nitrogenous waste products, such as ammonia, urea, or uric acid; typical efficiency values are 25 to 35% with urea synthesis (Table 3–6).

Anaerobic Metabolism

The complete oxidation of glucose to carbon dioxide and water requires oxygen, for the regeneration of NAD^+ and FAD and synthesis of ATP. However, oxygen is sometimes in limited supply (hypoxia) or unavailable (anoxia) for some animals. Consequently, these animals have alternative pathways for **anaerobic metabolism** to form ATP in the absence of oxygen.

Many animals rely on aerobic metabolism for at least their resting ATP requirements; they are strict, or obligate, aerobes. However, even strict aerobes can use anaerobic metabolic pathways for supplemental ATP formation under some circumstances, e.g., intense activity. For example, almost all vertebrates rely on aerobic metabolism to sustain their resting metabolic rate, but can produce ATP during activity by anaerobic metabolism.

Some animals rely on anaerobic metabolism for extended periods of time; they are facultative anaerobes. For example, many intertidal invertebrates experience cyclic submersion and emersion and are facultative anaerobes.

Some animals are strict, or obligate, anaerobes. For example, many parasitic invertebrates are obligate anaerobes and cannot survive in the presence of significant amounts of oxygen.

Anaerobic Metabolic Strategies

Glycolysis is essentially the only important anaerobic metabolic pathway for higher vertebrates. Some invertebrates use glycolysis for anaerobic metabolism, but many have alternate anaerobic pathways for ATP production. Some of these pathways produce more ATP/glucose, and some can be maintained as steady-state processes.

Glycolysis. Glycolysis is one anaerobic metabolic pathway that is commonly used by vertebrates. Two ATP are formed per glucose at an efficiency of about 50% of the free energy change. The coenzyme NAD^+ accepts two hydrogen atoms during glycolysis to form $NADH/H^+$, but is rapidly depleted because it is present in very low concentrations. Glycolysis would quickly stop unless the NAD^+ were regenerated. In glycolysis, this regeneration involves the conversion of pyruvate to lactate. Pyruvate acts as a hydrogen acceptor.

$$\begin{array}{lcl} \text{pyruvate} + \text{NADH/H}^+ & \underset{}{\overset{\text{lactate dehydrogenase}}{\rightleftharpoons}} & \text{lactate} + \text{NAD}^+ \\ CH_3 & & CH_3 \\ C{=}O & & HCOH \\ COO^- & & COO^- \end{array} \quad (3.28)$$

Consequently, glycolysis can continue unabated, at least in terms of NAD^+ availability.

The equilibrium between pyruvate and lactate favors lactate ($\Delta G° = -25$ kJ mole^{-1}), so high concentrations of lactate can accumulate before the reverse reaction (lactate $\rightarrow$ pyruvate) becomes significant. However, lactate production is not a steady-state anaerobic pathway, and lactate can accumulate to such high levels that pyruvate also accumulates and inhibits further glycolysis.

Linkage with the Citric Acid Cycle. Glycolysis can be linked with the metabolic pathways of the citric acid cycle to form a variety of end products and additional ATP (Figure 3–18).

Glycolysis proceeds normally to phosphoenolpyruvate, but formation of pyruvate is minimized by low levels of pyruvate kinase (PK). A low level of lactate dehydrogenase (LDH) minimizes lactate formation. The main metabolic pathway is formation of oxaloacetate by CO_2 fixation; high levels of phosphoenolpyruvate carboxy kinase (PEPCK) favor this pathway. Oxaloacetate is reduced to malate by malate dehydrogenase. Malate then enters the mitochondria, where about half is converted to pyruvate and CO_2 and the rest to fumarate. Fumarate conversion to succinate (by fumarate reductase) results in ATP generation. The pyruvate and succinate are converted (via CoA) to acetate and propionate which, in turn, are converted to 2-methylvalerate and 2-methylbutyrate; ATP may also be formed in these steps.

The ATP production is high for these pathways. The formation of succinate yields at most 4 ATP per glucose, with 2 ATP formed at the level of phosphoglycerate kinase, 2 ATP at PEPCK, and 2 ATP by electron transfer at fumarase reductase (with 2 ATP used at glucokinase and phosphofructokinase). The further conversion of succinate to propionate produces an additional 2 ATP per glucose, for a total of 6 ATP/glucose. Further synthesis of 2-methylbutyrate and 2-methylvalerate might yield an additional ATP.

Pyruvate Condensation with Amino Acids. The general reaction for the combination of pyruvate (or any R—C=O compound) with an amine (NH_2) allows the regeneration of NAD^+ from $NADH/H^+$, to sustain anaerobic metabolism;

$$\mathrm{R{-}\underset{R'}{C}{=}O + H_2N{-}R'' + NADH/H^+ \rightleftharpoons R{-}\underset{R'}{CH}{-}NH{-}R'' + H_2O + NAD^+} \tag{3.29}$$

Clearly, any amino acid could readily combine with pyruvate and at the same time regenerate NAD^+ via this reaction. In fact, a variety of amino acids are used by various animals (principally mollusks) to achieve this (Table 3–7).

Aspartate Metabolism. Aspartate can be an important anaerobic metabolic substrate (Figure 3–19). The aspartate is deaminated by α-ketoglutarate to form oxaloacetate, which is then converted to propionate (as described above via linkage to the citric acid cycle). To sustain this pathway, the deaminating mechanism must be maintained, i.e., α-ketoglutarate must be re-formed from glutamate. This is accomplished by coupling transamination of

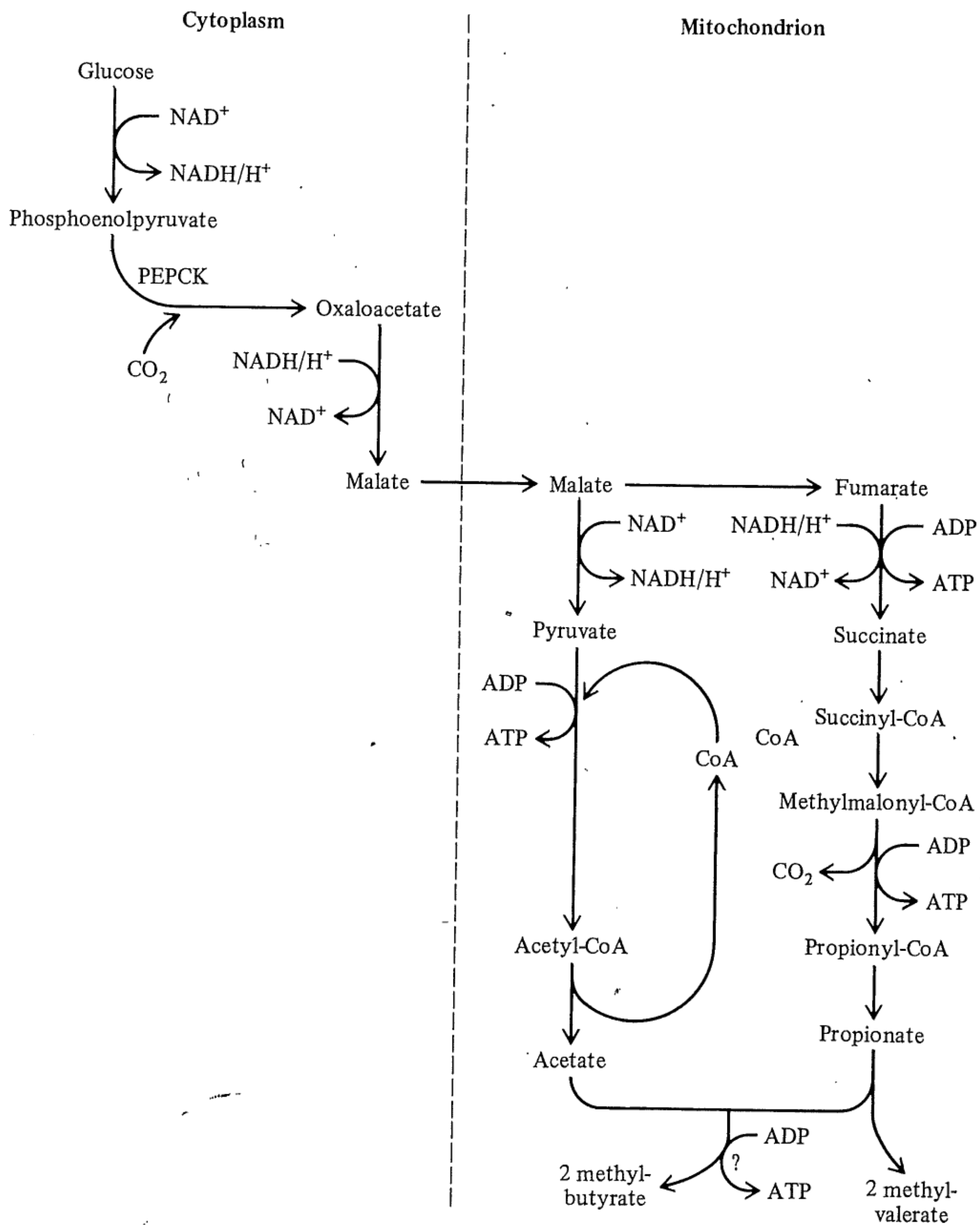


FIGURE 3–18 Linkage of glycolysis with citric acid cycle pathways is an anaerobic metabolic pathway that provides additional ATP formation. These pathways are found, for example, in many platyhelminth worms.

TABLE 3–7

Amino acids (H_2N-R) are used by bivalve and cephalopod mollusks for removal of pyruvate ($CH_3COCOOH$) and regeneration of NAD^+ from $NADH/H^+$ during anaerobiosis, using the general reaction illustrated below.

Amino Acid	Enzyme	End Product
$H_2N\text{-R} + NADH/H^+ + \begin{matrix} CH_3 \\ C{=}O \\ COOH \end{matrix}$	enzyme $\rightleftharpoons$	$\begin{matrix} CH_3 \\ HC\text{-NH-R} \\ COOH \end{matrix} + H_2O + NAD^+$
Alanine[1]	Alanine dehydrogenase	Alanopine
Glycine[1]	Strombine dehydrogenase	Strombine
Lysine[1]	Lysopine dehydrogenase	Lysopine
Arginine[2]	Octopine dehydrogenase	Octopine

[1] Bivalves.

[2] Cephalopods.

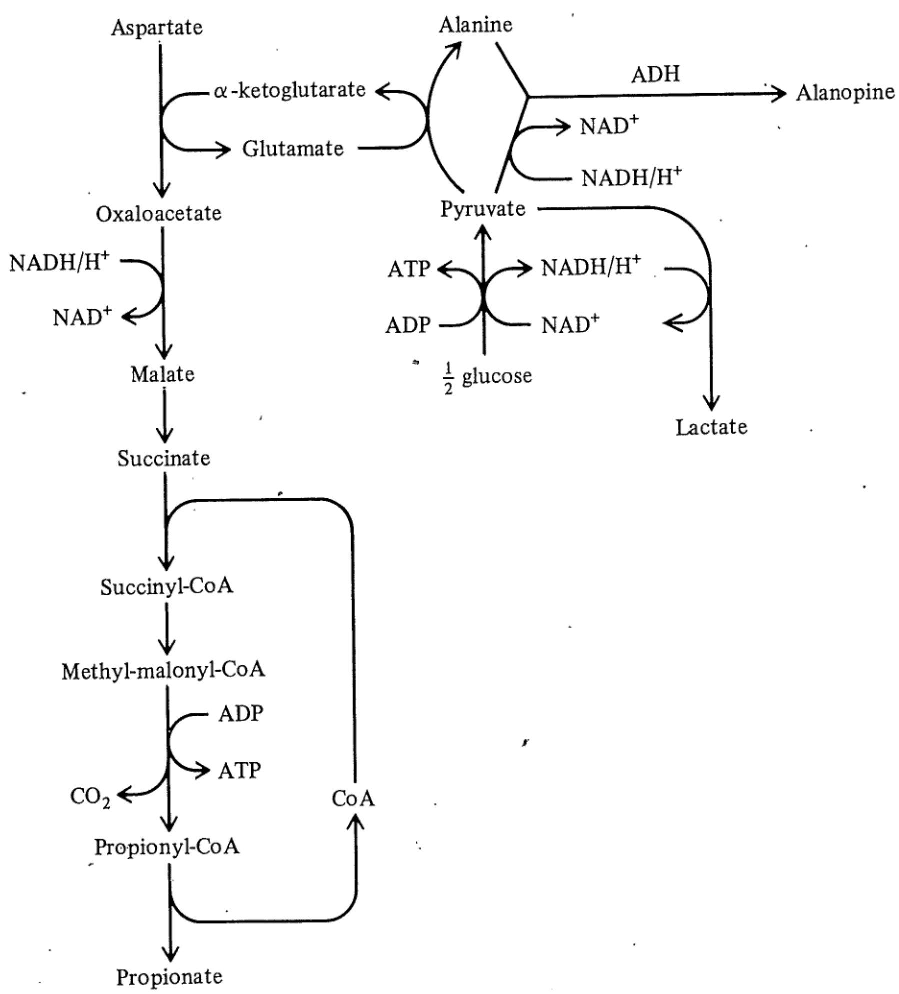


FIGURE 3–19 Anaerobic metabolic pathway of bivalve mollusks showing coupling of aspartate and glucose metabolism to produce propionate and alanopine.

pyruvate to alanine. Low concentrations of LDH and PEPCK channel pyruvate from glycolysis towards alanine, rather than oxaloacetate (which is formed from aspartate) or lactate. Glycolysis proceeds without pyruvate or lactate accumulation, but alanine would eventually accumulate and inhibit both aspartate and glucose metabolism.

