

Theory of Physiological

Adaptation of

Poikilotherms

to

Heat and Cold

C. Ladd Prosser

Brody Memorial Lecture V



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Some of many publications are:

Prosser, C. L. and S. J. F. Weinstein. Comparison of blood volume in animals with open and with closed circulatory systems. *Physiol. Zool.* 23:113-124. 1950.

Prosser, C. L. Physiological variation in animals. *Biol. Rev.* 30:229-262. 1955.

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Prosser, C. L. Conduction in non-striated muscles. *Physiological Reviews.* 42 (Suppl.):193, 1962.

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Theory of Physiological Adaptation of Poikilotherms to Heat and Cold

C. Ladd Prosser

"It is with real pleasure that I recall discussions that I had with Professor Brody concerning problems of physiological adaptation. In his rough and ready manner, which many of you well know, he brushed aside trivial considerations and came directly to significant questions. What I have to say to you this evening concerns environmental physiology in a very broad biological sense. It has little immediate application to thermal and respiratory physiology of mammals, on which Professor Brody worked. However, most of the general principles which I should like to discuss with you can very readily be applied to many kinds of animals.

"The detailed understanding of the intimate nature of interactions between the environment and organisms is at the heart of general biology; it is of the essence of life. Organisms are at once independent of their environment and yet a part of their ecosystem. How organisms maintain this independence, while at the same time they are environmentally dependent, is a major biological problem. It is sometimes said that living organisms in their net synthetic reactions run counter to the second law of thermodynamics; also that the informational content of an intact organism is less than that of its components in isolation because of the restraints imposed by its organization. These statements are valid only if an organism is considered in isolation and as a closed thermodynamic system. But if the ecosystem as a whole is considered, the organism has input and output, the entire environment-organism complex clearly follows thermodynamic laws, and the environment effectively is one of the "parts". The environment gives instruction in an informational sense. I do not wish to pursue this matter of informational theory further but I think we must recognize that organisms are in dynamic equilibrium with their environment and the maintenance of this equilibrium may be considered as a definition of homeostasis irrespective of whether the internal state of the organism is maintained constant or is allowed to vary with the environment.

"In all living things, the internal environment is narrower than is the physico-chemical range of the tolerable external environment. This is quite obvious.

For example, the internal temperatures tolerated by organisms for enzymatic reactions are narrower than the extremes of temperature which exist on earth.

"I like to think of an organism as interacting reciprocally with its environment, receiving more than it gives for some components of the environment and the converse for others. An organism, for example a higher animal, is played upon by the physical components of its environment: water, ions, oxygen, carbon dioxide, organic nutrients, non-nutrient organic compounds, temperature, light, mechanical factors, both oscillatory and fixed. Also, it is played upon by its biotic environment, especially in the behaviour of other animals.

"It is self-evident that every organism is the result of a long evolutionary history; that where an organism lives today depends on where its ancestors lived. The adaptational physiologist can contribute not only to our understanding of how organisms survive as they are now constituted but also as how organisms came to be as they are now. Unfortunately, it is very difficult for us to do experiments on fossils. On the other hand, I think there is much that the environmental physiologist can contribute to the understanding of evolution because the evolution of organisms is based primarily upon natural selection of physiologically adaptive characters.

"We may use the word 'species' in several different ways. I would first suggest three meanings of the word 'species.' First, there is the species of the systematist who, by recognizing key characters which need not by themselves be adaptive, delimits and names species. Second, is the biological species, the populations or series of populations of similar organisms between which there is no gene flow. There is reproductive isolation among them which may be attained by various means. Finally, I would suggest that there is the physiological species which takes account of unique adaptiveness of organisms to their environment. If we accept the premise that no two species occupy identical ecological niches

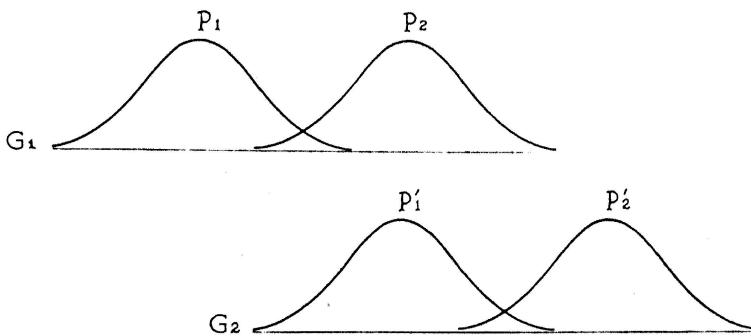


Fig. 1—Schematic representation of distribution of a character in two genotypes, G₁ and G₂, and in two populations of each genotype indicated by phenotypes P₁ and P₂ for G₁ and P'₁ and P'₂ for G₂. From Prosser, 1957.

Fig. 2

SPECIATION SEQUENCE

1. Environmentally induced variation
2. Behavioral extension
3. Random genic variation
4. Selection of varieties, races and subspecies by adaptive characters
5. Reproductive isolation established
6. Intensification of isolation by non-adaptive characters

or geographic ranges through their life cycles, and that each species is uniquely adapted to its niche and range, then a complete physiological description of its adaptive properties would give a meaningful description of a species in terms of natural selection. There is great need for description of populations in terms of isolating mechanisms which are physiological.

"At the outset we must distinguish two bases or types of physiological adaptation. We know that there are measurable differences among individuals, among natural populations, subspecies or varieties, species, and genera. These variations may be environmentally induced or may be genetically determined. Figure 1 schematically represents two genotypes, each with two populations living in different environments and differing phenotypically, for example, in a character such as temperature tolerance, which I shall discuss later in more detail. The genotype sets the limits within which a developing individual can vary and two populations, P1 and P2 with identical genotypes for a given character, may grow in different environments and be quite different. A genetic change which is adapted toward G2, a different genotype, in the direction of a particular environmental stress, is more likely to become fixed by selection if it occurs in the population living near the limits where that change is adaptive than in a population living far from these limits.

"Whether an observed difference between two populations is genetic or environmentally induced can be ascertained by acclimatization—that is, by putting individuals from one environment into the environment of the other population. I use the word 'acclimation' to refer to long term adaptive changes observed under laboratory conditions where only one environmental factor is varied and I use the 'acclimatization' to refer to those changes under natural conditions where many factors vary, as in geographic, climatic, and seasonal differences of environment.

"In the commonly accepted sequence of speciation, both genetic and environmentally induced variations are important (Figure 2). First, in the process of speciation, individuals of similar genotypes vary initially according to their environmental conditions, the conditions under which they develop and live. This is the environmentally induced variation which is so much studied by physiologists. Second, there may be in association with this, according to the environment, behavioral extension of the range. Here I am referring to behavior

which is related to physical factors in the environment, which is to be distinguished from the behavior which is so important in establishing reproductive isolation at a later stage in speciation. An example is the extension of ranges of insects to new food plants. Third, there is random genetic change—mutations and rearrangements—which may be adaptive in specific environments and which by selection may lead to formation of local races and subspecies. It may be asked whether the environmentally-induced variations are of any evolutionary value since they occur in somatic tissues rather than in germinal tissues. I would insist that they are of value when they put populations into environments where the genetic variation can become fixed by selection. This is particularly true of the behavioral adaptations.

“Following this, there is spatial separation and establishment of reproductive isolation, frequently behavioral, which may become intensified so that when two populations become sympatric they remain isolated. I would suggest then that the physiologist may make important contributions at several levels, in understanding the environmentally-induced variation, in understanding the physiological differences between closely related forms which are related genetically, and finally, in understanding the reproductive isolation which is established in speciation.

“We can examine adaptive variation in two ways. First, we can study the changes in individual organisms when the environment alters, especially with respect to stressful factors; second, we can compare the biochemistry and physiology of organisms which differ genetically in respect to some adaptation to the environment.

“In the first approach we must distinguish between a direct response to an environmental change and later compensatory acclimation which tends to counteract the stress. I would emphasize this distinction between a direct response, the initial reaction to an environmental stress, and the later compensatory acclimation. For example, a fish may respond to temperature change by increasing or decreasing its metabolism in accord with well known Q_{10} relations, yet over periods of days or weeks of acclimation, this fish tends to compensate in its metabolism for the temperature change. A plant may die of direct exposure to cold in the summer but may resist the same cold once it is acclimatized or winter-hardened.

