Table 2. Effect of distending the reticulum on food intake by sheep after being deprived of their normal diet for 5.6 h

Time after deprivation (min)	Analyses of variance of 5×5 Latin square Mean food intakes (g) Distension (ml)					F+	RSD (g)	Regression analyses Intercept (g)	Slope (g/ml)	F+	r
	0	200	400	600	800						
0–10	223[a]	191[a]	163[ab]	104[b]	116[b]	****	37	220	−0.151	****	−0.55
0–20	305[a]	278[ab]	208[ab]	157[b]	183[ab]	**	64	299	−0.182	****	−0.48
0–30	371[a]	329[ab]	220[ab]	177[b]	236[ab]	*	83	351	−0.211	****	−0.48
Rebound feeding with source of distension removed											
30–40	102[bc]	89[c]	147[ab]	178[a]	166[a]	***	34	92	+0.109	****	+0.60
30–50	133[b]	143[b]	254[ab]	303[a]	259[ab]	***	66	136	+0.206	****	+0.63
Total intakes for the experiment											
0–50	504	471	474	480	494	NS	72	–	–	NS	–

+ Significance levels; NS, not significant; * $p < 0.05$; ** $p < 0.025$; *** $p < 0.01$; **** $p < 0.005$. Means with different superscripts differ significantly by a sequential variant of the Q method.

Distending the rumen did not depress the intake of food according to Student's paired t-test ($p > 0.05$). The quantities consumed with and without distension were 217 and 231 g after 10 min of feeding, 362 and 360 after 20 min and 392 and 417 after 30 min respectively. The intakes of food for 20 min after the balloons were removed from the animals were also not significantly different, the values being 136 and 105 g respectively. The depression of food intake by ruminal distension in cattle[7] but not in goats[8] and sheep may be due to the type or form of the diet as the cattle were given hay, the goats a concentrate diet and the sheep in this study pelleted alfalfa. However the suppression of intake by 0.211 g/ml distension of the reticulum is remarkable, exceeding considerably even the suppression of 0.05 g/ml distension of the rumen in cattle[7]. The greater suppression of intake seen when the reticulum was distended may have been due to its relatively small size and to its greater innervation with tension receptors[9,10] compared to the rumen. Reticular distension suppressed food but not water intake so the effect was a specific response. The receptors involved may signal satiety but the physiological significance of the results will not be appreciated dimensions of the reticulum are measured in association with the intake of food.

Simultaneous monitoring by optical techniques of respiratory chain and intracellular pH in toad ventricle strip[1]

J. C. LaManna[2], J.-J. Saive, V. W. Macdonald[3] and F. F. Jöbsis

Department of Physiology and Pharmacology, Duke University Medical Center, Durham (North Carolina 27710, USA), 28 June 1977

Summary. Intracellular pH and oxidative metabolism can be measured in toad ventricle strips simultaneously by the use of the pH indicator dye, neutral red, and a rapid scanning spectrophotometer. The effects of hypoxia and acidification on mechanical function are approximately additive. The decrease in tension due to slight acidification is probably through an effect on the portion of the twitch tension supported by anaerobic metabolism.

During hypoxia and ischemia of cardiac tissue there is a failure of the oxidative production of energy and a decrease in mechanical function. In these pathological states there is also a shift to a more acidic intracellular pH, especially in ischemia where the acid metabolites of anaerobic metabolism are not washed out. Since many of the enzymes involved in energy production are pH sensitive, such as phosphofructokinase[4] and the nicotinamide adenine dinucleotide linked substrate shuttle systems[5], it has been suggested that the fall in pH is responsible for the failure of anaerobic metabolism to supply sufficient energy to maintain mechanical function[6–8]. In this regard, it would be of interest to reliably measure intracellular pH (pH_i), mechanical function, and oxidative energy metabolism non-invasively during the development and recovery from hypoxia.

