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Molecular and Integrative Biology

THE PHYSIOLOGY OF CHANGE

The body does not always seek constancy in its internal environment. It does not always react in ways that prevent change; sometimes physiological mechanisms actively promote change. This is no new revelation. The scientific literature already contains numerous explanations couched in terms of changing of set-points. One may read of the resetting of baroreceptors, osmostats, chemostats, and alphastats. Adjustment to thermostats, gonadostats, mechanostats, and lipostats have already been proposed. Some name is needed to recognize the generality of these phenomena. *Rheostasis* is a convenient term for designating change in regulated levels.

What qualifies as an example of rheostasis depends on how extensive or rigid are the criteria adopted and whether the preference is for errors of commission or errors of omission when information is lacking. Table 8–1 lists examples discussed in this book, dividing them into those for which the evidence for their being a defense of a different level of a variable seems fairly persuasive and those for which little more than a few suggestive hints and speculations about rheostasis exist. At the bottom come cases where obvious open-loop factors are present. This classification is provisional, arbitrary, and subjective. Some of the examples may perhaps not be justified and others could doubtless have been included. Table 8–1 is presented just by way of a recapitulation of the phenomena discussed in previous chapters and as a reminder that, whatever one may think of particular cases, rheostasis is not confined to a few esoteric difficulties encountered by atypical species. On the contrary, it contributes to major and common themes of life: maturation, the challenge of the seasons, survival and breeding, the care of the young, feeding, and the alternation of day and night. The subject merits study—but how?

Table 8.1. Summary of Examples of Change Discussed in This Book*

Programmed	*Reactive*	*Second Order*
CATEGORY 1		
Incubation weight loss	Infection: fever	Irradiation: growth trajectories
Seasonal weight changes: hibernation	Infection: cryexia	Brain lesions: growth/fattening rates
Seasonal weight changes: premigration	Psychogenic fever	Precocious and delayed puberty
Seasonal weight changes: other	Paraplegia: thermal set-point	Circadian cycle amplitudes
Estrous cycle: weight	Skin temperature: thermal set-point	
Menstrual cycle: temperature	Undernutrition: gonadostat resetting	
Estrous cycle: gonadostat resetting	Undernutrition: temperature and torpor	
Seasonal cycles: gonadostat resetting	Undernutrition: thyrostat resetting	
Puberty: gonadostat resetting	Undernutrition: glucocorticoid levels	
Aging: gonadostat resetting	High-fat diet: persistent obesity	
Lactation: elevated temperature	Dehydration: elevated temperature	
Pregnancy: osmostat resetting	Inappropriate osmostat resetting	
Pregnancy: CO_2 chemostat	Hypovolemia: osmostat resetting	
Hibernation: lowered temperature	Blood pressure in hypertension	
Hibernation: alphastat resetting	Acute baroreceptor resetting	
Slow-wave sleep: lowered temperature	Heat stress: thermal set-point	
Slow-wave sleep: CO_2 chemostat	Physical training: thermal set-point	
Circadian cycles: temperature	Local thermal regulation	
Circadian cycles: glucocorticoid levels	High altitude: CO_2 chemostat	
Reproductive frenzy: glucocorticoid levels	Hypoxia: thermal set-point	
	Low-salt diets: CO_2 chemostat	
CATEGORY 2		
Pre-egglaying: calcium stores	Infection: weight loss	
Pregnancy: calcium stores	Shock: hypothermia	
Menopause: mechanostat change	Anorexia nervosa: temperature	
Antler growth: cyclical osteoporosis	Blood pressure: effects on temperature	
Incubation: egg cooling	Temperature: effects on blood pressure	
	Scrotal warming: body temperature	
CATEGORY 3		
	Exercise: hyperthermia	
	Diet-induced weight changes	

*See Chapters 4, 5, and 6 for details and species. In Category 1 are cases where there is some evidence for a change in regulated level. Category 2 comprises the more speculative cases. Category 3 contains examples where there are obvious open-loop factors that might be responsible for the changes.

MOLECULAR AND REDUCTIONIST APPROACHES

Once a regulatory system exists, it should be a relatively simple task for evolution to modify its setting by adjusting the strength of one of its components. For example, simply a change in the number or affinity of receptors for a feedback substance could produce a shift in regulated level. Small quantitative modulations in an existing system could be linked to biological clocks and programmed temporally. Increased transcription of a single substance might be sufficient to produce rheostasis. How could such changes be tracked down?