“ My colleague in this field, Professor Herbert Precht of Kiel, has distinguished two kinds of adaptation. First is what he calls ‘capacity adaptations’, in German ‘Leistungsadaptation’. These are the changes which permit survival or relatively ‘normal’ life in the equable or usual range, for example, the changes in metabolic rates within the normal temperature range of animals. Second, there is ‘resistance adaptation’ which determines survival of an organism, of a tissue, of an enzyme at the environmental limits. The mechanisms of capacity adaptation may be quite different from those of resistance adaptation;

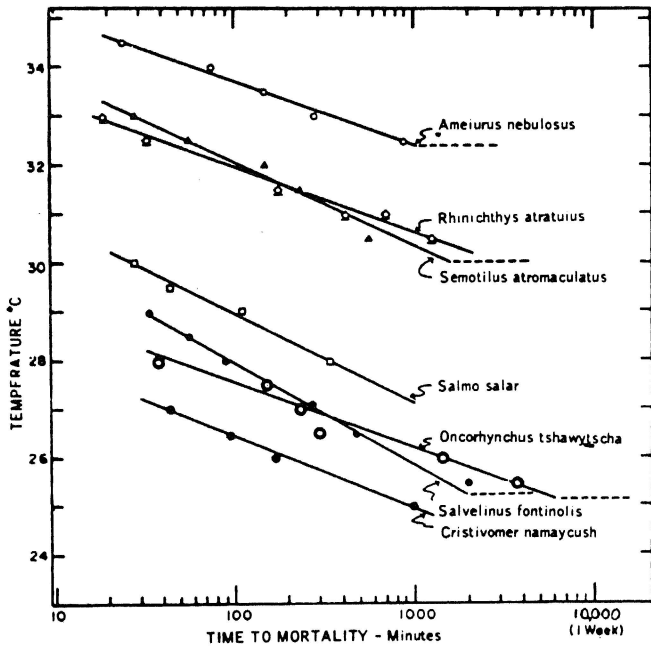


Fig. 3—From Brett, 1956.

in fact, the two may not go together at all. We should distinguish these two general classes of adaptation and I shall try to point out examples of each of these for both environmentally-induced variations and genetically-determined variations.

“After this rather lengthy theoretical introduction, I shall now move to a consideration of some examples of physiological variation as studied among individuals, populations and species. Since I have been working extensively with poikilothermic animals, I shall pick most of my examples from these, although I hope that the general principles are applicable to whatever kind of organisms may interest you as individuals.

“The first of these criteria of variation which I will discuss is a category of resistance to environmental extremes—these would come under Professor Precht’s grouping of Resistance Adaptations. These, for animals, are the lethal limits; for enzymes the denaturation limits. In the first place I should emphasize the self-evident fact that the survival limits for a whole animal are much narrower than the survival limits for most of its tissues in isolation and certainly than for its enzymatic proteins. An intact fish dies at both high and low limits, which its tissues in isolation survive. This we do not entirely understand. We can say that this is a matter ‘integration’ of the system but this is essentially ‘passing the buck’ and admitting that we do not understand it. Also, the measurement of lethal limits is more difficult than it may seem at first glance. One has to use actuarial statistics, and Figure 3 gives data from some of our Canadian colleagues

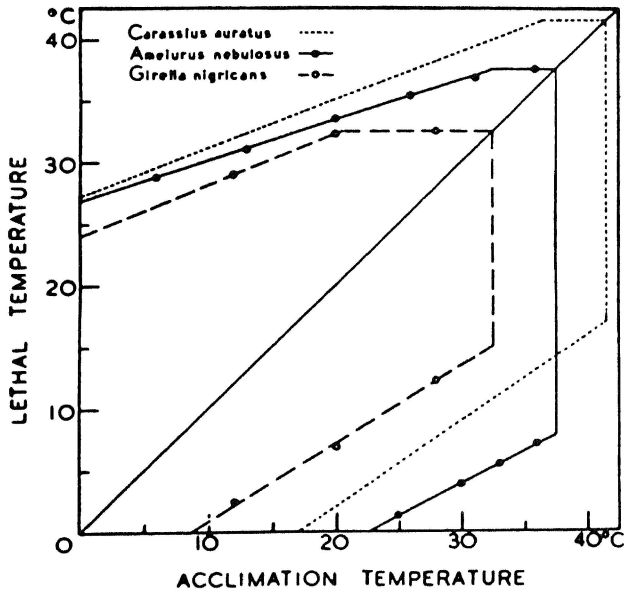


Fig. 4—From Brett, 1944.

which show the time of death of 50 percent of populations at various temperatures for different species. It is apparent that the high lethal temperature is higher for a short time to death than for a long time, but the important point here for our consideration is that there is a tremendous range according to genotypes, or species and these are ecologically meaningful. Professor Fry of Toronto has plotted the high lethal temperatures and low lethal temperatures for several kinds of fish and has pointed out that for a given fish the lethal temperature is not a fixed quantity. It is determined by the prior temperature history of that fish, that is, by its acclimation. The lethal temperature for a goldfish, for example, either the high or the low lethal, rises approximately one degree for every three degrees difference in temperature acclimation. Professor Fry connects these high lethal temperature curves with the low lethal temperature curves and produces what he calls a 'temperature tolerance polygon' (Fig. 4). You will note that each species has its own temperature tolerance polygon and when populations of the same species are compared, one finds that there are genotypes which differ even within the species and which are strictly adaptive. We summarize then that whole animals have narrower temperature tolerance limits than their parts; that the temperature tolerance limits can be varied according to acclimation; and that the limits are essential to the genetic history of the species.

"When we come to enzymatic proteins, we find, as you well know, that there are temperatures of denaturation above which the proteins are inactivated if held at these high temperatures long enough. Figure 5 is an experiment taken from Professor Ushakov's work in Leningrad on cholinesterase from leeches which had been acclimated at three different temperatures—5°, 15° and 25° C.

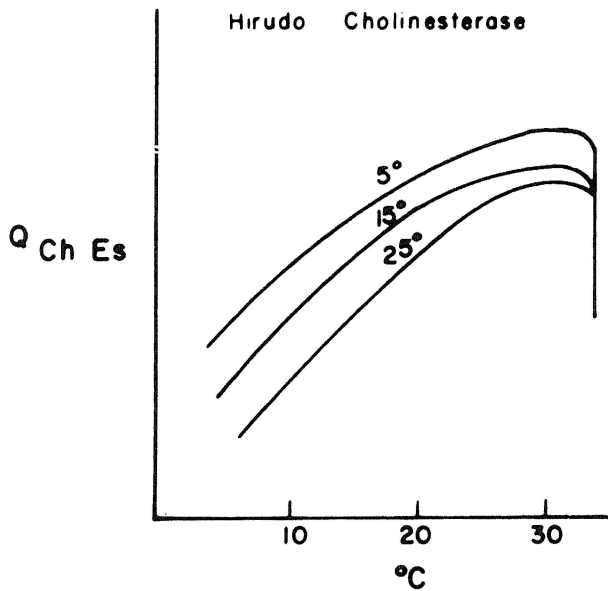


Fig. 5—Modified from Ushakov, 1964.

There are differences in rates of activity in the low temperatures. However, all of these enzymes were inactivated at the same high temperature. There is disagreement as to whether thermal limits, or as Ushakov calls them, the 'thermostability' of the tissues and enzymes can be altered by acclimation. The Russians of the Leningrad school maintain that they cannot be altered by acclimation. They do find differences among species in the thermostability of muscles and these differences are very well correlated with the ecology of these species. They find, for example, that the high lethal limits of arctic species are much lower than those of temperate zone species for isolated tissues and isolated enzymes. But all the differences they find are genetic and are not inducible by acclimation. Not all of us agree with this. Figure 4 presents data for whole fish where the lethal limits are certainly affected by acclimation. Also, in the plant material and in protozoa, which the Russians have studied, there are differences with acclimation or temperature hardening. I have suggested that perhaps one of the reasons that Ushakov failed to find acclimation differences in respect to enzymes has been that he has been looking mainly at enzymes from muscle tissue. I am quite certain that in the case of these animals, and in poikilothermic animals in general, the lethal limits are determined by the central nervous system and perhaps if they had looked at enzymes from the nervous system rather than from muscles they might have found acclimation effects.

