Figure 1 Fourteen frames from an animated sequence illustrating the interaction of actin filaments with myosin heads protruding from a thick filament. The final sequence contained 35 frames which can be single-stepped or smoothly animated using the techniques described in the text

been able to animate sequences containing up to 35 separate frames (Figure 1).

The method is not difficult to implement on other microcomputers and the author has developed the machine code routines to allow the method to be used on an IBM *Personal Computer* (Intel 8088 processor) and a Sinclair *ZX Spectrum* (Zilog Z80A processor).

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Teaching Metabolic Pathways

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Introduction

Some students consider biochemistry to be an exercise in memorization. Even worse, some teachers present the subject in this manner. Modern biochemistry is much more than a catalogue of molecular structures and chemical reactions. The chemistry of life has unifying principles and concepts, eloquently described by Lehninger[1] as the "molecular logic of living organisms." Nevertheless, familiarity with the field of biochemistry does require some memorization. A case in point would be the pathways of metabolism.

The purpose of memorizing a metabolic pathway is to acquire some understanding of the strategy used by a cell to accomplish a particular goal. Pathways should be taught with the emphasis on general principles and concepts. For example, the citric acid cycle (and its various inputs) demonstrates the generality of converging catabolic pathways. It also illustrates the principles of extracting energy from food molecules by oxidation, and the concept of a cyclic pathway in which an essential acceptor molecule is regenerated.

It is not necessary, desirable, or even possible, to teach all of the pathways of metabolism. Teachers must choose how many pathways to teach, and which ones. The decision will depend upon the type of course, its level and depth. It is my belief that it is better to teach fewer pathways in greater detail, than to risk obscuring the forest with trees.

Approaches to the teaching of certain specific pathways have been described.[2,3,4,5] I suggest here a general method which is applicable to almost all pathways. It is a sequential approach in which the pathway is considered on several levels of increasing sophistication. Proceeding step-wise through these levels provides an appreciation of the overall strategy of the pathway, as well as a framework to assist in committing its details to memory. The approach is illustrated below with the pathway of glycolysis.

Level 1 — The purpose of the pathway

Every metabolic pathway exists for a reason. The purpose of the pathway, whether obvious or subtle, is of primary importance. It is necessary to understand what the goal of the pathway is before attempting to comprehend how that goal is accomplished.

The purpose of a metabolic pathway can usually be deduced from its overall equation. In the case of glycolysis, this equation is
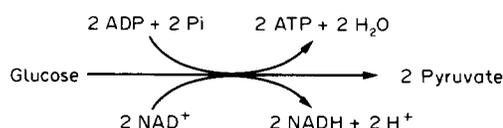
Inspection of this equation reveals that the pathway converts glucose to pyruvate, and that this conversion is accompanied by the synthesis of ATP and NADH. ATP is well-known as the universal energy 'currency' of living systems. NADH carries electrons, whose energy can be readily converted into the ATP currency. With this knowledge, it can be surmised that one purpose of the glycolytic pathway is to extract energy from glucose molecules, and convert it into forms which can be used to do cellular work.

Carbon atoms from pyruvate can be converted into amino acids and lipids. Thus, another purpose of glycolysis is to provide raw material for biosynthesis of cellular components.

Level 2 — The major stages of the pathway

Most metabolic pathways can be divided into two or more major stages. These stages often reflect the overall strategy of the pathway, with each stage having a particular goal. Thus, it is useful to consider what has been accomplished in each stage.

The ten reactions of glycolysis can be divided into three major stages. These are:

(1) *The activation stage* In the first three reactions of the pathway, ATP energy is used to 'activate' the glucose molecule. Apparently, it must be 'primed' before its energy can be tapped. The molecule is also re-arranged slightly, so that it can be cleaved more readily in the next stage.

(2) *The cleavage stage* In the next two reactions, the activated molecule is cleaved into two different fragments, and one of the fragments is converted into the other. The single product of this stage can be handled by a single set of enzymes in the next stage. It seems that energy can be more efficiently extracted from the products of the cleavage stage, than from the larger uncleaved precursor molecule.

