Biochemical Adaptation to the Environment

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SYNOPSIS. Biochemical adaptation to environmental parameters such as temperature appears to involve two distinct types of changes in the organism's chemistry. On the one hand, the quantities of certain molecular species present in the cells may change. Alternatively, the actual types of molecules present may vary. Rainbow trout (Salmo gairdneri) acclimated to warm and cold temperatures exhibit a striking example of this latter type of adaptation. For all enzymes we have examined in this species, distinct "warm" and "cold" isozymes are present. The isozymes found in warm-acclimated (18°C) trout function well only at temperatures above 10-12°C. The isozymes present in cold-acclimated (4°C) trout function optimally at 2-5°C, temperatures this species normally encounters in winter. These data, plus information on comparable changes in membrane lipids, lead us to propose that adult poikilotherms may undergo a considerable degree of "biochemical restructuring" on a seasonal basis. The factors which control this "restructuring," and the rates at which the process occurs at high and low temperatures, are topics for future investigation.

Although we shall focus our attention almost entirely on adaptation to a single physical parameter, temperature, it would seem useful to preface this essay with a brief discussion of a well understood biochemical adaptation to the chemical environment. Perhaps the classical example of environmental adaptation, at least in the opinion of biochemists, is the adaptation of micro-organisms to changes in the nutrient content of their environment. For example, the addition of a simple sugar to the culture medium in which a micro-organism has been growing will soon result in the induction of sets of enzymes necessary for the transport of this sugar into the cells and for the subsequent utilization of the sugar in energy metabolism. We cite this example in order to lay a firm foundation for what we feel might be termed a "paradigm of biochemical adaptation to the environment." That is, biochemical adaptation to the environment is effected by producing the proper molecules in the proper quantities at the proper time to ensure satisfactory biological function in the face of new environmental conditions. We shall show that much as bacteria are capable of producing the right enzymes to take advantage of changes in their nutritive environment, so can cold-blooded (poikilothermic) organisms produce the necessary enzymes to enable the organisms' metabolic functions to continue under quite extreme conditions of thermal stress.

Before we begin a detailed examination of temperature adaptation in poikilotherms, it is essential to emphasize that the mechanisms we shall discuss may be characteristic only of those poikilotherms which are incapable of avoiding thermal stress through such avenues of escape as partial heterothermy and behavioral regulation of temperature. That such escape mechanisms do exist in numerous forms among poikilotherms should be abundantly clear from the papers of others in this symposium (e.g., Carey and Teal; Heath et al.).

The types of thermal stress encountered by true poikilotherms can be conveniently grouped into three classes on the basis of the time-course of the stress. At one extreme is evolutionary adaptation to temperature, a process by which a species adapts to a new thermal regime over many generations. The end result of this long term process is a high degree of similarity in the rates of physiological processes among organisms from widely different
FIG. 1. In vitro oxygen consumption levels (Q_{O_2}) of *Trematomus* gill filaments, expressed as μl O_2 consumed/mg dry wt tissue/hr. From Somero et al. (1968) and Ekberg (1958).

latitudes (Scholander et al., 1953; Wohlenschlag, 1964; Somero et al., 1968). An excellent example of evolutionary cold adaptation is found in the Antarctic Nototheniid fishes such as *Trematomus bernacchii*. The whole organism and tissue respiratory rates exhibited by this species at its normal habitat temperature of −1.9°C are roughly the same as the rates characteristic of temperate zone species at their much higher habitat temperatures (Fig. 1).

The second time-course of thermal stress to be discussed is also illustrated in Figure 1. Much as different species may exhibit similar rates of physiological activity in spite of widely different habitat (body) temperatures, so may an individual of a single species show compensatory adjustments in physiological rates on a seasonal basis. This process, which occurs during the life span of the individual organism, is commonly termed "acclimitization," if the changes occur in the organism's natural habitat, or "acclimation," if the changes occur in the laboratory and in response to variation in a single parameter, e.g., temperature. At this point it seems pertinent to raise the following question: Is the similarity in the physiological end-results of the evolutionary adaptation process and the acclimitization (or acclimation) process due to an identical biochemical basis for these two temporally disparate processes? We shall indicate later that at least a partially affirmative answer can be given to this question.