"A second criterion of physiological variation and one which is related to the first, is reproduction and growth. Embryos and developing organisms in general have narrower limits of survival than adults. We find frequently that

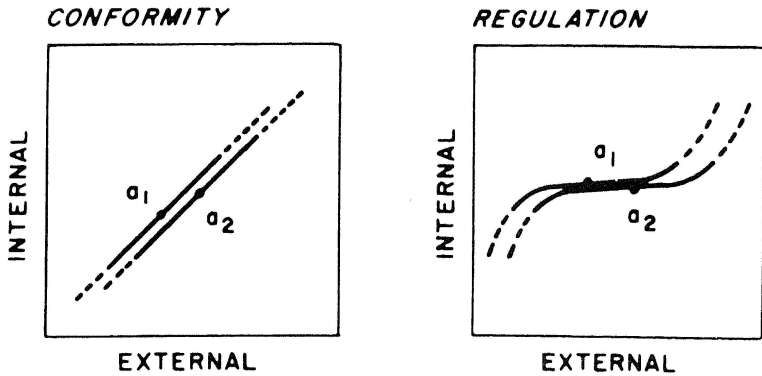


Fig. 6—From Prosser and Brown, 1961.

there are geographic clines of populations of a species which show striking differences in the tolerance, particularly for temperature, of their embryos. One of the best known of these is for the common grass frog, *Rana pipiens*, studied in America by Professor Moore at Columbia. The frogs of Wisconsin develop at such different rates at given temperatures than those of the frogs of Texas and northern Mexico that while they are the same species and their eggs can be cross-fertilized by sperm from the other populations, the hybrid embryos will not develop because of differences in the rates of growth.

“A third criterion is what I have called the characterization of the internal state as a function of the external state or of the environment. By this I refer to any property of the internal medium, for example, the internal body temperature, internal osmotic concentration, concentration of specific ions, and the like, as a function of the same parameter in the environment.

“There are two basic patterns by which organisms respond to their environment (Fig. 6). They can conform, that is, the internal state may be the same as that of the external. In this case the internal state of the organism is a linear function of the external state, as for example, in poikilothermic animal the body temperature is essentially that of the environment. Beyond certain temperature limits which are genetically determined, the animal can survive for only a short time. The range within which an animal can function normally can be altered by acclimation, hence we may have two states of acclimation which have different limits and these can be shifted only as far as the genotype permits.

“The second pattern is that of regulation (Fig. 6), in which the organism maintains relative constancy of its internal state in varying environments. This is brought about by feed-back reactions which tend to stabilize the internal state, as for example, in warm blooded animals. At certain limits these feed-backs or homeostatic reactions fail and the organism does not long survive. Acclimation can shift the mid-point for these regulatory curves and can shift the points of threshold or the limits of failure of the feed-back reactions. Animals which con-

form tend to have much wider tolerance of internal variation; whereas animals that regulate have a narrower internal variation but a very wide tolerance of external variation. A moment's thought will show you how true this is for such factors as temperature and some others. Figure 7 gives data for osmotic regulations. The osmotic concentration in terms of the lowering of the freezing point for body fluids of a few crustaceans is given as a function of environmental osmotic concentration. Some crustaceans, like the spider crab, *Maja*, are conformers throughout. There are others such as some of the shore crabs, which regulate on the dilute side and conform on the high side (Fig. 7a). Still other crabs regulate on the dilute side and also regulate on the concentrated side (Fig. 7b). They are hyper-osmotic in a dilute medium and hypo-osmotic in a concentrated medium and the mechanisms at the two ends are very different. We can, therefore, have combinations of conformity and regulation. This type of analysis can be extended to various physico-chemical parameters, oxygen levels, glucose, and so on as well as osmotic, ionic concentrations and temperature.

"A related phenomenon is the recovery from deviated state. This applies particularly for regulators such as homeotherms. Dr. Johnson has been interested in the problem of extreme heat and Professor Brody made important contributions to the general question of heat loads. This concept can be applied to a variety of parameters—heat load, water load, sugar load. When an organism is deviated from its 'optimal' load with respect to heat, water, blood sugar and other parameters, and is then removed from the stress back to an equable or normal environment, it returns over a definite pattern and this pattern of return is in itself usually genetically determined and may be used to characterize physiological variations.

"A fourth general category of physiological variation is what I would call 'rate functions'. These bring us back to Precht's classification of capacity adaptations. Heart rates, rates of breathing, enzymatic rates of various sorts, metabolic rates, have been used very much. To understand this, we must first follow the time course of oxygen consumption in poikilotherms in an altered temperature (Fig. 8.) If we consider that the animal has a definite level of oxygen consumption initially, and its temperature is raised, usually there is an initial overshoot and then a period of what we have referred to as 'stabilified state'—this is the time when we measure the rate for a Q_{10} measurement. This would be a matter of hours. If the animal is then left for some time—days, weeks, months—at the new temperature, it gradually comes back toward its original O_2 consumption level. If it compensates completely it returns to its original rate but usually compensation is only partial. The animal is in a different biochemical state in these two conditions. If it returns to its initial temperature from the stabilized state it comes back directly to the original metabolism, but if it returns after compensation it under-shoots considerably below its original metabolic rate. Fig. 9 is an example from the work of one of my students with goldfish; when the temperature was raised the metabolism rose and then over a period of days it gradually returned toward the original value. The time course of these changes was unaffected by several endocrine treatments.

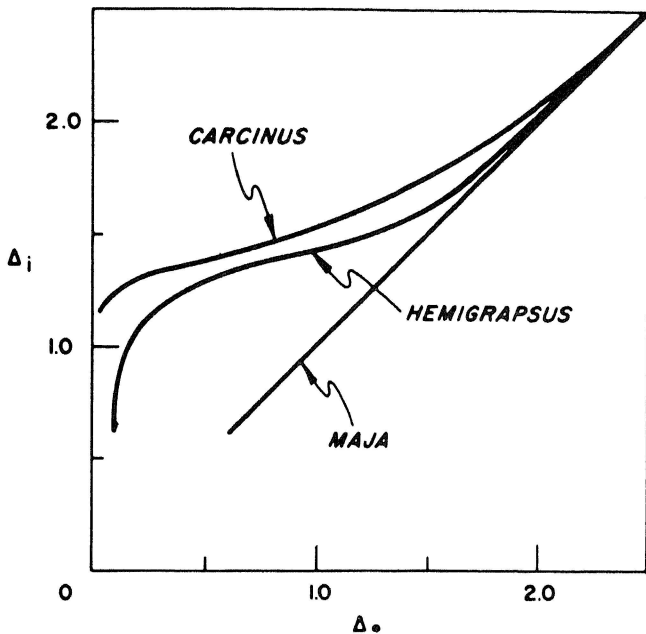


Fig. 7a—From Prosser and Brown, 1961.

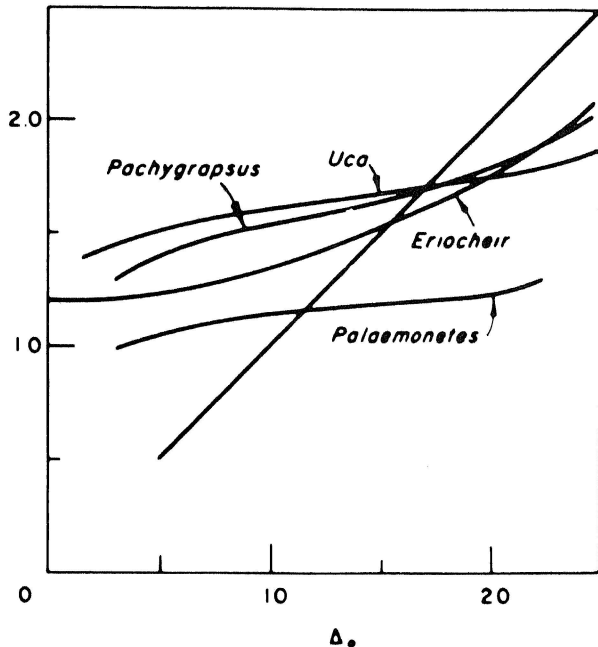


Fig. 7b—From Prosser and Brown, 1961.

