output. Although blood pH measurements were not made throughout the duration of each of these experiments, it appears reasonable to us that changes seen subsequent to 60 min of sampling may be due to the development of acidosis. In other experiments we have done on dogs anesthetized with pentobarbital we have noted the development of an acidosis with time.

In summary the data presented herein on preliminary experiments in vivo lend support to work done in vitro to demonstrate enhanced renal gluconeogenesis in acidotic rats[5] and dogs[6].

While our experiments were being analyzed a report by STEINER et al.[8] utilizing different methodology indicated also that renal gluconeogenesis occurs in vivo in acidotic dogs to a greater extent than in normal dogs. We have not presented any evidence to clarify the mechanism by which acidosis effects renal gluconeogenesis. However, our data are not inconsistent with the hypothesis put forth by STEINER et al.[8] that metabolic acidosis may accelerate a rate-limiting reaction in renal gluconeogenesis between α-ketoglutarate and glucose. The resulting enhancement in the conversion of α-ketoglutarate and glutamate to glucose would produce an increased renal venous glucose and a decrease in intracellular glutamate. The reduction in glutamate could in turn activate glutaminase 1 or reduce its inhibition[9] and thus result in an increase in ammonia production from glutamine.

Table I. Renal glucose uptake (+) and output (−) in control and acidotic dogs

	Control	Acidotic
Renal arterial mg/100 ml	84.2 ± 2.18	84.5 ± 1.65
Renal venous mg/100 ml	78.7 ± 1.82	85.0 ± 1.55
RBF ml/g kidney min	4.9 ± 0.45	4.2 ± 0.70
Output or uptake μ*M*/g min	1.95 ± 0.22	−0.215 ± 0.07
Blood pH	7.30 − 7.39	7.17 − 7.22

Averages for first six 10-min periods.

Table II. Average renal uptake (+) or output (−) of glucose (μmoles/g kidney · min)

Time	Control (n = 3)	Acidotic (n = 4)
0	1.22	− 0.17
10	1.62	− 0.17
20	1.47	− 0.35
30	2.72	− 0.09
40	1.96	0.09
50	2.64	− 0.47
60	2.04	− 0.35
70	0.21	− 0.80
80	0.98	− 0.41
90	0.28	− 0.07
100	− 0.03	− 0.15

Zusammenfassung. Bei durch Ammoniumchlorid 10 Tage azidotisch gemachten Hunden übertraf die venöse Glukosekonzentration in der Niere die arterielle. Der gesamte Blutdurchfluss blieb indessen konstant.

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Effect of Autonomic Drugs on Ca-Induced Contractures of the Frog's Ventricle

To clarify the action of autonomic drugs on cardiac muscle, their effect on contractures was studied by recording tension and membrane potential. Potentials were determined by the sucrose gap method. Modified Ringer solution contained in m*M*: NaCl 115; $CaCl_2$ 1; KCl 2; *Tris* chloride (pH 7.4) 2. Contractures were produced by Ringer solution containing 25–40 m*M* Ca, made by substituting $CaCl_2$ for NaCl. Strips of the ventricle of the frog (*Rana pipiens*) were used. The following results were obtained:

(1) Acetylcholine increased the contracture induced by high Ca Ringer solution or initiated a response at Ca concentrations slightly below threshold. A maximal effect was produced at a concentration of 10^{-8} g/ml. Atropine subsequently caused rapid relaxation.

(2) During high Ca contracture, epinephrine at concentrations of 10^{-8} g/ml induced relaxation (Figure) which was nearly complete in some experiments. The subsequent application of the β-blocking agent MJ 1999 caused contracture again.

(3) When a muscle was washed in Ca-free Ringer solution containing 2 m*M* EGTA for 10–30 min, then transferred to Ringer solution, a transient contracture was produced, perhaps due to an influx of Ca. Acetylcholine under these conditions produced rapid relaxation.

(4) Membrane potentials did not change during contractures induced by acetylcholine or during relaxation induced by epinephrine or atropine, but during relaxation caused by acetylcholine the membrane potential was usually increased by a few mV.