Arginine Phosphate Stores. Arginine phosphate is an important phosphagen in some animals. It can be an important source of energy over short periods of anaerobic metabolism, although the amount of arginine phosphate present in muscle cells is insufficient to maintain ATP supplies for more than brief periods (even though it can be as high as 70 μmole g^{-1}).

Hydrolysis of arginine phosphate stores to arginine is energetically coupled to the regeneration of ATP from ADP. However, a high concentration of arginine would have detrimental effects on cells, since arginine is a very basic amino acid. It would disrupt intracellular acid-base balance and might inhibit the catalytic properties of enzymes. However, arginine is an amino acid and participates in the general reaction outlined above (Equation 3.29) for the elimination of pyruvate and regeneration of NAD^+. The end product is octopine, and the catalytic enzyme is octopine dehydrogenase (Table 3–7). Thus, utilization of high-energy arginine phosphate stores and anaerobic metabolism can be conveniently coupled (Figure 3–20). A small amount of lactate also accumulates as some LDH is present in cephalopod muscle.

Acetate, Acetaldehyde, and Ethanol. Another anaerobic metabolic strategy is to form ethanol as the end product. Pyruvate is converted to acetyl-CoA, releasing CO_2. Subsequent synthesis of acetate (by acetate thiokinase) regenerates CoA and forms ATP.

$$\text{pyruvate} + \text{CoA} \xrightarrow{CO_2} \text{acetyl-CoA} \xrightarrow{\text{ADP}\ \text{ATP}} \text{acetate} + \text{CoA}$$

$$\begin{array}{l} CH_3 \\ C{=}O \\ O^- \end{array} \qquad (3.30a)$$

Acid-base balance is not disturbed as much by acetate formation as by lactate production. Acetic acid has a higher pK (4.8) than lactic acid (3.7); the pK is the pH at which 50% of an acid, HA, is present as the anion A^-, and 50% as acid HA. Furthermore, acetate can be further metabolized to acetaldehyde, then ethanol.

$$\underset{\begin{array}{l} CH_3 \\ C{=}O \\ O^- \end{array}}{\text{acetate}} \xrightarrow[\text{NADH/H}^+\ \text{NAD}^+]{\text{acetaldehyde dehydrogenase}} \underset{\begin{array}{l} CH_3 \\ C{=}O \\ H \end{array}}{\text{acetaldehyde}} + H_2O \xrightarrow[\text{NADH/H}^+\ \text{NAD}^+]{\text{ethanol dehydrogenase}} \underset{\begin{array}{l} CH_3 \\ CHOH \\ H \end{array}}{\text{ethanol}} \qquad (3.30b)$$

Each step regenerates NAD^+ from $NADH/H^+$.

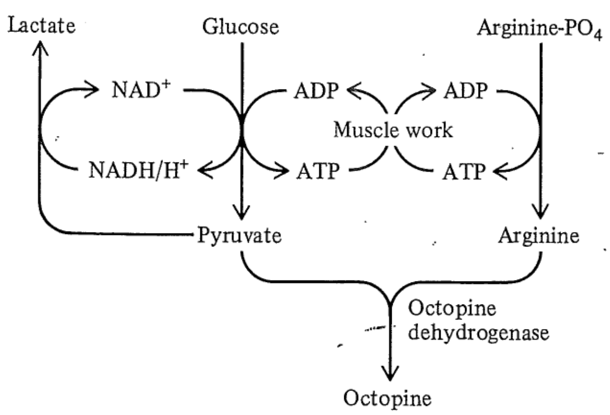


FIGURE 3–20 High-energy phosphate stores (arginine-PO_4) and glycolysis are coupled as an anaerobic metabolic pathway, with octopine as a major metabolic end product.

Invertebrates

Invertebrate animals use a variety of the above described strategies for anaerobic metabolism. In general, there is an evolutionary trend towards a greater activity level in the higher invertebrates, hence a greater reliance on aerobic metabolism. Associated with this trend is the tendency for a lesser reliance on sustainable anaerobic pathways, and greater utilization of phosphagen stores.

Free-living platyhelminthes (turbellarian worms such as planarians) have little need for a well-developed anaerobic capacity, but parasitic platyhelminths (flukes, tapeworms) often encounter hypoxic or anoxic environments (e.g., the gut) and have well-developed anaerobic metabolic pathways (Saz 1981). Parasitic helminths can be obligate aerobes (e.g., *Litomosoides*, *Nippostrongylus*), can support resting metabolism anaerobically but require aerobic metabolism for motility (e.g., microfilarial stages of *Litomosoides*), or do not require oxygen (e.g., *Ascaris, Schistosoma*). The anaerobic pathways of parasitic helminthes are citric acid pathways linked with glycolysis, and there are various end products. For example, the common metabolic end products for *Ascaris* are succinate, propionate, acetate, 2-methylvalerate, and 2-methylbutyrate.

Many mollusks, especially intertidal bivalves (mussels, oysters, clams), routinely experience prolonged hypoxia. Some bivalves, such as oysters, can live indefinitely in the complete absence of oxygen! These bivalves obviously have a well-developed anaerobic capacity. The amino acid aspartate is a major metabolic substrate for anaerobiosis, with succinate and alanine as end products, e.g., the oyster ventricle is a well-studied anaerobic molluskan tissue (Collicutt and Hochachka 1977). Aspartate and glucose metabolism can continue in many bivalve mollusks because alanine combines with pyruvate to form alanopine; this also regenerates NAD^+ from $NADH/H^+$. The enzyme that catalyzes this reaction is alanopine dehydrogenase. Littoral, sessile bivalves rely on carbohydrate rather than aspartate as their anaerobic energy substrate. Glycolysis is linked to the citric acid cycle via oxaloacetate and malate, by conversion of phosphoenolpyruvate to oxaloacetate. The latter reaction is made possible by the important enzyme, PEPCK. The normal (aerobic) energy pathway is replaced by different anaerobic pathways during short-term and long-term hypoxia (de Zwaan 1983).

Squid and octopus are considerably more active than bivalve mollusks, and their greater metabolic demands suggest a greater reliance on aerobic metabolism. Nevertheless, they have a considerable anaerobic capacity because periods of relative hypoxia still occur, e.g., during strenuous swimming. The primary phosphagen of cephalopod muscle is arginine phosphate (rather than creatine phosphate); hydrolysis of arginine phosphate is coupled with ATP formation. The arginine is removed by its combination with pyruvate to form octopine. There are general similarities in aerobic metabolism of cephalopods and vertebrates despite differences in their specific anaerobic reactions. Glucose (or glycogen) is the only significant metabolic substrates, not amino acids. Only 2 ATP are produced per glucose. Anaerobic metabolism is not a steady-state process; there is an accumulation of end products (octopine and lactate) that eventually inhibit further glycolysis.

Vertebrates

The only significant anaerobic metabolic pathway of most vertebrates is lactate formation. However, some fish have other important anaerobic metabolic pathways for periods of inactivity coupled with hypoxia.

Fish, like other vertebrates, are generally active and aerobic animals. Their resting metabolism is maintained by aerobic pathways (with a few exceptions; see below), but a significant anaerobic capacity is advantageous in two circumstances: during bursts of strenuous activity and during hypoxia. However, very different anaerobic strategies are employed by fish in these two different situations. Bursts of intense activity are generally of short duration with an extremely high rate of ATP demand. Periods of hypoxia (such as being frozen under ice during winter) are typically of much longer duration with a low ATP demand rate.

Fish utilize the lactate anaerobic pathway during periods of strenuous activity. There is a positive relationship between the level of activity and anaerobic capacity for fish muscle, as shown by their concentrations of glycolytic enzymes and metabolite levels. The white muscle of active fish, such as tuna, has considerably higher levels of some glycolytic enzymes, including LDH, and citric acid cycle enzymes than does white muscle of an inactive fish (Table 3–8).

There is also adaptive variation for enzyme and metabolite levels in different muscles of individual fish. Red muscle of tuna has lower levels of some anaerobic glycolytic enzymes (PK and LDH) and higher levels of some aerobic citric acid cycle

TABLE 3–8

Levels for anaerobic (glycolytic) and aerobic (citric acid cycle) enzymes of white and red muscle for an inactive and active species of fish. Units for enzyme activity are μmoles per minute per gram wet tissue weight. *(Values for Hochachka et al. 1978; Guppy, Hulbert, and Hochachka 1979.)*

	Inactive Fish Arapaima *Arapaima*		**Active Fish Skipjack tuna *Euthynnus***	
	White	***Red***	***White***	***Red***
Anaerobic				
Pyruvate kinase	103	134	1295	195
Lactate dehydrogenase	260	263	5492	514
Aerobic				
Citrate synthetase	1.7	3.3	2.1	20.6
Malate dehydrogenase	140	221	718	723
Glutamate dehydrogenase	1.3	3.1	3.0	5.9
Glutamate-oxaloacetate transaminase	11.2	54.4	43	102

enzymes (CS, GDH, and GOT) than does white muscle (Table 3–8). These enzymatic differences are reflected by changes in the metabolite levels of tuna red and white muscle, at rest and after burst activity (Table 3–9). There is a substantial depletion of muscle energy stores (glycogen, creatine phosphate, and ATP) and an accumulation of anaerobic end product (lactate), but only small changes in the concentration of glycolytic and citric acid cycle intermediary metabolites. White muscle has a greater depletion of energy stores and greater accumulation of lactate than red muscle. Red muscle also has a higher myoglobin content and is more vascular than white muscle; it is used for sustained aerobic metabolism. White muscle has a high anaerobic capacity and is used for bursts of anaerobic metabolism.

Fish that survive long periods of environmental hypoxia rely on alternate anaerobic pathways. Apparently, lactate anaerobiosis is not suitable for long-term bouts of hypoxia. For example, lactate accumulation would eventually inhibit glycolysis. The capacity for anaerobic metabolism would determine the likelihood and duration for survival of extreme hypoxia. Carp, goldfish, and some other cyprinid fish can, under certain circumstances, survive hypoxia for 100 days or more. Two observations of fish kept under hypoxic conditions indicate that lactate accumulation is not the major source of ATP synthesis, and there are alternate metabolic pathways for long-term tolerance of anoxia. First, blood lactate levels of goldfish after five days of anoxia are only 20 mM; this level of glycolysis is apparently insufficient to support resting metabolism (Hochachka 1980). Second, anoxic fish excrete carbon dioxide, but CO_2 is not evolved by the glycolytic pathway to lactate. Acetaldehyde dehydrogenase and ethanol dehydrogenase are present in goldfish tissues at sufficient concentrations to form ethanol during anoxia. The ethanol is then excreted into the environment, avoiding the accumulation of a metabolic end product.

