GENERAL AND COMPARATIVE PHYSIOLOGY

Third Edition

William S. Hoar

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Preface

In a broad sense, there can be little quarrel with the argument that comparative physiology is precisely what the term implies—a comparative study of differences and similarities in the functional processes of animals at selected levels in phylogeny. Beyond this, however, individuals can be expected to fit these facts into different patterns. Moreover, some presentations may have distinct advantages for students who must remember the facts. My own choice has been to discuss the adaptive significance of life processes in an environment that has evolved with the animals and is rarely static. To quote from the preface of the first edition: "This book is written with the conviction that a story of phylogeny in animal functions can now be sketched and that this will provide a framework into which the many details of physiology can be interestingly fitted." Twenty years later I find little to justify this conviction. Eager comparative physiologists have accumulated volumes of new facts, demonstrated unsuspected processes, and revealed surprising biological adaptations, but the facts rarely fall on neat phylogenetic lines nor reveal systematic evolutionary patterns. On the contrary, there are often several solutions to a particular problem; the evolutionary process seems to have utilized limited biological materials in a somewhat fortuitous manner. The solutions are as often compromises as they are logical steps in a phylogenetic progression (see Waterman, 1975a, and Ross, 1981).

Although the broad coverage of previous editions has been retained, the subject matter has been reorganized. The separate chapter on regulatory mechanisms has been dropped and the material is included at relevant points in discussions of the organ-systems. Likewise, the section on environmental relations has been dispersed throughout the text or differently organized in new chapters. For my own part, I find it more logical and easier for the students to deal with stimulus-response mechanisms

and nervous activity at the beginning of the course. Consequently, these topics have been discussed immediately after the introduction and chapters on transformation of energy and biomembranes.

Again, the references include some names of pioneers who made fundamental contributions to comparative physiology but concentrate on a selected list of monographs and critical reviews; through these, interested students can readily locate pertinent original references. Once more the index shows the taxonomic position for the scientific names of the animals; the hypothetical phylogenetic tree facing the title page should orient readers with limited knowledge of animal taxonomy.

Throughout this project, I have enjoyed the friendly cooperation and support of my colleagues in our Department of Zoology. Professor G. G. E. Scudder, Head of the Department, provided advice, facilities, and space after my retirement; P. W. Hochachka, D. R. Jones, J. E. Phillips, J. D. Steeves, and M. A. Ali (Université de Montréal) read particular sections in depth, while several anonymous readings arranged by the publishers proved invaluable in making revisions. There are fewer errors because of these careful readings; I hope those that remain do not prove troublesome to students, for whom this book is written. The final production of this book as well as the previous edition and the laboratory companions that accompany them were skillfully managed by Zita de Schauensee. I am most grateful for her meticulous care, cooperation, and patience.

Vancouver, Canada William S. Hoar

Contents

1 The Animal and Its Environment

The Russian biochemist Oparin, about 1922, first developed those ideas which have become basic to the modern concepts of the origin of life. Oparin (1936) emphasized two alternatives. One might postulate that life arose in a world much like ours, or one might assume that it originated under very different conditions. Until about half a century ago only the first alternative seemed likely. Oparin argued that all the evidence pointed to the second alternative and that the earth, in the beginning, was probably very different. He maintained that, although spontaneous generation is now impossible, it might have been inevitable before the origin of life. The environment has been gradually changed with the evolution of life. Living organisms are inseparable from their environment and our present world, with its abundance and variety of life, precludes spontaneous generation.

This argument now seems sound. Geochemists and biologists agree that there has been a series of irreversible steps while the earth cooled, generated an atmosphere, and produced the first simple organic compounds which gradually increased in complexity and formed the self-duplicating living systems. Each step has changed conditions and reduced the likelihood of a repetition of earlier events.

Miller (1953, 1955) was the first to test this hypothesis. In his classic experiments, mixtures of methane, ammonia, hydrogen and water were subjected to electrical sparks in a closed system for about 1 week and the chemical changes in the apparatus were monitored by radiochemical and chromatographic techniques. Small quantities of glycine, alanine, aspartic acid, and α-amino-*n*-butyric acid were readily identified. These exciting experiments stimulated investigators in many laboratories (Calvin, 1969; Miller and Orgel, 1974). Syntheses of all the building blocks required for the formation of the complex organic substances were subsequently effected under

abiological conditions, using various mixtures of gases and energy sources thought to have been present during the prebiotic period.