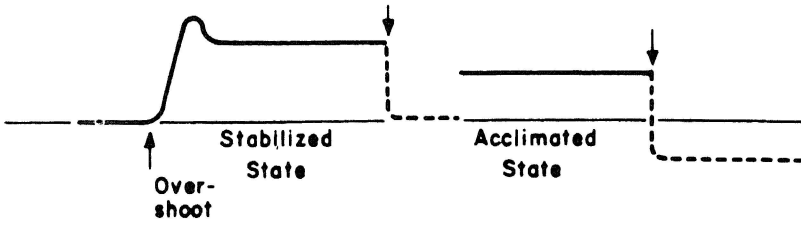


Fig. 8—From Prosser, 1964.

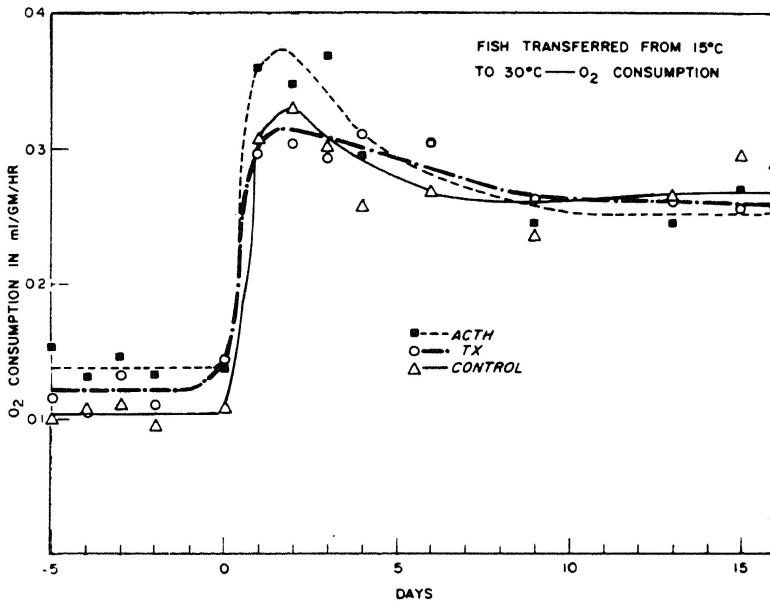


Fig.9—From Klicka, 1962.

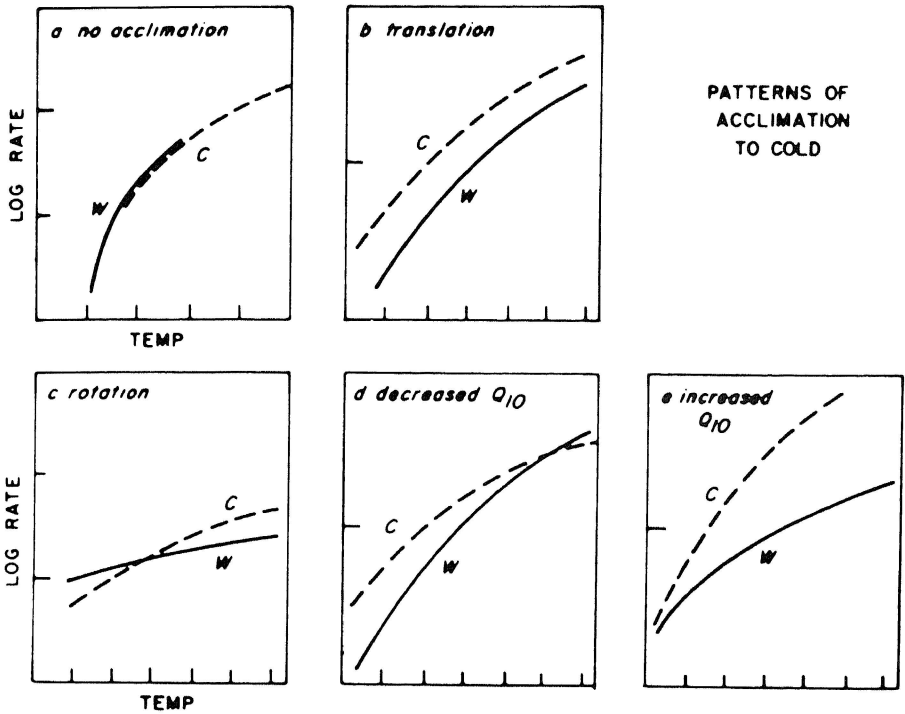


Fig. 10—Patterns of acclimation of rate functions in cold. Rate functions measured at different temperatures. W, warm acclimated animals; C, cold acclimated animals. Modified from Prosser and Brown, 1961.

“When one measures a rate function at different temperatures, for example, the rate of metabolism plotted on a log scale against temperature, we find a variety of patterns of acclimation. There may be no acclimation at all (Fig. 10a); the warm acclimated and the cold acclimated curves may be continuous. There may be a simple translation of the curve (Fig. 10b), and the effect of this is that the metabolism of the cold acclimated is at a higher rate at intermediate temperature than the metabolism of warm acclimated. There may also be a rotation of the curves, the curves intersecting at a mid-point (Fig. 10c). Often one finds a combination of translation and rotation (Fig. 10d, e). The second pattern or the translational change, would imply quantitative alterations in the enzyme systems involved. The rotation implies qualitative changes in the enzymes. There may be combination patterns in both directions.

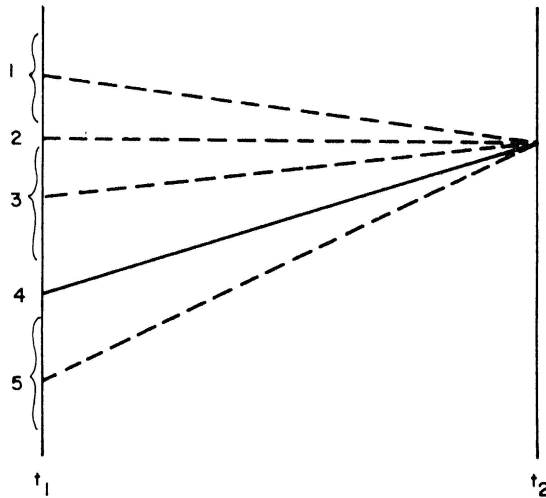


Fig. 11—From Precht, 1958.

“We can look at this in a simpler way by the scheme of Figure 11, which is from Precht. If we consider the metabolism of a cold-blooded animal at one temperature t_2 , and if the animal is then cooled to temperature t_1 , the metabolism drops directly to point 4—and this would be the Q_{10} determining point. If there is no acclimation, no compensation, the metabolism will remain at that point. If there is complete acclimation, it will go up to point 2. This would be complete or perfect compensation. Usually it falls in group 3, somewhere between 4 and 2. There are known cases of over-acclimation, type 1, and there are a few cases, a few specific enzymes, where it goes in the opposite direction, that is, the so-called paradoxical acclimation (type 5).

“Figure 12 shows data on goldfish where the oxygen consumption of the cold-acclimated and the warm-acclimated fish in activity and in standard conditions are compared. The active metabolism curve of warm-acclimated fish lies to the right of the curve for the cold ones and the same is true for the standard metabolism. This says that at an intermediate temperature, the cold-acclimated animal has a higher metabolism than the warm-acclimated—that is, the cold-acclimated animal is compensating for the cold. This phenomenon of compensatory acclimation in the cold is a very common one and the differences between cold- and warm-acclimated poikilotherms can be seen not only in whole animals but in isolated tissues and in relatively purified enzymes.

