indicated by the length of coagulated albumin digested in glass tubing of standard diameter under specified conditions[3]. All experiments were randomized in each dog with no animal having more than 2 test days per week.

Results and discussion. Figure 2 shows the effects of AHR-2438B on pepsin proteolysis as determined by the 2 procedures. It is evident that the lignosulfonate markedly inhibited digestion of egg albumin under the conditions described. The maximum effect occurred 15 min postdosing with some inhibition apparent at 60 min. The intragastric administration of 10 ml of water had no effect. The pH values of the gastric samples ranged from 1.0 to 1.3. While the pH of the environment in which pepsin proteolysis occurs is of utmost importance, we have repeatedly demonstrated that such small fluctuations as those observed in these experiments would not be an important factor. Any sample of gastric juice containing bile was discarded.

Results of the present experiments confirm in another animal species that AHR-2438B is an effective inhibitor of pepsin proteolysis and may be useful in the medical treatment of peptic ulcer.

Zusammenfassung. Die Wirkung eines Lignolsulphonates auf die Pepsinproteolyse im Hund mit totaler Magenfistel wurde untersucht und geronnenes Eialbumin als Substrat der Pepsinverdauung verwendet. Es ergibt sich, dass der Pepsin-Inhibitor AHR-2438B – wie früher bei der Ratte nachgewiesen – auch beim Hund die Proteolyse im Magen wirksam hemmt.

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Theophylline-Stimulated Aerobic Glucose Uptake by Rat Thymocytes Exposed to Anoxia[1]

Methyl xanthines have been shown to exert influences on glucose metabolism, but the effects have varied with the tissues and other conditions used. Inhibition of uptake or oxidation of glucose by methyl xanthines has been reported using rat epididymal adipose tissue[2,3], isolated adipose cells[4], polymorphonuclear leucocytes[5], and dog and pig thyroid slices[6]. A biphasic dose-response to theophylline was demonstrated both in sheep thyroid slices[7], and beef thyroid homogenates[8]. This response involved stimulation of $^{14}CO_2$ production from glucose-1-^{14}C at a specific theophylline concentration, and inhibition of the oxidation at higher concentrations.

During studies of aerobic glucose metabolism of isolated rat thymus cells, it was observed that theophylline, at a concentration of 0.5 m*M*, stimulated glucose uptake if the cells had been previously exposed to anoxia. In the present investigation the dose-response curve to theophylline was found to be biphasic, and the effect dependent on both the time of incubation and the glucose concentration.

Materials and methods. Thymocytes were prepared from 150 to 200 g Sprague-Dawley derived male rats (Sasco, Omaha, Nebr.), which were bilaterally adrenalectomized 4 or 5 days before each experiment to remove the source of endogenous steroids which can vary and cause inhibition of the carbohydrate metabolism of these cells[9]. Following decapitation of the rats, thymuses were quickly removed and chilled in Krebs-Ringer bicarbonate (KRB) buffered medium[10], equilibrated with 5% CO_2 in N_2. Cells were released into the medium by gentle teasing, and then filtered through a 200 mesh stainless steel screen, washed twice, and resuspended in KRB buffer to give a 10% (w/v) suspension based on the wet weight of thymus. All measurements are referred to cell number, which was determined by hemocytometry.

Anoxic treatment consisted of a 1 h 37°C incubation of 3×10^8 cells per ml of KRB buffer, gassed with 5% CO_2 in N_2. Aerobic incubations were conducted with 5% CO_2 in O_2 and U-^{14}C-glucose (18 μCi/nmole, New England

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Effect of 0.5 m*M* theophylline on aerobic glucose metabolism of thymocytes following anaerobic preincubation

Theophylline addition	Incubation time (h)	Glucose utilization (μmoles)[a]	Lactate production (μmoles)[a]	$^{14}CO_2$ production (dpm)[a]
0	1	16.52 ± 1.17	15.50 ± 0.15	160,400 ± 4,600
+	1	34.23 ± 1.55[b]	19.43 ± 0.19[b]	165,100 ± 2,00[d]
0	2	28.63 ± 2.86	21.53 ± 1.38	384,400 ± 3,300
+	2	27.50 ± 2.38[d]	26.00 ± 0.54[c]	387,600 ± 5,000[d]
0	3	38.37 ± 1.76	22.07 ± 0.54	564,000 ± 17,900
+	3	34.37 ± 1.87[d]	28.86 ± 0.90[b]	596,500 ± 3,800[d]

[a] Per 10^{10} cells. [b] $p < 0.001$. [c] $p < 0.01$. [d] Not significantly different from the control. Thymocytes (10^8 per ml) in KRB medium were preincubated 1 h at 37°C under 5% CO_2 in N_2 without substrate. Aerobic incubation: 10^8 cells per ml of KRB medium were incubated with 2.78 m*M* U-^{14}C-glucose (49,300 dpm per μmole) at 37°C under 5% CO_2 in O_2. Values are mean of triplicate flasks ± standard deviation.

Nuclear), with or without theophylline. Erlenmeyer flasks (25 ml) fitted with a hanging center well were used with a total incubation volume of 3.0 ml and 10^8 cells per ml. Radioactive carbon dioxide was collected in 1 *M* Hyamine hydroxide in methanol in the center well, and was counted by liquid scintillation spectrometry at an efficiency of 78% as determined by external standardization. Production of $^{14}CO_2$ was expressed as dpm per 10^{10} cells. Lactate and glucose concentrations in the supernatant fluid were measured without neutralization by standard enzymatic analysis[11]. Glucose uptake was defined as glucose lost from the medium. Results are expressed as μmoles per 10^{10} cells. Significance of difference between means was evaluated by Student's *t*-test.

