3. PROTEINS polymers of amino acids (N,C,H,O)
--polypeptide chains with peptide bonds
--20 amino acids

Protein: a macromolecule that consists of one or more polypeptide chains folded and coiled into specific **conformations** (unique 3-D shape)

Functions:

1. Structural support
2. Storage (of amino acids)
3. Transport (e.g. hemoglobin)
4. Signaling (chemical messengers)
5. Cellular response to chemical stimuli (receptor proteins)
6. Movement (contractile proteins)
7. Defense against foreign substances & pathogens (antibodies)
8. Catalysis of biochemical reactions (enzymes)

Amino acid – monomer of protein; most are asymmetric carbon, termed the alpha carbon which is covalently bonded to a(n):

1. Hydrogen atom
2. Carboxyl group (COOH)
3. Amino group (NH2)
4. Variable R group (side chain specific to each amino acid)

Amino acids are grouped by properties of side chains

1. Nonpolar side groups (hydrophobic)
2. Polar side groups (hydrophilic)
   c) uncharged polar
   d) charged polar
   - acidic side groups (- charge)
   - basic side groups (+ charge)

Protein conformation: 3-D shape of a protein
4 levels of protein structure:

1. <u>Primary Structure</u> – the amino acid sequence

2. <u>Secondary Structure</u> – folds & coils due to hydrogen bonding α alpha and β beta pleated sheet
   c. (α) alpha helix – helical coil, H bonds between every fourth peptide bond. (Fibrous proteins such as keratin & collagen)
   d. (β) beta pleated sheet – sheet of antiparallel chains folded into accordion pleats. Globular proteins such as lysozyme and some fibrous such as fibroin

3. <u>Tertiary Structure</u> - 3-D shape of a protein
   2 types of 3-D shapes:
   1. Fibrous
   2. Globular

4. Quaternary Structure- a protein with 2 or more polypeptide chains (Ex. Collagen, a fibrous protein with three helical polypeptides supercoiled into a triple helix)

Denaturation
PH, salt concentration, temperature, and chemical agents can cause a protein to unravel and lose its shape. This is called **denaturation**. ☆A protein that denatures is biologically inactive.

Types of proteins
c) Binding proteins
d) Structural proteins

Enzymes: special proteins that are made by cells that act as catalysts (chemical that help to speed up reactions without themselves being changed). Catalysts work by lowering the "start-up" energy of a reaction

Enzyme Facts:
--almost all are proteins
--most end in –ase
--act as catalysts
--they are specific. Each enzyme reacts with a specific substance called the enzyme's substrate
--act upon substrates (help change and break them down)

E + S → E-S complex → E + products

sucrase + sucrose→sucrase-sucrose complex→ sucrase + glucose + fructose

--active site is on the enzyme
--reactive site is on the substrate
--involved in digestion, respiration, reproduction, vision, thought, movement, and even making other enzymes

Induced Fit Model – the active site of the enzyme must adjust to fit the reactive site of the substrate

ENZYME KINETICS; Rate of Enzyme Reactions
How do temperature and pH affect the rates of enzyme reactions? In what other ways can enzyme activity be regulated?

a. **Temperature**

When temperature ↑ up to 40°C, the rate of reaction ↑. Above this temperature, hydrogen bonds break – enzyme loses its 3D shape---active site can not bind---no products can form

b. **pH**

Different enzymes have different optimal conditions. Pepsin (in stomach) breaks down protein works best at a pH of 2. Trypsin (in small intestine) works best at a pH of 8. If there is a ↓ in pH because more acid is added, hydrogen bonds break and the enzyme is denatured and no longer effective.

c. **Cofactors**

Nonprotein, most vitamins that are required for proper enzyme catalysis.

d. **Negative Feedback**

A common method of regulation in living systems. As the product is formed, it hinders the mechanism that produced the product. If there's too much product being formed, then excess product remains in the active site. When this happens, the substrate can no longer enter the active site. When the level of product decreases, the product leaves the active site, and the reaction can resume.

e. **Competitive Inhibitors**

They resemble the enzyme's substrate and competes with it for the active site. Blocks the active site from the substrate.

f. **Allosteric Control**

Sometimes an enzyme may have a second binding site called an allosteric site. When a small molecule binds to the allosteric site, the enzyme's active site changes, the enzyme can no longer bind to the substrates. (* may act as metabolic poisons –DDT, many antibiotics)

