Ammonia production by hind limbs of rat was studied in perfused preparations during rest, exercise, and hypoxia, and while the perfusate contained epinephrine or cyanide. Ammonia production by hind limbs was also studied in situ during rest and exercise. Strong correlations were observed between ammonia, IMP, and lactate formation. Adenylosuccinate was not present in detectable amounts in resting muscle (<1 nmol/g dry weight) but it appeared and rose sharply in exercising muscle, and then declined during recovery. The maximum found was about 18 nmol/g dry weight. When IMP accumulation exceeded 2.8 pmol/g dry weight, the formation of adenylosuccinate diminished, presumably because of an inhibition of adenylosuccinate synthetase by excess substrate.

Exercise resulted in a greatly increased output of ammonia and a decreased output of alanine and glutamine. The tissue contents of aspartate and glutamate were decreased, while that of alanine was increased. The content of malate increased 3-fold during exercise.

Epinephrine, hypoxia, and cyanide each caused an increased output of ammonia at the expense of the amino acid content of the tissue.

These results provide evidence for the operation of the purine nucleotide cycle in skeletal muscle under various conditions that are associated with an increased rate of glycolysis.

Extracts of skeletal muscle produce ammonia from aspartate under conditions that mimic muscle working. The conversion of aspartate to fumarate and ammonia occurs via the purine nucleotide cycle, which consists of the reactions catalyzed by adenylosuccinase, adenylosuccinate synthetase, and adenylosuccinase (1, 2). In the present paper we report a study of ammonia production and associated metabolic changes in intact skeletal muscle of rat under various conditions. Exercise causes a drop in total adenine mononucleotides that is accompanied by accumulation of ammonia, IMP, and adenylosuccinate. The levels of IMP and adenylosuccinate decline again during recovery. The time course of these changes is consistent with the operation of the purine nucleotide cycle in skeletal muscle.

MATERIALS AND METHODS

Animals-Male rats of the Sprague-Dawley strain and laboratory chow were obtained from Charles River Breeding Laboratories, Wilmington, Mass. The animals received food and water ad libitum, and weighed 180 to 230 g at the time of use.

Perfusion Technique-The perfusion apparatus, anaesthetization, and surgical procedure were as described by Ruderman et al. (3) with minor modifications. The spermatic artery and vein were ligated and the testis and seminal vesicles were removed. The perfusate was pumped from a central mixing reservoir through a nylon filter into a water-jacketed oxygenator maintained at 38° (P. A. Brooks, Witney, Oxon, OX8 5HT, England). The gas phase oxygenator was gassed with humidified 95% O₂, 5% CO₂ at a flow rate of 300 ml/min. From the oxygenator the perfusate was sent to the preparation using a multichannel pump (Buchler Instruments, Fort Lee, N. J. 07024). The flow rate was approximately 12 ml/min. The entire apparatus and animal were enclosed in a cabinet maintained at 38°.

The perfusate consisted of Krebs-Ringer bicarbonate buffer (4) containing 3% bovine serum albumin (Fraction V, fatty-acid poor, Miles Laboratory, Kankakee, Ill.), washed bovine erythrocytes (hematocrit 20%, hemoglobin 6 to 7 g/100 ml), substrates, and hormones. Endogenous lactate (0.3 to 0.6 mM) was always present after washing and diluting the erythrocytes; because of this, pyruvate was added initially to obtain a [lactate]/[pyruvate] ratio of approximately 10. The pH of the perfusion medium was monitored and kept between 7.35 and 7.40. The initial volume of perfusion medium was 150 ml. The first 20 ml of perfusate that passed out of the venous drainage were discarded; thereafter the perfusate was recirculated. At the end of each perfusion a portion of the right musculature, mainly gastrocnemius and posteroinferior thigh muscle, was rapidly frozen in situ with aluminum clamps cooled in liquid nitrogen (5).

Exercise Studies-The sciatic nerve of the perfused hind limb was exposed and a Dasarte's electrode (Palmer Co., London, England) was attached around the nerve in its gluteal course. The leg was fixed to the perfusion platform at the ankle with adhesive tape. Isometric contractions were induced by square-wave electrical pulses from a stimulator (Grass Instruments, Quincy, Mass.). The pulses were applied for a duration of 10 ms with a frequency of 1, 4, or 6/s. The voltage was usually maintained between 0.5 and 10 V; however, at times it was necessary to increase the voltage to as much as 50 V in order to maintain vigorous contractions at high frequencies of stimulation. The intensity of the contractions was assessed by palpation of the exercising muscles. Approximately one-third to one-half of the muscle tissue of the hindquarter contracted when the nerves were stimulated.

