ACTIONS OF HORMONES ON MOLECULAR PROCESSES

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Experimental Biochemistry: A Laboratory Manual

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Cholesterol

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Preface

The purpose of this book is to stimulate interest in, and to suggest new approaches to, the problem of hormonal regulation of molecular reactions and to gather in one source information presently available in this area of interest. Wherever possible we have encouraged speculation primarily to give to the contributor a wide latitude in expressing his viewpoint. The coverage of the overall subject of hormonal interactions is necessarily selective mainly because of the degree of advancement in specific areas.

Owing to the lapse of time between submission of manuscripts and publication and to the activity in the field, some contributors have found it necessary to bring their chapters up to date by adding material in proof. Such material will be found in the form of Addenda at the end of certain chapters.

Because regulation in biochemistry is a rapidly developing field, it is attracting more interest, and it is a field that is ready for the application of methodology which has been employed with great success in advancing our knowledge of other areas of biochemistry. Thus it is not surprising that an attack on the problem of hormonal regulation should be made at the molecular level. Although it is evident that this information may not readily explain the systemic effects of hormones in the physiological state as we now interpret them, it is equally clear that only by a molecular approach can the primary event triggered by hormonal action in the organism be understood. The molecular approach will inevitably also lead to a clearer understanding of the reactivity of hormonally active compounds. It is with this philosophy in mind that this collection is tendered.

G. L.
D. K.

Philadelphia, Pennsylvania
July, 1964

List of Abbreviations

DPN or DPN^+, DPNH diphosphopyridine nucleotide and its reduced form.

TPN or TPN^+, TPNH triphosphopyridine nucleotide and its reduced form.

NAD or NAD^+, NADH nicotinamide adenine dinucleotide and its reduced form.

NADP or $NADP^+$, NADPH nicotinamide adenine dinucleotide phosphate and its reduced form.

FAD, $FADH_2$ flavin adenine dinucleotide and its reduced form.

GSH, GSSG glutathione and its oxidized form.

CoA, acyl-CoA coenzyme A and its acyl derivatives.

AMP, GMP, IMP, UMP, CMP the five prime phosphates of ribosyladenine, guanine, hypoxanthine, uracil, cytosine. Other abbreviations for nucleotides are similar to those outlined in *The Journal of Biological Chemistry.*

RNA, DNA ribonucleic acid, deoxyribonucleic acid.

sRNA, mRNA soluble ribonucleic acid, messenger ribonucleic acid.

RNAase, DNAase ribonuclease, deoxyribonuclease.

UDP-glucose, UDP-galactose, etc. uridine diphosphate glucose, uridine diphosphate galactose, etc.

P_i, PP_i orthophosphate and pyrophosphate.

TRIS tris-hydroxymethylaminomethane.

EDTA ethylenediaminetetraacetic acid.

The abbreviations used for amino acids, the units of measurement including those for mass, the contractions which are used for polynucleotides, and the units of length, area, and volume as well as those of concentration are similar to those outlined in *The Journal of Biological Chemistry.*

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Hormones and the Transport of Nutrients across Cell Membranes

Thomas R. Riggs

The flow of chemical substances and electrons that we know as metabolism has been studied extensively in the test tube one step, or a few steps, at a time. But, in the living cell, a very large number of reactions occur at one time; often these reactions are inherently incompatible with one another. The organization of these reactions within the cell depends not only on the mutual reactivities of the hundreds of substances involved but also on their controlled access to one another. Although many of the scalar aspects of metabolism have now been described, the accompanying three-dimensional or vectorial aspects (Mitchell, 1) are as yet little known. Without a system of barriers and segregating and desegregating processes the ordered flow of metabolism would be impossible.

Developments, especially since about 1950, have suggested that the control of metabolic relationships by hormones may frequently be expressed on the vectorial rather than on the scalar aspects of metabolism; that is, hormones may often effectively modify the actions of barriers and transports at levels lower than are needed to modify scalar enzymatic reactions. The inherent simplicity of controlling metabolic events by modifying access between reactants is obvious. The specificity of the modification could be determined by the transport properties of the individual membranes.

For the most part, studies on hormonal modifications of membrane transports of nutrients have been carried out on intact cells and the

effects measured across the membrane surrounding the cell. This membrane is an ideal site of action for hormones, since hormones could exert their control here without entering the cell—a somewhat difficult and time-requiring occurrence especially for those large protein hormones that must be supplied intact to produce their activities. In addition, hormonal alteration of substrate levels inside cells can, under certain conditions, provide selective and delicate control over metabolism; for example, the profound changes occurring in insulin lack can be related directly to an inadequate transport of glucose into tissues. Control of nutrient entry or loss from the body may likewise be exerted by hormonal regulation of intestinal transport and kidney tubular reabsorption or secretion. The very critical sodium, potassium, and water balance of the body, for example, is under delicate hormonal control at the kidney tubule.