Finally, at the other extreme of the time-course scale, one observes thermal stresses which occur on a diurnal basis. Stresses of this frequency are particularly acute in the case of intertidal organisms which may encounter 10-20°C changes in body temperature over periods of a few hours. Quite surprisingly, at least on the basis of the dogma of classical physiology, many intertidal organisms have been shown to exhibit essentially temperature-independent rates of physiological activity (Newell, 1966; Newell and Northcroft, 1967; Baldwin, 1968; Newell and Pye, 1970a,b). What biochemical mechanisms can possibly permit Q_{10} values approximating unity?

In this essay we shall attempt to summarize the known information on the biochemical mechanisms by which physiological processes are maintained at relatively temperature-independent rates, regardless of the time-course of thermal stress. Our summary of the existing data should serve not only to illustrate what is known, but also to point out what gaps remain in our knowledge and what avenues of attack on the remaining questions would seem most fruitful.

If we equate metabolic processes with enzymic reaction sequences, then we can readily list the basic types of biochemical changes which could possibly serve to elevate metabolic processes at low temperatures. First, and perhaps most simply, the quantities of enzymes present in the cell may increase during cold adaptation. Changes in the concentrations of rate-limiting enzymes would seem of particular importance. Quite surprisingly, in spite of the inherent appeal of this hypothesis, no unequivocal measurements of enzyme concentrations in warm- and cold-acclimated organisms have been made. Indeed, there are many studies in which the gross activities of enzymes in tissues from warm- and cold-acclimated organisms have been estimated (e.g., Hochachka and Somero, 1971). In no case, however, have changes in enzymic activity been shown to be due to actual changes in enzyme concentration. Thus, verification of this hypothesis remains a
A second important manner in which enzymic activity can be altered involves changes in the intracellular environment in which the enzymes function. One can list a large number of intracellular changes which can affect enzymic activity: (1) alterations in the concentrations of enzyme activators and inhibitors; (2) changes in intracellular pH; (3) changes in the membranes to which enzymes are bound; (4) changes in substrate concentrations; and (5) changes in hormone levels. The occurrence of such changes in temperature adaptation is incompletely charted. It is known that major alterations occur in membrane phospholipids (Johnston and Roots, 1964; Roots, 1968; Anderson, 1970) and that such alterations can drastically modify the properties of lipoprotein transport systems in the membrane (Wilson et al., 1970). Changes of this nature may be particularly important in adaptation of the nervous system. Intracellular pH also varies with temperature due to the temperature-dependence of the pK of water (Rahn, 1965); pH changes could therefore be of importance in all time-courses of adaptation. The concentration of tissue and serum ions may also change dramatically during acclimation (Hickman et al., 1964). These kinds of modification of intracellular microenvironment can lead to important metabolic adjustments and can provide at least a partial answer to our question about the biochemical basis of temperature adaptation.