Composition of Perfused Tissue and Expression of Results—Analyses of a series of 15 perfusions with rats weighing 125 to 280 g showed that the perfused material consisted of 81% soft tissue (mainly...
Adenylosuccinate in tissue extracts was measured on a Perkin-Elmer model 456 dual wave length spectrophotometer by following absorbance changes at 292 nm minus absorbance changes at 310 nm using an E₅₀₀ of 10.0 (2). The assay mixture contained 50 mM triethanolamine, pH 7.4, 1 mM EDTA, 0.6 ml of tissue extract, and about 0.1 unit of enzyme, in a total volume of 2.0 ml. Known amounts of adenylosuccinate were recovered quantitatively when added to tissue extract and to the frozen tissue powders prior to extraction.

Adenylosuccinate in neutralized tissue extracts stored at -20° was stable for at least 6 months.

**RESULTS**

**Ammonia, Alanine, and Glutamine Output by Perfused Muscle** - Resting perfused hind limbs slowly released ammonia into the perfusate. The rate of release of ammonia was not affected by glucose or insulin (Table I). The ammonia released was produced by the muscle, and was not due to washout of pre-existing intracellular ammonia, since the muscle content of ammonia was maintained or increased during the course of the perfusion. For example, in a set of control perfusions the ammonia content of resting muscle was 0.47 ± 0.07 (n = 5) and 0.83 ± 0.13 (n = 4) μmol/g dry weight after 10 and 45 min of perfusion, respectively. The initial concentration of ammonia in the perfusate was between 50 and 100 μM. It was contributed in part to by the erythrocytes and reagents used for preparing the perfusate. Arterial blood of humans and rats normally contains 25 to 75 μM ammonia (20–22). When the initial concentration of ammonia in the perfusate was raised to between 0.20 and 12 mM by adding ammonium chloride, rest muscle preparations always took up ammonia, in accordance with results reported previously (22–25).

Exercise resulted in a greatly increased output of ammonia and a decreased output of alanine and glutamine (Table I). Enhanced release of alanine, which has been demonstrated during exercise in man (26), was not observed in our perfusion experiments. As was already mentioned under “Materials and Methods,” approximately one-third to one-half of the muscle tissue of the hind limb contracted intensely when the sciatic nerve was stimulated. Contractions of lesser intensity also occurred in the remaining muscle not innervated by the sciatic nerve, owing to direct stimulation of the muscle by the electrodes. Since rates of uptake and output of metabolites in Table I were calculated on the basis of the whole hind limb, the results shown probably do not represent maximum capacities during exercise.

Skeletal muscle produces glutamine from glutamate and ammonia (23, 25, 27); because of this the amount of glutamine released and the change in the amount of glutamine stored must be taken into account when determining the amount of ammonia produced by the muscle. In resting muscle perfused with glucose and insulin for 2 h there was no change in the ammonia and glutamine stored, but the output of ammonia plus glutamine was 0.43 μmol/30 g/min (Table I). Addition of 2 mM methionine sulfoximine, an inhibitor of glutamine synthetase (28), increased ammonia output by resting muscle to 0.037 ± 0.005 (n = 4) to 0.307 ± 0.047 (n = 4) μmol/30 g/min; the increase persisted during 2 h of perfusion. In the same experiments methionine sulfoximine decreased glutamine output by 15% during the 1st h and by over 90% during the 2nd h of perfusion. The tissue content of ammonia increased 3.8-fold. The total increment in ammonia (Δtissue + aperfusion)
in the presence of methionine sulfoximine was 47 μmol over 2 h, which is equal to a production rate of 0.38 μmol/30 min. This result is in close agreement with the increment in ammonia plus glutamine obtained in the absence of methionine sulfoximine (0.43 μmol/30 min).

Addition of 5 mM aspartate, glutamate, or leucine, or of 2 mM glutamine to the perfusate did not alter the rate of ammonia output by resting muscle (data not shown).

