis and hepatocyte proliferation in the presence of insulin and glucagon[12]. Nothing is known about the production and activity of these or other growth-stimulating factors in cold acclimation, but, as assays for at least some of them are now available, the induction of organ growth in cold-exposed rats is open to further analysis.

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Effects of 2-deoxy-D-glucose, glucose and insulin on efferent activity in gastric vagus nerve[1]

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Summary. Intracarotid injection of 2-deoxy-D-glucose and insulin increased the efferent activity in the gastric vagus nerve of anesthetized rats, while glucose injection transiently decreased vagus activity.

2-Deoxy-D-glucose (2-DG) and insulin are powerful stimulants of vagal gastric secretion[2,3]. Since 2-DG is converted by phosphorylation to 2-DG-6-phosphate but is not metabolized further, this product competes with glucose-6-phosphate and inhibits its phosphorylation[4-6] resulting in glucopenia in the cell. 2-DG causes glucopenia in neurones in the lateral hypothalamic area which have been shown to initiate vagally mediated gastric secretion[7]. Little work has been done in studying the effect of 2-DG on efferent activity of the gastric vagus nerve. The present experiments were designed to investigate this aspect.

Materials and methods. Experiments were carried out on 28 male rats weighing 250–400 g. The animals were fasted for 18 h but had free access to water. Animals were anaesthetized with sodium pentobarbital (45 mg/kg, i.p.). A fine filament dissected from the central cut end of the vagus nerve innervating the stomach was placed on a pair of silver wire electrodes. Efferent nerve activity was amplified with a differential amplifier and was integrated after converting the spikes to standard pulses by a window discriminator and presented as vertical deflections. Spontaneous activity is expressed as mean spikes per 5 sec over 50 sec (i.e. mean of 10 samples) just before the injection of the drugs. Responses to 2-DG and insulin are indicated by the

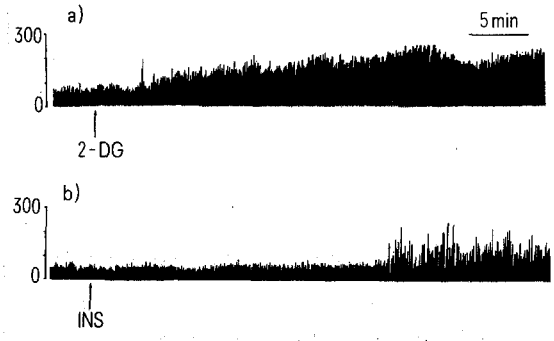

Fig. 1. Effect of intracarotid injection of 2-deoxy-D-glucose and insulin on the efferent activity of the gastric vagus nerve. *a*) Nerve activity of gastric vagus in response to an injection of 2-DG (50 mg/kg, 2-DG). *b*) Insulin (5 units/kg, INS) similarly increased the activity of the gastric vagus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

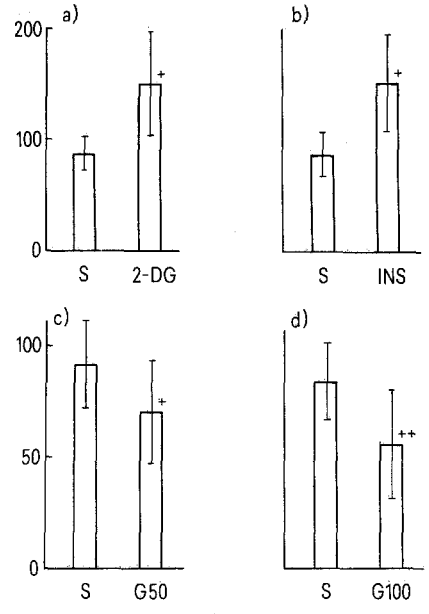

Fig. 2. Graphic presentations of the effect of 2-DG (a), insulin (b) and glucose (c) (d) on the efferent activity of the gastric vagus nerve. *S*, spontaneous activity; 2-DG, maximum activity within 60 min after the injection of 2-DG (50 mg/kg); INS, highest activity within 60 min after insulin injection (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-