Finally, there is a third important way in which enzymic activity may be regulated for thermally-independent function. Adaptation to temperature may involve the production of enzymes which are particularly well suited for function at the temperature to which the organism is adapted. Thus, an enzyme from a cold-adapted organism might be a better catalyst at low temperatures than the homologous form of the enzyme found in a warm-adapted organism. To appreciate how the catalytic function of an enzyme might be improved, it is helpful to consider a simplified version of an enzymic reaction sequence, symbolized: E + S = ES = E + P. The first step, or equilibrium, in this reaction involves the reversible formation of an enzyme-substrate complex (ES) from free enzyme (E) and free substrate (S). To cause an improvement in function, one would predict that the propensity for ES complex formation could be increased. As an approximation of this propensity for ES complex formation, biochemists customarily refer to the "apparent enzyme-substrate affinity," which is often equated with the reciprocal of the apparent Michaelis constant (Km) of substrate. This latter parameter is the concentration of substrate necessary to yield half the maximal velocity of the reaction; when the Km (determined by kinetic studies) is identical with the binding constant (determined by binding tests), it is a good measure of enzyme substrate affinity. Intuitively, it seems clear that the smaller the amount of substrate necessary to half-saturate the enzyme, the higher the affinity between enzyme and substrate molecules (Fig. 2). It is known, in fact, that ES affinity is a vitally important factor in the modulation of enzyme activity in the cell (Atkinson, 1969). Most positive modulators (activators) of enzymes exert their influence by increasing ES affinity (lowering the apparent Michaelis constant of
substrate); negative modulators usually have the opposite effect. With these considerations in mind, it became clear that the effect of temperature on ES affinity could be a most important factor in thermal adaptation. This expectation has been fully realized.

As illustrated in Figure 3, ES affinity normally increases as the temperature decreases over the species' biological temperature range. In other words, the following important analogy is seen to hold: Over the biological temperature range, temperature decreases act analogously to positive modulators of the enzyme. The biologically important result of this relationship between temperature and ES affinity is that, at the low substrate concentrations normally found in vivo (roughly $10^{-4}$ M and below), rates of enzymic activity may be largely independent of temperature. It is important to stress that, were substrate concentrations in the cell not below saturating levels, $K_m$ changes, whether in response to modulators or to temperature changes, could exert no influence on rates of enzymic function. Reduction in $Q_{10}$ through temperature-dependent changes in ES affinity may obviously be of major importance to some organisms, such as those in intertidal zones, which encounter large diurnal changes in habitat (body) temperature.

Although the apparent $K_m$ usually exhibits a direct relationship to temperature over the biological temperature range, we have found that sharp increases in this parameter often occur at temperatures at or below the organism's normal temperature range. For example, the catalysis of pyruvate kinase of rainbow trout acclimated to temperatures of 10-17°C (Fig. 4) would appear to be severely limited at temperatures much below 10°C. This enzyme certainly would be largely incapable of binding substrate, under conditions of physiological substrate concentration, at temperatures normally experienced by Antarctic fishes. In addition, one can see that the summer trout would also have greatly reduced pyruvate kinase function at this species' winter temperatures of 2-4°C. The fact that trout remain active over a temperature range in which an enzyme such as pyruvate kinase (Fig. 4) would function poorly led us to examine enzymes from...
Temperature, °C

FIG. 5. The influence of temperature on the Km values of phosphoenolpyruvate (PEP) for pyruvate kinases of 2° and 18°C acclimated trout. Adult rainbow trout weighing approximately 175 g were acclimated for 4-5 weeks. Specimens were fed ad libitum during holding. To prepare the enzymes white epaxial muscle was homogenized with 4-5 vols. of 0.01 M tris/HCl buffer, pH 7.4, containing 1 mM EDTA. The crude homogenate was centrifuged for 20 minutes at 12,000 g and the pellet discarded. The supernatant was brought to 40% saturation with solid ammonium sulfate. The suspension was centrifuged as above and the resulting supernatant was brought to 75% saturation with ammonium sulfate. The precipitate was collected and redissolved in 0.01 M tris/HCl buffer, pH 7.4. Dialyzed aliquots of this preparation were used in the assays. Enzyme activities were assayed spectrophotometrically. Km values were determined by double-reciprocal (1/velocity versus 1/S) plots. At all temperatures pH values were held constant.

cold-acclimated specimens. For all enzyme systems examined (Figs. 5, 6, 7, 8, 9), distinct "summer" and "winter" variants of the enzymes were present. In each case, acclimation to low temperature led to the appearance of a new enzyme variant which exhibited a minimal Km value at temperatures 5-10°C lower than the minimal Km temperatures observed for the "summer" variants.