The effects of these drugs on contractures are quite unexpected on the basis of our present knowledge of their inotropic action in conducted responses. The epinephrine-induced relaxation may be related to the diminution of the K contracture observed by GRAHAM and LAMB[1,2] and KAVALER and MORAD[3]. GRAHAM and LAMB[2] proposed that epinephrine diminishes the K contracture by lowering Ca influx. In a similar fashion, the relaxing

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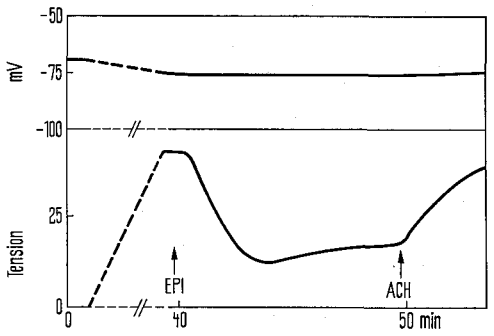
effect of epinephrine and atropine on Ca contractures can be explained if it is further assumed that the contracture depends on a continuous influx of Ca. Since the membrane potential does not change significantly, this influx must be very small or be associated with other ion movements. The increase in contracture produced by acetylcholine can then be explained by assuming that influx of Ca is increased. That epinephrine lowers Ca influx in the frog's ventricle [4] supports this explanation, but the opposite effect has been reported for the heart of other species [5–7].



Effect of epinephrine (10^{-7} g/ml) and acetylcholine (10^{-7} g/ml) on tension (lower line) and membrane potential (upper line) during contracture induced by high Ca (34 m*M*) Ringer solution. High Ca Ringer solution produced a slow increase in resting potential and a contracture. Arrows: application of epinephrine (EPI) and acetylcholine (ACH).

Zusammenfassung. Kontrakturen des Froschventrikels in Ringerlösung mit hoher Ca-Konzentration werden durch Azetylcholin verstärkt, während Adrenalin Erschlaffung herbeiführt. Diese Wirkungen sind nicht mit Änderungen des Membranpotentials verbunden und sind vielleicht die Folge sehr kleiner Änderungen des Ca-Austausches. Bei niederer Ca-Konzentration kann Azetylcholin auch Erschlaffung erzeugen.

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Lack of Effect of *Tris*-(Hydroxymethyl)Aminomethane Upon Myocardial Contractility

The cardiovascular actions of *Tris*(hydroxymethyl)-amino-methane (THAM) have been reported previously [1–7]. This amine has been found to increase ventricular contractile force and the reponse of the heart to catecholamines in acidotic dogs [3]. It has been suggested that the cardiovascular actions of THAM are not dependent on changes in arterial blood pH [6]; however, Wang and Katz found no increase in ventricular contractile force and coronary blood flow when THAM titrated to pH 7.40 was injected to dogs [7].

When THAM is injected in the bloodstream, 2 separate phenomena occur; on one side, there is a decrease in pCO_2 of the blood that is in immediate contact with THAM; later on, after blood has been equilibrated in the lungs, pH will remain high, its value obviously depending upon the amount of THAM injected.

It was our intention to study whether or not THAM has any inotropic action upon isolated heart muscle, in a system in which acid base changes could be avoided.

Methods. The experiments were performed using isolated heart muscle; in 5 experiments, strips of the right ventricle of rat, and in 7 experiments, right ventricular cat papillary muscle, was used. The muscles were mounted in a chamber, thermostatized at 30 °C, through which Ringer solution, previously equilibrated with a gas mixture of 5% CO_2 in O_2, was allowed to flow at a rate of 5 ml/min. The muscle, attached to a force transducer, was stimulated at a rate of 12/min. The isometric developed tension (DT), as well as the rate of rise of tension (dp/dt), and the temperature of the solution, were recorded in a polygraph.

The pH and pCO_2 of the solutions were measured anaerobically with electrodes thermostatized at 30 °C.

The experimental sequence was the following: since the beginning of the experiment, the muscle was immersed in a solution containing $NaHCO_3$ 30 m*M*/l, and equilibrated with a gas mixture with a pCO_2 of approximately 40 mm Hg. The pH of the solution was approximately 7.40. After a steady state was achieved in DT and dp/dt, the solution was changed to a second one in which $NaHCO_3$ was replaced by THAM, in a concentration of 40.8 m*M*/l, that, when equilibrated with a pCO_2 of 40 mm Hg, yielded a pH of about 7.40. The muscle was immersed in this solution for approximately 15 min, and then switched again to the first solution. During the 3 periods samples for pH and pCO_2 and fast recordings were obtained. The results obtained during the first and second exposure to the solution of $NaHCO_3$ were essentially the same, and were averaged.

Results and discussion. Figure 1 shows the effect of changing the solution containing $NaHCO_3$ to another containing THAM at the same pH and pCO_2. In this

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