of these results revealed that the negative association between flatworms and mosquitoes could not be explained by chance alone [$\chi^2(2) = 13.3$, $P < .05$]. The seven rice fields that lacked flatworms but still yielded few (less than six) mosquitoes either had been stocked with the mosquito fish *Gambusia affinis* or were cool-water fields with a summer midday temperature usually below 21°C (*3*).

Our data show that rice fields with large numbers of flatworms produce few mosquitoes, even if the fields contain warm water and are not stocked with *Gambusia*, and that in the laboratory the flatworms are directly responsible for the death of larvae of *C. tarsalis*. Exclusion of the flatworms by the floating field cages causes a fourfold increase in larval survival but survival under these conditions is still lower than that in other fields that lack flatworms altogether. Perhaps in fields infested with flatworms the toxin is able to diffuse into the floating cages in sufficient quantities to adversely affect the mosquito larvae. An alternative explanation is that some other microorganism in flatworm-infested fields may also be detrimental to mosquitoes but may lack virulence under our laboratory conditions (*3*).

Also puzzling is the question of why some rice fields support flatworms and others do not. Most of the fields in this study that had inordinate numbers of mosquitoes and lacked flatworms were fields that had just been turned over to rice production. Typically, farmers in the Sacramento Valley will rotate rice to safflower or some other crop every 3 to 5 years. Perhaps flatworms have difficulty dispersing to new rice fields but once established they are able to overwinter and control mosquitoes for the successive years the field is in rice. Further study should reveal the validity of this hypothesis.

TED J. CASE

Department of Biology, University of California, San Diego, La Jolla 92093

ROBERT K. WASHINO

Department of Entomology, University of California, Davis, Davis 95616

References and Notes

1. In the Sacramento Valley of California, typically over 400,000 acres of land are devoted annually to rice production. These fields are usually flooded in early May. When the rice emerges, populations of larval *Culex tarsalis* develop in the warm water and usually reach a peak abundance in mid-June. By July or August the rice increasingly shades and cools the water and populations of *Anopheles freeborni* then predominate. Both *C. tarsalis* and *A. freeborni* are serious pests and pose a threat to public health as vectors of Western equine and St. Louis encephalitis and malaria, respectively [S. F. Bailey and P. A. Gieke, *Proc. Calif. Mosq. Control Assoc.* **6**, 53 (1968)].
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10. The floating emergence cages were constructed from 1-gallon size plastic buckets with organdy screen windows (approximate mesh size 200 μm) on the sides and bottoms. Three fishing bobbers were placed near the top to keep the buckets afloat.
11. Available from Mardel Laboratories, Coral Stream, Ill.
12. Available from Aquarium Pharmaceuticals, Perkasie, Pa. Each tablet contains 120 mg of thiamine, 0.10 mg of riboflavin, 2.0 mg of niacin, 0.20 mg of pyridoxine, 1.50 mg of pantothenic acid, 0.003 mg of biotin, 0.20 mg of folic acid, 0.025 mg of aspartic acid, 0.025 mg of glutamic acid, 0.03 mg of choline, and 0.02 mg of inositol.
13. Aquarium plant food, Aquarium Pharmaceuticals, Perkasie, Pa.
14. As an independent check on flatworm occurrence, micro-rice field ecosystems were made by filling 1-gallon plastic aquariums with soil, algae, macrophytic plants, and unsieved water samples from each of the fields. A 300-ml plastic cup with 200-μm mesh windows was floated on each aquarium. Fifteen third-instar *C. tarsalis* were placed in each cup and the presence or absence of flatworms in the cup was noted after 1 week. In all but 2 of the 32 fields these two methods gave the same results concerning the presence or absence of flatworms. For the two fields for which the results differed, flatworms were found in the field but not in the laboratory aquariums.
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Vascular Smooth Muscle: Aerobic Glycolysis Linked to Sodium and Potassium Transport Processes

Abstract. *Under aerobic conditions, glucose is primarily catabolized by vascular smooth muscle to lactate, in spite of an adequate oxidative capacity. Although this is often considered to be indicative of some nonspecific metabolic insufficiency, there is evidence that aerobic glycolysis is specifically coupled to sodium and potassium transport processes, whereas oxidative metabolism is coupled to contractile energy requirements.*

Under fully oxygenated conditions, lactic acid is a major end product of vascular smooth muscle (VSM) metabolism. Such aerobic glycolysis has long been an enigma since it is normally associated with relatively few cell types, such as tumor or retinal cells, which generally are poorly oxygenated in comparison with VSM (*1*). Although it is sometimes considered to be an artifact of tissue preparation (*2*), substantial aerobic glycolysis is a consistent finding in reports of vascular metabolism and indeed of smooth muscle in general (*3*). The mismatch of glycolytic and oxidative metabolism in VSM (50 to 90 percent of the glucose entering the cell is lost as lactate) has been the source of considerable speculation.