Results and discussion. Preliminary experiments revealed that theophylline increased aerobic glucose uptake by thymocytes that had been previously exposed to varying periods of anoxia during preparation of the cells. To explore this finding further, thymocytes were exposed to the standard anaerobic preincubation and the aerobic glucose uptake was measured with or without theophylline present. Typical results in the Table show that theophylline at a concentration of 0.5 m*M* increased glucose uptake to 210% of the control during the first hour of incubation. The response to theophylline varied from one experiment to another, with the degree of stimulation of glucose uptake ranging from 125% to 400% of the control. In each case, however, glucose in the medium was the same at 2 h in the presence or absence of theophylline. Increased glucose uptake was reflected in a small, but not always significant, increase in lactate production. The presence of 0.5 m*M* theophylline had no effect on $^{14}CO_2$ production from U-^{14}C-glucose.

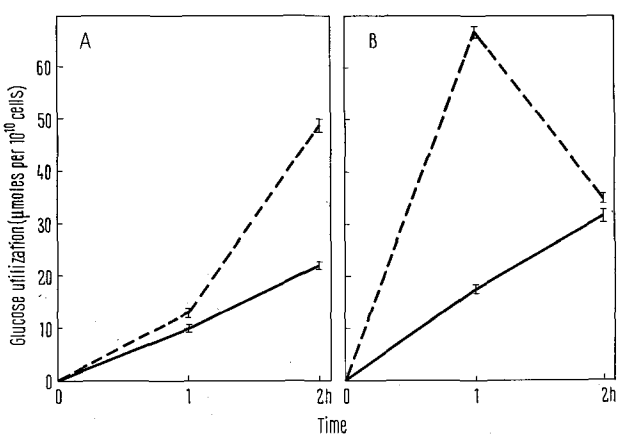

Fig. 1. Time course of glucose utilization in the presence and absence of 0.5 m*M* theophylline. Experimental conditions are described in the Table, except that substrate concentration is varied. A) 1.1 m*M* glucose substrate. B) 2.78 m*M* glucose substrate. ——, no theophylline; – – –, 0.5 m*M* theophylline added. Points are average of duplicate flasks. Vertical bars represent ranges.

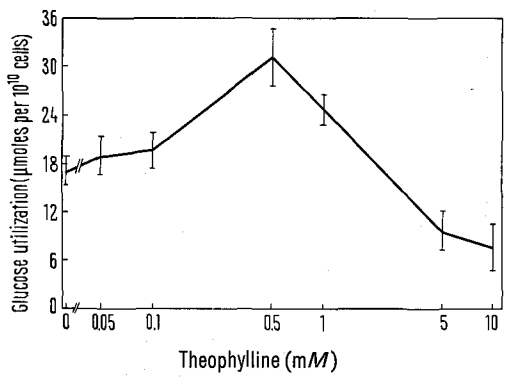

Fig. 2. Influence of various theophylline concentrations on glucose utilization by thymocytes. Experimental conditions are described in the Table. Points are mean of 6 flasks. Vertical bars represent standard deviations.

The stimulation of glucose uptake was found to be dependent on time and substrate concentration. Figure 1 illustrates the time course of glucose utilization with and without 0.5 m*M* theophylline at 2 concentrations of glucose substrate. The peak stimulation occurred at 1 h with 2.78 m*M* glucose substrate (Figure 1B) whereas stimulation was not apparent until 2 h using the lower substrate concentration of 1.1 m*M* (Figure 1A). At neither substrate concentration was there a significant change in lactate or $^{14}CO_2$ production in the presence of theophylline (data not shown).

The effect of varying the theophylline concentration is illustrated in Figure 2. Concentrations of theophylline less than 0.5 m*M* had no effect on glucose uptake and higher amounts inhibited glucose utilization, and both lactate and $^{14}CO_2$ production. The response is similar to that observed with the oxidation of glucose by thyroid preparations[7,8].

Theophylline at a level of 0.5 m*M* did not influence anaerobic glucose metabolism, and no effect could be detected if the anoxic treatment was omitted. Following 1 h of anaerobic preincubation at 37°C without substrate, thymocytes were devoid of glycogen; however, increased glucose uptake in the presence of 0.5 m*M* theophylline did not result in an enhancement of the glycogen level[12]. The fate of the increased increment of glucose consumed is of interest. Failure of lactate, $^{14}CO_2$ or glycogen to increase concomitantly with the glucose uptake can only mean that pools of some carbohydrate intermediates must be increased by theophylline.

The specific conditions required to observe the transitory but pronounced increase in glucose uptake suggest that some oscillatory changes are induced by the anaerobic-aerobic treatment which consequently permit the effect of theophylline to become manifest. Because theophylline is an inhibitor of cyclic-4′5′-nucleotide phosphodiesterase it is widely used to study hormone action[13]. The results from the present investigation indicate that the experimental conditions are of great importance if theophylline is to be used as an agent to study the effects of hormones on glucose metabolism.

Zusammenfassung. Untersuchung der Wirkung von Theophyllin auf den aeroben Glukosestoffwechsel isolierter Thymuszellen. Nach sauerstoffreier Inkubation zeigten die Thymocyten einen Anstieg der Glukoseaufnahme, der jedoch nicht zu einer gleichzeitigen Erhöhung von Lactat oder $^{14}CO_2$ führt.

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