TABLE 3–9

Changes in metabolite concentrations of red and white muscle from skipjack tuna (*Euthynnus pelamis*) after burst activity. Units for concentrations are μmole g wet weight^{-1}. *(Data from Guppy, Hulbert, and Hochachka 1979.)*

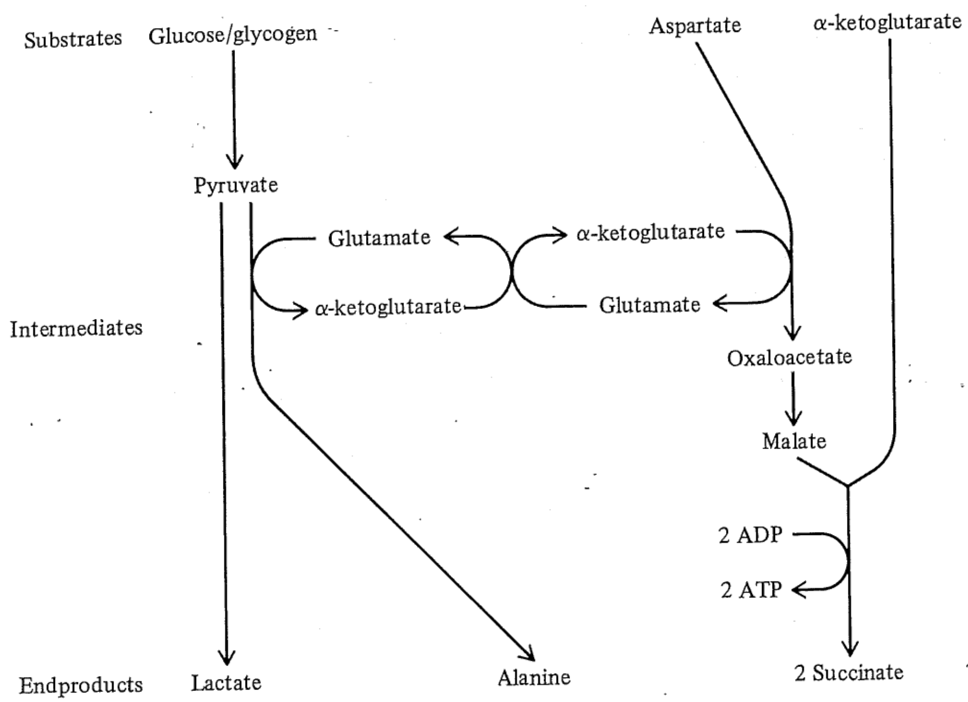
Metabolites	Red	White
High-Energy PO_4 Stores		
Creatine PO_4	−1.73	−12.90
ATP	−0.87	−2.60
Substrate Stores		
Glycogen	−1.70	−22.8
Glycolytic Intermediates		
Glucose	−0.13	+2.01
Glucose-6-PO_4	+0.41	+2.30
Fructose-6-PO_4	−0.21	−0.45
Fructose-1,6-diPO_4	−0.06	−0.34
Di(OH)acetonePO_4	−0.05	−0.02
Glyceraldehyde-3-PO_4	−0.04	−0.01
Citric Acid Cycle Intermediates		
Citrate	+0.08	−0.05
α-Ketoglutarate	−0.02	−0.07
Malate	+0.13	+0.12
Anaerobic End Products		
Lactate	+5.9	+70.95

Other possible anaerobic end products for fish include succinate, lipids (elongation of fatty acids by condensation with acetyl-CoA), and ammonia. The fatty acids could be derived from carbohydrate (oxidation of glucose or glycogen to acetyl-CoA) and the NH_3 from amino acid deamination, in coupled reactions.

There may be some partitioning by different organs within the fish with respect to specific pathways occurring in specific organs (Hochachka 1980). For example, peripheral tissues receiving a low blood supply (such as white muscle) might predominantly produce lactate as their anaerobic end product. The lactate could diffuse to more highly perfused and aerobic tissues (such as liver, red muscle, heart) and be further oxidized to other anaerobic end products, such as ethanol or fatty acids. There may be coupling of anaerobiosis with amino acid metabolism, particularly in the liver.

Anaerobic metabolism in tetrapods (i.e., amphibians, reptiles, birds, and mammals) is almost completely limited to glycogen utilization and lactate accumulation. Only small amounts of other anaerobic end products, such as glycolytic or citric acid cycle intermediates, pyruvate, succinate, alanine, and ethanol have been reported (Hochachka et al. 1975; Felig and Wahren 1971). For example, preliminary studies of diving vertebrates (a sea turtle, seal, sea lion, and porpoise) indicate a minor role of amino acid metabolism compared with glycolytic metabolism (Figure 3–21). Although the quantitative contribution of this form of anaerobic metabolism has not been evaluated, it is likely to be low (1 to 2% of the total anaerobic energy production).

The accumulation of lactate by amphibians during activity well illustrates the important role of muscle glycogen as the primary source of anaerobic energy (Table 3–10). The production of lactate not only



Changes in Metabolite Levels:

	μm before dive	μm after dive	μM change
Aspartate	96	73	– 23
α-ketoglutarate	200	110	– 90
Succinate	40	280	+ 240
Alanine	300	650	+ 350
Lactate	90000	160000	+ 70000

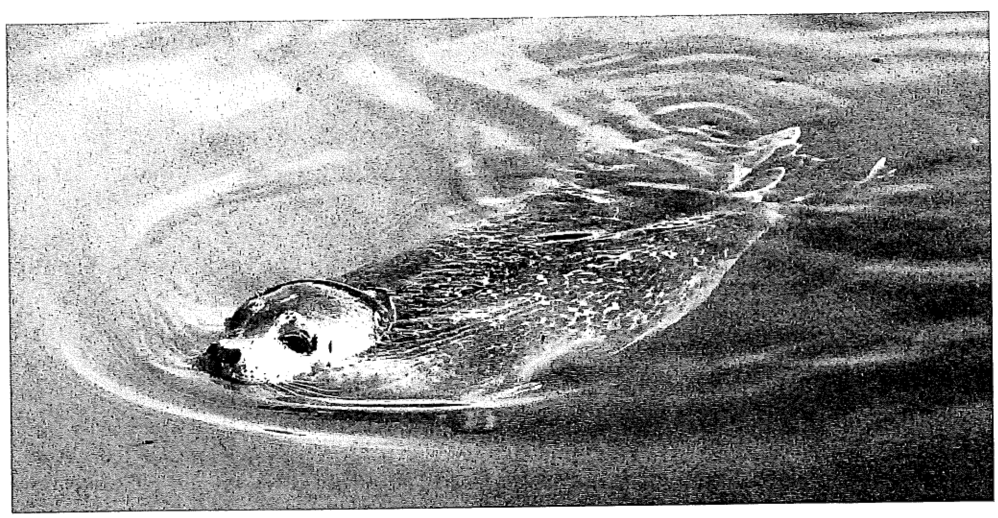


FIGURE 3–21 Schematic for metabolism of substrates to anaerobic end products (lactate, alanine, succinate) for higher vertebrates, and levels of blood metabolites (μm) in seal blood before and after a dive, illustrating the predominance of lactate as the anaerobic end product. *(Values from Hochachka et al. 1975; Hochachka 1980. Photo by George Whitely/Photo Researchers, Inc.)*

TABLE 3–10

Comparison of whole body and tissue lactate levels, glycogen stores, and blood pH for a resting and fatigued clawed frog (*Xenopus laevis*). *(Data from Putnam 1979a; Putnam 1979b.)*

	Rest	Fatigued
Liver glycogen (g%)	10.4	9.3
Gastrocnemius glycogen (g%)	1.8	0.7
Whole body lactate (mg%)	11	213
Blood lactate (mg%)	42	177
Liver lactate (mg%)	29	144
Gastrocnemius lactate (mg%)	98	289
Blood pH	7.62	6.89

eventually leads to end product inhibition of glycolysis, but also has profound effects on acid-base balance. The dissociation of 20 mM lactic acid would be accompanied by formation of 20 mM H^+, and pH would decline by 5 units if the excess H^+ were not buffered! Fortunately, the body has well-developed buffer systems (see Chapter 15), and so the decline in pH due to lactate accumulation is considerably less than 1 pH unit. Nevertheless, pH does decline and this could be an important contributing factor to onset of muscle fatigue.

Anaerobic metabolism is a graded rather than an all-or-none process. There are significant levels of lactate in the blood even at rest, indicating limited lactate formation in muscle and its diffusion into the blood stream. The blood lactate level of humans during graded activity remains at a low, resting level (1 to 2 mM) until about 70% of the maximal work load. After this **anaerobic threshold** is reached, the lactate level increases linearly with work load. The elevated blood lactate levels at high work loads are indisputably the result of muscle anaerobiosis and loss of lactate from muscle, but the "anaerobic threshold" does not necessarily represent the work load at which the muscle first becomes anaerobic. At rest, blood lactate is converted in the liver to glucose for recirculation to the muscle (the Cori cycle). A decrease in blood flow to the liver at moderate work loads could diminish the removal of resting lactate levels from the blood and result in blood lactate accumulation. The anaerobic threshold might also reflect a change in the types of muscle cells that are recruited or a change in substrate from fat to carbohydrate (Davis 1985; Brooks 1985). Consequently, the anaerobic threshold is perhaps more appropriately called a "hyperlactemia threshold," reflecting the observation of elevated blood lactate levels without implying a mechanism for lactate buildup.

The cycle of lactate production in one tissue (e.g., muscle) and its reconversion to glucose in another (e.g., liver) can be of great significance during long-term anaerobic conditions, such as diving (Hochachka 1980). In the Weddell seal, for example, lactate produced in the brain and muscle during diving enters the circulation and is reconverted to glucose in the liver and kidney; the glucose then recirculates to the brain and muscle.

Anaerobic metabolism is important to higher vertebrates not only during physical activity. Some amphibians and reptiles are very tolerant of long-term anoxia, and their lactate levels can be much higher during chronic anoxia than during intense and exhaustive activity. This extreme hyperlactemia is accompanied by a massive acid-base imbalance, which has numerous physiological consequences, including dissolution of bone and elevation of plasma calcium. For example, blood lactate levels exceed 200 mM in freshwater turtles *Chrysemys* after 180 days of submergence in nitrogen-equilibrated water, and total plasma calcium increases markedly from normal levels of about 4 mM, to 120 mM or more (Ultsch and Jackson 1982; Jackson and Ultsch 1982). Up to 2/3 of the total calcium may be bound to lactate, so that as little as 1/3 of the calcium is present in the free, ionized form (Ca^{2+}). Nevertheless, such ionized Ca^{2+} levels would be expected to markedly modify nerve cell excitability and synaptic transmission (see Chapter 6) and muscle contractility (see Chapter 9). For example, elevated Ca^{2+} levels increase the cardiac muscle contractility of the turtle heart *in vitro* (Yee and Jackson 1984).

Energetics of Anaerobic Metabolism

No anaerobic pathways produce as much ATP from a substrate as aerobic metabolism. Anaerobic metabolic pathways have potential ATP yields of 2 to 6 ATP per C_6 unit (Table 3–11). Anaerobic metabolism of glucose to lactate produces 2 moles lactate/mole glucose and 2 moles ATP/mole glucose, i.e., 1 mg lactate $\equiv$ 11 μmole ATP. For glucosyl subunits, the yield is 2 lactate/glucosyl and 3 ATP/glucosyl, i.e., 1 mg lactate $\equiv$ 17 μmole ATP. Aerobic metabolism can potentially yield 38 ATP mole^{-1} of glucose, or 140 to 150 mole ATP mg^{-1} of CO_2. Aerobic metabolism of glucose consumes 6 moles O_2/mole glucose and yields 38 ATP, i.e., 1 ml O_2 (STPD) $\equiv$ 283 μmole ATP. Consequently, anaerobic energy production is generally used to either support a low metabolic rate for a long period or a high metabolic rate for a short period.

TABLE 3–11

Stoichiometry of ATP synthesis by various anaerobic metabolic pathways for glucose (Glu) and glycogen (Glc) compared with aerobic metabolism; the ATP yield is expressed as µmole mg^{-1} of the end product. *(Modified from de Zwaan 1983.)*

		µm ATP mg^{-1}
Anaerobic Metabolism		
Glu + 2 ADP →	2 lactate + 2 ADP	11.2
Glc + 3 ADP →	2 lactate + 3 ATP	16.7
Glc + 3 ADP →	2 lysopine + 3 ATP	7.3
Glc + 2 Arg + 3 ADP →	2 octopine + 3 ATP	6.4
Glc + 2 Gly + 3 ADP →	2 strombine + 3 ATP	11.1
Glc + 2 Ala + 3 ADP →	2 alanopine + 3 ATP	10.1
Glu + 4 ADP →	2 acetate + 4 ATP	33.9
Glc + 2 Asp + 4.71 ADP →	1.71 succinate + 1.14 CO_2 + 2 Ala + 4.71 ATP	23.7
Glc + 0.86 CO_2 + 4.71 ADP →	1.71 succinate + 4.71 ATP	23.7
Glc + 6.43 ADP →	1.71 propionate + 0.86 CO_2 + 6.43 ATP	51.5
Aerobic Metabolism		
Glu + 6 O_2 + 38 ADP →	6 CO_2 + H_2O + 38 ATP	144
Glc + 6 O_2 + 39 ADP →	6 CO_2 + 5 H_2O + 39 ATP	148

Metabolic Fates of Anaerobic End Products

So far we have considered the pathways for anaerobic metabolism and the nature of the end products, but what is the fate of these end products after anaerobic metabolism stops?