It would help greatly to start with information on the site of the changes producing rheostasis. For example, suppose it were known that modulation of activity in a particular area of the brain is responsible for a shift in the regulated level of a variable, then one could search for correlated phenomena in that area. Perhaps a unique mRNA or great quantities of a particular mRNA would be found. Antibodies to the corresponding proteins could then be injected to narrow down the site of action to particular cell types or receptors. However, to discover a unique mRNA would probably require comparing a cDNA library for a particular brain area to that for some control site. This could be very laborious. It would also require having a good idea in the first place of where in the brain to look for different mRNAs.

For an initial crude localization, lesion and stimulation methods still have their place. The realization that the preoptic area of the hypothalamus was important in thermoregulation stemmed in part from experiments involving lesions and local thermal stimulation. Of course, "localization" experiments of this kind must be interpreted cautiously. Presumably the preoptic region plays some role in thermal rheostasis, but other systems may be involved—for instance, in the case of fever, cytokines from the white blood cells. However, finding any manipulation at all that knocks out rheostasis of a regulated variable would at least give one a starting point, a thread to trace. The ideal would be a manipulation that eliminates rheostasis but leaves the ability to regulate intact. The lesion approach does not always yield answers easily. For example, attempts to eliminate cyclical changes in body weight in hibernators have been failures. The manipulations tried so far include vagotomy, castration, and pinealectomy, lesions of the ventromedial hypothalamic nuclei, lateral hypothalamus, paraventricular nuclei, suprachiasmatic nuclei, ascending noradrenergic bundles, and olfactory bulbs (see Zucker and Dark, 1986; Mrosovsky, 1985; Powley and Fox, 1986). In all these cases, programmed changes in body weight continued.

A different strategy for localizing the physiological mechanisms that underlie rheostasis would be to put away the electrodes and stimulators and take a correlational approach. Species that exhibit changes in regulated levels are naturally occurring preparations making their own manipulations. Instead of lesioning and stimulating particular brain areas and studying the effects, one may let the animals make their own manipulations and then try to discover where and what these are. Perhaps sufficiently salient and detectable changes exist; perhaps combinations of manipulative and correlational methods will be the most successful. Presumably, in some way or another, either by stumbling

around or by inspired insight, sufficient knowledge about the location of changes producing rheostasis eventually will be obtained. It would then become worthwhile to undertake systematic work on RNAs in particular areas.

A different approach would be to find a genetic marker in a strain of animals with an elevated set-point for a variable. If one could also find a probe for a closely linked site—an obstacle in genetically unstudied species—then it might be possible to locate the gene involved in the elevated set-point. Again, implicit in this general approach is the assumption that the adjustment of the set-point is controlled in a relatively simple way by products coded for by only one or a few genes. This is not perhaps unrealistically optimistic. It is a matter of modulating an existing system, not creating a new one. The role of the *period (per)* gene in biological rhythms of fruit flies *(Drosophila)* provides an analogy.

The *per* gene is sometimes referred to as a clock gene. In fact, what it does is to control the periodicity of circadian rhythms: mutations of the wild type (per^+) give rise to strains with longer (per^l) or shorter (per^s) cycles. Another mutation at the same allele lacks a circadian rhythm (per^0). There is no strong evidence that the *per* gene is responsible for the creation of circadian clocks. A variety of points are more consistent with the view that its main role is controlling the speed of the clock. First, the amount of transcription of *per* product is important. When rhythmicity is restored to arrhythmic (per^-) *Drosophila* by inserting exogenous *per* locus DNA, the frequency of the rhythm obtained varies greatly but correlates with the amount of *per* RNA produced. The variation in *per* RNA titer among different transformed strains presumably depends on the exact position of the inserted *per* gene, since location within the genome affects transcription rate (Baylies et al., 1987). Second, while expression of *per* gene products is important for the expression of biological rhythms, and for the periodicity of those rhythms, it is not essential for the development of the pacemaker mechanisms in the first place. This has been shown by controlling the expression of the *per* gene coding region by fusing it to a heat shock promoter (Ewer et al., 1988). At high temperatures there is expression of the *per* gene but at low temperatures there is little or no expression. The *per* gene, with its heat shock switch, is then inserted into the arrhythmic (per^0) strain. This restores rhythmicity in the right temperature conditions. It does not matter if heat is not applied until after development is completed; rhythmicity still appears when the flies become warm. "The rapidity with which rhythms can be turned on or off . . . seems to argue that *per* regulates the ongoing operations of oscillator functions, as opposed to being involved in the construction of the fly's circadian pacemaker" (Ewer et al., 1988).

A tentative idea about how *per* might influence periodicity is beginning to emerge. The *per* locus codes for a proteoglycan (Reddy et al., 1986). These proteins are important in the extracellular matrix (but could have other roles also; see Hall and Rosbash, 1988). Possibly, then, the periodicity of circadian rhythms in multicellular organisms depends on interactions between different cells. The demonstration that *per* enhances spread of dyes across the gap junctions in salivary glands of *Drosophila* suggests a general role in intercellular interactions, mediated through effects on the extracellular matrix (Bargiello et al., 1987).