Table 1. Steady-state values of metabolic and contractile parameters in porcine coronary arteries. Measurements were made during the second 1/2 hour of the experimental period. Metabolic functions are expressed as a fraction of the values obtained in the absence of stimulation: $J_{O_2} = 0.095 \pm 0.006$ μmole/min-g ($N = 29$); $J_{lac} = 0.150 \pm 0.006$ μmole/min-g ($N = 77$). Active isometric force (ΔP_0) is expressed as a fraction of that induced by 80 m*M* KCl. Contraction induced by the pharmacological agonist histamine is included for comparison. Number of tissue measurements is included in parentheses after each value.

Condition	J_{lac}	J_{O_2}	ΔP_0
KCl*	1.67 ± 0.08 (28)	1.68 ± 0.07 (23)	1
K^+ substituted for Na^+	0.28 ± 0.05 (8)	1.93 (1)	1.88 ± 0.14 (3)
K^+ substituted for Na^+ + 50 m*M* sucrose	0.34 ± 0.03 (8)	1.57 (2)	1.60 ± 0.19 (3)
K_2SO_4 substituted for NaCl	0.60 ± 0.05 (12)	1.66 ± 0.12 (7)	1.50 ± 0.10 (4)
Ouabain ($10^{-5}M$)	0.53 ± 0.05 (12)	1.21 ± 0.035 (11)	0.62 ± 0.14 (10)
K^+-free PSS	0.54 ± 0.04 (10)	.99 ± 0.09 (9)	0.22 ± 0.07 (7)
K^+ restored	1.18 ± 0.17 (6)	1.04 ± 0.09 (7)	−0.02 ± 0.07 (6)
Histamine ($10^{-4}M$)	1.85 ± 0.12 (8)	1.84 ± 0.12 (7)	1.51 ± 0.31 (5)

*Added to cause an 80 m*M* increase in bath concentration.

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It has been suggested that aerobic glycolysis is beneficial as well as destructive to vascular tissue (*4*). In fact, many investigators have attempted to use the ratio of glycolytic to oxidative metabolism as an index of vascular myopathy related to aging, atherosclerosis, or hypertension (*5*).

Our evidence suggests that aerobic glycolysis in VSM does not reflect metabolic failure, but is specifically coupled to processes related to Na-K transport. In earlier studies (*6*), both oxygen consumption and lactate production were found to be linearly related to isometric force, such that the ratio of lactate production to oxygen consumption remained constant under a wide variety of conditions, including stimulation by epinephrine, norepinephrine, and histamine. However, in a more recent study (*7*) it was reported that an isometric contraction achieved by substituting K^+ for Na^+ in the physiological saline solution (PSS) increased the rate of oxygen consumption (J_{O_2}) while inhibiting the rate of lactate production (J_{lac}). This finding suggested that aerobic glycolysis is potentially coupled to specific processes rather than to the overall cellular energy demands.

In this study, porcine right coronary and left anterior descending arteries were obtained from a slaughterhouse and used within 1 hour of the death of the animal (*6*). The J_{O_2} was measured polarographically in a chamber that allowed for simultaneous measurement of isometric force. Lactate production was estimated at intervals from the lactate content of the bathing solution in the chamber. As measurements of oxygen are made continuously, they are inherently more precise than the lactate measurements. To improve the precision of lactate production measurements, separate measurements on additional segments of vessel used in the polarographic chamber were made. These segments were inverted, cannulated on rods, and incubated in test tubes in such a way that they were isometric, with their luminal side toward the bathing medium. The PSS contained (in millimoles per liter): NaCl, 130; $NaHCO_3$, 14.9; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$, 1.17; $CaCl_2$, 1.6; and glucose, 5.5. Solutions were bubbled with gas mixtures containing 5 percent CO_2, resulting in a *p*H between 7.3 and 7.4 at 37°C. Consumption of O_2 was measured between 0.15 and 0.40 atm of O_2, always greater than the critical partial pressure for diffusion, which was 0.078 ± 0.013 atm of O_2 [mean ± standard error (S.E.); $N = 9$]. Separate measurements of lactate were made at 0.95 atm of O_2 to ensure that the rod-mounted tissue was not diffusion-limited (*8*). The results for the right coronary and left anterior descending arteries were indistinguishable.