Some bivalve mollusks accumulate anaerobic end products in their muscle tissues during anaerobiosis, and these are later reconverted *in situ* to the original substrates when O_2 is available. In other bivalves, however, the anaerobic end products (especially propionate and acetate) are distributed by the hemolymph to other body organs, and they may be used by these other tissues as substrates for aerobic metabolism or for reconversion to the original substrate. Gastropod mollusks, which use the lactate, opine (octopine, alanopine, and strombine), and succinate pathways during anaerobiosis, may also distribute these end products via the hemolymph for subsequent utilization by other tissues (such as liver).

The octopine produced anaerobically in muscle by cephalopods during activity can be used by other tissues as their aerobic metabolic substrate (Storey and Storey 1983). At least some octopine is metabolized *in situ*, but some fraction (perhaps high) is released into the blood after the cessation of muscle anaerobiosis. The octopine is taken up by tissues that either aerobically metabolize the arginine and pyruvate portions (e.g., kidney) or resynthesize glucose and recycle it to muscle (e.g., hepatopancreas). These processes are analogous to the fates of lactate in the Cori cycle of vertebrates.

Vertebrates can accumulate large quantities of lactate during intense activity, and this can result in muscle fatigue either through end product inhibition of glycolysis or pH imbalance. There clearly is adaptive significance to the rapid restoration of normal acid-base status and cellular metabolite levels and replenishment of muscle glycogen stores after activity. However, the removal of lactate after the cessation of activity poses certain problems, particularly for ectothermic animals with low resting metabolic rates. The amounts of lactate that accumulate during activity would sustain resting metabolism for many hours if all of the lactate were slowly oxidized to CO_2 and H_2O for maximum energy economy. For example, the lactate accumulated during a short activity period by the salamander *Amphiuma* would sustain resting metabolism for well over a day (Table 3–12)! A further disadvantage of this strategy for lactate elimination would be the eventual depletion of body glycogen stores. An alternative strategy would be to reconvert all of the lactate to glycogen and glucose, but this requires 6 ATP per glucose and there would be a net loss of 4 ATP in the biochemically futile cycle.

$$\text{glucose} \underset{\substack{\text{recovery} \\ \text{6 ATP}}}{\overset{\substack{\text{2 ATP} \\ \text{activity}}}{\rightleftharpoons}} \text{2 lactate} \qquad (3.31)$$

TABLE 3–12

Comparison of resting oxygen consumption rate, lactate levels accumulated after activity, and time calculated for elimination of lactate via resting aerobic metabolism for an amphibian (salamander, *Amphiuma*) and a mammal (rat, *Rattus*). Each has a body mass of about 100 g.

	Salamander	Rat
Aerobic Metabolic Rate[1]		
ml O_2 hr^{-1}	3.9	120
mmole ATP hr^{-1}	1.09	33.6
Anaerobic Metabolism[2]		
Lactate accumulated (mg)	153	90
ATP from lactate (mmole)	2.6	1.5
Aerobic Recovery[3]		
ATP from aerobic lactate metabolism (mmole)	30.6	18
Time to eliminate lactate	28 hr	32 min

[1] Assuming 1 ml O_2 (STPD) yields 0.28 mmole ATP.

[2] Assuming 1 mg lactate formed from glycogen yields 0.017 mmole ATP.

[3] Assuming 1 mg lactate yields 0.20 mmole ATP when metabolized to CO_2 + H_2O.

The ATP required for glucose resynthesis could be derived from fat or protein metabolism. An advantage of this strategy would be the conservation of body carbohydrate stores for subsequent anaerobic metabolism; remember that glucose and glycogen are the only significant anaerobic metabolic substrates for tetrapod vertebrates.

Another strategy would be the oxidation of some of the lactate to CO_2 and H_2O, to provide the ATP necessary for reconversion of the remaining lactate to glucose or glycogen. The optimal stoichiometry for reconversion of lactate to glucose, by ATP produced from some lactate oxidation, is to oxidize about 0.15 mole of lactate per 0.85 mole reconverted to glucose. This ratio of moles lactate reconverted/mole lactate oxidized is the Meyerhof quotient, e.g., 0.85/0.15 = 5.7.

In endothermic mammals and birds, most lactate is probably oxidized because the high resting metabolic rate can rapidly deplete the lactate (Table 3–12) and restore normal conditions; this has been demonstrated for the rat. In ectotherms, the low resting metabolic rate would only slowly deplete the lactate and the conservation of body carbohydrate stores for further anaerobic activity might be a high priority. There is less oxidation of lactate and more reconversion to glucose/glycogen in toads and lizards.

Bioluminescence

Bioluminescence is the emission by animals of visible light derived from the chemical release of photons. For example, the "flashlight" fish *Photoblepharon* has a large bioluminescent organ under each eye (Figure 3–22). Incandescence, in contrast to bioluminescence, is the emission of visible light at high temperatures. All animals emit nonvisible infrared radiation by incandescence (see Chapter 5), but only some animals are able to emit visible light by the chemical processes of bioluminescence.

The phylogenetically widespread yet scattered distribution of bioluminescence among animals and the diversity of bioluminescent mechanisms indicate the probable independent evolution of light emission many times among these diverse groups of organisms. Nevertheless, there are three basic patterns of bioluminescence among animals: those of bacterial origin, those that are extracellular, and those that are intracellular.

Some squid and teleost fish rely on symbiotic bacteria for light production. For example, the rat fish *Malacocephalus* has anterior and posterior light organs containing bacteria (Figure 3–23A). The

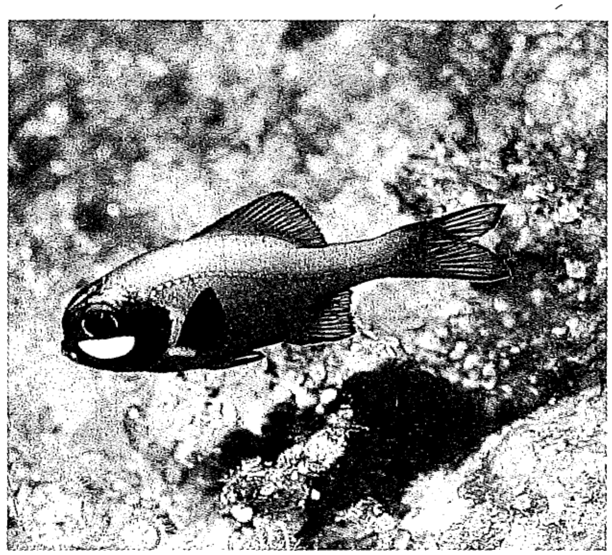


FIGURE 3–22 The bioluminescent "flashlight" fish, *Photoblepharon*, has a light-emitting organ under each eye. The organ emits light continuously by bacterial action, but the organ can be covered and uncovered by a shutter mechanism. *(Photo by David C. Powell, Monterey Bay Aquarium)*

bacteria generally luminesce continuously, and so the light-emitting organs of these animals continuously glow. However, some animals have a mechanism to cover the light-emitting organ. The "flashlight fish" can cover and uncover the large light-emitting organ under each eye with a shutter.

Many marine invertebrates, myriapods, oligochete worms, and teleost fish exude luminescent secretions, and the bioluminescent reaction occurs extracellularly. For example, the earthworm *Eisenia submontana* produces a luminous slime from oral, anal, and dorsal pores in response to irritating stimuli; the source of the secretion is coelomic fluid.

Bioluminescence of some animals is intracellular and may be associated with very specialized structures, including reflecting surfaces and lenses. This pattern of bioluminescence is most developed in teleost fish, cephalopods, and terrestrial insects. The toad fish *Porichthys* has dorsal, lateral, and ventral rows of light-emitting photophores (Figure 3–23B).

Bioluminescence appears to have evolved as a fortuitous by-product of the exogenous metabolic pathways of organisms. The general principle for most forms of bioluminescence is the oxidation (usually, but not always, involving oxygen) of a complex-structured, high-energy organic molecule ("**luciferin**"), catalyzed by a specific enzyme ("**luciferase**") to release photons of light (see Supplement 3–2, page 76). Luciferin and luciferase are general terms used to describe a wide variety of high-energy organic molecules and enzymes.

A well-studied example of extracellular bioluminescence is that of a crustacean, the ostracod *Cypridina*. The luciferin and luciferase are produced by two different kinds of secretory cells in a large light-producing organ located near the mouth; both are simultaneously ejected by muscular contractions. The luciferin is oxidized by O_2 to produce oxyluciferin and carbon dioxide; light is also released (λ_{max} = 460 nm). The *Renilla* coelenterate-type luciferin has a similar reaction sequence (see Supplement 3–2). Accumulation of end products is not a problem because they are secreted into the external medium. All luminescent crustaceans and many fish use this *Cypridina*-type mechanism, although the luminescence is intracellular in some animals (e.g., the fish *Porichthys*).

The energy released as light by bioluminescent reactions is considerable

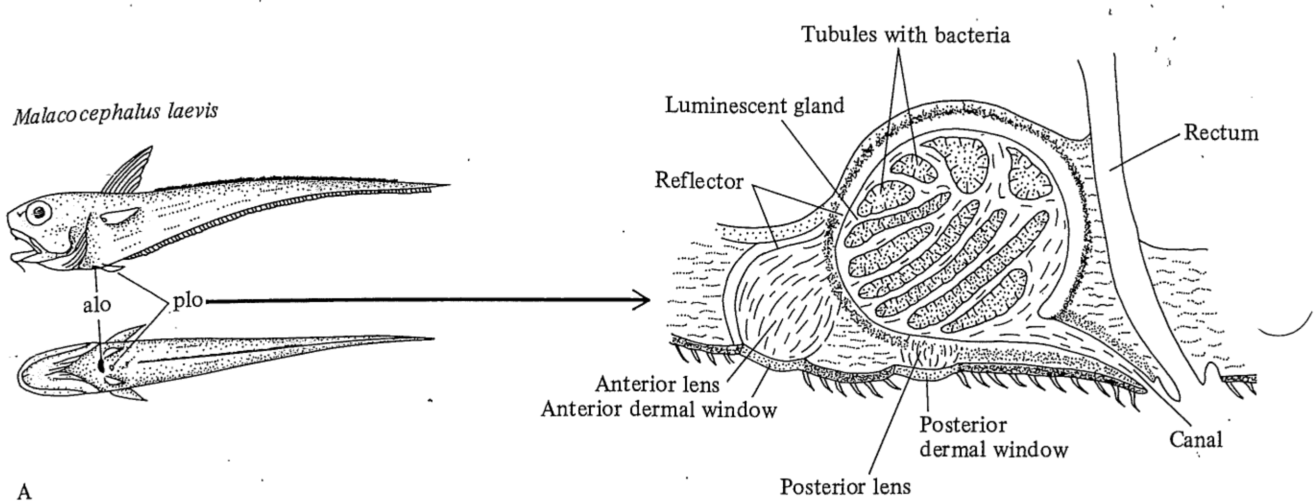
$$E = h\nu = hc/\lambda = 119560/\lambda \text{ kJ mole}^{-1} \quad (3.32)$$

where E is the light energy (kJ mole photons^{-1}), h is Planck's constant, ν is the frequency, λ is the wave length (nm), and c is the velocity of light. There is a considerable variation in the λ and E emitted by bioluminescence (Figure 3–24). E varies from about 254 kJ mole photons^{-1} for blue light (λ = 470 nm) to 234 kJ mole^{-1} for green light (λ = 510). The rarer red bioluminescence of the "railway worm" (λ = 700 nm) has the lowest energy (of about 171 kJ mole^{-1}).

The chemical energy for even the lowest energy bioluminescence cannot come from ATP on a mole ATP/mole photons basis ($\Delta G^{\circ\prime}$ for ATP hydrolysis is 30 to 60 kJ mole^{-1}). In bacteria, only 0.15 to 0.20 moles of photons are produced per mole of substrate ($FMNH_2$). The chemical energy yield of $FMNH_2$ is about 150 kJ mole^{-1} and that of the emitted light is about 40 to 50 kJ mole^{-1}, and so this bioluminesce system is energetically feasible without additional energy input. *Cypridina*-type and coelenterate-type bioluminescence require O_2 for the direct reaction with luciferin (not for the cellular production of ATP). *Renilla* has a quantum yield of about 0.055 moles photons per mole substrate. This is equivalent to light production of about 0.61 J per ml O_2 used (*cf* 20.1 J ml O_2^{-1} for aerobic metabolism). The *Cypridina* luciferin has a quantum yield of about 0.3, which is equivalent to about 3.5 J ml O_2^{-1}. For the firefly, each of the reacting luciferins may release a photon (214 kJ mole^{-1}), with the hydrolysis of only 1 ATP (30 to 40 kJ mole^{-1}), to form oxyluciferin. The synthesis of luciferin and reconversion of oxyluciferin to luciferin must therefore require considerable energy expenditure.