Perhaps less obvious but equally important is the differentiation of embryonic tissues, which might be aided and controlled by selective accumulation of certain nutrients and exclusion of others from the surrounding medium. In addition, the nutritional maintenance of a multicellular organism depends on the proper distribution of nutrients to all tissues so that one does not feast while the others starve, a situation which can be realized only if a balance is achieved among the tissues for capture of nutrients. Cancerous growth in an animal, for example, represents a nutritional problem in that the tumor tissue captures nutrients at such a rapid rate for its own growth that the normal tissues cannot compete. The relative abilities of the various cells to take up nutrients from a common extracellular fluid could well help to determine the differentiation, development, and maintenance of the balance of the organism. By modifying the nutrient distribution in the body, hormones might control the body economy in many and yet highly specific ways.

In the following section the current evidence for hormonal modification of transport of nutrients across cell membranes is discussed.

DEFINITION OF TRANSPORT

In order to understand better the scope of the topic to be discussed here, we must first consider carefully the concept of transport. Over recent years this term has come to have a rather precise meaning which is not always recognized. For a more extended discussion of the subject from the present viewpoint, the reader is referred to the reviews of Ussing (2, 3) and of Rosenberg (4) and to the recent mono-

graph by Christensen (5). For our purposes here, only a brief outline will be needed.

Transport may be defined as the transfer of a chemical entity from one phase to another, usually across a membrane, the substance appearing in the same state in both phases. Such a definition distinguishes between translocations of chemical groups across membranes and translocations of molecules and ions. Both of these types of translocation may conceivably be acted on by hormones, and they should perhaps not be considered fundamentally different. Nevertheless, the distinction seems useful and necessary in order to limit the subject.

Substances are generally believed to be able to move into or across cells by one or more of several general ways: (*a*) by diffusion of the free molecule across the cell membrane; (*b*) by movement in combination with a membrane carrier—a mediated movement which may or may not occur by free diffusion; and (*c*) by movement in combination with a carrier in such a way that energy is required and active or uphill transport can take place and a chemical gradient can be established. Entry by pinocytosis is excluded from discussion here because it does not explain movements of nutrients into most cells (see 5).

All chemical substances of small molecular weight might be expected to cross cell membranes to some extent by diffusion as free molecules or ions. Nutrients, being generally hydrophylic, do not appear to enter cells to any great extent by free diffusion, however—an indication that some barrier minimizes free passage, a fact that has led to the concept that transport into cells is a matter of breaking down barriers, and that hormones and other agents may control entry of nutrients into cells by controlling the permeability of the barrier.

That transport is more than removal of barriers is clear, however, from the fact that many substances (amino acids, inorganic cations) are found at much higher levels in cells than they are found externally. These levels can be further raised by hormones. One frequent explanation for the high internal levels, that of internal binding of the nutrients, is not satisfactory for many solutes for several reasons:

1. Good evidence has been presented to show that at least the neutral and basic amino acids and the potassium and sodium ions are osmotically free within cells. If much potassium were bound internally, large osmotic gradients would be expected across the cell membrane unless one also proposed that the cell water is bound. This assumption, however, leads to many further difficulties.

2. The movement of substances *out of* cells against concentration gradients, as shown by Kernan (6) to occur for Na^+ in the presence of insulin, for example, cannot be explained as a result of binding of the substance in the extracellular fluid.

3. In addition, the present concept of transport will apply to movement of nutrients across cell layers, as in intestinal transport or in kidney tubular secretion or reabsorption. Here, again, binding cannot account for the gradients found, since the transported substance is free on both sides of the cell layer. The experiments of Oxender and Christensen (7) suggest that transcellular transport is only a special case of transport into cells, that is, that transport *across* a cell layer can be accomplished as a result of greater transport *into* cells at one surface than at the other. At present there is no clear evidence for separate mechanisms for the two processes.

Most of the available evidence for movement of dissolved nutrients into cells fits well the theory of a transport carrier, which binds the nutrient transiently in the cell membrane and releases it in the free state internally. A large number of models of this general type have been proposed, but most of these can be illustrated by a single example as shown in Fig. 1.1. Both mediated diffusion and uphill transport can be explained by this scheme. The important feature of such a