“The net effect of these changes in poikilotherm is shown in Fig. 13, as follows: The animal’s temperature slides up and down with environment—it is a conformer with respect to temperature. However, the metabolism initially drops when it is cooled and on acclimation it gradually increases; or, its metabolism when it is warmed goes up and then gradually falls, so that there is then a tendency for a cold-blooded animal to have a varying temperature but a relative-

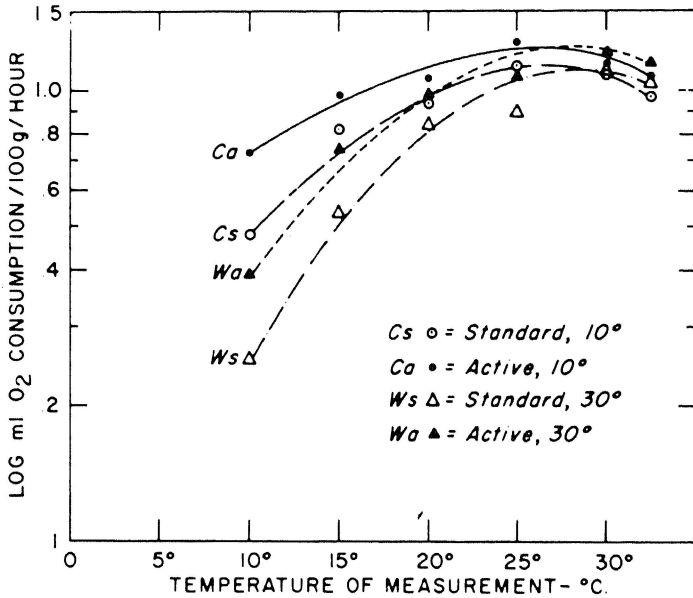


Fig. 12—Oxygen consumption of fish acclimated to 10° and 30° C. From Kanungo and Prosser, 1959.

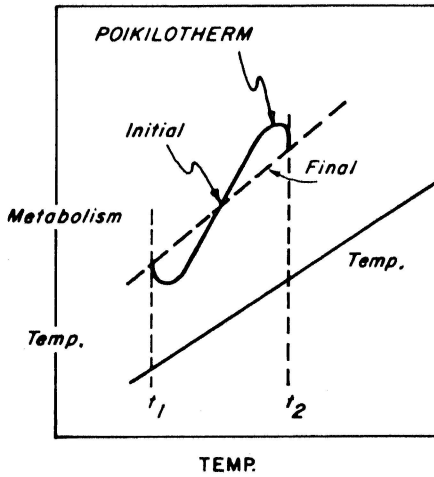


Fig. 13—Schematic representation of temperature relations in a poikilotherm. Metabolism decreases more steeply than body temperature. Acclimation results in a rise in metabolism at low temperature and a fall at high temperature, thus tending toward relative constancy of metabolism as the environmental temperature changes. From Prosser, 1962.

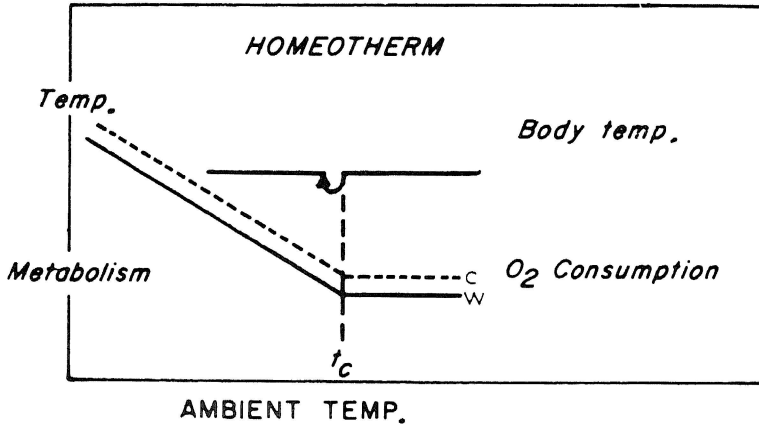


Fig. 14—From Prosser, 1962.

ly constant metabolism. This is to be contrasted (Fig. 14) with a warm-blooded animal where over a range of environmental temperature, compensation is brought about initially by insulating mechanisms, but at some critical value the metabolism increases—heat production rises, so that the homeotherm is just the opposite of the poikilotherm metabolically in the cold. In the cold, the homeotherm maintains a constant temperature with varying metabolism; while the poikilotherm keeps a relatively constant metabolism in a varying temperature. At high temperatures, as some of Dr. Johnson's work has shown, the homeotherm behaves more like the temperature conformer.

"We may conclude that rate functions, particularly those of metabolism, do show compensatory acclimations and also variations which are genetically related to the ecology of the organism.

"A fifth category of physiological variation which I would list is somewhat different from the others that I have named. This is animal behavior. This is probably the most important of all variations in terms of isolating mechanisms in nature. Initially, I would refer to orientation behavior, the *taxes* of which are related to physical factors in the environment: this is behavior which puts the animal into an environmental limit. In later stages of speciation, reproductive behavior appears—sex and species recognition, courtship, territoriality, mating, care of young, and so on. Part of this behavior is genetically determined, the so-called instinctive behavior. Part of it is environmentally determined, the so-called learned behavior. Let me just cite one example from a recent paper by Konishi from Marler's laboratory at Berkeley on the songs of the white-crowned sparrows. These sparrows were collected from different valleys around the San Francisco Bay and phonograms of the songs of this sparrow show that each valley has its own dialect of the song for that particular species. On the other hand, there is a basic pattern of song for the species. When the young were reared from eggs which were hatched in the laboratory and the young did not hear

another bird until they came to the singing stage, they sang and with difficulty the song could be recognized as that of the white-crowned sparrow. On the other hand, if they had heard any one of the various other birds for a short time during their early development, long before they themselves could sing and then heard no more, when they reached the stage of singing they showed the dialect that they happened to have heard. Many such examples could be cited, so that here there is a genetically determined character, in this case a behavioral pattern, which must be dependent upon certain neural circuits and superimposed upon this is the environmentally-induced variation, the learned pattern. Thus our theoretical approach to the problem of animal variation can be carried over even to such things as animal behavior.

“Finally, in this listing of criteria of physiological variations, I would refer to chemical specificities. All of you are familiar with the striking species differences with respect to antibodies, specific proteins; these by and large are genetically determined. There is a whole host of compounds in this field of chemical specificity, which is a very popular one at the moment. Many kinds of compounds are being used for tracing genetic relationships and evolutionary history. For example, I would like to cite the work of Margoliash on the cytochromes. Finger-printing of cytochromes in terms of the number of variant residues of amino acid sequences shows that for muscle cytochrome, between horse and pig there are 3 such different residues; between pig and chicken 12; and between pig and tuna 19; between pig and yeast 43. The time between horse and man is estimated from the fossil records as having taken 130 million years which for the number of differences gives 11 million years per residue difference retained. This would suggest then that the time for divergence between pig and horse was 33 million years; for rabbit and yeast, 500 million years. Those of you who are familiar with paleontological data will recognize that these values are not too far from the figures which we get from other kinds of evidence. The same sort of data is shown for hemoglobins in Figure 15, which indicates the evolutionary sequence for hemoglobins which apparently have evolved by a step-wise fashion from myoglobin to the A_2 hemoglobin, which is present in all hemoglobins, to the gamma hemoglobin which is in fetal and then to the adult alpha and the

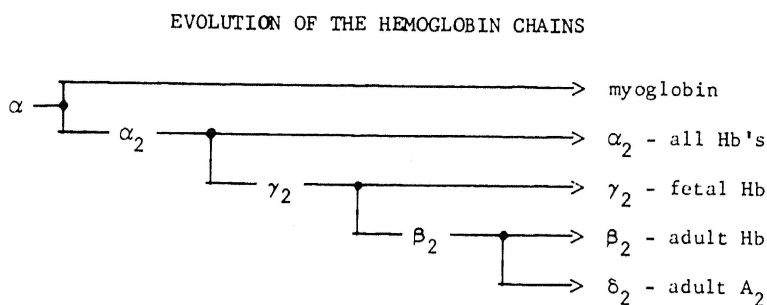


Fig. 15—From Ingram, 1963.

adult delta. Ingraham has, in a similar fashion to that for cytochromes, estimated the times at which these might have separated.

