PHYSIOLOGY OF THE AMPHIBIA

Edited by JOHN A. MOORE

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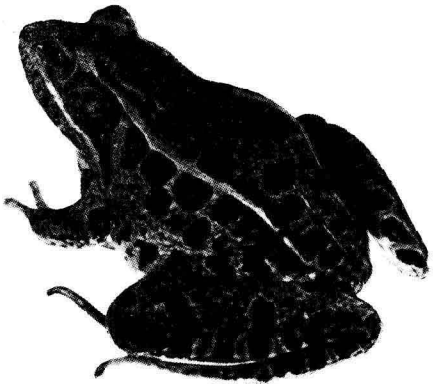

PHYSIOLOGY

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PREFACE

In many branches of animal biology it is assumed that *the* animal is *the* frog. This is less so today than a generation ago, now that the techniques and equipment for experimentation with homothermic vertebrates have improved. Nevertheless, a variety of species of amphibians still play an enormously important role in physiology—both in research and in the training of students. It is surprising, therefore, that there are no recent and general books covering the physiology of the amphibians. This lack is the reason for our volume.

Considering the importance of the amphibians in biological investigations, we have sought to provide a treatment that will be useful for the advanced student, for specialists in fields other than amphibian physiology (who will wish to relate information about the amphibians to their own studies), and for the physiologist concerned largely or entirely with amphibians.

The domain of physiology is interpreted here somewhat more broadly than is usual. In addition to customary topics such as digestion, respiration, and metabolism, we have other aspects not usually included in physiology. We have chosen to consider the special usefulness that amphibians have served in answering questions about the physiology of development, metamorphosis, and regeneration. The treatment of other topics ordinarily expected in a discussion of physiology, and originally intended to be considered here, will be taken up in a future volume.

One of the more interesting things that impressed the contributors to this volume is the many and large gaps that still remain in our knowledge of amphibian physiology. Far from closing the field with these chapters, therefore, we hope to have shown how really open it still is.

March, 1964 JOHN A. MOORE

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THE METABOLISM OF AMPHIBIA

George W. Brown, Jr.

"...all vital action may...be said to be the result of the molecular forces of the protoplasm which displays it." Thomas Henry Huxley (1868).

I. Introduction

The present chapter is devoted to a discussion of certain aspects of the metabolism of Amphibia. This is a prelude to the special phases of the physiology and biochemistry of this class which are treated in subsequent chapters. All physiological processes ultimately must be explained in terms of the numerous reactions taking place in various cells, and it is the purpose

here to provide a broad view of the metabolic activities which support these processes.

At the present time there are some ten thousand original papers dealing with material which could be included under the heading of this chapter. These are currently being sifted for a longer presentation (Brown, 1963). In a treatise such as this, selection of material and reference citations was inevitable and difficult, and the subject matter of many noteworthy papers had to be omitted. Unfortunately, it has not been possible to include many structural formulas which would aid the reader in following metabolic sequences. For these and for the more intimate details of metabolic processes, reference should be made to standard biochemical texts such as those of Fruton and Simmonds (1958), Cantarow and Schepartz (1962), White *et al.* (1959), and West and Todd (1961).

A list of the abbreviations used in the present writing is given.*

Thermodynamic considerations. A portion of the material presented deals with energy metabolism. For this reason it was deemed advisable to include wherever possible throughout the chapter data on the free energy change for reactions cited. The free energy (Gibbs' free energy) change for any reaction is given by

* The following are abbreviations used in this chapter:

AMP, adenosine-5′-monophosphate
ADP, adenosine-5′-diphosphate
ATP, adenosine-5′-triphosphate
cal, gram-calories
CoA, coenzyme A
DNA, deoxyribonucleic acid
DOPA, dihydroxyphenylalanine
DPN^+ and DPNH, oxidized and reduced forms of diphosphopyridine nucleotide
E.T.S., electron transport system
FAD and FAD·2H, oxidized and reduced forms of flavin adenine dinucleotide
FMN and FMN·2H, oxidized and reduced forms of flavin adenine mononucleotide
Kat-*f*, katalase fähigkeit
kcal, kilogram-calorie
M, molar (moles per liter)
P_i, inorganic orthophosphate
PP_i, inorganic pyrophosphate
Q_{O_2}, microliters O_2 per hr per mg dry weight of tissue
q_{O_2}, microliters O_2 per hr per mg wet weight of tissue
Q_{10}, ratio of (rate of reaction at $t + 10°$)/(rate of reaction at t)
RNA, ribonucleic acid
R.Q., respiratory quotient (volume of CO_2/volume of O_2)
R.T., room temperature
(s), solid substance
STP, conditions of 0° centigrade and 1 atmosphere pressure
t = degrees centigrade
T = °Kelvin (absolute)
TPN^+ and TPNH, oxidized and reduced forms of triphosphopyridine nucleotide
Tris, tris(hydroxymethyl)amino-methane
μ, 10^{-6} meter
μg, 10^{-6} gram
μliter, 10^{-6} liter
$\sim$, energy-rich bond or approximate sign
$\cong$, approximately equal to
Δ, change in the quantity.
F, free energy
H, heat content (enthalpy)
S, entropy

$$\Delta F = \Delta H - T\Delta S \quad (1)$$

where ΔF = change in free energy
ΔH = change in heat content (enthalpy)
ΔS = change in entropy
T = absolute temperature (°Kelvin)

