A minority of the slowly conducting afferents showed an expected[7], infrequent (less than 1 impulse/sec) and sporadic spontaneous activity. All spontaneously active, and a minority of non-spontaneously active afferents responded to suxamethonium by an increase or appearance of activity.

A powerful though threshold excitation of the slowly conducting afferent was obtained with a suxamethonium chloride (Scoline, Allen Hanburys) concentration of $1:10^4$ (f), a value 10 times the threshold concentration for the spindle afferent in the same muscle. The different sensitivity of the 2 forms of receptor was revealed also by the latencies of the 2 responses (c–f). A maximum activity of 45 impulses/sec was attained by the slowly conducting afferent 5 min after application of the drug (g). The remaining records of the Figure illustrate control procedures.

The sensitivity of the slowly conducting afferents to suxamethonium might be due to its depolarizing action on either the receptor ending or on a nodal region of the axon, so permitting impulse propagation in response to a natural but subthreshold depolarization of the receptor ending. Alternatively, the response could be a secondary effect due to evoked changes in the tissues in which the free endings lie[8]. The changes could be either a response to suxamethonium by smooth muscles of the vascular system, or a response by the ending to K^+ released from muscle cells by the suxamethonium[9,10].

Résumé. Des afférences myéliniques non-proprioceptives, à vitesse de conduction lente, en provenance des muscles de la queue du rat, sont excitées par le suxaméthonium d'une concentration de $1{,}0 \times 10^4$. On considère les mécanismes possibles de cette excitation, dont la signification est discutée.

G. L. Kidd and J. Kučera

The Physiological Laboratory, The University, Liverpool, 3 (England) and The Institute of Physiology, Czechoslovak Academy of Sciences, Prague 4 (Czechoslovakia), 12 September 1968.

[7] A. S. Paintal, J. Physiol. *152*, 250 (1960).
[8] D. Barker, in *Ciba Foundation Symposium* (Ed. A. de Reuck and J. Knight; Churchill, London 1967).
[9] H. Kluff and O. Krauff, Archs int. Pharmacodyn. Ther. *98*, 340 (1954).
[10] Acknowledgments. This work is supported by a Medical Research Council Grant. Dr. Kučera gratefully acknowledges a grant from the Wellcome Trust.

An in vivo Study of the Effect of Chronic Metabolic Acidosis on Renal Gluconeogenesis

It is well established that ammonia production by kidneys increases markedly in metabolic acidosis[1]. Pitts[1,2] has demonstrated that a major source of ammonia production is renal tubular glutamine. The exact mechanisms responsible for stimulation of increased glutamine deamidation are still unclear. At various times, increased synthesis or activity of glutaminase I[3,4] as well as inhibition[2] of the citrate condensing enzyme (citrate synthetase) have been implicated in augmented renal ammonia production. More recently Goodman, Fuiz and Cahill[5] demonstrated that in rats metabolic acidosis increased, whereas metabolic alkalosis decreased gluconeogenesis of renal cortical slices. Also, Goorno, Rector and Seldin[6] demonstrated that metabolic acidosis in dogs increased gluconeogenesis as well as ammoniagenesis of renal cortical slices. The purpose of the present study was to determine whether the increased renal gluconeogenesis described in in vitro studies for dogs with metabolic acidosis is in fact seen in vivo.

Seven mongrel female dogs weighing 9–15 kg were used in these studies. Each dog was fed a standard diet (Friskies dog food, Carnation Co., 16 oz/day). All animals were injected twice daily with 50 mg cortisone acetate (i.m.) to suppress endogenous steroid secretion[6]. Four of the dogs received 10 g NH_4Cl daily in their meal. The animals were maintained on this schedule for 10 days. Blood pH determinations were made periodically during this time. On the eleventh day, the dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Cannulae were placed in a femoral artery and vein for blood pressure monitoring and sampling as well as for infusion of drugs. The left renal artery was exposed by retroperitoneal approach and an electromagnetic flow probe (Medicon) placed around the vessel. The left renal vein was catheterized via the ovarian vein. Each animal was allowed to recover from the surgical procedure for 45 min before sampling was initiated. Samples of venous blood from the renal vein and arterial blood from the femoral artery were drawn at 10 min intervals for 90 min. At completion of each experiment, location of the catheter in the renal vein was verified and the electromagnetic flow probe on the renal artery calibrated in situ. Blood samples were placed immediately in cold 10% TCA, and stored overnight at 4 °C. Glucose determinations were made using a standard glucose oxidose method[7].

The data obtained in these experiments are summarized in Tables I and II. The data in Table I are reported as averages of the mean values for each animal for the first 60 min of sampling. Renal arterial blood glucose levels as well as blood flow are essentially the same for both groups. However, the A–V differences as well as the net renal uptakes or outputs of glucose for both groups are quite different. In contrast, data for the control animals show a mean uptake of glucose of 1.95 μmoles/g kidney · min.

An interesting change in renal glucose uptake and output appears to occur in time in the control animals. Table II shows that the glucose uptake by the control dogs decreases after 60 min and in fact changes to an

[1] R. F. Pitts, L. A. Pilkington and J. C. M. Deffaas, J. clin. Invest. *44*, 731 (1965).
[2] R. F. Pitts, Physiologist *9*, 97 (1966).
[3] B. M. A. Davies and J. Yudkin, Biochem. J. *52*, 407 (1952).
[4] F. C. Rector jr., O. W. Seldin and J. H. Copenhaver, J. clin. Invest. *34*, 20 (1955).
[5] A. O. Goodman, R. E. Fuisz and G. F. Cahill jr., J. clin. Invest. *45*, 612 (1966).
[6] W. E. Goorno, F. C. Rector jr. and D. W. Seldin, Am. J. Physiol. *213*, 969 (1967).
[7] A. St. G. Hugget and D. A. Nixon, Lancet *2*, 368 (1957).

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.

S. CASSIN and C. E. BUNNEL

Department of Physiology,
College of Medicine, University of Florida,
Gainesville (Florida 32601, USA), 9 September 1968.

[8] A. L. STEINER, A. D. GOODMAN and D. H. TREBLE, Am. J. Physiol. *215*, 211 (1968).
[9] L. GOLDSTEIN, Am. J. Physiol. *210*, 661 (1966).

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

[1] J. A. GRAHAM and J. F. LAMB, J. Physiol. *183*, 25P (1966).
[2] J. A. GRAHAM and J. F. LAMB, J. Physiol. *197*, 479 (1968).
[3] F. KAVALER and M. MORAD, Circulation Res. *18*, 492 (1966).