On the basis of these pioneer studies, it was assumed that the prebiologic atmosphere contained hydrogen, methane, ammonia, and water. Under these highly reducing conditions, methane and not carbon dioxide furnished the carbon for the first organic syntheses (Fox and Dose, 1972; Dickerson, 1978). Concepts have been radically modified by recent work and the early atmosphere of the earth is now thought to have been dominated by carbon dioxide, water, and nitrogen with small amounts of ammonia and possibly some carbon monoxide (Windley, 1976; Eglinton *et al.*, 1981; Wigley and Brimblecombe, 1981). There remains agreement that the primary source of energy for the first organic syntheses was the sun. The major components of this energy budget were photochemical and ionizing radiations with lesser contributions from volcanism, meteorite impact, and lightning (Miller and Urey, 1959). It is also agreed that free oxygen was absent from the prebiologic atmosphere; indeed, its presence would have quickly oxidized and destroyed the first organic molecules, while their synthesis would have been very unlikely if an ozone blanket had curtained the more energetic ultraviolet radiation of the sun from the earth as it does today.

It follows that the first forms of life were probably anaerobic and heterotrophic, exploiting the organic compounds synthesized abiotically in the rich primordial soup. Their low energy demands were satisfied by processes of fermentation (Chapter 2). Subsequently, at some point in this vast expanse of time, mutants appeared that were independent of this mode of nutrition, with the capacity of synthesizing organic substances from the inorganic compounds of their environment. These are the autotrophic organisms, and the most effective synthetic system to have appeared in their phylogeny is that of chemically reducing carbon dioxide to carbohydrate in the process of PHOTOSYNTHESIS (Broda, 1975). In this process, the green plants and the blue-green algae use water as the hydrogen donor and produce carbohydrate according to the following simplified reaction:

$$CO_2 + H_2O \xrightarrow{h\nu} CH_2O + O_2$$

In a thermodynamic "uphill" reaction, powered by low-energy visible solar radiation, these autotrophs synthesize organic matter (carbohydrates) from carbon dioxide and water; molecular oxygen is released as a by-product. One oxygen molecule is released into the environment for each carbon atom fixed in carbohydrate. The gradual accumulation of oxygen in our atmosphere changed the anerobic into an aerobic world, provided the basis for aerobic respiration with the release of much larger amounts of energy than was possible with simple fermentation processes (Chapter 2), and generated the ozone blanket that surrounds the earth and screens the damaging radiation of the sun from the delicate organisms that inhabit the earth. Thus, the environment has been gradually altered as life evolved; the organisms and their environment have evolved together.

The forces of natural selection that shape the living organisms involve constant biological interactions among different species: the flowering plants have evolved with their insect pollinators, cryptically colored animals with their colorful environments, and warning signals with predator attacks. These interactions between animals and

their environment, both physical and biological, have effected habitat changes and altered ecological systems as well as the individual species (May, 1978). Thus, in a very real sense, the animal and its environment have evolved together.

PHYSIOLOGICAL COMPENSATION FOR ENVIRONMENTAL CHANGE

An animal rarely lives under constant conditions. Some habitats, such as the depths of the ocean or the interior of a warm-blooded animal which houses its parasites, provide relatively constant conditions. Yet even in the depths of the ocean supplies of food may be uncertain, while the habitat of the parasite can change with the health and nutrition of its host. Most animals face not only nutritional uncertainties but also marked diurnal and seasonal oscillations which alter rates of metabolism and activity; sudden extremes may tax the physiological machinery to the limit. An animal does not exist apart from its environment; the comparative physiologist recognizes this association and attempts to describe and explain the varied mechanisms by which animals compensate for all sorts of environmental alterations and stresses.