(3) *The harvest stage* In the next five reactions, energy is harvested with the synthesis of ATP and NADH. First, the energy investment of the activation stage is recovered. Then, the molecule is rearranged so that additional energy can be extracted. Finally, the product pyruvate is formed, with the synthesis of more ATP.

Level 3 — The pathway of the carbon skeletons

More detail is introduced at this level, as the intermediates in the pathway are represented by the number of carbon atoms that they contain. The description of glycolysis would be

Activation Stage   — all C_6 compounds
Cleavage Stage   — a C_6 compound is cleaved to produce two identical C_3 compounds, or $C_6 \rightarrow 2\,C_3$
Harvest Stage   — all C_3 compounds.

In the case of glycolysis, this level is only marginally more informative than the previous one. It is more useful when applied to complicated pathways in which carbon

skeletons are extensively altered, such as the Calvin cycle, as shown below.
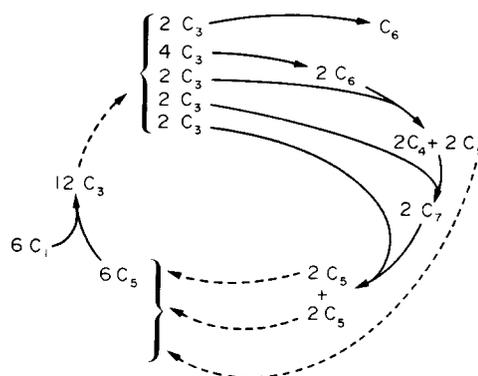
In some cases, it is also useful to describe the pathways of other elements found in biomolecules, in particular oxygen, nitrogen, sulphur and phosphorus. For example, the pathway of nitrogen can be used to illuminate certain aspects of amino acid metabolism, such as the urea cycle.

Level 4 — Energy inputs and outputs

Most metabolic pathways are concerned with energy. One main purpose of the catabolic pathways is to provide the energy required by the anabolic pathways for the work of biosynthesis. Thus, it is important to analyse pathways in terms of their energy inputs and outputs. Energy released by the catabolic pathways is usually conserved in the phosphoanhydride bonds of ATP. Energy required by the anabolic pathways is often invested as a transfer of phosphate from ATP to an acceptor molecule, forming an activated phosphate ester. Thus, energy inputs and outputs can often be described as the pathway of phosphate.

Oxidation/reduction reactions are also important in the energy profile of a pathway, since the energy of high potential electrons can readily be converted into ATP by oxidative phosphorylation. The pathway of reducing equivalents can usually be described as the involvement of electron carriers, such as NADH and $FADH_2$.

In glycolysis, all the intermediates between glucose and pyruvate have at least one phosphate group. In the first stage of the pathway, the C_6 molecule is activated by two phosphate transfers from ATP. These occur in the first and third reactions.

The second stage cleavage reactions do not involve energy transfers. The doubly-phosphorylated C_6 molecule is cleaved such that each of the C_3 products has one phosphate.

The final harvest stage involves three energy transfers. The first reaction of this stage is in oxidation/reduction in which inorganic phosphate is taken up to yield oxidized C_3 molecules with two phosphates each, and NADH. The reaction is called a substrate-level phosphorylation, and is discussed in more detail later. In the next reaction of the pathway, one of these phosphates is transferred to ADP to form ATP. The molecule is then rearranged and dehydrated to facilitate transfer of the second phosphate

to ADP. This transfer occurs in the last reaction of the pathway, yielding ATP and pyruvate.