The metabolic cost of *Cypridina*-type luminescence has been estimated for photophores of the teleost fish, *Porichthys* (Mellefet and Baguet 1984). Quiescent photophores have a VO_2 of about 0.07 nmole O_2 min^{-1} photophore^{-1}, which is about 2% of its resting O_2 requirement. Luminescence increases the O_2 requirements of the photophores to about 0.25 nmol O_2 photophore^{-1} (about a five times increase). About 1 nmol O_2 photophore^{-1} provides for the production of about 76 megaquanta of photons (76 10^6 photons) at a wavelength of about 470 nm; this is equivalent to about 1.4 J ml O_2^{-1}. The deep sea fish *Argyropelecus* appears to maintain a prolonged luminescence of photophores, without an increase in O_2 requirement (Mellefet and Baguet 1985). The bioluminescence of *Argyropelecus* may involve a coelenterate-like, preactivated Ca^{2+}-sensitive photoprotein intermediate, rather than the direct oxidation of luciferin by O_2, so that the metabolic cost of "preactivation" is expended over long periods between luminescence activity.

The many different functions proposed for bioluminescence (Buck, 1978) can be divided into three general categories: (1) roles of light emission by an



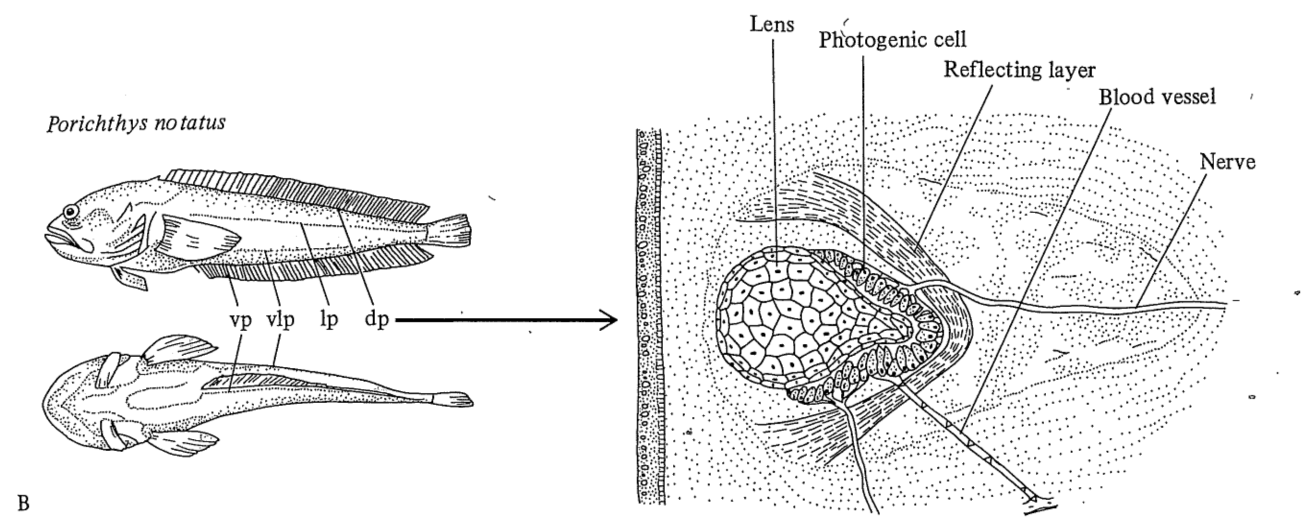


FIGURE 3–23 **(A)** Bacterial light organ of the rat-tail fish, *Malacocephalus laevis*; alo, anterior light organ; plo, posterior light organ. **(B)** Intracellular bioluminescent organ of the toad fish, *Porichthys notatus*; vp, ventral photophore row; vlp, ventro-lateral photophore row; lp, lateral photophore row; dp, dorsal photophore row. *(From Herring and Morin 1978.)*

individual; (2) intraspecific communication; and (3) interspecific interactions. Emitted bioluminescence can be used for food gathering by attracting prey to light (e.g., deep-sea angler fish have a luminous "lure," and various fish have luminous photophores on their lips or within their mouth). Some bioluminescent animals might benefit during food gathering by improved vision owing to their emitted light. A variety of animals use emitted light for defense, either by startling and confusing predators (e.g., the flashlight fish *Photoblepharon* has a "blink-and-run" behavior to confuse predators), as camouflage, as concealment by counter-shading the ventral surface to match the surroundings, as a decoy to confuse predators, or as a repellent to light-avoiding predators. Many examples of bioluminescence are involved with intraspecific communication for either courtship (e.g., many female fireflies attract males by a species-specific flashing pattern) or congregation of many individuals into large groups (e.g., some Oriental fireflies aggregate into courtship congregations with synchronized light-flashing; many schools or shoals of fish, squid, and crustaceans have nonsynchronized bioluminescence). The pred-

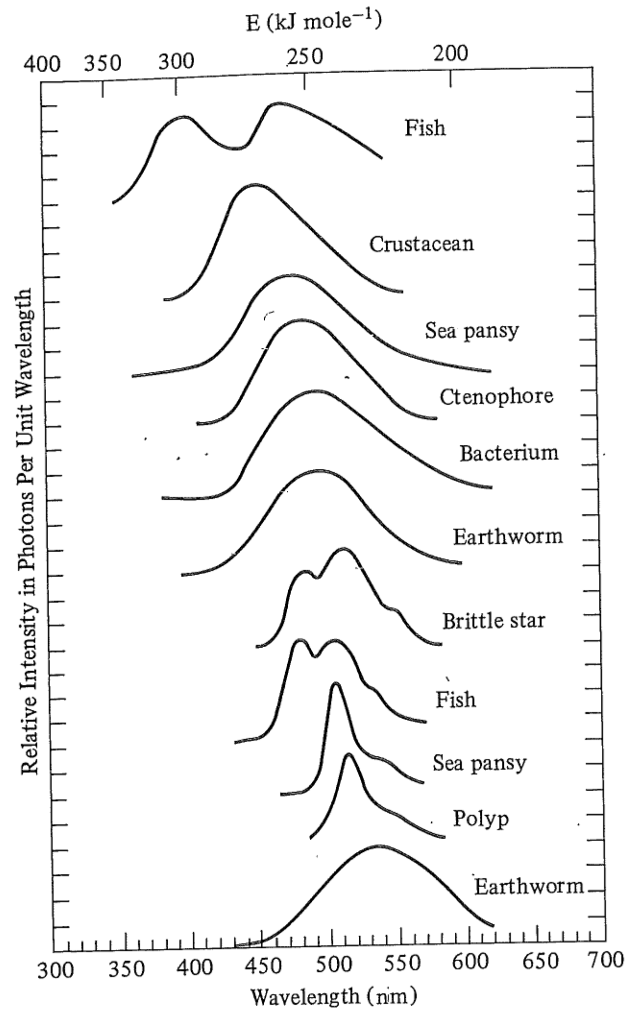


FIGURE 3–24 Wavelength (λ; nm) and energy (kJ mole photons^{-1}) emitted by bioluminescent organisms. Searsid fish exudate, crustacean *Cypridina* reaction *in vitro*, sea pansy *Renilla* reaction *in vitro*, ctenophore *Mnemiopsin* emission *in vivo*, photobacterium emission *in vivo*, earthworm *Diplocardia* emission *in vivo*, brittle star emission *in vivo*, fish *Porichthys* emission *in vivo*, sea pansy *Renilla* emission *in vivo*, hydropolyp *Clytia* emission *in vivo*, earthworm *Diplotrema* emission *in vivo*. *(From Wampler 1978.)*

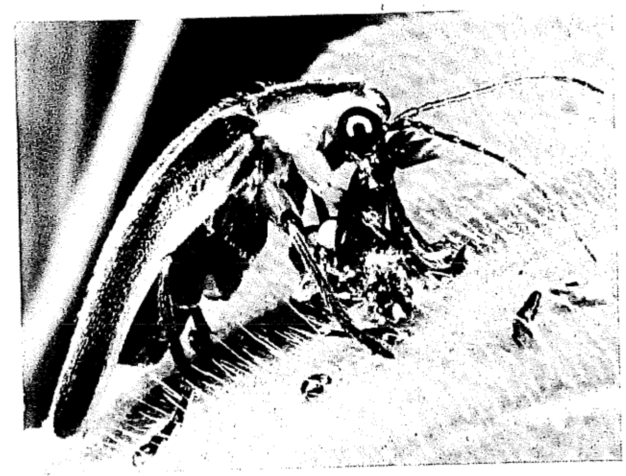


FIGURE 3–25 A female firefly *Photuris versicolor* consumes a male firefly *Photinus tanytoxus*, which she has attracted with false signals that mimic that of the female *Photinus tanytoxus*. *(Photograph by J. E. Lloyd.)*

atory use of bioluminescence by some female fireflies is an example of interspecific communication; females of several species of *Photuris* fireflies lure male *Photinus* fireflies by mimicking the courtship flash pattern of the female *Photinus* (Figure 3-25).

Thermogenesis

Endothermic animals use chemical energy to generate heat for thermoregulation. A high rate of heat production enables some animals (mammals, birds, a few reptiles, many insects, and some large elasmobranch and teleost fish) to elevate body temperature above ambient temperature and often to precisely regulate body temperature independent of ambient temperature (see Chapter 5). Insects and fish produce heat when flying, walking, or swimming by contracting locomotory muscles. Nonmuscular sources of heat are also very important in mammals and birds for the regulation of body temperature since they are continuously endothermic (although some abandon endothermy for short periods of daily torpor or hibernation).

There are at least two nonmuscular mechanisms for the specialized production of metabolic heat: futile cycles of ATP synthesis and degradation, e.g., Na^+-K^+-ATPase and leaky cell membranes, and "proton leaks" in the inner mitochondrial membrane, e.g., brown adipose tissue (BAT).

Leaky Membranes

Endothermic mammals and birds have about 4 to 5 × the metabolic rate of similar-sized ectotherms (e.g., lizards) at the same body temperature (see Chapter 4). This difference in metabolic rate is also evident *in vitro* for tissue homogenates and slices. A large portion of the energy expended by the tissues of endotherms appears to be used by the Na^+-K^+-ATPase to actively pump K^+ into and Na^+ out of the cells. However, the transcellular membrane K^+ and Na^+ gradient is similar for endotherms and ectotherms (despite the higher energy expenditure of endotherm cells for ion pumping) and so the membranes of endotherm cells appear to be considerably more "leaky" to K^+ and Na^+ (Else and Hulbert 1987). Thus, a futile cycle of passive influx of Na^+ and K^+ efflux, and active transport of Na^+ out and K^+ in, provides a major source of metabolic heat. This futile cycle, and perhaps other similar ATP-requiring futile cycles, provide metabolic heat for thermoregulation. In contrast, brown adipose tissue provides heat without requiring the futile synthesis and degradation of ATP.

Brown Adipose Tissue

Brown adipose tissue (BAT) is a highly specialized form of fat, found only in certain mammals. Some hibernators, some cold-adapted mammals, and some newborn mammals (including humans) have brown fat. BAT is the only animal tissue with the sole function of heat production. Brown fat is generally deposited in the thorax, and its vascular drainage is directed to the vital organs (heart, lungs, etc). The BAT content of mammals can vary with age and between species, but it is generally present in those mammals, and at those stages of development, when the capacity for metabolic heat production is of critical importance, e.g., hibernating mammals that need to warm up and newborn mammals that have a high surface/volume, hence high heat loss.

BAT cells are quite distinctive from normal white lipid deposits; they have numerous small lipid droplets and are packed with circular mitochondria, characterized by many cristae (Figure 3–26A). The normal coupling of electron transport and the proton gradient across the inner mitochondrial membrane, resulting in ATP synthesis, can be short-circuited in BAT mitochondria. Heat can therefore be produced by electron transport without concomitant ATP synthesis.

Activation of BAT is primarily hormonal, through the β-adrenergic pathway (Cannon and Nedergaard 1985). Norepinephrine (noradrenalin) binds to β-adrenergic receptors on the cell membrane and causes cyclic-AMP synthesis (Figure 3–26B). The cyclic-AMP activates protein kinase, which in turn activates a hormone-sensitive lipase (HSL) that hydrolyzes intracellular triglyceride stores to free fatty acids. The cyclic-AMP also activates lipoprotein lipase, which hydrolyzes circulating triglycerides to fatty acids. Thus, fatty acids are made available to the cells for β-oxidation to acetyl-CoA, which then enters the citric acid cycle and produces $NADH/H^+$ and $FADH_2$.

