According to Macnicol[15] sulphur deficiency severely affects the composition of the free amino acid pool of developing *Pisum sativum* seeds, causing a marked decrease in cysteine but no change in methionine. In the present study the response to exogenous amino acids varied with the amino acid, i.e. decrease in protein content with cysteine and increase with methionine.

Glutathione treatment caused a reduction in protein content on day 10 by about 1.3% while treatment with glutathione and chloramphenicol caused a further reduction (14.5%). However, glutathione could partially reverse the inhibition by chloramphenicol (29.3 to 14.5%). Thus, when compared to methionine and cysteine, glutathione appears to be weak in counteracting the effect of chloramphenicol. Glutathione disulphide was found to be involved in reversing inhibition of glucose-6-phosphate dehydrogenase by NADPH[16].

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PYRUVATE OXIDATION IN TOAD GASTROCNEMIUS MUSCLE AFTER ANODIC OR CATHODIC STIMULATION

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THE anatomical basis of metabolic compartmentation in the central nervous system[1-6] is attributed to neuronal elements, glial components and astrocytes. Within the neuron, regionalized compartmentation, involving perikaryon with central functions, mitochondrial elements in the axoplasm with supporting functions and terminal units with synaptic functions, has been suggested[7-9].

The metabolic systems involving oxidoreductases possess cathodal characteristics[10], while those involving protein turnover, proteases, transaminases and acetylcholinesterase activities possess anodal characteristics. These systems are demarcated in terms of cathodal or anodal charge-based metabolic compartments[11-13]. If the activity patterns of these metabolic systems are under the control of the respective polarity characteristics, exogenous supplementation of identical characteristics is expected to activate the respective metabolic events. This has been tested in the present study on toad gastrocnemius muscle.

Medium-size (30 ± 5 g) *Bufo melanostictus* were collected in and around Tirupati and were maintained in clean glass troughs containing wet sand and leaves. The bed in the troughs was changed once every day. The toads were fed cockroaches *ad libitum* and acclimatized to laboratory conditions for one week. For electrical stimulation the toads were positioned on a Perspex base with a soft rubber band and made immobile. One platinum electrode (0.2 mm thickness) was placed superficially on the skin in the region of the gastrocnemius muscle of the right leg and the other electrode (0.2 mm) was placed in the midventral region of the abdomen. The skin of the animal between the electrodes was wiped to minimize surface wetness and reduce the possibility of short-circuit effects across the skin. An electric gradient of 0.8 V/cm (DC) was applied for 5 min every day for five days. The electrode contact regions

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were wetted with potassium chloride (0.75%). In one batch of toads, the electrode in the proximity of the muscle was made cathode and the midventral electrode was made anode. In the second batch of toads, the polarity was reversed, i.e. the muscle electrode was made anode and the midventral one was made cathode. On day 6 the toads were weighed and sacrificed quickly by decapitation. The gastrocnemius muscle of the right leg and the contralateral muscle, liver, heart and brain were excised in the cold, washed in amphibian Ringer medium[14], and allowed to stand for 10 min to recover from shock effects. The tissues were homogenized in 0.25 M sucrose and the homogenates centrifuged at 1500 *g* for 15 min. The clear supernatants were used in the studies. Pyruvate was estimated by the method of Reitman and Frankel[15], lactate by the method of Barkar and Summerson[16], and the activities of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) by the method of Nachlas *et al.*[17] The results were statistically analysed by Student's *t* test.

The animals in which the cathode was placed near the gastrocnemius muscle gained weight (+5.3%) while those in which the anode was placed near the gastrocnemius lost weight (−9.7%). Such weight changes after cathodic/anodic stimulation have been discussed earlier[18, 19] and shown to involve tissues such as liver, heart, brain and gastrocnemius muscle.

Pyruvate and lactate levels in the various tissues examined are given in table 1. In untreated animals, pyruvate level was lower than lactate level in all the tissues examined except heart, suggesting rapid mobilization of pyruvate into lactate. On cathodic stimulation, pyruvate level increased while lactate level decreased in all the tissues except heart, suggesting that lactate formation was diminished. This results when LDH preferentially acts in the direction lactate to pyruvate. This normally occurs when NADH content is low and NAD content is high, suggesting rapid oxidation of NADH and consequent active TCA cycle and associated oxidoreductase system.

On anodic stimulation, pyruvate content of all tissues decreased while lactate level increased (except in heart). Such a system needs a free supply of NADH and is possible when pyruvate oxidative processes are slow.