Recently, the pH sensitive indicator dye, neutral red has been applied to amphibian skeletal muscle enabling the comparison of optically detected metabolic and pH changes[9,10]. We have now been able to apply this technique to amphibian cardiac muscle[11]. The toad ventricle

1 Acknowledgments. This work was supported by PHS grants HL 17391, Am 17876 and HL 05208. J.-J.S. is aspirant au Fonds National de la Recherche Scientifique (Belgium).

2 Present address: Department of Neurology, University of Miami Medical School, P.O. Box 520875 Biscayne Annex, Miami, Florida 33152.

3 Present address: Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pa. 19104.

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strip preparation was chosen because the respiratory chain components are present in high concentration and myoglobin is absent[12]. This results in clearly defined spectra of the oxidative mitochondrial enzymes.

Methods. Toads (*Bufo marinus*, 10 cm body length, kept at 21 °C) were decapitated and the exposed heart perfused retrograde through the aorta to remove hemoglobin. The ventricle was extirpated and the apex removed, resulting in a muscular ring. The ring was subsequently cut open to provide a strip that was then mounted in a chamber suitable for optical monitoring. Tension was recorded by a strain gauge. The strips were allowed to equilibrate for 90 min in phosphate Ringer solution at 15 °C. They were continuously bubbled with 100% O_2 and stimulated at a rate of 0.5 Hz with 2 msec supramaximal pulses. The toad Ringer solution contained NaCl, 113 mM; KCl, 5 mM; $CaCl_2$, 2 mM; Na_2PO_4, 8 mM; pH 7. When bubbled with 95% O_2 and 5% CO_2 the pH was measured at 6.15; the pH was 5.7 when 10% CO_2 in O_2 was bubbled; with 75% O_2 and 25% CO_2, 5.4. For dyeing, the muscles were soaked in the chamber for 90 min in Ringer plus 0.25 μM neutral red. The fluid in the chamber was then replaced with undyed Ringer for 10 min. The undyed solution was changed again and the experimental observations began. Optical measurements were made using a rapid scanning vidicon detector spectrophotometer[13] capable of scanning 150 nm in 33 msec, data is stored and later processed by a PDP-8E mini-computer.

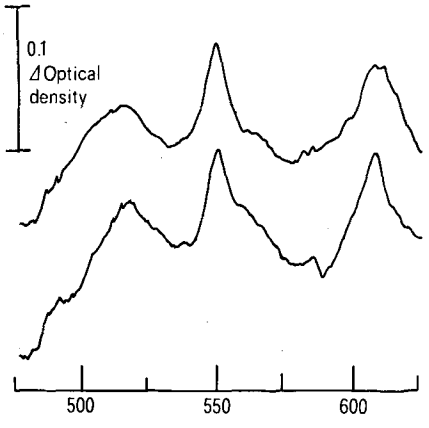

Fig. 1. Top: Difference spectrum produced by subtracting the absorption spectrum of undyed isolated toad ventricle strip in a bath bubbled with 100% oxygen from the spectrum obtained in 100% nitrogen. The scale is in nanometers of wavelength. Bottom: As in figure 1, top, except in the presence of neutral red dye.

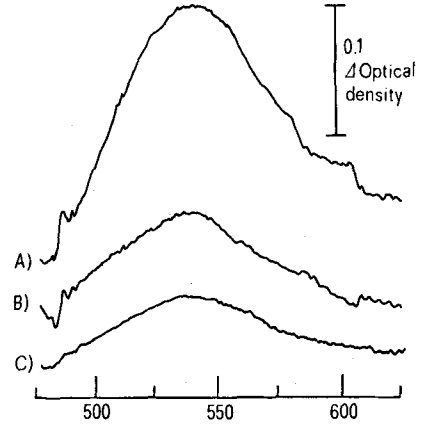

Fig. 2. *A* Difference spectrum from dyed toad ventricle strips produced by subtracting the absorption spectrum measured in 100% oxygen from that measured in 25% CO_2 in oxygen. *B* As in figure 2A, except 5% CO_2 is used insteady of 25% CO_2. *C* As in figure 2A, except the spectrum measured in 100% nitrogen is subtracted from that in 95% nitrogen, 5% CO_2. The scale is in nanometers of wavelength.