Studies of the physiological compensations for environmental oscillations and stresses are often considered together as a subdivision of comparative physiology—ENVIRONMENTAL OR ECOLOGICAL PHYSIOLOGY. This subdivision is largely one of convenience. It does, however, recognize a changing emphasis in comparative physiology. The pioneers of this science were usually taxonomically oriented and excited by the variations in physiological processes found at successive levels in phylogeny; present-day comparative physiologists are more often environmentally oriented and find their problems in the curious adaptations to unusual habitats and in the physiological capacities which permit life under changing and adverse circumstances. Environmental physiology focuses on this latter group of problems but inevitably merges with other areas of comparative physiology, particularly with studies of physiological adaptation in the evolutionary sense. In this section, principles concerned with physiological compensations for environmental change will be discussed; in succeeding sections, compensations for specific environmental variables will be considered.

NATURE OF THE INTERACTION WITH THE ENVIRONMENT

Tolerance and Resistance

As a part of its genetic endowment, every animal has a capacity to compensate for environmental change. It can live within a certain range of variations, whether the variable is temperature, humidity, oxygen supply, or any other environmental factor. This is its TOLERANCE, and it will not be killed or damaged by any particular environmental factor, provided this does not exceed the tolerance limits. Beyond these limits, however, the organism is damaged. Although it may RESIST the change for a longer or shorter period, it will eventually succumb as a result of the change. Thus, an

organism has a certain capacity for both TOLERANCE and RESISTANCE. Under appropriate conditions a catfish may live at temperatures ranging from 1 to 35°C. This is its RANGE OF TOLERANCE, with a lower and an upper INCIPIENT LETHAL LEVEL (Fry, 1947, 1971); exposure to temperatures less than the lower or greater than the upper lethal level will kill the animal after a RESISTANCE TIME which depends on the magnitude of the temperature differences.

Acclimation and Acclimatization

An animal has not only a capacity for tolerance and resistance but also one for ACCLIMATION and ACCLIMATIZATION. This means that its previous history with respect to any factor may modify its subsequent tolerance and resistance to changing conditions of this factor. Again, explanations are easiest in terms of familiar temperature effects, but it should be noted that the same principles apply to many of the other variables. If a catfish is maintained for a week or more at 30°C instead of 25°C, then its upper and lower incipient lethal levels are elevated by 2 to 3°C, as shown in Fig. 1.1. In short, there is a whole family of upper and lower incipient lethal levels, and the range of tolerance is really a ZONE OF TOLERANCE bounded by a ZONE OR REALM OF RESISTANCE (Fig. 1.2).

Many of the vital functions change in response to altered environmental conditions, whether the environmental changes fall within the zone of tolerance or the zone of resistance. These zones should not be thought of as static physiological areas. Temperature acclimation has, for example, been shown to alter the nature of the body fats of goldfish, the oxygen-binding of the blood of frogs, the heat resistance of the proteolytic enzymes in the stomach juices of snails (*Helix*), the excitability of the isolated foot of the gastropod (*Lymnaea*), and the pathways and kinetics of several different metabolic enzymes in fish. Precht (1958) and Hochachka and Somero (1971, 1973) have documented these as well as many other physiological and biochemical responses to temperature change. Similar examples of responses to other environmental factors will be discussed in subsequent sections. An animal makes a dynamic

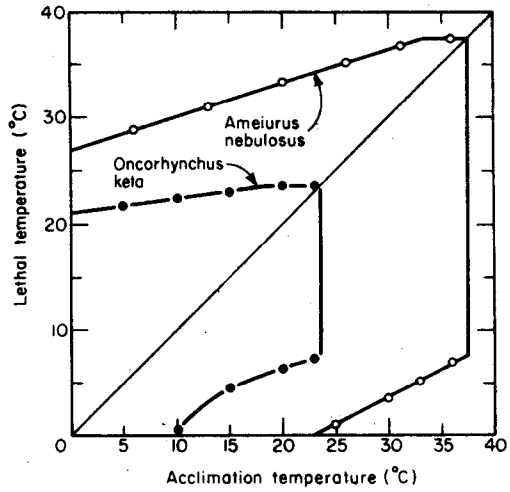

Fig. 1.1 Relation between the acclimation temperature and the upper and lower lethal temperatures for the catfish *Ameiurus nebulosus* and for the chum salmon *Oncorhynchus keta*. The areas bounded by the trapezoids are the zones of tolerance. [From Brett, 1956: Quart. Rev. Biol., *31*:76.]