LDH is involved where anaerobic conditions favour reduction to lactate while aerobic conditions promote entry of pyruvate into the TCA cycle. Thus LDH indicates prevailing emphasis on EMP and TCA cycle systems. SDH was selected since it is the parent enzyme of the citric acid cycle. MDH was chosen in view of the catalysis involving malate where the substrate is either dehydrogenated into oxaloacetate, or undergoes decarboxylation, leading to the formation of pyruvate (catalysed by pyruvate carboxylase). Thus MDH activity reflects relative diversion of malate oxidation from the TCA cycle.

Activities of the three oxidoreductases are given in table 2. In gastrocnemius muscle from unstimulated toads, the activities were in the order SDH > LDH > MDH, suggesting that succinate oxidation takes precedence over other oxidations. The low level of oxidation of malate in terms of low MDH activity indicates a bottle-neck in the TCA cycle. The low LDH activity, compared to SDH, indicates inherent preference for TCA cycle oxidations over glycolysis under normal resting conditions.

Table 1 *Pyruvate and lactate in tissues of Bufo melanostictus after electrical stimulation of gastrocnemius muscle*

		Stimulated muscle	Contralateral muscle	Brain	Heart	Liver
Pyruvate	Control	5.64 ± 0.05	5.12 ± 0.06	3.86 ± 0.06	4.89 ± 0.075	4.36 ± 0.11
(μmol/g wt)	Cathode	7.26 ± 0.06* (+28.72)	6.20 ± 0.07* (+21.09)	4.59 ± 0.05* (+18.91)	3.81 ± 0.07* (−22.09)	5.52 ± 0.101* (+26.61)
	Anode	4.32 ± 0.06* (−23.4)	4.19 ± 0.09* (−18.16)	3.23 ± 0.06* (−16.32)	5.75 ± 0.07* (−17.59)	3.38 ± 0.102* (−22.5)
Lactate	Control	6.84 ± 0.06	6.45 ± 0.074	3.94 ± 0.056	4.28 ± 0.074	8.26 ± 0.085
(μmol/g wt)	Cathode	5.22 ± 0.064* (−23.68)	5.27 ± 0.69 (−18.29)	3.10 ± 0.051* (−21.32)	5.02 ± 0.081* (+17.29)	6.13 ± 0.091* (−25.78)
	Anode	8.05 ± 0.057* (+17.69)	7.47 ± 0.067* (+15.82)	4.56 ± 0.048* (+15.74)	3.67 ± 0.068* (−14.25)	9.94 ± 0.084* (+20.34)

Each value is mean ± SE of six observations; numbers in parentheses are per cent change over control.
*Significantly different from control ($P < 0.05$).

Table 2 *Succinate dehydrogenase, lactate dehydrogenase and malate dehydrogenase activities in tissues of Bufo melanostictus after electrical stimulation of gastrocnemius muscle*

		Stimulated muscle	Contralateral muscle	Brain	Heart	Liver
SDH	Control	1.246 ± 0.052	1.234 ± 0.035	0.986 ± 0.035	1.839 ± 0.042	1.487 ± 0.06
	Cathode	1.674 ± 0.055* (+ 34.34)	1.546 ± 0.057* (+ 25.3)	1.320 ± 0.039* (+ 33.87)	1.243 ± 0.044* (− 32 4)	1.952 ± 0.056* (+ 31.27)
	Anode	0 867 ± 0.051* (− 30.41)	0.956 ± 0.056* (− 22.52)	0.697 ± 0.037* (− 29.3)	1.198 ± 0.046* (− 34.85)	1.096 ± 0.06* (− 26.31)
LDH	Control	0.751 ± 0.016	0.737 ± 0.013	0.639 ± 0.02	1.216 ± 0.056	0.837 ± 0.016
	Cathode	0.978 ± 0.019* (+ 30.23)	0.912 ± 0.016* (+ 23.74)	0.854 ± 0.016* (+ 33.64)	0.834 ± 0.052* (− 31.4)	1.137 ± 0.018* (+ 35.84)
	Anode	0.535 ± 0.017* (− 28.76)	0.548 ± 0 015* (− 25.64)	0.466 ± 0.017* (− 27.07)	0.854 ± 0.055* (− 29.76)	0.617 ± 0.021* (− 26.28)
MDH	Control	0.429 ± 0.022	0.416 ± 0.014	0.314 ± 0.026	0.516 ± 0.019	0.542 ± 0.007
	Cathode	0.547 ± 0.026* (+ 27.5)	0.507 ± 0.038* (+ 21.87)	0.397 ± 0.025* (+ 26.43)	0.390 ± 0.018* (− 24.40)	0.735 ± 0.01* (+ 35.61)
	Anode	0.320 ± 0.025* (− 25.4)	0.317 ± 0.013* (− 23.79)	0.238 ± 0.024* (− 24.2)	0.415 ± 0.018* (− 19.57)	0.365 ± 0.011* (− 32.65)

Activity expressed as μmol of formazan/mg protein/h.
Each value is mean ± SE of six observations; numbers in parentheses are per cent change over control.
*Significantly different from control ($P < 0.05$).