But how is electron transport uncoupled from ATP synthesis? Free fatty acids appear to control the activity of a special protein, **thermogenin**, which is found only in the inner mitochondrial membrane of BAT mitochondria. Thermogenin can bind purine nucleotides (e.g., ATP) but in particular binds GDP; it also modifies the ionic permeability of the inner mitochondrial membrane by providing channels for Cl^- and H^+ exchange. A low concentration of GDP increases the permeability of the thermogenin protein to H^+ and Cl^-, but the GDP concentration of the cells is probably always sufficiently high to maintain minimal H^+ and Cl^- permeabilities. However, the presence of even submicromolar concentrations of fatty acids affects thermogenin and increases the permeability of the inner mitochondrial membrane to H^+ and Cl^-, effectively short-circuiting the proton gradient established by the electron transport chain. This allows β-oxidation and maximal rates of heat production without concomitant ATP synthesis.

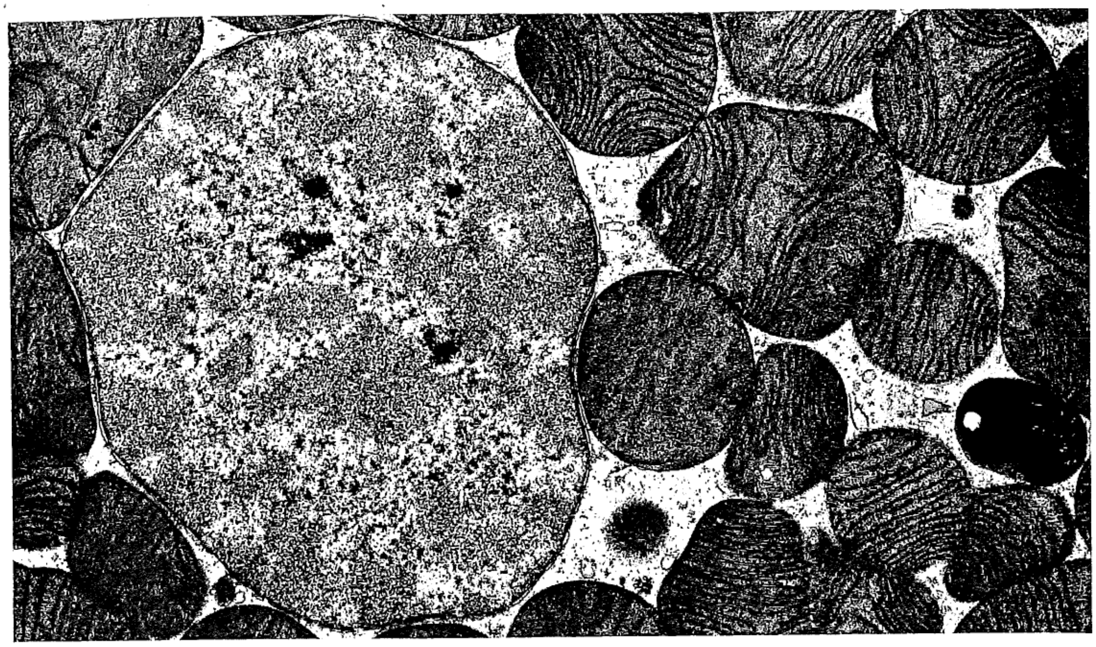
BAT is an important component of the elevated capacity of cold-acclimated mammals to produce heat by nonshivering thermogenesis. There is both an increased thermogenin content of BAT mitochondria and increased mitochondrial density with cold acclimation (Sundin et al. 1987).

Defense

Thermal energy can be used as a defense mechanism as well as for thermoregulation. For example, the defensive noxious quinone spray of carabid "bombardier" beetles (Figure 3–27A) is made more effective by its high temperature, 60 to 100° C! The explosive discharge of the hot spray is clearly audible, hence the name bombardier beetle.

Most carabid beetles discharge a cold defensive secretion, but bombardier beetles belonging to three separate subfamilies—the Carabinae (*Brachinus*), Paussinae (*Goniotropis*), and Metriinae (*Metrius*)—have a hot discharge. It is not clear if the bombardier strategy evolved independently in these three separate subfamilies, since the biochemistry and thermodynamics are similar for bombardier beetles of each subfamily (Aneshansley et al. 1983).

The secretory portions of the bombardier beetle's glands (Figure 3–27B) synthesize hydroquinone (and methylhydroquinone); hydrogen peroxide; and a hydrocarbon, pentadecane (Schildknecht and Holoubek 1961). The secretion is stored in a reservoir portion of the double-chambered gland. The second chamber of the gland is the "reaction" chamber, which contains a mixture of catalase and peroxidase enzymes. To discharge a hot, noxious spray, the beetle contracts a small muscle to open a valve between the reservoir and reaction chambers. This



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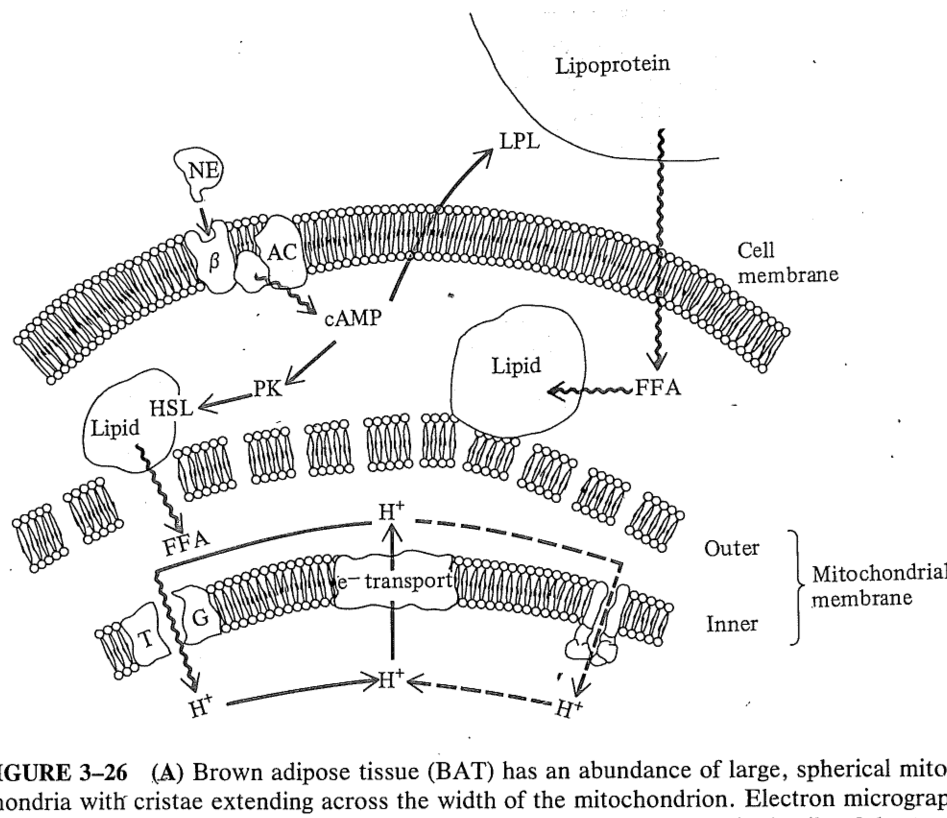


B

FIGURE 3–26 (**A**) Brown adipose tissue (BAT) has an abundance of large, spherical mitochondria with cristae extending across the width of the mitochondrion. Electron micrograph is of BAT from a recently aroused bat. *(From Fawcett 1986.)* (**B**) Schematic details of the β-adrenergic pathway for thermogenesis by brown adipose tissue (BAT) showing norepinephrine (NE) stimulation of surface receptors, activation of lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL), formation of free fatty acids (FFA), and activation of thermogenin (TG) to increase the permeability of the inner mitochondrial membrane to H^+ and Cl^-. *(Modified from Cannon and Nedergaard 1985.)*

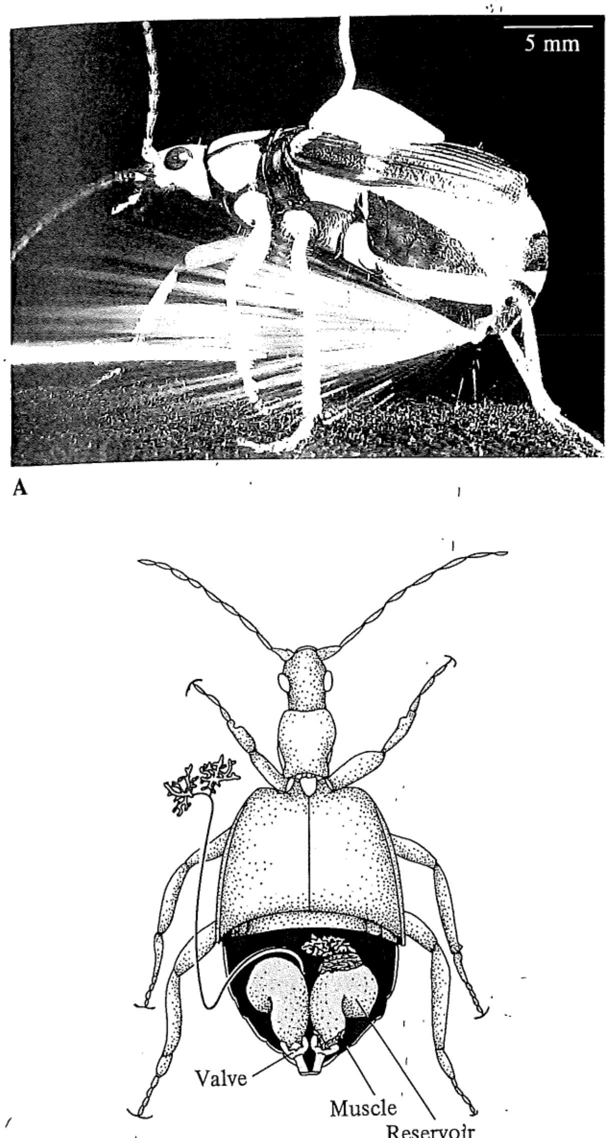


FIGURE 3–27 (A) The Bombardier beetle can accurately discharge a hot, noxious defensive spray of p-benzoquinones in pulses of 500 sec^{-1}. *(From Dean et al. 1990.)* **(B)** The Bombardier beetle has paired defensive glands, each with a secretory gland (uncoiled on the left) and reservoir. A muscle controls the release of hydroquinones and hydrogen peroxide into a reaction chamber where the enzymatic catalytic production of quinones, oxygen, and water releases heat. Sufficient heat is released to vaporize some of the reactant solution. *(Modified from Eisner 1970.)*

allows some of the reactant fluid (25% hydrogen peroxide, 10% hydroquinones) to enter the reaction chamber where peroxidases promote oxidation of the hydroquinones to quinones, and catalases decompose the hydrogen peroxide to water and oxygen.

The reactions that occur in the reaction chamber are very exothermic. For example, the reaction of hydroquinone with peroxide to form quinone and water has a $\Delta G°$ of -203 kJ $mole^{-1}$. The actual heat production of the reaction solution is about 790 J g^{-1} solution. This is sufficient heat to warm the solution to 100° C (about 334 J g^{-1}) and to vaporize about 1/5 of the solution (about 455 J g^{-1}).

Bombardier beetles are not only able to emit a hot, noxious spray, but they have a very accurate aim! *Brachinus* rotates the tip of its abdomen (where the glands open) to effect an accurate aim! *Goniotropis* has a "high technology" targeting system, consisting of a pair of curved and grooved abdominal flanges. The ejected spray follows the curvature of the flanges and is directed forward towards the target (Eisner and Aneshansley 1982).

The release of cornicle wax by aphids is another even more bizarre defense system, utilizing an exothermic reaction (Edwards 1966). The aphid secretes from the cornicles a globule of liquid wax, which crystallizes on contact and deters or immobilizes predators. The waxes have a sufficiently high melting point (38° C for *Aphis* to 48° C for *Macrosiphum*) that they appear to be secreted in a supercooled state. The supercooled wax is released on contact with a predator, such as the hymenopteran wasp *Aphidius*, by a reflex contraction of the cornicle muscle that opens the terminal cornicle valve. A positive abdominal hydrostatic pressure is thought to then expel the liquid wax. A foreign object such as the wasp acts as a nucleating point to immediately crystallize the wax. The thermodynamics of this spontaneous change of state from liquid to solid wax is similar to that for supercooled water crystallizing to ice, when it is "nucleated" by a small ice crystal. Such a charge of state for a supercooled solution is an exothermic reaction and is irreversible.

Summary

Animals use chemical energy to support energy-requiring cellular processes (e.g., ion pumps, biosynthesis) and to do work on the external environment (e.g., muscle, ciliary movement).