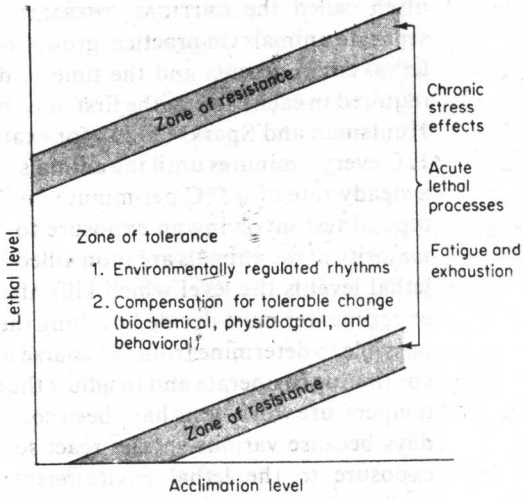

Fig. 1.2 Summary of factors involved in physiological compensation for environmental variation. Further description in text. [From Hoar, 1967: Trans. Roy. Soc. Canada Ser. IV, *5*:135.]

physiological response, whether the environmental change is within its normal (zone of tolerance) or more extreme range (zone of resistance). In temperature work, Precht (1958) uses the term CAPACITY ADAPTATION for the compensations which take place within the range of normal temperatures (Fig. 17.3) and RESISTANCE ADAPTATION (cold and heat resistance) for compensations to extremes which alter the lethal level of the environmental factor (Prosser, 1969; Alderdice, 1972; Vernberg and Vernberg, 1972).

Although the terms ACCLIMATION and ACCLIMATIZATION have essentially the same meaning in the English language and are frequently used interchangeably in biological literature, there is a tendency on the part of environmental physiologists to restrict their usage to somewhat different compensatory changes. In the current terminology, "acclimation" is the descriptive term applied to compensatory changes which occur in the laboratory where animals are maintained under controlled conditions of one particular environmental variable, while "acclimatization" refers to the more complex situation in nature. With reference to temperature, the catfishes described in Fig. 1.1 were acclimated in the laboratory by holding them in aquaria at different constant temperatures. In nature, these animals also show seasonal changes in their temperature tolerance. These are partly due to the seasonal temperature cycle but may also be associated with photoperiod and other seasonally changing conditions. The term "acclimatization" is reserved for compensatory changes occurring under such natural conditions. It is the sum of the adjustments which follow repeated and prolonged exposure to natural environmental change. There is also a third level (the species level) on which temperature compensation is possible. Zones of tolerance may also be altered by natural or artificial selection through changes in genotypes.

Measurement of the Lethal Level

The precise boundaries of the apparent zone of tolerance will depend on the method of determining the lethal levels. Two techniques have been commonly used. In one, the level of the environmental variable is gradually altered until the animal dies or becomes seriously incapacitated; in lethal temperature measurements, this value is

often called the CRITICAL THERMAL MAXIMUM (CTM). In the second technique, separate animals (in practice, groups of animals) are placed in a series of constant but lethal environments and the time to death is noted. Certain arbitrary decisions are required in each case. In the first, it is the RATE OF CHANGE which must be standardized. Huntsman and Sparks (1924), for example raised the temperature of the sea water by 1°C every 5 minutes until the animals died; Tsukuda (1960) altered the temperature at a steady rate of 0.5°C per minute until temperature coma was observed. The second type of test involving an exposure to one lethal level presents no problem when the majority of the animals are soon killed by the lethal agent. However, if by definition the lethal level is the level which kills after an indefinite exposure, then, obviously, the experimenter must decide how long the lethal tests are to last. In practice, it is usually possible to determine from the course of the mortality curve whether the lethal agent is continuing to operate and to adjust the experimental procedures accordingly. In lethal temperature work, tests have been continued for 12 to 24 hours or even as long as seven days because various species react so differently. In general, tests involving sudden exposure to the lethal environment are preferred since there is less chance of acclimation during the test and since the data are more readily susceptible to standard statistical analysis. Most determinations of lethal levels follow this technique (Fry, 1971; Brett, 1972).

