in older cells are probably due to alterations in the rates of transport of these compounds, and differences in the patterns of age-related changes of various amino acids may reflect different rates of inactivation of individual amino acid carriers. Another explanation might involve a more intensive proteolysis in young than in mature erythrocytes[10]. The decrease in the total concentration of amino acids determined during intravascular erythrocyte ageing (fig. 2) can contribute to the diminution in the volume of old erythrocytes via osmotic water efflux from the cells. Since the magnitude of the decreases in the concentrations of some amino acids was comparable to that of creatine, they could also serve as sensitive indices of red cell age when using less elaborate methods of estimation.

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## Effects of DL-propranolol on exercise heart rate and maximal rates of oxygen consumption in *Scaphiopus intermontanus*[1]

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*Summary.* Lymphatic injection of propranolol (0.2–10 µg) into toads decreased exercise heart rate in a dose-dependent manner. There was a significant linear correlation between exercise heart rate and maximal oxygen consumption rates ($\dot{V}O_2$ max). These data are consistent with the hypothesis that blood oxygen transport is the limiting process for $\dot{V}O_2$ max in anuran amphibians.

The quantity of oxygen transported to tissues per unit time (ml $O_2 min^{-1}$) is, by Fick Principle, the product of blood oxygen content (ml $O_2$ · ml $blood^{-1}$) and blood flow rate (ml blood · $min^{-1}$). If the maximal rate of oxygen consumption ($\dot{V}O_2$ max) is limited by blood oxygen transport, then $\dot{V}O_2$ max should be influenced by either changing the blood oxygen content and/or the blood flow rate. Evidence that intraspecific differences in $\dot{V}O_2$ max for an anuran amphibian was proportional to blood oxygen content has been previously presented[2]. The purpose of these experiments was to evaluate the proportionality of $\dot{V}O_2$ max and maximal heart rate in the anuran amphibian *Scaphiopus intermontanus*. By using the β-adrenergic blocker propranolol maximal heart rate during activity could be reduced. Since maximal heart rate is reduced so presumably is blood flow rate since blood flow rate is the product of heart rate and stroke volume. The relationship of $\dot{V}O_2$ max and maximal heart rate should provide a key test of the hypothesis that blood oxygen transport limits $\dot{V}O_2$ max in amphibians.

*Materials and methods.* Great Basin Spadefoot toads, *Scaphiopus intermontanus*, were collected in Central Oregon (Oregon scientific collecting permit 9, 1981). Experiments were performed in the spring 1–2 weeks after collection of the toads. Toads were not fed during the experiments and were maintained in containers with access to both water and dry areas. Only males were used with a mass of $18.2 \pm 0.6$ ($\bar{X} \pm SE$). Maximal oxygen uptake rates were determined as previously described[3]. The procedure consisted essentially of forcing the animal to right itself constantly in a closed container for 3 min of activity, then a gas sample withdrawn and analyzed polarigraphically (Beck-

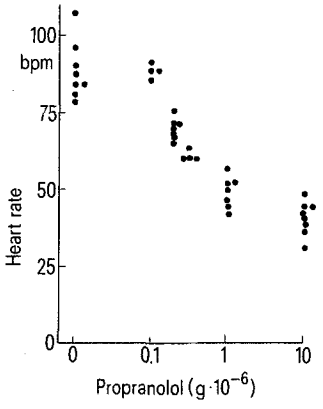


Figure 1. The effect of various doses of DL-propranolol on heart rates immediately post exercise. Points represent individual toads.

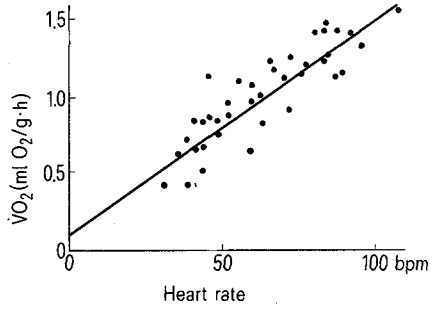


Figure 2. The relationship between post exercise heart rate and $\dot{V}O_2$max. The line of the regression equation relating these 2 variables is drawn in ($\dot{V}O_2 max = (0.014 \times$ heart rate$) + 0.1$).

man OM-14). This method has proved to be the most reliable method for measuring $\dot{V}O_2max$[4]. Maximal heart rates were determined as previously described[5] essentially by electronic recording or manual palpatation immediately after exercise. Excessive electrical noise from skeletal muscle activity forced the use of manual palpatation periodically. No difference in heart rate was noted in animals measured by both methods. All determinations were made at 20 °C. DL-propranolol (Sigma) treatment groups were 0.1, 0.2, 0.4, 1, 10 µg/toad. Toads were run through a sequence of control then 2 propranolol treatment in 3 successive days. The doses of propranolol were sequentially increased in concentration. Fresh propranolol solutions were made before the experiments in distilled water and injection volume was 0.1 ml into the dorsal lymph sac. Metabolic and heart rate measurements were made an hour post injection.