The Gibbs free energy change (ΔG) for a chemical reaction depends on the change in heat energy, or enthalpy (ΔH), temperature (T), and degree of disorder, or entropy (ΔS). A negative ΔG (i.e., energy released by the reaction) can be used in biological systems to do mechanical work ($f.\Delta s$), to transport molecules against a chemical gradient ($\mu.\Delta n$), to transport ions against an electrical gradient ($E.\Delta q$), or to emit light ($h\mu$);

$$\Delta G = H - T.\Delta S = f.\Delta s + \mu.\Delta n + E.\Delta q + h\nu$$

Diffusion is passive mass transport from a high concentration to a low concentration. Fick's first law of diffusion states that the diffusion flux (J) is dependent on the diffusion coefficient (D), the area for diffusional exchange (A), the concentration difference (ΔC), and the path length for diffusion (Δx). For 1-dimensional planar diffusion, we have the following.

$$J = -DA\Delta C/\Delta x$$

The standard Gibbs free energy change ($\Delta G°$) for complete oxidation of typical metabolic substrates is considerably negative, e.g., for glucose, -2854 kJ mole^{-1}; palmitate, -9791 kJ mole^{-1}; and alanine, -1619 kJ mole^{-1}. The $\Delta G°$ for individual biochemical reactions is considerably less, about $+40$ to -40 kJ mole^{-1}. Biochemical reactions with negative $\Delta G°$, about -20 to -40 kJ mole^{-1}, provide the energy for active cellular reactions. Adenosine-triphosphate (ATP) hydrolysis is the most common energy-providing cellular reaction ($\Delta G° = -30.5$ kJ mole^{-1}).

Oxidation of glucose to CO_2 and water yields about 2854 kJ mole^{-1} of energy; about 38 mole of ATP are formed from ADP per mole of glucose. Two ATP are formed by the Embden-Meyerhof pathway (glucose $\rightarrow$ 2 pyruvate). A further 2 GTP are formed by the citric acid cycle and 34 ATP are formed via the electron transport system from $NADH/H^+$ and $FADH_2$. About 1693 of the total 2854 kJ mole^{-1} of energy is released as heat by cellular metabolism of glucose, and 1161 is converted to the chemical energy of ATP; the efficiency of cellular glucose metabolism is about 41%. Cellular metabolism of lipids (e.g., fatty acids) and proteins (and amino acids) has an efficiency of about 60% and 30%, respectively.

The cellular metabolic pathways of many animals are adapted for ATP synthesis in the absence of oxygen, due to either limitations of the O_2 delivery system during intense activity or to environmental hypoxia or anoxia. Glycolysis is a common anaerobic pathway. Glucose is metabolized to pyruvate, then the end product lactate; 2 ATP are synthesized per glucose. Most vertebrates and many invertebrates utilize mainly glycolysis for anaerobic metabolism. Many invertebrates however, have other anaerobic pathways for increased ATP synthesis from glucose, utilization of additional substrates (e.g., amino acids), and to form other nonaccumulated end products (e.g., propionate, succinate, acetate, methyl-butyrate, and ethanol).

In some biochemical reactions, part of the free energy change is released as photons of light, rather than as heat. The chemical reactions generally involve an activated, or high-energy, substrate (luciferin) and an enzyme (luciferase) that oxidize the substrate; light is released by the oxidation reaction. Bioluminescence has evolved independently in a number of diverse protozoans and animals. Some animals utilize symbiotic luminescent bacteria for their light production. Some exude mixtures of luciferin and luciferase for extracellular light production. Some animals have intracellular biochemical pathways for light production.

The biochemical production of heat has no useful role in ectothermic animals, but endothermic animals use metabolic heat to regulate body temperature. These endotherms can augment metabolic heat production by increasing the activity of ATP-requiring cellular processes, such as muscle contraction (e.g., shivering) or ion pumping (e.g., cellular Na-K ATPase). Some mammals have a special lipid store, brown adipose tissue (BAT), the sole function of which is metabolic heat production. In BAT, heat is produced by short-circuiting the synthesis of ATP in mitochondria, rather than by using ATP hydrolysis to release heat. A protein, thermogenin, is stimulated by fatty acids to increase the permeability of the inner mitochondrial membrane to H^+, thereby short-circuiting the proton gradient across the membrane.

Biochemical heat production is also important as a defense mechanism for the bombardier beetle, which ejects a hot, noxious spray. The heat produced in the defensive secretion by the reaction of hydrogen peroxide with hydroquinones is sufficient to vaporize about 1/5 of the secretion.

Supplement 3–1

Diffusion in Various Geometries

The general form of Fick's law of diffusion is

$$J_j = -D_jA\frac{\delta C}{\delta x} - D_jA\frac{\delta C}{\delta y} - D_jA\frac{\delta C}{\delta z},$$

where J_j is the flux of species j; D_j is the diffusion coefficient for species j; and $\delta C/\delta x$, $\delta C/\delta y$, and $\delta C/\delta z$ are the partial derivatives of the concentration with respect to the x, y, and z coordinates. The negative sign indicates that the flux is from the high to the low concentration. The exact geometrical form of the diffusional exchange surface determines the complexity of the diffusion equation. For example, in simple 1-dimensional planar geometry, the general diffusion equation reduces to

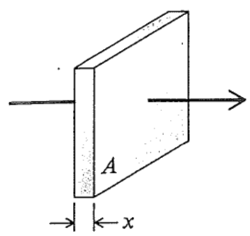
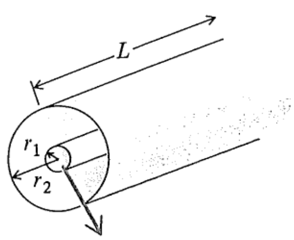
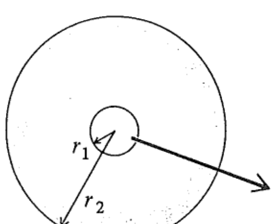
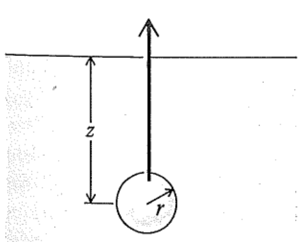
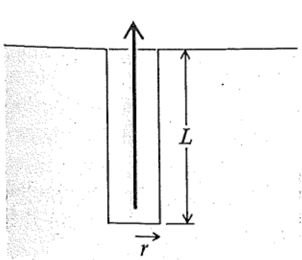
$$J_j = -D_j A \Delta C / \Delta x.$$

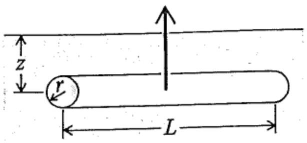
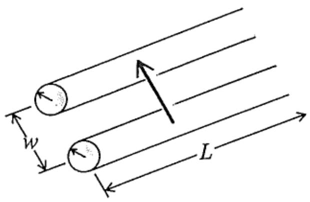
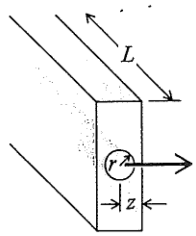
Biological examples of such simple diffusion geometries would be gas exchange across the lung alveolar surface in mammals or diffusional exchange of ions across the body surface of aquatic animals.

The equations for diffusional flux across a variety of physiologically relevant spatial organizations of exchange surfaces are given below, with some biologically relevant examples of such exchange geometries. Note that all equations are of the same general form

$$J_j = -FD\Delta C,$$

where F is the diffusion factor and ΔC the concentration difference. The same equations apply to heat transfer as well as mass flux by diffusion.

Configuration	Flux	Biological Examples
	$J = -\frac{A}{x} D\Delta C$	Alveolar surface exchange in higher vertebrates (not birds). x = thickness of alveolar membrane; A = area.
	$J = -\frac{2\pi L}{ln(r_2/r_1)} D\Delta C$	Cutaneous gas exchange in long, thin animals; r_2 is the boundary layer thickness.
	$J = -\frac{4\pi}{(1/r_1) - (1/r_2)} D\Delta C$	Cutaneous gas exchange in spherical animals; r_2 is the boundary layer thickness.
	$J = -\frac{4\pi r}{1 - r/2z} D\Delta C$	Gas exchange from subterranean animal nest chamber ($z > r$).
	$J = -\frac{2\pi L}{ln(2z/r)} D\Delta C$	Insect tracheal system; air capillaries of bird lung (L >> r).

Configuration	Flux	Biological Examples
	$J = -\frac{2\pi L}{ln(2L/r)}D\Delta C$	Blood vessel under surface of skin ($L >> r$; $z >> 3r$).
	$J = -\frac{2\pi L}{\cosh_{-1}\frac{(w^2 - r_1^2 - r_2^2)}{(2\, r_1\, r_2)}}D\Delta C$	Countercurrent exchange between parallel artery and veins ($L >> r_1, r_2$; $l >> w$)
	$J = -\frac{2\pi L}{ln(4z/\pi r)}D\Delta C$	Capillary in fish gill lamella ($z > r$).

Supplement 3–2

Mechanisms of Bioluminescence

Bioluminescence occurs when some of the chemical energy of biochemical reactions is released not as heat but as photons of light. This conversion of chemical energy to light is typically due to highly strained chemical structures of luciferins, often with peroxide bonds (—O—O—). The light energy is emitted when the chemical structure returns from an excited, high-energy state to a lower energy, stable ground state. Different bioluminescent organisms have various types of luciferins that are used in different biochemical pathways to release light.

There are a variety of types of luciferin, the bioluminescent substrate for light production, although there are some general similarities in structure for some luciferins from some very different animals. For example, note the basic similarity of the reactive portions of the *Renilla*, *Aequorea*, and *Cypridina* luciferins (shaded regions).

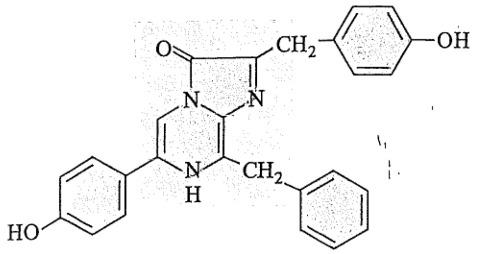
The luciferin of bioluminescent bacteria is a complex formed from a flavoprotein ($FMNH_2$) and a long-chain aldehyde (R—CHO; R is longer than C_6). The FMN-RCHO complex is oxidized to FMN and a carboxylic acid in the presence of a luciferase and oxygen, producing H_2O and releasing photons of visible light (A). Most of the photons have a wavelength of about 480 nm (λ_{max}) with energy ($h\nu$) of 249 kJ mole photons^{-1}. The overall stoichiometry of the reaction is

$$FMNH_2 + O_2 + RCHO \xrightarrow{\text{luciferase}} 0.15\, h\nu + FMN + H_2O + RCOOH.$$

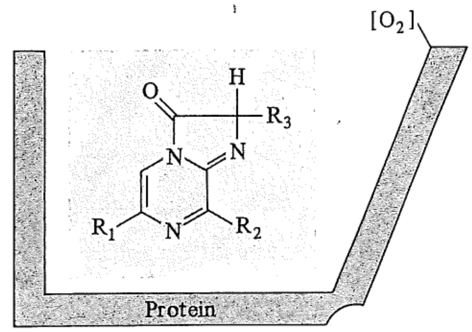
The bioluminescence system of an anthozoan coelenterate, the sea pansy *Renilla*, requires oxygen but not ATP. The oxidation of reduced oxyluciferin releases light. In some coelenterates, including *Renilla*, the emitted light is green (λ_{max} = 510 nm) *in vivo* but is blue (λ_{max} = 470) *in vitro*, indicating a transformation of the chemical energy from the oxyluciferin complex by a green fluorescent protein (GFP).