**Effect of Exercise on Ammonia, Nucleotide, Nucleoside, and Hypoxanthine Content of Perfused Skeletal Muscle**—The ammonia and IMP contents of muscle increased upon exercise, in a manner that was related to the severity of the exercise (Table I). There was no stoichiometry between the changes in ammonia and IMP contents, and none was to be expected since ammonia but not IMP was diffusing from the tissue. Exercise resulted in a large decrease in the content of creatine phosphate, a proportionately smaller decrease in ATP, and a small increase in the content of AMP. Similar changes in creatine phosphate and ATP contents have been described previously (1, 16, 29–31) and are repeated here simply to facilitate comparison. Exercise led to decreases in the total adenine mononucleotide pool of 7, 19, and 32% for 1, 4, and 6 shocks/s,

### Table I

**Effect of exercise, epinephrine, hypoxia, cyanide, and insulin on ammonia, alanine, and glutamine output by perfused muscle**

<table>
<thead>
<tr>
<th>Metabolite outputs into the perfusate</th>
<th>Controls</th>
<th>Exercise</th>
<th>Epinephrine (25 mM)</th>
<th>Hypoxia</th>
<th>Cyanide (1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia output</td>
<td>0.073 ± 0.014</td>
<td>0.058 ± 0.019</td>
<td>0.079 ± 0.017</td>
<td>0.200 ± 0.056</td>
<td>0.622 ± 0.093</td>
</tr>
<tr>
<td>Alanine output</td>
<td>0.309 ± 0.032</td>
<td>0.393 ± 0.040</td>
<td>0.183 ± 0.028</td>
<td>0.225 ± 0.025</td>
<td>0.807 ± 0.021</td>
</tr>
<tr>
<td>Glutamine output</td>
<td>0.387 ± 0.065</td>
<td>0.332 ± 0.043</td>
<td>0.227 ± 0.039</td>
<td>0.101 ± 0.002</td>
<td>0.232 ± 0.022</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>0.68 ± 0.124</td>
<td>5.70 ± 0.213</td>
<td>8.41 ± 0.761</td>
<td>8.06 ± 0.391</td>
<td>8.54 ± 0.200</td>
</tr>
<tr>
<td>Lactate output</td>
<td>2.05 ± 0.474</td>
<td>2.50 ± 0.316</td>
<td>3.53 ± 0.282</td>
<td>6.35 ± 0.852</td>
<td>16.4 ± 1.005</td>
</tr>
<tr>
<td>Pyruvate output</td>
<td>0.149 ± 0.029</td>
<td>0.157 ± 0.019</td>
<td>0.227 ± 0.016</td>
<td>0.201 ± 0.043</td>
<td>0.202 ± 0.017</td>
</tr>
</tbody>
</table>

**Table II**

**Effect of exercise, epinephrine, and hypoxia on content of selected metabolites of perfused muscle**

Metabolite contents are expressed as means ± S.E. The lower case letter designation of the perfusion groups identifies identical groups in Tables I and II. The number of preparations analyzed is given by n. Only five samples each from perfusion Groups b and c were used for metabolite analyses. All perfusions were started with 6 mM glucose. Where indicated, 5 milliunits of insulin/ml were added at the start of the control period for Groups c and 14 min after the start of the control period for Groups d, e, and f.