Results. Neutral red shows differential absorption peaks when in an acid or basic medium which can be perceived as color changes. The acid form of the dye exhibits strong absorption at a wavelength of approximately 535 nm. The dye apparently equilibrates with an intracellular compartment of the muscle since the tissue becomes highly colored within a short period of soaking. When the tissue is removed to undyed solution there is almost no washout of the dye for the length of the experiment. The dye is subjected, however, to photodecomposition from the intense light source used in the spectrophotometer and this necessitated the use of an electronically controlled shutter that only allowed light to fall on the tissue during the brief periods when spectra were being scanned by the vidicon. There was no perceptable effect of the dye on the resting or stimulated tension of the ventricle strip.

When an undyed toad ventricle strip is made hypoxic by changing the bubbling gas from 100% O_2 to 100% N_2, a clearly identifiable cytochrome difference spectrum is obtained (figure 1, upper spectrum). The peaks indicate the α absorption bands of cytochrome a,a_3 at 605 nm, cytochrome b at 564 nm and cytochrome c at 550 nm; also shown is the β absorption band of cytochromes at 517 nm. The lower spectrum in figure 1 shows the same muscle under the same conditions except after being dyed. The cytochrome peaks are still observable together with a small broad absorption increase at about 535 nm. This indicates an acid shift of intracellular pH.

During this hypoxia, stimulated tension in the undyed muscle was decreased to 42% of control and, in the dyed muscle, to 41% of control. The effect of bubbling carbon dioxide with oxygen in the muscle bath was also a decrease in stimulated tension. For mixtures of 5%, 10% and 25% CO_2 in oxygen, tension was decreased to 64%, 34% and 8% of control respectively.

The spectra shown in figure 2 were produced by minimizing the change in cytochrome redox state during CO_2 bubbling. Figure 2, upper spectrum, is the neutral red difference spectrum obtained from the toad ventricle strip absorption spectrum in 25% CO_2 minus the spectrum obtained in oxygen alone. There is a large absorption peak evident at approximately 540 nm which we identify with neutral red. Small changes in the cytochrome spectrum at 605 nm and 564 nm are just visible. Figure 2, middle spectrum, is the difference spectrum between oxygen and 5% CO_2. Again, an acid shift is indicated by the dye, but much smaller than in figure 2A. Figure 2, lower spectrum is the difference spectrum obtained by subtracting the spectrum in 95% nitrogen-5% CO_2 from that in nitrogen. This acid shift is smaller than the shift in figure 2B which indicates that some acidification has occurred with nitrogen alone as also suggested in figure 1, lower spectrum. The decrease in tension observed under the conditions of figure 2, lower spectrum, was to 16% of the control.

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Discussion. Changes in intracellular pH induced by changes in perfusate pCO_2 can be monitored in intact, toad ventricle strips by optical monitoring of neutral red absorption spectra. This technique allows simultaneous measurement of cytochrome chain redox state and pH_i. While quantitative values are not accurate, calibration of the changes in pH can be accomplished by comparison with changes in bath pCO_2.

In these muscles, almost half of the developed tension can be supported by the small amount of residual oxygen in the bath during nitrogen bubbling and anaerobic metabolism. The effect of the acidification of the tissue by bubbling with 5% CO_2 in oxygen on the developed tension is probably due to an effect on that part of the tension supported by anaerobic metabolism since there is little change in cytochrome redox state (figure 2B). Larger acidification has an effect on both aerobic and anaerobic processes as suggested by figure 2A.

The suggestion that the effects of hypoxia and acidosis on mechanical function are additive[14] is supported by the almost additive decrease in tension which accompanies both nitrogen bubbling and 5% CO_2 in nitrogen. This is probably due to the decreased uptake of substrates such as glucose and lactate[14]. The combination of both aerobic and anaerobic support of mechanical function may explain the finding that the oxidative metabolic change and decrease in mechanical function begin prior to the fall in intracellular pH[15].