Calcium-activated photoproteins emit light in the presence of calcium ions. Examples are aequorin from the hydrozoan coelenterate *Aequorea* and mnemiopsin from the ctenophore *Mnemiopsis*. The photoprotein consists of protein and a coelenterate-type luciferin. Oxygen is incorporated into the structure of the photoprotein during its synthesis, and so free O_2 is not required for the luminescence process (unlike other coelenterate-type luminescent reactions). A model for Ca^{2+}-activated bioluminescence of aequorin and mnemiopsin is essentially identical to *Renilla* bioluminescence except for the preincorporation of O_2 and the excitatory role of Ca^{2+}. The fluorescent properties alter only slightly in the absence of Ca^{2+} to a bright blue (*in vitro*) or green (*in vivo*) in the

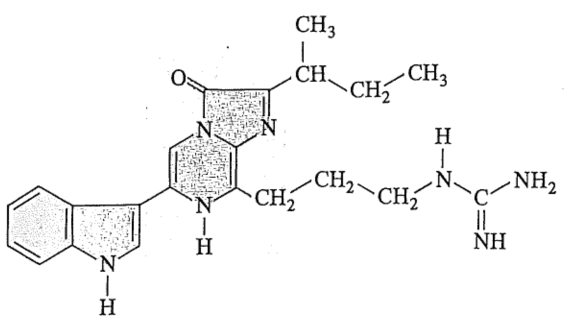
Renilla (sea pen)



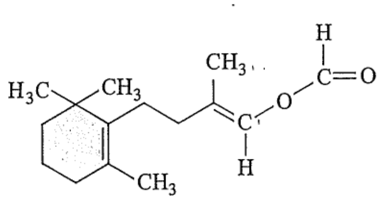
Aequorea/Mnemiopsin (hydrozoan/comb jelly)



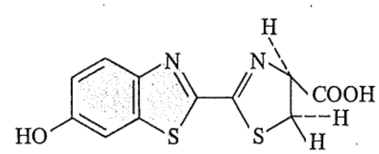
Cypridina (Ostracod)



Latia (Limpet)



Photinus (firefly)

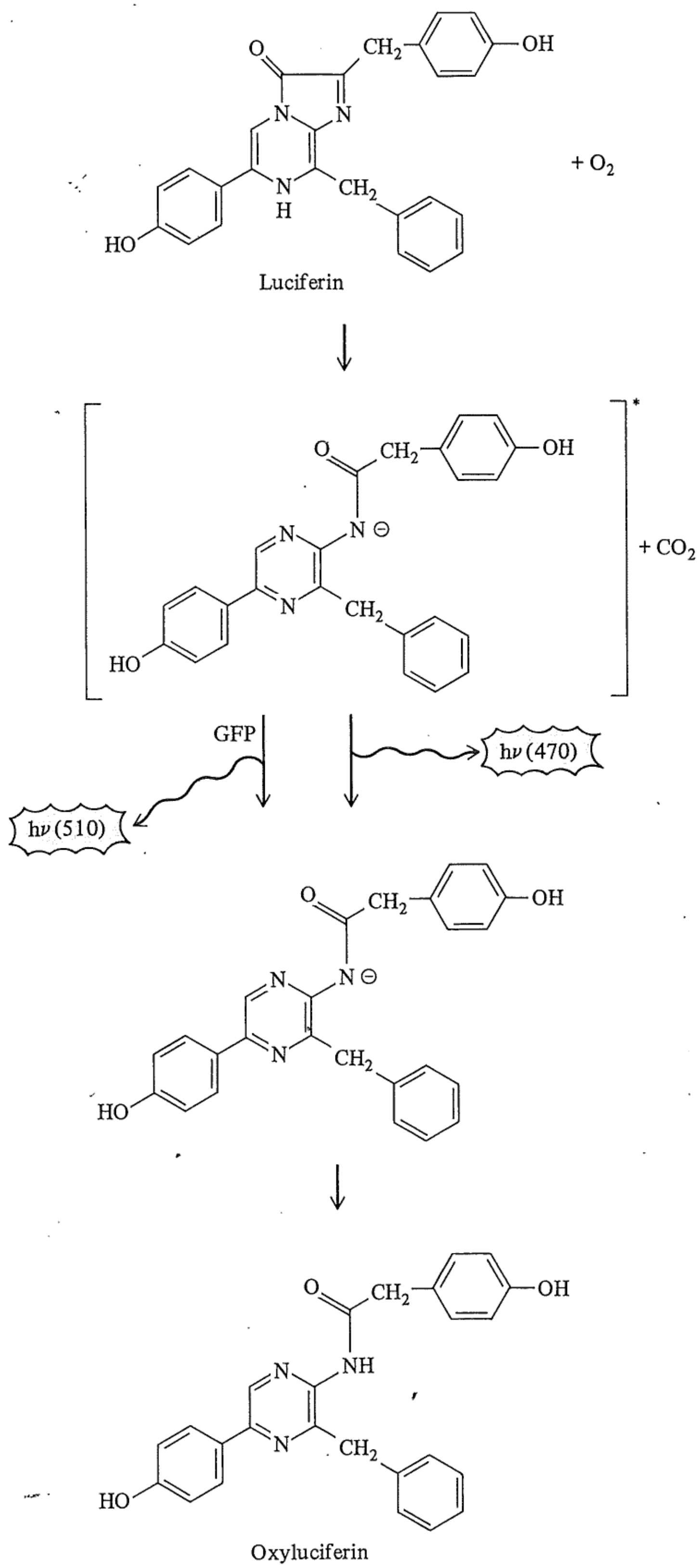


Structure of luciferins.

presence of Ca^{2+}. This mechanism has enabled physiologists to measure the amount of Ca^{2+} present inside cells by injecting aequorin into the cell and then measuring the light emission.

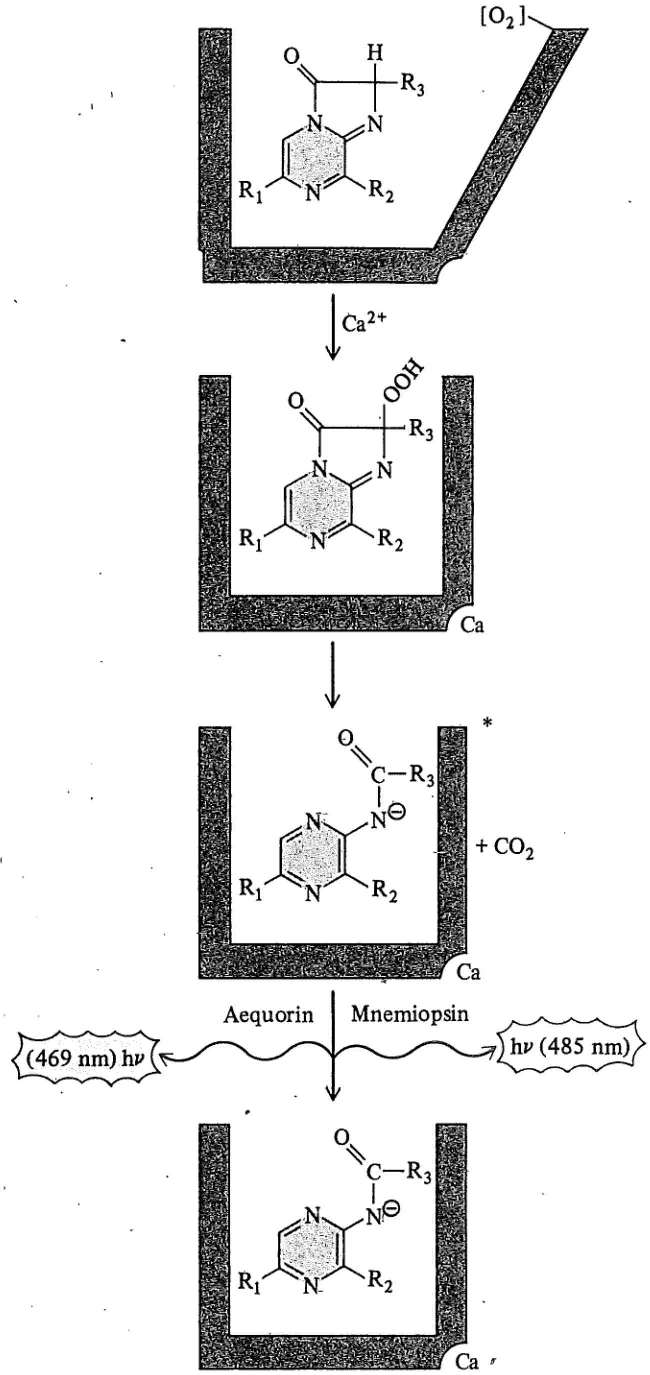
The bioluminescence systems of terrestrial earthworms such as *Diplocardia* are poorly known. Hydrogen peroxide reacts with the luciferin to release light, and it is likely that O_2 is converted to H_2O_2 by an oxidase enzyme when coelomic cells containing the luciferin are lysed. This occurs prior to the exudation of coelomic fluid from the mouth, anus, and dorsal pores. Hydrogen peroxide also activates the bioluminescent system of the freshwater limpet, *Latia*. The *Latia* luciferin combines with purple protein, then reacts with H_2O_2 to release light (λ_{max} = 535 nm).

A very different series of reactions requiring ATP is seen for insect luminescence, typified by that of the firefly beetle *Photinus* (family Lampyridae). The reduced form of the luciferin combines with ATP in the presence of a luciferase to form a luciferyl adenylate complex. This complex then decomposes to produce the oxidized luciferin carbon dioxide and light (λ_{max} = 560 nm). The luciferin reacts with ATP to form a luciferyl-adenylate intermediate, which then reacts with O_2 to form oxyluciferin and CO_2, and releases light. (See Cormier 1978; McElroy and DeLuca 1981; Hastings 1978; Lehninger 1982.)



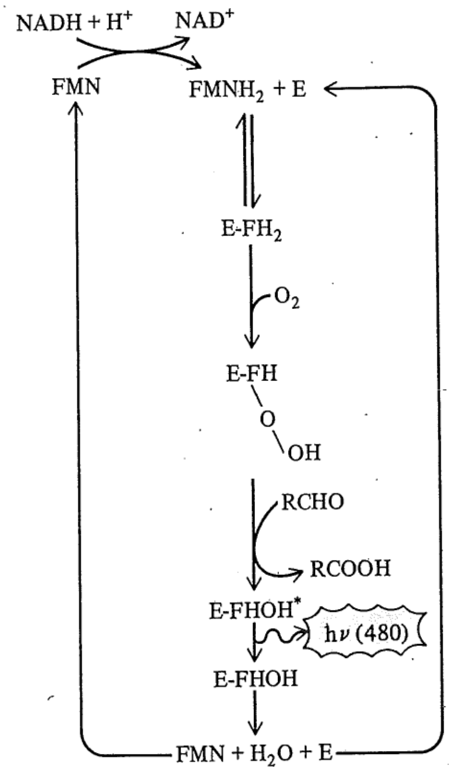
Bioluminescence in the coelenterate *Renilla* emits either blue light (470 nm) directly from the blue fluorescent photoprotein or green light (510 nm) by the green fluorescent protein associated with the luciferin complex.

Luciferin (reduced)

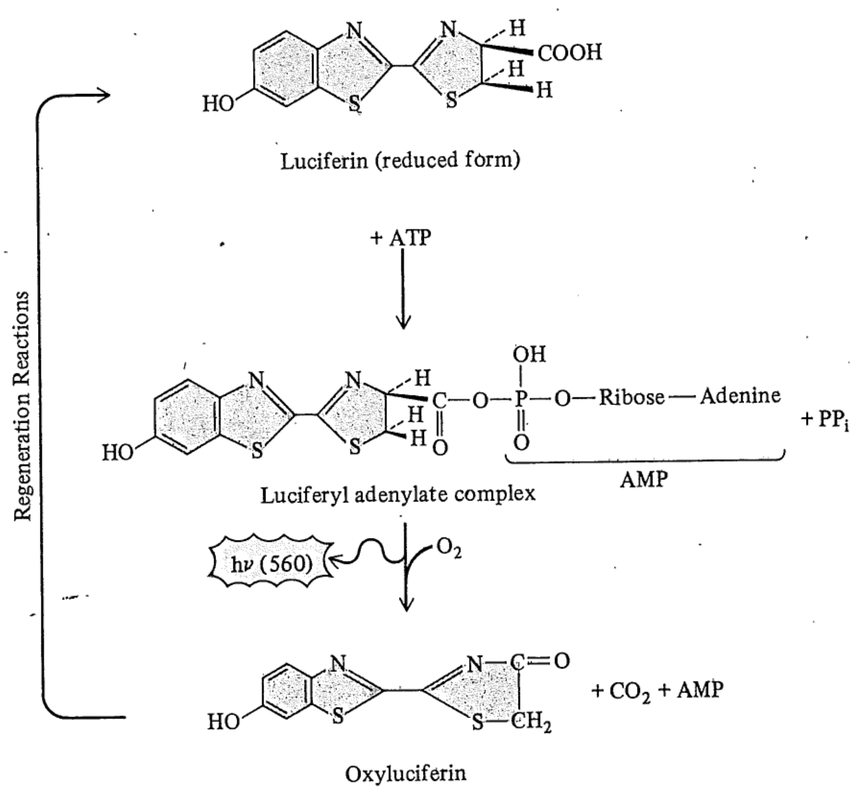


Oxyluciferin

Bioluminescent system of coelenterate-type calcium-activated photoproteins, such as aequorin and mnemiopsin. Light is continually emitted from the high-energy activated complex as long as calcium is present.



Reaction sequence for bacterial bioluminescent release of light (hν).



Bioluminescent reactions for firefly luciferin and ATP to release light.