<table>
<thead>
<tr>
<th>Metabolite contents</th>
<th>Controls</th>
<th>Exercise</th>
<th>Epinephrine</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine phosphate</td>
<td>81.0 ± 4.17</td>
<td>76.0 ± 5.5</td>
<td>43.8 ± 3.8</td>
<td>30.2 ± 2.0</td>
</tr>
<tr>
<td>ATP</td>
<td>24.4 ± 1.1</td>
<td>26.6 ± 0.7</td>
<td>23.8 ± 0.4</td>
<td>20.2 ± 0.8</td>
</tr>
<tr>
<td>ADP</td>
<td>9.78 ± 0.13</td>
<td>3.71 ± 0.27</td>
<td>3.98 ± 0.66</td>
<td>4.07 ± 0.41</td>
</tr>
<tr>
<td>AMP</td>
<td>0.315 ± 0.018</td>
<td>0.38 ± 0.001</td>
<td>0.752 ± 0.062</td>
<td>0.675 ± 0.090</td>
</tr>
<tr>
<td>Sum of AMP, ADP, and ATP</td>
<td>27.5 ± 1.3</td>
<td>30.7 ± 0.8</td>
<td>28.6 ± 0.9</td>
<td>24.9 ± 1.0</td>
</tr>
<tr>
<td>IMP</td>
<td>0.075 ± 0.027</td>
<td>0.085 ± 0.033</td>
<td>0.451 ± 0.098</td>
<td>4.73 ± 0.61</td>
</tr>
<tr>
<td>Sum of AMP, ADP, ATP, and IMP</td>
<td>27.6 ± 1.1</td>
<td>30.7 ± 0.8</td>
<td>29.0 ± 0.8</td>
<td>29.6 ± 1.2</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.028</td>
<td>0.046</td>
<td>0.084</td>
<td>0.052</td>
</tr>
<tr>
<td>Insosine</td>
<td>0.018</td>
<td>0.015</td>
<td>0.028</td>
<td>0.159</td>
</tr>
<tr>
<td>Ammonia</td>
<td>1.12 ± 0.11</td>
<td>1.61 ± 0.01</td>
<td>2.41 ± 0.52</td>
<td>4.96 ± 0.85</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.27 ± 0.13</td>
<td>0.86 ± 0.03</td>
<td>0.86 ± 0.15</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>Glutamate</td>
<td>6.00 ± 0.14</td>
<td>5.80 ± 0.05</td>
<td>4.49 ± 0.42</td>
<td>4.13 ± 0.30</td>
</tr>
<tr>
<td>Glutamine</td>
<td>15.5 ± 1.2</td>
<td>18.5 ± 1.7</td>
<td>12.2 ± 0.7</td>
<td>12.5 ± 1.4</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.42 ± 1.35</td>
<td>8.42 ± 0.14</td>
<td>9.97 ± 0.56</td>
<td>8.87 ± 0.61</td>
</tr>
<tr>
<td>Lactate</td>
<td>8.04 ± 0.50</td>
<td>8.55 ± 0.21</td>
<td>45.7 ± 0.61</td>
<td>8.32 ± 4.7</td>
</tr>
</tbody>
</table>

*Only two samples were analyzed from each group.*
respectively. The adenine nucleotides lost were largely accounted for by the IMP formed. Adenylosuccinate was not detected in the resting preparations (<1 nmol/g dry weight) and was only detected in some of the exercised preparations (Table II, Groups d, e, and f). On the other hand, adenylosuccinate could readily be demonstrated in muscle of intact animals immediately after exercise and during recovery (Table III).

Exercise also caused increases in the content of inosine and hypoxanthine, but these were small compared to the changes observed for IMP and ATP. Low but significant amounts of IMP, inosine, and hypoxanthine were found in resting muscle. Adenosine could not be detected under our assay conditions, indicating that its tissue content was <1 nmol/g dry weight.

Exercise resulted in a decrease in the tissue content of aspartate and glutamate, and in an increase in the tissue content of alanine (Table II, Group c, d, and e, data not shown). On the other hand, adenylosuccinate could readily be demonstrated in muscle of intact animals immediately after exercise and during recovery (Table III).

Effect of Exercise on Muscle Metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Resting control</th>
<th>Exercise</th>
<th>Recovery after exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 5</td>
<td>n = 5</td>
<td></td>
<td>2.5 min, n = 4</td>
</tr>
<tr>
<td>Creatine phosphate</td>
<td>84.0 ± 3.6</td>
<td>28.7 ± 3.0</td>
<td>70.4 ± 4.2</td>
</tr>
<tr>
<td>ATP</td>
<td>25.9 ± 1.2</td>
<td>18.9 ± 0.8</td>
<td>20.7 ± 1.0</td>
</tr>
<tr>
<td>ADP</td>
<td>3.12 ± 0.21</td>
<td>3.01 ± 0.15</td>
<td>2.67 ± 0.12</td>
</tr>
<tr>
<td>IMP</td>
<td>1.96 ± 0.04</td>
<td>0.36 ± 0.089</td>
<td>0.003 ± 0.023</td>
</tr>
<tr>
<td>AMP</td>
<td>0.931 ± 0.182</td>
<td>1.0 ± 0.32</td>
<td>1.41 ± 0.13</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1.40 ± 0.22</td>
<td>1.87 ± 0.25</td>
<td>1.42 ± 0.40</td>
</tr>
<tr>
<td>Lactate</td>
<td>8.24 ± 0.89</td>
<td>7.84 ± 0.63</td>
<td>7.08 ± 0.80</td>
</tr>
<tr>
<td>Glutamine</td>
<td>15.9 ± 1.0</td>
<td>14.1 ± 0.7</td>
<td>14.2 ± 1.3</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>4.82 ± 1.59</td>
<td>25.3 ± 3.1</td>
<td>15.0 ± 2.8</td>
</tr>
<tr>
<td>Alanine</td>
<td>28.7 ± 1.3</td>
<td>25.7 ± 0.6</td>
<td>26.2 ± 0.74</td>
</tr>
</tbody>
</table>