The importance of these changes in the seasonal acclimation process can best be appreciated by considering how a "summer" enzyme variant would function at the trout's winter temperatures. The first disadvantage of the "summer" variant is...
apparent: The rate of catalysis per se is lower than in the case of the “winter” variant. Second, it should be noted that the “summer” variant also has a limited ability to vary its rate of catalysis as the concentration of substrate varies in the cell. Thus, at low temperatures the “summer” enzyme is poorly equipped to regulate its activity in response to changes in substrate levels which might occur during vigorous metabolism.

A third disadvantage of the “summer” variants at low temperatures stems from the fact that, over the winter temperature range (generally below 10°C), decreases in temperature promote decreases in enzyme-substrate affinity. Consequently, the temperature-dependence of the reactions catalyzed by the “summer” enzymes is extremely large. For example, consider the temperature-dependence of the “summer” and “winter” pyruvate kinase reactions at low temperature and at low substrate concentrations (Table 1). At winter temperatures, variations in habitat (body) temperature would be accompanied by changes in pyruvate kinase activity characterized by Q_{10} values approximating 20 if only the “summer” variant of the enzyme were present. Clearly, the maintenance of relatively stable rates of enzymic function in the face of sudden changes in body temperature is compromised.

Table 1. The influence of temperature on the pyruvate kinase reactions catalyzed by the “summer” and “winter” variants at different substrate concentrations.

<table>
<thead>
<tr>
<th>Concentration of substrate (PEP)</th>
<th>V'/V°</th>
<th>( Q_{10} )</th>
</tr>
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<tbody>
<tr>
<td>“Summer” Pyk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 \times 10^{-3}M</td>
<td>2.5</td>
<td>9.8</td>
</tr>
<tr>
<td>1 \times 10^{-4}M</td>
<td>3.2</td>
<td>19.8</td>
</tr>
<tr>
<td>5 \times 10^{-5}M</td>
<td>3.6</td>
<td>24.6</td>
</tr>
<tr>
<td>“Winter” Pyk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 \times 10^{-3}M</td>
<td>1.4</td>
<td>2.2</td>
</tr>
<tr>
<td>1 \times 10^{-4}M</td>
<td>1.6</td>
<td>3.5</td>
</tr>
<tr>
<td>5 \times 10^{-5}M</td>
<td>1.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>

FIG. 9. Effect of temperature on the Michaelis constant of oxaloacetate and acetyl Co A on liver citrate synthases, which were partially purified from trout acclimated to 2°C and 18°C. At temperatures above 9°C, estimates of Km were highly reproducible for both enzyme preparations. For example, in the case of the 2° enzyme, three independent estimates of the Km for oxaloacetate at 22°C, pH 7.5, yielded 0.022 mM in each case. The Km estimates at temperatures below 9°C were from different enzyme preparations. (From Hochachka and Lewis, 1970.)
Table 2. Biochemical changes associated with temperature acclimation in poikilothermic organisms. See Hochachka and Somero (1971) and Precht (1968) for literature dealing with these topics.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes</td>
<td>Different variants in winter and summer rainbow trout</td>
</tr>
<tr>
<td>Lipids</td>
<td>Changes in saturation, chain length and quantity of lipid</td>
</tr>
<tr>
<td>Metabolic pathways</td>
<td>Increases in activities of pathways associated with biosynthesis in cold-acclimated fish, e.g., increase in hexose monophosphate shunt</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Change in rate</td>
</tr>
<tr>
<td>Nucleic acid synthesis</td>
<td>Change in rate</td>
</tr>
<tr>
<td>Blood ions</td>
<td>Changes in relative concentrations</td>
</tr>
<tr>
<td>Tissue ions</td>
<td>Same as above</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>Differences in ribosome melting temperatures between summer and winter trout</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Changes in O2 affinity likely due to changes in modulator concentrations</td>
</tr>
</tbody>
</table>

Temperature demands that “winter” forms of the enzyme be present.