A schematic representation of the glycolytic pathway through the first four levels of description is shown below.
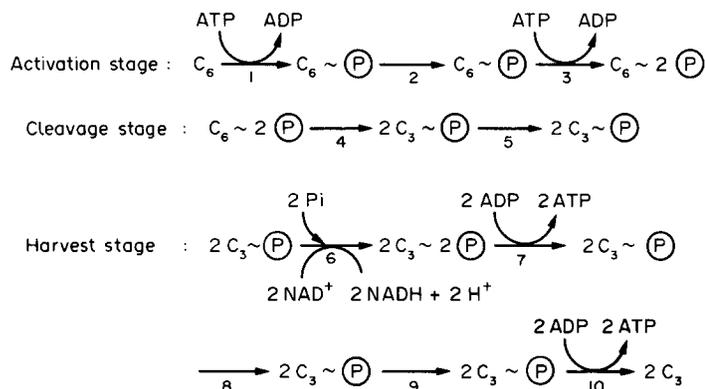
Level 5 — Special features of the pathway

Some reactions in a metabolic pathway are of special interest, and should be pointed out to students. An example in glycolysis would be the substrate-level phosphorylation which occurs in the first reaction of the harvest stage. This reaction shows that 'high energy' phosphate bonds can be synthesized in ways other than the chemiosmotic mechanism of oxidative phosphorylation. It is also noteworthy because it illustrates how energy can be transferred from one reaction to another if the two reactions are coupled by a common intermediate. Part of the energy released by the oxidation of an aldehyde to an acid is conserved in the synthesis of a 'high energy' mixed phospho/carboxylic anhydride bond. The two sub-reactions are coupled by the participation of a covalent enzyme-substrate complex.

Level 6 — Control of the pathway

It is important for students to realize that all metabolic pathways are not active at the same time. The flux of material through a particular pathway is usually regulated by modulating the activities of enzymes at key control points. These ideas should be reinforced by identifying the control points in each pathway studied, and by pointing out the strategy of the control mechanism. The main control point of a pathway is usually the first irreversible reaction which is unique to the pathway, called the 'committing' reaction. Thus, the flow of material down the pathway can be reduced before it has proceeded very far, yet can be regulated without affecting other pathways. In glycolysis, the committing reaction is the third reaction of the activation stage. The pathway is also fine-tuned by control at the first and last reactions of the pathway.

Teachers should point out that control mechanisms usually make good physiological sense. In the case of glycolysis, the enzyme at the main control point is feedback inhibited by ATP, one of the products of the pathway (as well as by other effectors). Thus, when the pathway is not required because ATP levels are high, it is turned down.

Level 7 — The details of the pathway

For a complete understanding of a pathway, especially its relationship to the rest of metabolism, it is necessary to know the structures of all the intermediates, the features of all the reactions, and the properties of all the enzymes. It is easier to memorize these details if one has proceeded sequentially through the previous levels of description, and acquired a framework into which the details can be fitted.

It is useful to keep in mind some general characteristics of metabolic pathways. First, the intermediates of pathways are often derivatives of a smaller number of 'parent' compounds. Stryer[6] points out that in glycolysis, the C_6 compounds are all derivatives of either glucose or fructose. The C_3 compounds are all derivatives of either dihydroxyacetone, glyceraldehyde, glycerate or pyruvate. Furthermore, the substrates and products of each reaction are derivatives of each other, and must have some structural relationship to one another.

Second, although there may be many reactions in a pathway, there are usually fewer different types of reactions. In the ten reactions of glycolysis, there are only six different types of reactions. These are (1) transfer of phosphate to or from ATP; (2) shift of phosphate from one oxygen to another within the same molecule; (3) isomerization between aldose and ketose sugars; (4) dehydration; (5) aldol-type cleavage; and (6) the substrate-level phosphorylation. Living systems catalyse only a limited number of types of reactions. Often, a reaction type will be already familiar to students from their studies of other pathways. This should be pointed out by teachers.

Memorizing structures of intermediates and memorizing reactions are complementary approaches to retaining the details of a pathway. Usually, knowledge of one of these aspects can be used to reconstruct the other. Similarly, it is often not necessary to memorize the names of the enzymes involved in a pathway because they can usually be reconstructed from a knowledge of their substrates, products and reactions.

Different students will proceed to different levels in understanding and/or memorizing metabolic pathways, depending upon their interest, the time they have available, and other factors. I think that it is far better to understand a pathway and its strategy on a relatively superficial level, than to memorize its details on the day before the examination, forget them the day after, and never appreciate even its most salient features.

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