We can argue the same way with respect to some of the hormones—insulin, and the pituitary polypeptides. You are familiar, in the case of hemoglobins, with sickle cell hemoglobin which seems to give some adaptive value in terms of resistance to malaria.

“I have reviewed very quickly a set of criteria of physiological variations and have attempted to show you that we can apply some of the theoretical considerations which I stated initially in terms of environmentally-induced vs. genetic control to this whole series of criteria. I would suggest further that it is time we began to apply these not only in laboratory animals but under natural conditions and that the ecologist, systematist, physiologist and geneticist must work together to solve some of these real problems of natural selection.

“What may be the molecular basis for these variations? I should now like to take a few minutes to talk about what we might hope to find as we get into the next step in the analysis of physiological variation. This next step in the analysis is to elucidate the molecular bases for these different patterns of variation that I have already enumerated. First, there are three different levels at which protein differences might be expected to be found. First, we might find qualitative differences in the proteins, and here the ones I have just referred to in cytochromes and hemoglobins may serve as examples. Insulin is one which has been studied very extensively. These differences in proteins might be of two sorts—qualitative differences (1) in the primary structure or the amino acid sequence, and (2) in the tertiary or quaternary structure—in the degree of folding of the protein molecules. If we accept the dictum that the primary structure is determined by the genetic template, we could hardly expect that acclimation could alter it. We should not expect that there would be any environmentally-induced variation in the primary structure. As far as the degree of folding of the protein molecules is concerned, we are on less certain grounds. There are changes in tertiary structure according to the way proteins are extracted, but these conditions are usually non-biological. There are differences of opinion as to whether there can be qualitative changes in the tertiary structure induced by environmental variation, but certainly we can find differences in protein structure which are genetically determined and these differences may be adaptive in their value. For instance, Figure 16 gives the hydroxy-proline content of collagens from a variety of fishes from different habitats. This amino acid is plotted against the temperature of melting of the collagens and you will note that there is a very distinct trend of cold water fishes to have a relatively low hydroxy proline content of the collagen as compared with warm water fishes. Actually, there are more recent data which seem to correlate better with the content of hydroxy-proline plus proline. Here is a clearly adaptive value for the particular primary structure of this particular protein. There are other cases for this same sort of correlation in some of the enzymatic proteins, particularly in microorganisms. Data for amylases, for example, from thermophilic bacteria as opposed to meso-

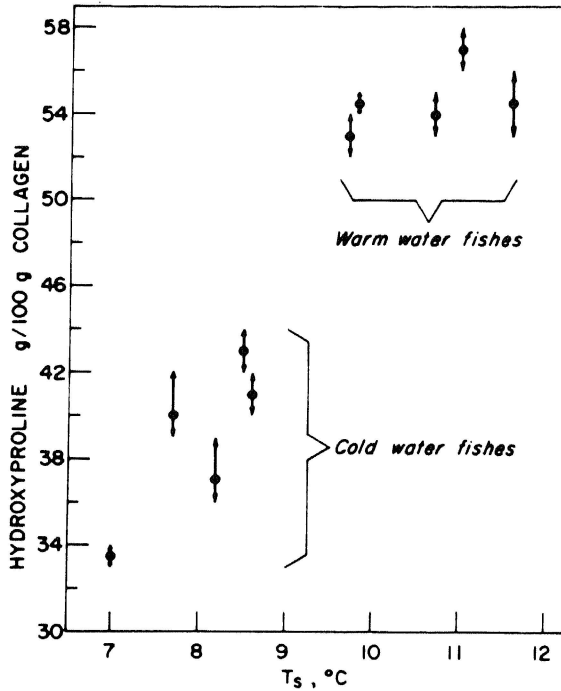


Fig. 16—From Gustavson, 1956.

philic bacteria show clear differences in amino acid sequences. The advantages of a particular sequence is not understood.

"A second aspect of protein variation is quantitative changes; that is changes in relative amounts of different enzymes and it is this to which I have referred in Figure 10 where I showed you translation of the rate function curves. This can occur in a variety of systems. Figure 17 gives data from current experiments of one of my students, Mr. Freed, on cytochrome oxidase from the muscle of fish that have been acclimated to different temperatures. Here is plotted the rate constant of the activity of the cytochrome oxidase measured at different temperatures. The broken line connects the points at temperatures of acclimation; the other points are temperatures of measurement other than temperatures of acclimation. You can see that the cytochrome oxidase curve is shifted in compensation, quite clearly a translational effect. This suggests that there is no change in the temperature coefficient but only a change in the amount of the enzyme. This sort of thing is found in many biological systems. At the present time we are measuring total protein turn-over in a variety of tissues from fish that are acclimated at different temperatures and we are finding the incorporation of labelled amino acids to show quantitative changes. The rate of incorporation at an intermediate or low temperature is greater for cold-acclimated fish than for warm-acclimated fish.

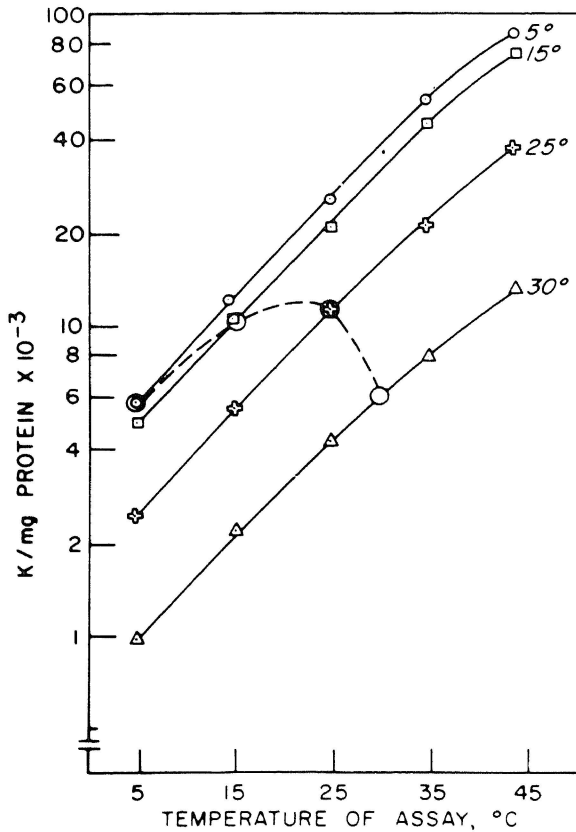


Fig. 17—From Freed, 1965.

“A third type of protein effect would be selection of one of the multiple proteins or one of the multiple pathways in an enzyme system. Many enzymes occur as isozymes, multiple forms, and we may find one of these to be selected preferentially over another. If, for example, we have parallel metabolic pathways and one of these may have a higher temperature coefficient than the other, this one may be reduced more in the cold and thus cause intermediates to be formed which can induce the enzyme of the alternate pathway. An example of this is found in metabolism of fish, from evidence that in cold-adapted fish there is a tendency to use the pentose phosphate shunt more than in warm acclimated fish. In general, the glycolytic pathways are more important in warm acclimation. There are many examples of this sort of thing, of the use of a particular form of protein under one environmental condition and another form of the same protein under a different environmental condition. An example, which is probably familiar to many of you, concerns the opsins of visual pigments. It is well known that rhodopsin is the visual purple of marine fish and of terrestrial verte-

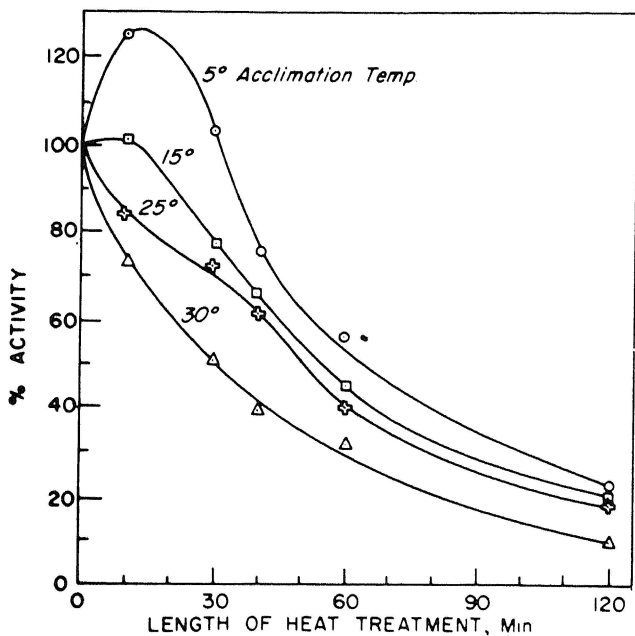


Fig. 18—From Freed, 1965.

brates. Porphyropsin, a slightly different protein, is the main visual pigment of fresh water fish, and those amphibians which start in fresh water and go to land, shift from the porphyropsin to the rhodopsin. Some few urodeles which go as young salamanders to land then back to water actually reverse the process back to a porphyropsin system. Why rhodopsin is adaptive for life in the sea and on land, and the porphyropsin to life in fresh water, we do not know, but it must have some adaptive significance. In summary, there are three types of protein variation—the structural changes which are certainly mainly genetic; the quantitative changes which can be environmentally induced, and the selection of isozymal proteins under environmental conditions.