Effect of Epinephrine and Hypoxia

Epinephrine increased the output and tissue content of ammonia plus glutamine by 35.4 μmol/30 g of muscle during 30 min. It decreased the output and content of aspartate, glutamate, and alanine by 29.0 μmol/30 g of muscle/30 min (Table I and II). Thus, the increases in ammonia and amide nitrogen are largely balanced by decreases in aspartate, glutamate, and alanine.1

Recently, a report appeared in which epinephrine was shown to diminish the output and content of alanine and glutamine by epitochori muscle of rat incubated in vitro (32).

Glycolysis by Perfused Muscle

Exercise stimulated glucose uptake and lactate output (Table I, compare Group c with Groups d, e, and f). The [lactate]/[pyruvate] ratio in the perfusate was 11 for muscle at rest; it rose to 15.7 and 25.3 μmol/30 g of muscle/min during exercise induced by 1, 4, or 6 shocks/s, respectively. (These ratios do not agree with ratios of lactate and pyruvate production rates (Table I) because the rates are calculated from initial and final perfusate concentrations of lactate and pyruvate.) At rest oxygen consumption was 12.7 μmol/30 g of muscle/min; it rose to 17.5 and 25.3 μmol/30 g of muscle/min during exercise induced by 1 and 4 shocks/s.

Addition of 25 mM epinephrine increased lactate and pyruvate output, and caused a small increase (from 11 to 14) in the [lactate]/[pyruvate] ratio. At the concentration used, epinephrine did not cause vasoconstriction, but it tended to do so at

---

1 These changes were obtained from the data in Tables I and II as follows (output is expressed as μmol/30 g fresh weight/30 min and content as μmol/30 g fresh weight):

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Output</th>
<th>Content</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>12.7</td>
<td>+6.60</td>
<td>+19.3</td>
</tr>
<tr>
<td>Glutamine</td>
<td>11.6</td>
<td>+4.69</td>
<td>+16.1</td>
</tr>
<tr>
<td>Ammonia plus amide N</td>
<td>24.3</td>
<td>+11.1</td>
<td>+35.4</td>
</tr>
<tr>
<td>Aspartate</td>
<td>-5.0</td>
<td>-5.0</td>
<td>-10.0</td>
</tr>
<tr>
<td>Glutamate</td>
<td>15.0</td>
<td>15.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.3</td>
<td>-26.4</td>
<td>-22.1</td>
</tr>
<tr>
<td>Asp + Glu + Ala</td>
<td>-49.4</td>
<td>-45.1</td>
<td>-94.5</td>
</tr>
</tbody>
</table>

Corrected for loss of glutamate into glutamine

-45.1 + 16.1 = -29.0

Thus only 35.4 - 22.0 - 6.4 μmol of the ammonia plus amide nitrogen output cannot be accounted for in terms of the amino acids measured.
higher concentrations. Hypoxia and cyanide increased glucose uptake and lactate output. The \[\text{[lactate]/[pyruvate]}\] ratio was 40 after onset of hypoxia.