On cathodic stimulation, all three activities increased in all tissues except heart, but the increase in MDH activity was smaller. This indicates that pyruvate oxidation through TCA cycle and LDH system favour NAD-dependent pyruvate formation.

Anodic stimulation resulted in decrease of activity of all three enzymes in all the tissues. The decrease was between 20 and 30%, and indicates pyruvate oxidation through TCA cycle and increased NADH-dependent lactate formation by LDH. The response of cardiac tissue, which showed decrease in LDH, SDH and MDH activities after both cathodic and anodic stimulation, was unique, probably indicating some fatigue resistance inherent in the heart.

In summary, cathodic stimulation elevated oxidoreductase activities, pyruvate oxidation and consequent energy production, with possible contribution to the endergonic reaction systems, while anodic stimulation caused decreased pyruvate oxidation and decreased oxidoreductase activity, and consequent diminished cellular energy production.

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MYOCARDIAL GLYCOGEN CONTENT OF SOME REPRESENTATIVE VERTEBRATES

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THE polysaccharide glycogen is the immediately available fuel reserve for energy metabolism along the glycolytic pathway. In muscle, including cardiac muscle, it has considerable physiological significance, especially during hypoxia, and is observed to vary in level under diverse physiological conditions[1-5]. Further, chamberwise variations in heart glycogen content are also reported in some vertebrates[2,6]. Nevertheless, data on glycogen content of myocardium of vertebrates adapted to diverse ecological conditions and activity levels are scant. Accordingly, it was thought that a study on the glycogen content of the different chambers of the heart of some representative vertebrates would yield valuable information regarding the adaptive physiology of vertebrate myocardium with reference to glycogen.

Nine representative vertebrates, adapted to diverse ecological conditions and activity levels, were chosen for the present study. Myocardial samples were excised from healthy adult animals of both sexes and comparable body size. Glycogen was estimated by the method of Seifter *et al.*[7] using anthrone reagent. The data are given in table 1.

In general, lower vertebrates show higher glycogen content in the myocardium than birds and mammals. Further, variation between the chambers is much more in lower vertebrates than in birds and mammals. This probably indicates that the carbohydrate fuel reserve, glycogen, has a greater significance in the physiological adaptation of the myocardium of lower vertebrates than that of birds and mammals.

Glycogen content of myocardium of *Cybium* is not so high in comparison with that of some other lower vertebrates. In fact, relatively higher glycogen values are seen in the myocardium of *Rana*, with significant regional variation, especially between the two ventricular halves, the right half showing a higher glycogen content than the left. This may be due to greater muscular effort of the right ventricular half in pumping venous blood to the pulmonary and cutaneous respiratory surfaces. Functional differentiation of the right and left halves

Table 1 *Glycogen content of myocardium of the different chambers of heart in nine vertebrates*

	Glycogen (μg/100 mg wet tissue) Atrium		Ventricle	
	Right	Left	Right	Left
Cybium guttatum	109.61 (6.52)		84.24 (5.34)	
Rana tigrina	354.16 (24.65)	329.46 (25.91)	758.58 (69.99)	473.14 (51.14)
Calotes versicolor	143.45 (13.63)	147.42 (14.41)	83.72 (15.51)	58.39 (9.02)
Geomyda trijuga	112.97 (7.21)	52.83 (5.11)	82.42 (6.24)	61.67 (4.51)
Lissemys punctata	2145.61 (121.07)	1605.23 (50.78)	1561.56 (70.88)	1904.14 (143.53)
Gallus domesticus	58.56 (3.71)	26.74 (2.63)	35.72 (2.59)	24.81 (1.21)
Columba livia	36.66 (2.28)	29.65 (2.82)	23.55 (3.57)	18.16 (1.21)
Carpa sp.	98.81 (5.58)	127.64 (11.58)	94.81 (7.02)	136.73 (9.57)
Pteropus giganteus	40.95 (5.21)	31.94 (2.97)	26.42 (1.73)	15.78 (1.21)

Values are the mean of ten assays with standard error in parentheses.
Variance analysis was carried out and the F ratio was calculated to test the significance of variations. Significant variation is observed between animals ($P<0.01$) and between chambers ($P<0.01$).