The relation between post exercise heart rate and $\dot{V}O_2max$ is shown in figure 2. There was significant linear correlation ($r=0.87$) between maximal heart rate and $\dot{V}O_2max$. These data are consistent with the hypothesis that blood oxygen transport is the limiting process for $\dot{V}O_2max$ in anuran amphibians. Previous studies dealing with both intraspecific variability in hemoglobin concentration[2] and interspecific variability in cardiovascular parameters[5] have both implicated a cardiovascular limit to $\dot{V}O_2max$ in anuran amphibians. These data are not consistent with the hypothesis that respiratory surface area represents the limit to $\dot{V}O_2max$ in anuran amphibians[6]. A similar decrease in $\dot{V}O_2max$ following $\beta$-adrenergic blockade in humans has previously been reported[7].

Propranolol aside from reducing heart rate is also known to decrease hemoglobin-oxygen affinity in mammals[8,9]. Decreased hemoglobin affinity for oxygen increases oxygen delivery to tissues. Therefore the observed decrease in $\dot{V}O_2max$ as blood oxygen transport decreases is at worst an underestimate, since right-shifted hemoglobin would tend to compensate for diminished blood oxygen transport capacity[10].

Since amphibian hearts have a poorly developed sarcoplasmic reticulum[11,12] and $Ca^{2+}$ for excitation-contraction coupling enters from the extracellular space, rather than being released from internal $Ca^{2+}$ stores[13], the kinetics of a contraction-relaxation cycle are slower in relation to the mammalian cardiac muscle cycle. Consequently maximal heart rates of similar sized mammals are much greater. Therefore from an evolutionary perspective amphibian maximal metabolic capacity may be ultimately limited by the kinetics of cardiac muscle $Ca^{2+}$ exchange, if blood oxygen transport does limit $\dot{V}O_2max$ in amphibians as these data suggest.

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## Relationship between the renal kallikrein activity and the urinary excretion of kallikrein in rats[1]

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*Summary.* Adrenalectomy reduces, and sodium depletion increases, both the daily urinary excretion of kallikrein and the kallikrein activity in the renal cortex. These 2 variables were found to correlate significantly in normal, sodium depleted and adrenalectomized rats, thus supporting the view that kallikrein excretion reflects the activity of the enzyme in the kidney.

It is known that glandular kallikrein is synthetized, among other organs, in the kidney[2], probably in the distal tubular cells[3]. Although it has been suggested that part of the kallikrein excreted in the urine might be of extrarenal origin[4], most of the urinary kallikrein appears to be secreted in the distal segments of the nephron[5]. Several investigations have been performed in the last 10 years in an attempt to elucidate the physiological role of the renal kallikrein-kinin system or its probable involvement in hypertension (for references see Levinsky[6]). Most of this work relied on the estimation of the urinary kallikrein excretion, assumed to reflect the activity of the enzyme in the kidney. However, until now there has been no proof for this assumption. Since in acute experiments we found an inverse relationship between the excretion of kallikrein and its activity in the kidney[7], we investigated whether this was also the case under 'steady state' conditions.

*Methods.* Rats from different experimental groups were placed in stainless steel metabolic cages for the collection of urine for 24 h. Apart from normal rats (200 g b.wt) fed a normal rat chow or a sodium deficient diet for 14 days (2 mmoles/kg dry food), adrenalectomized rats (19 days after operation) given 1% NaCl to drink were also used. The rats were placed in the metabolic cages at least 3 days prior to the urine collection. At the end of the experimental period the animals were anesthetized with pentobarbital (40 mg/kg b.wt, i.p.) and the kidneys were excised after rinsing them with an intraarterial perfusion of 150 mM NaCl until they were macroscopically free of blood. Kallikrein was brought into solution by mechanical homogenization and