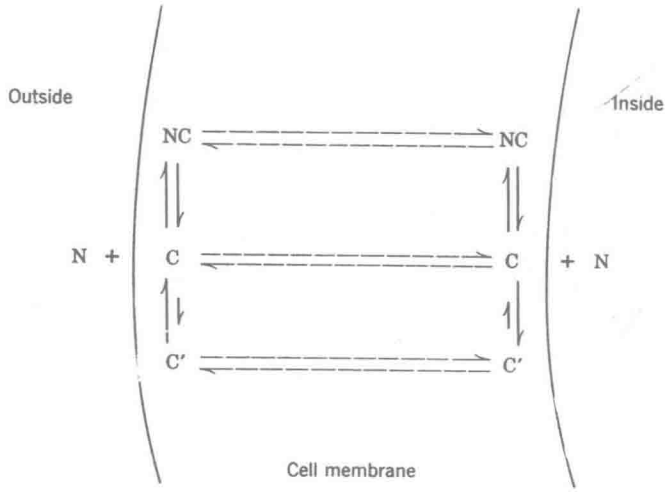

Fig. 1.1. A simplified model to demonstrate carrier-mediated transport of a nutrient across a cell membrane. Description in the text.

scheme is the facilitation of movement of the ion or molecule across the membrane by combination with the carrier, C, to produce mediated movement. The movement itself (represented by dashed arrows in Fig. 1.1) may be either by free diffusion or through some energy-requiring step. Gradients of the nutrient cannot be produced by free diffusion of the nutrient-carrier complex, NC (Fig. 1.1), without some other change, since the nutrient may be released from NC at either surface equally well. Uphill transport is possible, however, if energy is expended for the movement, or if energy is introduced at either surface to alter the affinity of N for carrier, as through a conversion of C to a modified carrier C′ only at the inner surface, or of C′ to C only at the outer. The last possibilities are suggested in Fig. 1.1 by the short arrows in the reverse reactions.

Although no carrier substance has been identified as yet, the scheme above accounts for two other facts that are necessary to characterize transport: (*a*) the entry process can be saturated by using large enough amounts of the substance in question; and (*b*) the transport of one substance can be inhibited competitively by a close structural analog. The scheme also provides a basis for explaining "exchange diffusion" (see 3, 8, 9). In addition, it has been used by Christensen et al. (10, 11) as a possible model for their postulated two-carrier mechanism for amino acid transport. Since both C and C′, as well as their possible complexes NC and NC′, are freely diffusible, C may represent one carrier for amino acids and C′ the other. Uphill transport could not, of course, occur in combination with C′ unless an elaboration were introduced into the scheme of Fig. 1.1.

Theoretically, changes in transport could be brought about in either of two ways:

1. By modifying the nature or rate of one or more of the chemical reactions of the sequence, such as the initial combination of substrate with carrier ($N + C \rightarrow NC$ in the scheme); a step in the breakdown of substrate-carrier complex to substrate + modified carrier ($NC \rightarrow N + C'$); or the conversion of modified back to original carrier ($C' \rightarrow C$). Each of these modifications would alter the concentration of one species and thereby change its total rate of movement.

2. By modifying the facility of movement of free N, NC, C′, or other species within the membrane in one direction but not in the other. This modification could result from removal or production of *physical* barriers, and its detection would require presence of the intact cell membrane. In this respect this modification differs from

the type of phenomena usually encountered in enzyme reactions, and, indeed, it may not involve an enzyme-catalyzed step. Therefore transport presumably could be modified without the requirement that any enzyme be influenced; or conceivably an enzyme directly involved in the transport could be modified. Changes in supply of either the nutrient or the carrier could, of course, change the rate of entry of nutrient into cells but would not necessarily represent a primary change in transport mechanism.[1,*]

Attention should also be called to suggestions (12–15) that hormones act at the cell surface to produce reorganization of the "cytoskeleton" or lattice system of the entire cell. According to these theories, such alteration would not only change the cell membrane but would also modify enzymatic activities and physical relations of structural components throughout the cell. Such a theory provides a method by which large molecules, such as protein hormones, could alter the activities of intracellular enzymes without the necessity of the hormones entering the cells. Unfortunately, experiments to test it have so far proved hard to devise.

Tables 1.1, 1.2, and 1.3 summarize briefly the reported actions of hormones on transport of inorganic ions, water, sugars, and amino acids. References are included in the tables as a general guide to the literature. Major evidence for these actions is discussed below for the individual hormones.

HORMONES THAT MODIFY TRANSPORT OF INORGANIC IONS AND WATER ACROSS CELLS

The most apparent changes in transport produced by hormones are those involving the movements of Na^+, K^+, Ca^{2+}, inorganic phosphate, and water across the kidney tubule or intestinal wall. Transport of these substances *across* cell layers can readily be measured, since binding can be excluded as a factor in establishing the gradients. In addition, levels of the inorganic ions cannot be altered by changes in their formation or utilization—a factor that may complicate transport studies with organic nutrients.

Aldosterone

At levels of 0.16 μg per animal, aldosterone can reduce the urinary excretion of Na^+ by 50% in adrenalectomized rats, whereas 6 μg can increase K^+ excretion by 50% (17). Since increases occur in

*Notes will be found at end of chapters, preceding the References.