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Enhancement of electrically stimulated guinea-pig gallbladder contraction by subthreshold concentrations of gastrointestinal hormones in vitro[1]

S. Foesel and K.-Fr. Sewing

Department of Pharmacology, University of Tübingen, Wilhelmstrasse 56, D-7400 Tübingen (Federal Republic of Germany), 14 July 1977

Summary. Concentrations of the octapeptide of cholecystokinin and pentagastrin, which alone do not contract the gallbladder, enhance the gallbladder contraction in response to electrical stimulation in vitro.

At present the neural and hormonal interaction in regulating the motor activity of the gallbladder is poorly understood and has been studied mainly in vivo[2,3]. The present study was carried out to investigate how subthreshold concentrations of cholecystokinin-octapeptide (CCK-OP), pentagastrin (PG) or secretin (S) influence the effects of transmural electrical stimulation (to mimick vagal excitation) on the isolated guinea-pig gallbladder.

Methods. Isolated guinea-pig gallbladders were longitudinally fixed at 37 °C in an organ bath of 100 ml with modified Krebs-Henseleit solution (NaCl 111, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 0.92, $MgSO_4$ 0.88, glucose 16.7 mM) aerated with 95% O_2 and 5% CO_2. A small platinum wire electrode was inserted through a hole in the fundus, fixed by a purse string suture and connected to an isometric strain gauge transducer (Grass FT 03 B). A circular platinum wire electrode (25 mm ⌀) was placed around the gallbladder. The preload was adjusted between 2.5 and 6.5 g so that an optimal ratio between spontaneous and electrically stimulated activity (<0.75) was obtained. The gallbladder was stimulated with rectangular electrical impulses of 20 V and 1 msec duration at a frequency of 10 Hz for 5 min followed by 5 min rest. After 6 stimulation cycles, CCK-OP, S, or PG was added in decreasing concentrations to determine the threshold concentration. 5 min after each test dose the bath was rinsed 2 or 3 times. Subsequently the first subthreshold concentration (approximately half of the threshold) was added and another 6 stimulation cycles were completed with the hormones remaining in the bath. To rule out the possibility that the contractile response to electrical stimulation alone changed during the course of the experiment, another group of 8 experiments were carried out where after 6 cycles the bath was simply refilled with fresh solution.

The effect of atropine on stimulated gallbladder contraction was studied as follows: after all preliminaries (see above) electrical stimulation (2 5-min cycles with 5 min interval) was applied as described. After 5 min rest, acetylcholine (ACh) was added to the bath to give a final concentration of 7×10^{-5} M, and after washing out ACh CCK-OP was added at a final concentration of 2×10^{-9} M. After further washing, starting with the lowest concentration of atropine, the next group of stimulation was applied until no further increase in atropine concentration seemed to be useful.

The average contraction of each 5-min cycle was evaluated planimetrically. For each group of tests, the means of the 6 contractions before adding the hormone were compared with the mean of the 6 contractions after addition of the hormones and analysed by t-test for paired comparison.

Materials. Pentagastrin = Gastrodiagnost® (kindly supplied by Dr Wendt, E. Merck, Darmstadt), acetylcholine chloride, and atropine sulphate (E. Merck, Darmstadt), natural secretin, batch No. 17561 (Karolinska Institutet, Stockholm), synthetic cholecystokinin-octapeptide (kindly supplied by the Squibb Institute for medical Research).

Results. All preparations displayed rhythmical spontaneous activity with a frequency varying between 10 and 20 min^{-1}. The amplitude increased with increasing preload to maximally 1 g, but was usually around 0.1 g. The basal tone varied from preparation to preparation, and was on the average during the 2nd 6 cycles of electrical stimulation higher than during the 1st 6 cycles. This phenomenon was seen whether the hormones were given or not. Electrical stimulation caused the gallbladder to contract with a force equivalent to 0.875 ± 0.099 g (mean ± SEM). This contractile response was always less than the maximal contraction induced by ACh or CCK-OP. The contraction in response to electrical stimulation was blocked by concentrations of atropine which were smaller than those

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