Table 2. Metabolic and contractile parameters measured after KCl-induced isometric force stabilized (see Fig. 1). Data give ratio of KCl to ouabain + KCl.

Item	Lactate production	Oxygen consumption	Isometric force
$\bar{X}$	2.54	0.88	0.93
S.E.	0.29	0.05	0.04
N	12	8	8

Stimulation of the coronary vessels with a saline solution in which K^+ was substituted for Na^+ (K^+ PSS) resulted in an increase in J_{O_2} and a considerable reduction in J_{lac} (Table 1). This agreed with the finding of Glück and Paul (*7*) for porcine carotid artery; however, when contraction was elicited with added KCl (to increase bath concentrations by 80 m*M*) both J_{O_2} and J_{lac} were increased. This aerobic glycolysis is unlikely to be an artifact of a population of smooth muscle cells that have lost their capacity for oxidative phosphorylation, since J_{lac} could be inhibited as well as stimulated under conditions when both isometric force and J_{O_2} were increased. These results suggest that aerobic glycolysis may be coupled to Na-K transport. In the case of stimulation by K^+ PSS, Na-K transport would be inhibited by the absence of external sodium, whereas the addition of KCl would stimulate Na-K transport (*9*).

An alternative explanation of these results is related to the transport of glucose. At the physiological concentration (5.5 m*M*) of glucose used in this study, glucose transport, glycogen content, and lactate production are all at or near the maximum levels that are found at saturating glucose concentrations in vascular tissue (*10*). Lactate elimination, however, can be significantly reduced at lower glucose concentrations, suggesting that glucose transport may play a role. In skeletal muscle, glucose utilization appears to be limited by membrane transport, which is rather sensitive to changes in cell volume (*11*). In K^+ PSS, VSM cells are known to swell (*12*); the inhibition of J_{lac} might be the result of decreased glucose transport. This hypothesis was tested in two ways. The J_{lac} measured in hypotonic PSS (65 m*M* NaCl) was not statistically different from the control value (fractional change, −0.1 ± 0.08; four measurements). Furthermore, when NaCl was replaced by equimolar KCl (with 50 m*M* sucrose added) or by K_2SO_4, conditions that are reported (*12*) to be isotonic for VSM, the inhibition of J_{lac} was similar to that found in the hypotonic PSS in which K^+ was substituted for Na^+ (Table 1). Therefore, volume changes and their potential effects on glucose transport appear to be of little significance compared to the effects of the presence or absence of external sodium.

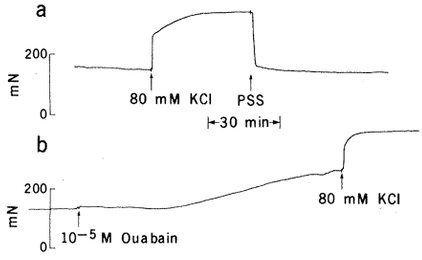

Fig. 1. Isometric myograms of porcine right coronary artery. (a) Trace showing a contraction-relaxation cycle induced by addition of KCl to cause an 80 m*M* increase in bath concentration. (b) Trace showing the effects of ouabain (10^{-5} *M*) and of KCl added in the presence of ouabain. Metabolic and contractile parameters from his type of experiment are summarized in Table 2.

To clarify the relation between lactate production and Na-K transport, the metabolism of VSM was studied under other conditions known to inhibit this ion pump. Ouabain (Fig. 1 and Table 2) was found to elicit a contraction that was slow to develop, compared to the depolarizing conditions given above. Lactate production was again reduced while J_{O_2} slowly increased. In the absence of K^+ ions, the coronary vessels again showed a slow development of tension, which was relaxed upon returning the K^+ concentration to normal. This behavior has been interpreted in terms of the inhibition and restarting of Na-K transport (*9*). The J_{lac} is inhibited by the absence of K^+ ions; it is initially stimulated by readmission of K^+ ions but after approximately 1/2 hour returns to the baseline value. The J_{O_2} in both these cases shows a relatively small increase that is temporally similar to the slow rise in isometric force.