“Another molecular basis for physiological variation lies in the co-factors, particularly co-enzymes, and lipids associated with enzymes. A possible example is given by the cytochrome oxidases to which I referred previously. Mr. Freed measured the activity after heating to 45° C. for different lengths of time and found that the enzymes from the 30° acclimated fish denatured pretty much as you would expect (Fig. 18). In the 15° acclimated fish it took a little time before denaturation occurred, and in the 5° acclimated fish the enzyme activity after 5 to 10 minutes of heating was actually greater than it was initially. This may represent the removal of some inhibitory substance which is present in greater amounts in the cold adapted fish.

“Another molecular difference might be in the nature of the lipids, both those associated with enzymes and with those in cell membranes. In general the

lipids which are deposited at low temperatures, even in domestic animals, have lower melting points and consist of more unsaturated fats than the lipids deposited at high temperatures. We are now finding very striking differences in this respect according to temperature acclimation of fish. This may be extremely important not only because of the conjugation of lipids with some enzymes but also because the central nervous system is important in adaptation and nervous membrane phenomena may be particularly important.

"As to mechanisms of production of biochemical changes, we postulate that there may be a number of mechanisms such as enzyme induction, repression, inhibition. The microbial biochemist is accustomed to thinking of induction, repression, inhibition by products and substrates. The microbial investigators have thought very little about physical factors in the environment as producing such changes and yet we in animal physiology know that these must be occurring.

"We may ask the question at this point—how many of these changes that occur in an animal, the environmentally-induced changes, are direct and how many are indirect? That is, are they effects of temperature on the tissue or are they produced via the nervous system and the endocrines? Professor Precht attempted to answer this question in a very ingenious experiment. He placed an eel, a long fish, in a glass tube with exits in the middle so that he could run cold water into one end and hot water into the other end. Thus he could have an eel with a hot tail and a cold head or a hot head and a cold tail. He had found previously that muscle removed from eels which had been acclimated to the cold and warm showed temperature acclimation of the sort that I have discussed heretofore and he found that when he had one end cold and the other one warm and he removed bits of the muscle, the activity of the cold was higher than the warm. Then one of his students, Schultze, found that the metabolism of the whole eel depended not so much on the temperature of the body as the temperature of the head, so that what was found in the isolated tissue might be opposite to what was found in *in vivo* conditions. He also found that if the entire eel was at one temperature, the enzyme activity of the tail was always higher than at the head. I came into the picture at this point and measured the tonic electrical activity of the muscles of the eel at the two ends. The oxygen consumption of the anterior end is expressed in Figure 19 as a percentage of that of the posterior end. When both ends of the fish were at the same temperature, the anterior was lower than the posterior end by about 25 per cent. When we measured the activity of the muscle electromyographically, we found that the number of spikes of muscle activity of the anterior end as per cent of those in the posterior end was almost identical to the value for oxygen consumption *in vitro*. When we had spinalectomized animals, the difference was somewhat greater. When the head was cold and the tail was warm, the difference in oxygen consumption diminished, also the electrical activity in the muscle. I am suggesting from this evidence that the nervous system, by tonic motor activity, can influence the enzyme levels in muscle. Second, I am suggesting that an important component in temperature adaptation in these fish may be brought about via the nervous system.

Tonic Electrical Spikes in Muscle and O₂ Consumption of Muscle Tissue with Standard Errors. In Parentheses Data from SCHULTZE ('61) and Number of Experiments [].
 Sp = Spinalectomized.

Conditions of Acclimation (°C)		O ₂ Consumption Relative to that of 14° Eels	O ₂ Consumption (%) $\frac{\text{anterior end}}{\text{posterior end}} \times 100$	Tonic Electrical Spikes in Muscle (%) $\frac{\text{anterior end}}{\text{posterior end}} \times 100$
Anterior	Posterior			
14	14	(100)	(74.1)	75 ± 3.7 [14]
25	25	(69)	(73)	83 ± 14.5 [6]
14	25	(100)	(95)	98 ± 14.2 [4]
25	14	(69)	(50.3)	-----
14	Sp 14	-----	61.8 ± 2.64 [7]	69.2 ± 3.6 [9]

Fig. 19—From Prosser, Precht, and Jankowski, 1965.

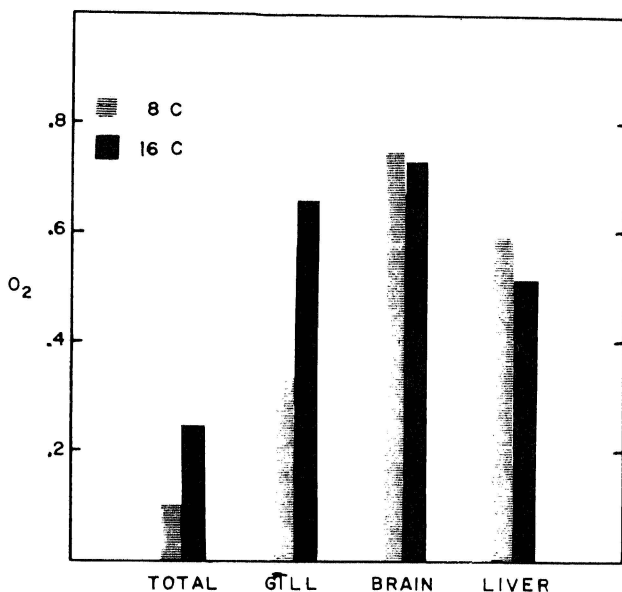


Fig. 20—Comparison of average total and tissue metabolic rates at 8° C and 16° C. Tissue oxygen consumption on a wet weight basis calculated from tissue dry weight (percentage water content: gill 81.7%; brain 81%; liver 75%). From Evans, Purdie, and Hickman, 1962.

“In addition, the temperature acclimation may be different in various tissues. Figure 20 gives data on oxygen consumptions by trout measured at the temperatures of acclimation of 8° and 16°. At the temperature of acclimation, the metabolism of the 16° fish is somewhat higher than that of fish acclimated to 8°. In the case of brain, the acclimation is complete—the oxygen consumption is essentially the same at both temperatures. In the gills, there is very little acclimation. In the liver there is an over-compensation in this particular species,

Conduction Block, Catfish Nerve

Acclimation Temperature	Number of Preparations	Lowest Temperature for Full Response	Partial Block	Complete Block (<10%)
23-25°	4	5°	4-4.5°	3°
15°	6	3-5°	3°	0.5-1.5°
10°	2	3°		1°
5°	2		0°	<0°

Fig. 21—From Prosser and Farhi, 1965.

the 8° fish having a higher metabolism than the warm fish. Thus we find a very complicated situation—direct effects, inequality of effects on different tissues, and finally control by nervous system and probably also by endocrines, although in fish the endocrines are much less important so far as metabolism is concerned than in mammals.

“Finally I wish to refer to another kind of temperature acclimation, one with which we have become increasingly concerned during the past few years. This has to do with behavioral adaptations. I have already referred to behavior as one of the types of physiological variation which is useful for characterizing populations and species. The question that we have raised has been this: to what extent are behavioral modifications under the influence of the environment important in individual acclimation with respect to temperature? We studied this by measuring the temperature of cold block of various nerves and in Figure 21 you can see that peripheral nerves are blocked at relatively low temperatures and that there is not much effect of acclimation.