Any interpretation of the new and rapidly growing findings about the molecular biology of rhythmicity could turn out to be premature. At present it appears that the effect of the *per* gene product on clock speed is a quantitative modulation of another mechanism. I am optimistic that, for at least some cases of rheostasis, the mechanisms will turn out to be relatively simple, perhaps of a purely quantitative nature. Finding these mechanisms will provide important therapeutic tools, enabling the clinician to alter the plans of a hostile regulatory system at its command post rather than doing battle with its effector armies in the periphery.

However, while the ability to manipulate set-points might perhaps—with luck, and with the aid of modern techniques—turn out to be relatively straightforward, that would remain far short of providing an understanding of homeostasis itself, just as the ability to alter the periodicity of rhythms in fruit flies has not explained how their clocks work. To fully understand the machinery underlying homeostasis and the interactions between homeostatic systems may also require analysis at levels not adequately encompassed in molecular biology.

INTEGRATIVE PHYSIOLOGY

The search for substances and molecular bases for rheostasis, the reductionist approach, may be only half, perhaps the easy half, of what needs to be undertaken. There may also be value in continuing to study rheostasis with concepts appropriate to understanding and conceptualizing events at levels removed from that of molecular biology. Complex processes may emerge from interactions in the whole organism that have no obvious counterpart in the genes. Differentiation of embryonic tissue into the adult form provides an illuminating example.

Although development usually proceeds in an orderly, predictable way, there is no need to assume that the genes contain developmental programs. For a phenomenon to be called programmatic, "it is a necessary condition that in addition to the phenomenon itself, there exists a second thing, the 'program,' whose structure is isomorphic with, i.e., can be brought into one-to-one correspondence with, the phenomenon" (Stent, 1982). An alternative way for regularity to arise in a series of events is for a particular stage of development to create a situation that promotes the move to the subsequent stage.

> To illustrate the difference between programmatic specification and stochastic history as alternative accounts of regular phenomena, we may consider the establishment of ecological communities upon colonization of islands, or growth of secondary forests. Both of these examples are regular phenomena in the sense that a more or less predictable ecological structure arises via a stereotypic pattern of intermediate steps, in which the relative abundance of various types of flora and fauna follow a well-defined sequence. The regularity of these phenomena is obviously not the consequence of an ecological program encoded in the genome of the participating taxa. Rather it arises via a historical cascade of complex stochastic interactions between various biota (in which genes play an important role, of course) and the world as it is. (Stent, 1982)

Knowledge of the genome is not enough for understanding such historical cascades. Because of the sheer number of components and interactions, study of historical interactions in the course of development is a daunting prospect. However, if concepts and experimentation at the appropriate level of analysis can be found, perhaps the task may be simplified. For instance, Larsen and McLaughlin (1987) believe that for morphogenesis, the appropriate level of analysis is the cellular level. There may be a relatively limited number of cellular processes, a small "morphogenetic alphabet" that determines the shape of a particular tissue and when it assumes that shape. The letters in this alphabet are cell division, cell growth, cell shape, cell change, cell movement, cell death, and cell membrane and extracellular matrix production. A change in one of these processes, as exemplified in mutant fruit flies, can have dramatic consequences for the development of an organ. Which organ is affected and when it is affected depends on the timing of these changes. Environmental events such as temperature and nutrition also influence timing. "The gene cannot 'master-mind' morphogenesis since it is only a part of an interactive epigenetic system, influenced by, as well as influencing, the cell and tissue in which it resides" (Larsen and McLaughlin, 1987).

Likewise, for homeostasis, the organizational and integrative aspects of physiological functioning may not be apparent in the DNA sequences. For homeostasis to be comprehensible, analysis must take place at different levels. One appropriate level is that of organ systems and hormonal and neural signals between those systems. The simplifying concepts are those from control systems theory. If this approach is valid, then a study of principles governing the switch between different effectors, of open and closed loops, of conflict between different regulatory systems, and in what sense set-points exist and can change, of homeostasis and rheostasis in general, is not just the last gasp of a superseded era in biology. It is a part of an integrative physiology that will be coming back into its own to tackle these problems, or to replace them by formulations and concepts better applicable to interactions at levels where a knowledge of all nucleotide sequences in the world would still leave darkness. If there is no biochemical homunculus to be found in the genes for the complexities of the whole organism, then integrative physiology must come back into its own, indeed will have to be greatly developed and elaborated if we are ever to make sense of ourselves.