Although these experiments demonstrate that aerobic glycolysis is coupled to Na-K transport, the extent of oxidative metabolism related to such transport is not clear. Although oxygen consumption was found to increase under conditions in which Na-K transport is inhibited, the increase in O_2 consumption due to the increased contractility also seen under those conditions could mask a decrease associated with inhibition of ion pumping. The following experimental

evidence suggests that oxidative metabolism is only marginally involved, if at all, with Na-K transport.

Addition of KCl to the PSS stimulated Na-K transport and development of isometric force; both O_2 consumption and lactate production were substantially increased (Table 1). The steady-state isometric force developed under stimulation by KCl, however, was found to be relatively unaffected by ouabain. Therefore one can investigate the effects of ouabain on stimulated Na-K transport at nearly constant levels of contractility. As Fig. 1 shows, steady-state J_{O_2} under stimulation by KCl in the presence of ouabain was virtually identical to the J_{O_2} under such stimulation in the absence of ouabain. On the other hand, lactate production, even in the presence of KCl, was again found to be greatly inhibited by ouabain. Thus, whereas stimulation and inhibition of Na-K transport is accompanied by parallel changes in aerobic glycolysis in VSM, oxygen consumption bears little relation to Na-K transport even though it is strongly associated with increases in isometric force.

Since aerobic glycolysis was estimated from measurements of lactate in the bathing medium, these experiments cannot distinguish between a direct coupling of glycolysis to the energetics of Na-K transport or to an effect on lactate permeability. The latter mechanism is unlikely, however, since the reported values of vascular lactate content, and changes in content with stimulation (*13*), are small compared to the rates of lactate efflux. Preliminary experiments measuring the lactate content of porcine coronary vessels indicate that, under conditions in which KCl has been added or potassium has been substituted for sodium, the change in lactate content from basal levels can be appreciable; however, the changes parallel those measured in the bathing medium. Thus the effects reported here for aerobic glycolysis would be minimal estimates, suggesting that glycolysis and Na-K transport are very tightly coupled.

Complete understanding of the nature of the coupling between Na-K transport and glycolysis may come with further experimentation. However, the coupling of glycolysis to Na-K transport via membrane-bound glycolytic enzymes, as postulated for erythrocytes (*14*), would appear to be a plausible model. A distribution of mitochondria suitable to the energy demands of membrane transport processes may not be possible in the smooth muscle cell, given that contractile force is transmitted by attachments of the myofilaments to dense bodies on the plasma membrane. Thus aerobic glycolysis in VSM, which derives most of its adenosine triphosphate from oxidative phosphorylation, may have evolved to favor the mechanical efficiency of the cell.

Although the exact nature of the coupling of aerobic glycolysis to Na-K transport-related processes is unknown, our results suggest that the reported increase in lactate production associated with vascular myopathy (*5*) may more reflect changes in Na-K transport processes than a nonspecific degradation of metabolism.

RICHARD J. PAUL
MARY BAUER
WILLIAM PEASE

Department of Physiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267

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A Direct Role of Dopamine in the Rat Subthalamic Nucleus and an Adjacent Intrapeduncular Area

Abstract. *The subthalamic nucleus, a clinically important component of the extrapyramidal motor system, and a lateral area extending into the peduncle contain catecholamine terminals and dopamine receptors coupled to adenylate cyclase. In addition, dopamine agonists administered in vivo enhance glucose utilization in the region. Thus, neuronal function in this region is directly affected by dopamine and dopaminergic drugs.*

The subthalamic nucleus (STN), a relatively small nucleus of the extrapyramidal motor system, is known for its vulnerability to stroke damage in humans and the resultant involuntary limb-flinging movements called hemiballismus (*1*). Previous neuroanatomical studies in animals have suggested that catecholamines do not play a direct role in the function of the STN (*2*). In a study of rats injected with [^{14}C]deoxyglucose as a metabolic tracer, however, we found that a dopamine (DA) agonist, apomorphine, markedly increased glucose utilization in the STN (*3*), suggesting either a direct action of apomorphine on DA receptors in the STN or an indirect action via the striatum, where a high density of DA receptors exists (*4*). We present anatomical and biochemical evidence here that the STN of rats is a DA receptor area with DA afferents, and that in addition, and unexpectedly, there is a region of neuropil within the cerebral peduncle, just lateral to the STN, which is also a DA receptor area.

Anatomical studies of the STN region were carried out with eight male Sprague-Dawley rats (200 to 300 g). Three rats were perfused with a glutaraldehyde solution for light and electron microscopy (*5*), and five rats were perfused with glyoxylic acid for catecholamine fluorescence histochemistry (*6*). Examination of the STN region by light and electron microscopy revealed

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