To conclude this section on enzymic adaptation to temperature two points should be stressed. First, it seems clear that evolutionary adaptation and seasonal acclimatization (or acclimation) are promoted by a common biochemical mechanism, namely the production of enzyme variants which exhibit minimal $K_m$ values at temperatures approximating the lowest temperatures experienced by the organisms in their habitats. Second, it is important to point out the limits to which enzyme-substrate affinity changes can promote rate compensation. For the majority of enzymes we have examined from winter and summer rainbow trout, the minimal $K_m$ values of the two seasonal variants are approximately equal. Thus, in the absence of changes in enzyme concentration on a seasonal basis, there would still be quite large differences in total enzymic activity between winter and summer specimens. For enzyme-substrate affinity changes to promote complete temperature compensation, the “winter” variant of the enzyme would have to exhibit higher absolute affinity for substrate than the “summer” variant. This type of difference does not appear to be the rule.

To promote rate compensation to temperature there may be a second important way in which an enzyme can be “improved” for low temperature function: The turnover number per se may be greater for a cold-adapted enzyme than for a warm-adapted enzyme. Tests of this hypothesis are very limited, albeit there are data which suggest that enzymes from poikilothermic forms have a higher catalytic efficiency, in terms of the substrate turnover number, than do enzymes from homeothermic species (Assaf and Graves, 1969). Comparisons of “winter” and “summer” enzyme variants would obviously be of great interest.

At the beginning of this essay we formulated a “paradigm of biochemical adaptation to the environment,” which stated that adaptation involves the production of the proper molecules in the proper amounts to ensure successful biological function under new environmental conditions. In all the enzyme systems we have examined in trout, changes of this sort have been observed. To indicate that qualitative changes are not restricted to enzymes alone, we have summarized in Table 2 the other types of biochemical changes which occur during temperature acclimation. It is clear that the acclimation process is characterized by a major biochemical restructuring of the organism. At the present moment the adaptive significance of certain of these changes is not clear. For example, how do “summer” and “winter” ribosomes differ in their protein synthetic abilities? How do altered phospholipids affect the activities of membrane-bound enzymes? However, we feel that when answers to these questions are sought, it will again be found that these other biochemical restructuring changes will be adaptive, much as we know that changes in enzyme variants are.

To conclude this essay, we now would like to briefly consider some of the major
unanswered questions concerning temperature adaptation. Clearly, we currently have a fair idea about what is occurring to promote temperature adaptation. The important questions remaining would seem to concern: (1) how are these changes brought about, and (2) how do these changes occur sequentially?

In Figure 10 we propose a hypothetical scheme for the time course of the acclimation process. Indicated in this scheme are several of the uncertainties remaining to be resolved. For example, the nature of the primary inducing stimulus is still uncertain. It is possible that photoperiod and temperature interact to induce the acclimation changes. We have noted that cold-acclimation in summer and warm-acclimation in winter are extremely difficult to effect.

Once the fact of environmental change is perceived by the organism, then it must respond by initiating the biochemical changes characteristic of acclimation. What mediates this response? Are neural stimuli important? Are hormone messengers involved? Is a direct effect of temperature on the cells enough to promote acclimatory changes in metazoan organisms?

After the biochemical changes are initiated, then one must ascertain which changes are truly characteristic of the final steady state and which changes merely serve to bring about the final state. For example, are the increased rates of protein and nucleic acid synthesis noted during cold-acclimation likely to be maintained after the new isozymes, new lipids, etc. have been synthesized? Might, for example, changes in the activity of metabolic pathways associated with biosynthesis, such as the hexose monophosphate shunt, be transitory events which will be observed only during the time before a new steady state is attained? If certain changes are merely effectors of the final steady state, then might not these transitory effects be characteristic of both warm and cold acclimation? Clearly, a critical examination of the factors inducing acclimation changes and of the time-course of the process itself is due.

REFERENCES