“When we go a little higher in the nervous system and start looking at reflexes, the temperatures of cold block are somewhat higher and there is a greater effect of acclimation temperature (Fig. 22). At still higher levels in the nervous system we find much more dependence upon temperature. My colleague, Dr. Roots, has measured the maximum rate of swimming of green sunfish at different temperatures. Figure 23 shows that there is a minimum temperature below which the fish will not swim. If the temperature is raised, the fish swims and the rate reaches a maximum and then there is a high temperature above which they will not swim. The minimum temperature, in other words, the temperature for cold block of this function, varies according to acclimation, so that we find higher temperatures for the blocking of swimming than for the blocking of spinal reflexes and much higher than for blocking of peripheral nerve.

“As a still more complex function we have conditioned the breathing movements of fish to a light, combining the turning off a light with an electric shock (Figure 24). After a few presentations, the light alone tends to interrupt the breathing. In other words, the fish has become conditioned to the light. If the fish was conditioned and then cooled from 25° to 15°, the light lost its effect,

Table 1. *Cold-blocking temperature of a simple reflex in goldfish*

Acclimation temperature (°C.)	Blocking temperature (°C.)	No. of fish
35	10	4
25	5	7
15	1	8
5	—	4

Table 2. *Cold-blocking temperature of a simple reflex in bluegills*

Acclimation temperature (°C.)	Blocking temperature (°C.)	No. of fish
30	10*	3
25	5	4
15	1	3
5	—	3

* Very slight response.

Fig. 22—From Roots and Prosser, 1962.

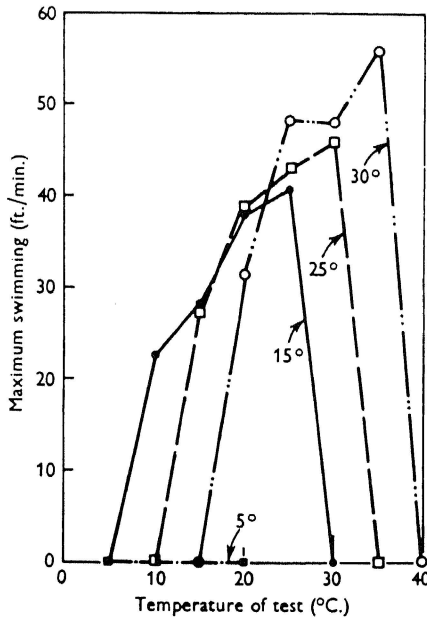


Fig. 23—Relationship between temperature and maximum swimming speed in green sunfish acclimated to different temperatures. The acclimation temperature is shown beside each curve. From Roots and Prosser, 1962.

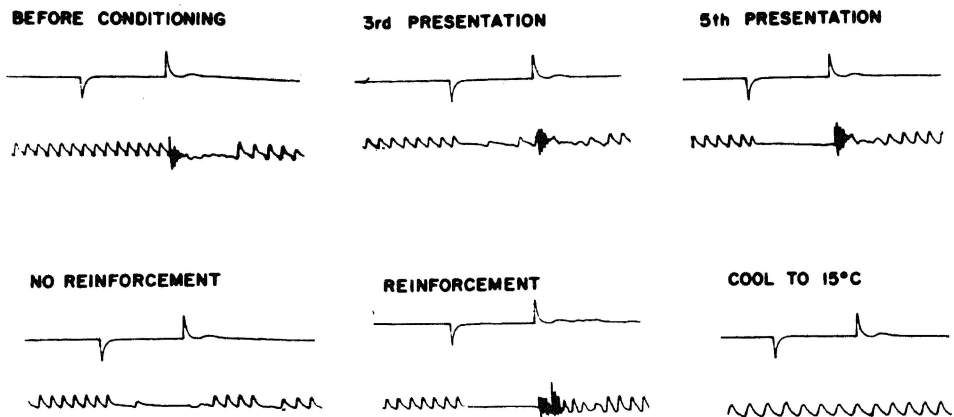


Fig. 24—From Prosser and Farhi, 1965.

that is, the fish forgot. Some fish are rapid learners and in only a few trials they are well conditioned, while other fish are poor learners and with many trials never do become conditioned. Usually, if we cool after we condition and then rewarm, we may get recovery of the response.

“The temperature at which the blocking occurs is a function of the temperature of conditioning, that is, the temperature of forgetting depends upon the temperature of learning (Fig. 25). In a fish that was conditioned at 25°, the blocking is at about 15°. Fish which were conditioned at 15° are blocked at 10°. In addition, we find that there is a minimum temperature below which they cannot be conditioned and this depends upon the temperature of acclimation. A fish which has been acclimated at 30° cannot be conditioned below 17-18°. A fish which has been acclimated at 15° can be conditioned down to 6-7°. Gain or loss of capacity for being conditioned occurs in 12 to 72 hours of temperature acclimation, much faster than metabolic acclimation. Thus we have three temperatures—the temperature of acclimation, the minimum temperature of conditioning, and the temperature of blocking—which are interrelated. I would suggest that these central nervous changes have a very important role in the temperature acclimation of fish. Such changes permit fish to carry out behavior at low temperature, behavior which they could not possibly carry out when first transferred directly to low temperature. Obviously, this must have a molecular basis in the nervous system. This is one reason we are so much interested at the moment in lipids in the nervous system. I am increasingly impressed with the role of the nervous system in the whole phenomenon of physiological adaptation.

“I may summarize in this way:

Adaptational physiology is the key to understanding of the basis for survival and reproduction of individuals, populations and species. I have listed a variety of categories of criteria of physiological variation. Each of these must be useful in natural selection. Each variation shows genetic limits within which environmen-

RELATION BETWEEN BLOCKING TEMP AND CONDITIONING TEMP

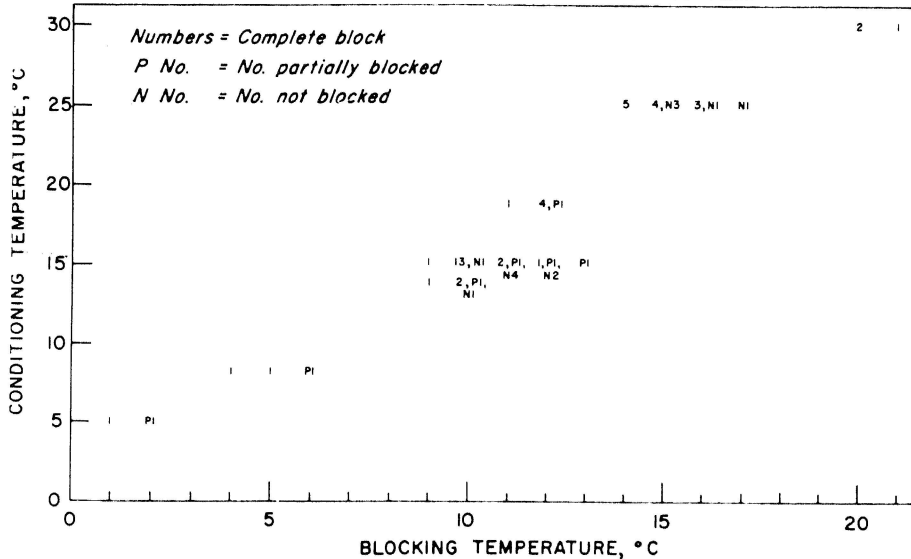


Fig. 25— From Prosser and Farhi, 1965.

tally-induced changes may occur. The mechanisms for these variations and for changes in them vary according to the organism or tissue and the kind of adaptive variation. In individuals, environmentally-induced variations can be based upon changes in protein synthesis, favoring of specific isozymes, co-factors, lipids and changes in the central nervous system. In genetic variation, in addition to these just named, one must add changes in protein structure, particularly primary and tertiary structure. I suggest that the analysis of environment-organism interaction in molecular terms may give us an ultimate explanation of the phenomenon of natural selection."

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