**Studies with Hind Legs in Situ**—Exercise depleted the creatine phosphate and ATP contents of hind legs in situ (Table II) to about the same extent as was observed with perfused hind legs (Table I). However, ammonia, IMP, and lactate contents were less elevated by exercise in situ. ADP was not significantly altered and AMP showed a slight rise. Recovery from exercise was fairly rapid, with most metabolites returning to resting values within 10 min, except for IMP levels which did not quite reach resting values after a recovery period of 15 min. Inosine and hypoxanthine increased during exercise and returned to resting values during recovery. Aspartate, glutamate, and alanine remained essentially unchanged, except for a fall in glutamate during exercise.

Adenylosuccinate was not detected (<1 nmol/g dry weight) in resting muscle but appeared during exercise, reaching a maximum of about 20 nmol/g dry weight. It disappeared again during recovery, becoming undetectable within 10 min after exercise. The appearance and disappearance of adenylosuccinate during exercise and recovery in relation to the appearance and disappearance of IMP constitutes evidence for the operation of the purine nucleotide cycle.

**Comparison between Ammonia, IMP, and Adenylosuccinate Contents**—Although there is no precise stoichiometry between the ammonia and IMP contents, there is nevertheless a strong correlation between these two parameters (Fig. 1). The slope of the line in Fig. 1 indicates that during the experimental periods an average of 0.56 \(\text{nmol of ammonia} \div \text{\mu mol of IMP formed}\) formed.

The relation between the adenylosuccinate and IMP contents of the tissue is more complex (Fig. 2). Adenylosuccinate accumulation reached a maximum when the IMP content was around 2 to 3 \(\text{\mu mol/g dry weight}\). Virtually no adenylosuccinate was found when the IMP content was low (<0.5 \(\text{\mu mol/g dry weight}\)), and very little was found above 8.5 \(\text{\mu mol of IMP/g dry weight}\). If it is assumed that the intracellular water accounts for 50% of the wet weight, and that the metabolites are evenly distributed throughout the intracellular water, then using the experimentally determined wet weight to dry weight ratio of

\[
\text{Wet weight} = \text{Dry weight} \times \frac{1}{1 - 0.5}
\]

4.97, it can be calculated that 2, 3, and 9 \(\text{\mu mol of IMP/g of dry weight}\) correspond to concentrations of 0.8, 1.2, and 3.6 mM.

The maximum activities of adenylosuccinate synthetase and adenylosuccinase in hind leg of rat are about the same (33). High [IMP] inhibits adenylosuccinate synthetase. The inhibition is competitive with respect to GTP, and the constant \(K_i\) for IMP is about 2 mM. An intracellular concentration of 3.6 mM IMP is therefore sufficient to cause a substantial inhibition of adenylosuccinate synthetase.

**Effect of Exercise on Malate and Citrate Content of Hind Limb**—The malate content of hind limbs in situ and perfused preparations rose from 0.373 ± 0.012 \((n = 8) \text{\mu mol/g dry weight}\) in resting controls to 1.120 ± 0.060 \((n = 8) \text{\mu mol/g dry weight}\) after 15 min of exercise. The citrate content did not change significantly in the same experiments \(0.71 ± 0.08 \text{\mu mol/g dry weight}\). The most probable source of the additional malate is the purine nucleotide cycle, the operation of which results in a net conversion of aspartate to fumarate and malate (1, 2).

**DISCUSSION**

The rate of ammonia production and glycolysis in skeletal muscle increases during exercise, and in response to epinephrine, hypoxia, and anoxia (Tables I to III) (1, 21, 34-38). These changes are accompanied by decreases in the size of the total adenylate pool (AMP + ADP + ATP), and by increases in the IMP content of the tissue. The relation between the appearance of ammonia and IMP shown in Fig. 1 is consistent with ammonia being produced by the adenylate deaminase reaction.

Adenylosuccinate accumulates during exercise and early recovery from exercise in situ (Table III), providing strong evidence for the operation of the purine nucleotide cycle. Although adenylosuccinate was detected reproducibly in the in situ studies, it was not observed to accumulate with consist

\[
K. \text{Tornheim and J. M. Lowenstein, unpublished observations.}
\]
ency in the inhibition of adenylosuccinase by high concentrations of IMP, suggest that failure to observe adenylosuccinate may be due to the accumulation of too much or too little IMP. The amounts of IMP found in the perfused muscle during and after exercise were much larger than those found in situ and this probably accounts for our failure to observe adenylosuccinate in most of the perfusion experiments. Indeed adenylosuccinate was found only in perfused muscles in which IMP accumulation was similar to those observed in situ (Table II, Groups d and e).