The convention of Lewis and Randall (1923) is employed whereby if at constant temperature and pressure ΔF is negative, the reaction tends to proceed from left to right as written. When ΔF is positive, the reaction tends to proceed in the reverse direction. If $\Delta F = 0$, the reaction is at equilibrium.

The notation, ΔF^0 ($= \Delta G^0$) represents the standard free energy change.* The notation, $\Delta F^{0\prime}$, represents the free energy change at pH 7 with all reactants and products except H^+ at unit thermodynamic activity. Knowledge of the standard free energy change for a reaction allows calculation of the equilibrium constant from the well known relationship:

$$\Delta F^0_{T^0} \text{ (calories)} = -RT\ln K_{eq} = -4.58T \log_{10} K_{eq} \quad (2)$$

In any system allowed to go from state A to state B at constant temperature and pressure, the maximum work, w', obtainable from the reaction which can be applied to useful purposes is given by

$$w' = F_A - F_B = -\Delta F \quad (3)$$

Thus, if the free energy change, ΔF, is negative, w' is positive and represents the maximum useful work which can be done.

Unless specified otherwise, the free energy change associated with a given reaction is that for the number of moles prefixed to reactants and products of specified ionic charge, in solution, as they appear in the chemical equation. Free energy changes cited should be regarded as best approximations only. The magnitude of free energy changes of most reactions occurring *in vivo* is not known because of meager information on concentrations of reactants and products at the site of the reaction. The application of free energy changes to problems in biochemistry is discussed in some detail by Pardee and Ingraham (1960) and by Klotz (1957).

Much of the thermodynamic data given is provided by values (or calculated therefrom) to be found in the literature (for example, see Lewis and Randall, 1923; Parks and Huffman, 1932; Latimer, 1938; Burton and

* The standard free energy change, ΔF^0, is the free energy change at 25° C (298° Kelvin) for a reaction in which reactants and products are in the standard state: solutes, 1 *M* thermodynamic concentration; gases, 1 atmosphere pressure (perfect gas); liquids, the pure liquid.

The symbol, $\Delta F^{0\prime}$, is used when all reactants and products are in the standard state except for H^+ which is at 10^{-7} *M*, i.e., pH 7. Subscripts are used for temperatures other than 298° K.

Krebs, 1953; Holzer, 1956; Krebs and Kornberg, 1957; Benzinger *et al.*, 1959; Cohen and Brown, 1960).

II. Over-all Energy Metabolism

The mature amphibian egg contains an energy-rich reserve of material in the form of carbohydrate, lipid, and protein—in amounts sufficient to carry the fertilized ovum through the embryonic stages of development. Moreover, it is equipped with the genetic information necessary to effect an orderly progression of transformations throughout this period. Carbohydrate (glycogen) and lipid account for about 8 % and 25 %, respectively, of the dry weight of mature *Rana* spp. eggs; an even greater percentage of the dry weight is accounted for by the yolk (Barth and Barth, 1954). The yolk material consists largely of phosphoprotein, but it contains, in addition, various other compounds, including RNA, phospholipids, acid-soluble organic phosphates such as nucleotides, and inorganic phosphate. All of the vitamins and cofactors required for the various enzymic reactions of embryogenesis are found within the egg. Probably most, if not all, of the various metal ions required for certain enzymic reactions are present as well.

The reserve materials of the egg are largely depleted by the time the larval stage with the onset of feeding is reached, except in those cases where the larval stages take place entirely within the egg such as with the Surinam toad, *Pipa pipa*. While it is not safe as yet to assume that the onset and relative rates of utilization of reserve materials are the same for all species of Amphibia, one can generalize from the observations already made: glycogen is broken down, probably at least to some extent at first anaerobically, then aerobically, followed by oxidation of fat. Inasmuch as embryogenesis involves the formation of new cells and is a growth process in spite of absence of external nutrition, it can be anticipated that the protein is largely spared an oxidative fate and is reorganized into new protein for the newly formed cells.

As pointed out by Barth and Barth (1954), the amount of "active" protoplasm which is directly responsible for the metabolism of the egg is insignificant in comparison with the stores of glycogen, fat, and protein; hence, the early energetic requirements of the egg are very small during the stages of cleavage, blastulation, and early gastrulation. G. ten Cate (1953) has summarized some of the metabolic events occurring during embryogenesis: The enzymes which are first active are hydrolytic (glycolytic) in nature. During the first few days carbohydrate is broken down as shown by a decrease in total carbohydrate with a resultant increase in lactic acid (anaerobic glycolysis). Subsequently, there is a rise in respiratory activity shown by increases in cytochrome oxidase, succinoxidase, and O_2 consumption.