The analytical techniques have been carefully studied by toxicologists and are described in many places (Litchfield, 1949; Litchfield and Wilcoxin, 1949; Finney, 1964; Bliss, 1967). They will not be detailed here, but several points are mentioned to facilitate understanding of the physiological literature. The analyses are based on the normal variability which every population shows with respect to its morphological and physiological characteristics. There are giants and dwarfs; there are also individuals which are extremely resistant and others which are particularly susceptible. In between there are the average individuals that make up most of the population. In short, we are usually dealing with a normal distribution curve, and its pattern is the same whether the measurements are sizes of animals or their incipient lethal levels.

The pioneer work was carried out by pharmacologists in their attempts to standardize drugs by bioassay techniques. In one of the earliest studies the lethal dose of digitalis was determined for each of 573 cats; the lethal doses were distributed around a mean value in the pattern of the normal distribution curve. In another very precise early investigation, 146 frogs were slowly infused with *k*-strophanthin until they died. When the frequency of deaths at different lethal doses was plotted, a distribution of the same type was obtained. These pioneer data are shown in Fig. 1.3.

The average individual is represented by the peak of the curve, and this dose (the median lethal dose or LD_{50}) is the best representation of the lethal dose for the sample. In actual practice it is not determined from data such as those shown in Fig. 1.3. On the contrary, relatively small samples are used; different samples are exposed to a single lethal dose, and a graded series of doses is used; the one which kills 50 per cent of the animals in the test period is recorded as the LD_{50}. Usually, five or six dose levels are sufficient to estimate the 50 per cent level when these are appropriately analyzed (Fig. 1.4). The mathematics has been carefully studied, and easy graphical methods are now available for the ready determination of lethal levels. These methods are based on the fact that the bell-shaped dose mortality curve becomes a sigmoid when cumulated

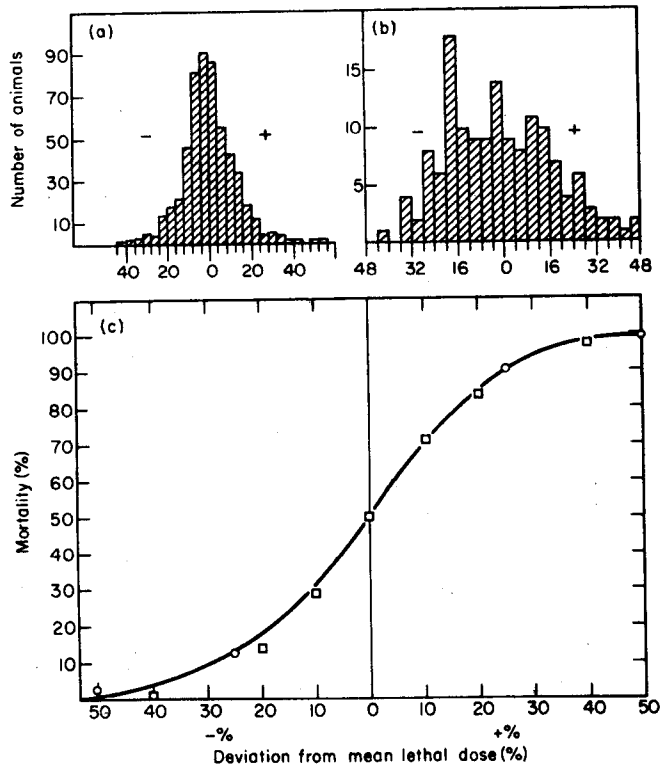

Fig. 1.3 Dose-mortality relationships. (a) Distribution about mean value of the lethal dose of digitalis for different cats. The abscissa O represents the mean with lethal dose given in percentages below and above the mean. (b) Same for lethal doses of strophanthin for frogs. (c) Data plotted as per cent mortality (ordinate) with circles for digitalis and squares for strophanthin. [After Burn, Finney, and Goodwin, 1950: Biological Standardization, 2nd ed. Oxford University Press, Oxford.]

deaths (or per cent dead) are plotted against the dose or the log dose, as was evident in the very early studies of cats and frogs (Fig. 1.3). A close approximation to the LD_{50} can often be obtained directly from such a sigmoid; for precise comparisons it is rectified through the probit transformation which converts it into a straight line (Fig. 1.4). The LD_{50} values can be obtained from these curves, or the lines can be compared by standard methods of linear regression analysis.