The activities of adenylosuccinate synthetase and adenylosuccinase in skeletal muscle of rat are 0.74 and 0.61 μmol/g fresh weight/min, respectively, at 38° (33). These activities can account for the rate of disappearance of IMP and the rate of appearance of adenylosuccinate and AMP. In addition to the inhibition by IMP, adenylosuccinate synthetase is inhibited strongly by GDP, and to a lesser extent by AMP, adenylosuccinate, and orthophosphate (1, 2, 39, 40). The Kₐ values for IMP, GTP, and aspartate are 200, 10, and 300 μM, respectively. Adenylosuccinate from mammalian skeletal muscle has a Kₐ for adenylosuccinate of about 9 μM (41).

ADP and AMP showed little or no change between rest, exercise, and recovery from exercise. The average ADP and AMP contents shown in Table III are 2.96 ± 0.08 and 0.52 ± 0.09 μmol/g dry weight, which correspond to 0.596 and 0.105 μmol/g wet weight, respectively. Skeletal muscle actin contains one tightly bound molecule of ADP per actin monomer; the binding is so tight that the ADP is not available to the pool of free ADP. The content of actin monomer is about 0.6 μmol/g fresh weight (42). This is about the same as the total ADP content. The standard error of these determinations is too great to use the total and bound ADP to calculate the capacity of adenylate deaminase even at the very low concentrations of AMP calculated to be freely available. This implies that the activity of adenylate deaminase is under control. The regulation of adenylate deaminase is complex; suffice it to say here that the muscle enzyme is activated by ADP and alkali metal ions and is inhibited by GTP and ATP (1, 45).

Contents of freely available AMP of 0.12 to 0.56 nmol/g fresh weight are equivalent to concentrations of about 0.24 to 1.1 μM (assuming that the intracellular water is 50% of the fresh weight, and that this AMP is evenly distributed throughout the intracellular water). Adenylate deaminase of rat skeletal muscle has an activity of about 200 μmol/g/mg protein (42). When such calculations are applied to the data shown in Table III, the contents of freely available ADP and AMP in the resting controls are 19 and 0.12 nmol/g fresh weight, respectively, assuming an intracellular pH of 7.0. After 15 min of exercise, the free AMP content is reduced to only about 5% of the total AMP (43).

5 The activities were determined at 30° (33) and were corrected to 38° by assuming a temperature coefficient of 1.8 per 10°.

6 The method of calculating ADP and AMP concentrations was that of McGilvery and Murray (42). In the equations given on page 5846 in Ref. 42, the concentration of ADP in terms of ATP₄⁺ should read

$$[\text{ADP}] = \frac{d[\text{ATP}]}{2K_{\text{ATP}}} \left[ \left( d^2 - 4K_{\text{ATP}} + 4K_{\text{ATP}} \left[ \frac{[\text{ATP}]}{[\text{ATP}]} \right]^{1/2} \right)^{1/2} - d \right]$$

The equilibrium constants for 38° used in the calculations were those given by Klibanov and Altenbach (43). In some cases they are the same as those used by McGilvery and Murray (42), but all are quoted here for clarity.

$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}] = 7.94 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{ADP}] = 5.01 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{AMP}] = 2.82 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{CrP}] = 3.16 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{CrP}] = 7.10 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{CrP}] = 7.10 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{CrP}] = 7.10 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{CrP}] = 7.10 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{CrP}] = 7.10 \times 10^{10} \text{ M}^{-1}$$
$$K_{\text{ADP}} = [\text{ATP}]/[\text{ATP}][\text{CrP}] = 7.10 \times 10^{10} \text{ M}^{-1}$$

Total adenine nucleotides used in the calculations were taken to be the amounts of ATP, ADP, AMP less 0.6 μmol/g fresh weight (the amount bound to actin). The free Mg²⁺ concentration used was 5 x 10⁻⁴ M (44).
Ammonia Production by Skeletal Muscle

The purine nucleotide cycle. Studies of ammonia production by skeletal muscle in situ and in perfused preparations.
M N Goodman and J M Lowenstein


Access the most updated version of this article at http://www.jbc.org/content/252/14/5054

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/14/5054.full.html#ref-list-1