The assumption of aerobic metabolism during embryonic development is accompanied by a decrease in lactic acid. At this point pyruvate presumably is converted to acetylSCoA and is oxidized via the Krebs tricarboxylic acid cycle rather than acting as the hydrogen acceptor for reduced DPNH generated from anaerobic glycolysis in the early stages (see Section IV,C,1).

A. RESERVE MATERIAL AND EMBRYOGENESIS

By the use of simultaneous equations, Løvtrup (1953a) finds with the axolotl, *Ambystoma mexicanum*, that the succession of energy sources during embryonic and larval development is carbohydrate, fat, and protein—the protein utilization not beginning until the carbohydrate is exhausted. Figure 1 shows the *rate* of utilization of these substances in this species as a function of embryonic development. It can be seen that the maximum rate of utilization of carbohydrate takes place during neurulation and that this utilization precedes that of fat and protein. Fat combustion begins as the rate of carbohydrate utilization falls off and reaches a maximum during the larval stages. Protein is not utilized until the larval stages. Of the carbohydrate which was initially present in the egg, some 65% is broken down anaerobically, according to Løvtrup.

In the case of *Ambystoma mexicanum*, Løvtrup (1953a) finds that the phosphoprotein of the yolk is not utilized before the tail-bud stage and is apparently not related to combustion of protein. During late larval stages at the time of maximum oxygen uptake, 41% of the total lipids and 16% of the protein have been utilized.

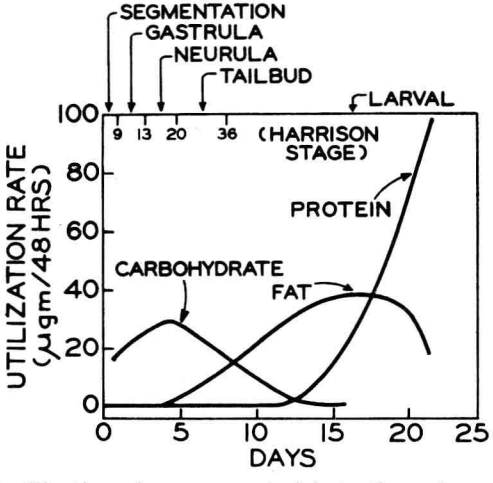

Fig. 1. Rate of utilization of reserve materials in the embryonic and larval development of *Ambystoma mexicanum*. After Løvtrup (1953a).

The reactions through which energy is made available for embryonic work are discussed in a later section (IV). While it is clear that such reactions make available the free energy of these storage or reserve foodstuffs, we still lack information on the efficiency of these processes in terms of the trapping of the theoretical amount of free energy available from them. It is a matter of observation that such energy carries the organism at least to the free-swimming stage, soon after which the larval amphibian becomes dependent upon food available in the environment for further growth, differentiation, and development.

B. OXYGEN CONSUMPTION

1. Consumption of Oxygen during Development

A considerable body of work on Amphibia deals with studies on the primary organizer or neural inductor of the embryo. An important phase of this work has been the analysis of chemical and respiratory changes in various parts of the embryo during development. Such studies were undertaken because it was thought that they would lead to a better understanding of biochemical events associated with the phenomenon of biological induction.

Brachet (1935a), carried out some early studies on the metabolism of frog eggs (*Rana fusca*) during development and showed that the act of fertilization did not result in demonstrable changes in oxygen uptake. Fertilized and unfertilized eggs (during a 1-hour period following fertilization) exhibited O_2 uptakes of 1.15 ± 0.02 μliters per hour per egg. However, respiration of blastulas was higher than that of eggs immediately before or after fertilization. The R.Q. (respiratory quotient) of unfertilized eggs was 0.99, while that of fertilized eggs was 0.66 (range, 0.55–0.73), and this decrease in R.Q. was attributed to an increase in the O_2 consumption by the spermatozoa. In addition, Brachet showed that mitotic events in frog eggs are accompanied by slight cyclic variations in O_2 uptake with at least two peaks corresponding to prophase and anaphase. The dorsal lip of the blastopore (organizer) was thought be the center of intense respiratory activity, more elevated than neighboring regions (Brachet, 1935b).

These early studies and those prior to 1948 are discussed at length in the excellent critique of Boell (1948). Boell has summarized the values of ten reports on the ratio of respiration by the dorsal lip region to that of ventral ectoderm. These data lead to a mean value (and mean deviation) for this ratio of 1.16 ± 0.14. It thus seems that if the respiration of the dorsal lip is higher than ventral ectoderm, it is only slightly so. Variations in experimental results are attributed by Boell to the failure of various workers to use corresponding areas of dorsal lip and ventral ectoderm for comparison.