The zone of tolerance illustrated in Figs. 1.1 and 1.2 is bounded by points representing temperatures at which 50 per cent of the sample died in tests lasting 14 hours. This provides the best representation of the way in which the population may be expected to respond to a single lethal factor. It is, in a sense, the mean reaction for a sample. However, there are times when the experimentalist may wish to evaluate the level which will kill all of the population or, alternatively, permit them all to survive. The trapezoid could just as well have been drawn for 5 per cent deaths and 95 per cent survival or any other level. The area would vary accordingly.

It should also be emphasized that the trapezoid (Fig. 1.1) is a zone of survival in the face of environmental change. It does not describe, in any way, the effects of this change on various vital processes such as growth, activity, or reproduction. It is known, for example, that young salmon fail to grow at temperatures slightly above the lower lethal level and slightly below the upper lethal level. Thus, a trapezoid representing tolerance levels for growth would be considerably smaller. Environmental limits for reproduction may be even narrower. Additional areas could be marked off to

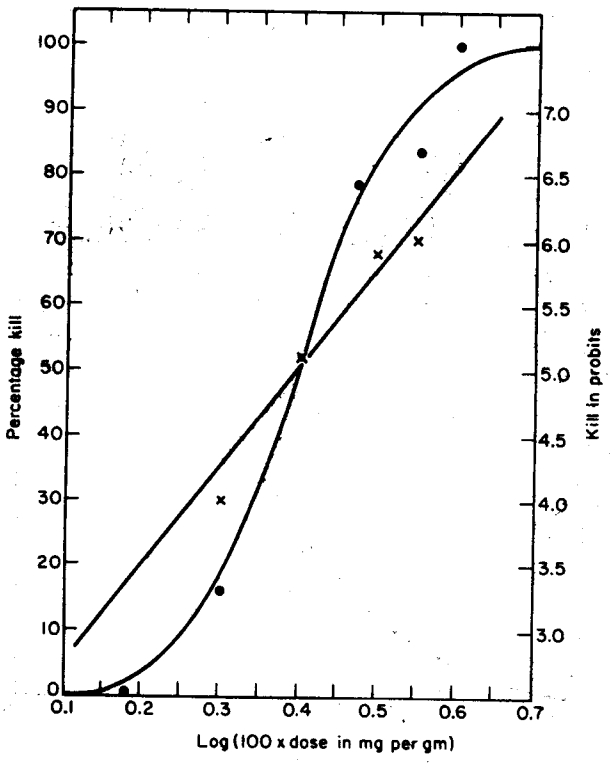

Fig. 1.4 Toxicity of cocaine hydrochloride to mice plotted as per cent kill (sigmoid curve) and as probits (straight line) against log dose. [From Burn, Finney, and Goodwin, 1950: Biological Standardization, 2nd ed. Oxford University Press, Oxford.]

show the environmental variation compatible with each of several different activities (see, e.g., Brett, 1979).

Physiological Compensation in the Zone of Tolerance

There are two different kinds of physiological compensation for successful living in altered environments. Many animals have tissues with the capacity to operate over wide ranges. In these the internal environment reflects the external environment, and changes in the latter are followed by corresponding alterations in the former. Thus, the body temperatures of many invertebrates and lower vertebrates fluctuate with their surroundings; these animals are said to be POIKILOTHERMIC (poikilos = manifold). Again, the osmotic content of the body fluids of some invertebrates like the polychaete worm *Arenicola* almost match that of the sea water over a range of dilutions down to about 12 per cent; these animals are said to be POIKILOSMOTIC. Their physiological processes operate well at a series of different temperatures or under varying osmotic conditions. They exemplify an environmental compensation referred to as CONFORMITY or ADJUSTMENT (Fig. 1.5).

In contrast to the conformers, many animals preserve relatively constant conditions in their tissues. They control or regulate their internal environment and are killed if this fluctuates beyond rather narrow limits. Temperature variations or fluctuations in osmotic and other environmental conditions activate the regulatory homeostatic