3. **NUCLEIC ACIDS :** largest organic molecule 2 Types: DNA & RNA

1. Deoxyribonucleic acid (DNA) Heredity material
   - ✓ Has coded info that programs all cell activity
   - ✓ Has directions for its own replication
   - ✓ Copied & passed from one generation of cells to another
   - ✓ In eukaryotic cells, is primarily in the nucleus
   - ✓ Makes up genes that has instructions for protein synthesis

     genes→DNA→mRNA→protein

2. Ribonucleic acid (RNA)
   - ✓ Functions in the actual synthesis of proteins coded for by DNA
   - ✓ Sites of protein synthesis are ribosomes in the cytoplasm
   - ✓ mRNA carries the encoded genetic message from the nucleus to the cytoplasm

Structure: Monomers of Nucleotides (3 parts to a nucleotide)

1. Pentose (5C) sugar: ribose or deoxyribose (1 less oxygen atom)

2. Phosphate group

3. Nitrogenous base
   a. pyrimidines: single ring cytosine, thymine (uracil in RNA)
   b. purines: double ring adenine, guanine

Bases held together by weak hydrogen bonds. Nucleotides are joined together by covalent bonds (phosphodiester linkages)

1953 WATSON & CRICK DNA STRUCTURE

- 2 nucleotide chains
- Double helix
- A – T
- C – G
- Two strands of DNA are complimentary
- Contains thousands of base pairs

IMPORTANCE OF NUCLEIC ACIDS

--DNA – heredity material genes→DNA→mRNA→protein

--ATP (adenosine triphosphate) cell's energy source ☆between the phosphate groups are bonds which can be broken to yield usable energy 7 kcal/mole

--CAMP (cyclic adenosine monophosphate) a second messenger in many hormonal reactions

--Use DNA and proteins as tape measures of evolution ☆closely related species have more similar sequences of DNA and amino acids

II. Enzymes ***

A. Enzymes speed up metabolic reactions by lowering energy barriers

Free energy change indicates whether a reaction will occur spontaneously, but does not give information about the speed of reaction.

- A chemical reaction will occur spontaneously if it releases free energy ($-\Delta G$), but it may occur too slowly to be effective in living cells.
- Biochemical reactions require *enzymes* to speed up and control reaction rates.

Catalyst = Chemical agent that accelerates a reaction without being permanently changed in the process, so it can be used over and over.

Enzymes = Biological catalysts made of protein.

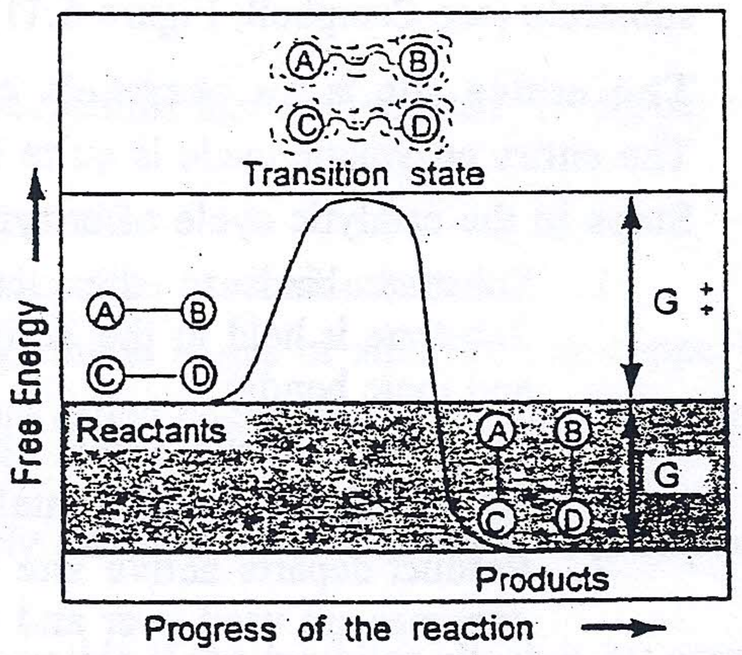
Before a reaction can occur, the reactants must absorb energy to break chemical bonds. This initial energy investment is the *activation energy*.

Free energy of activation (*activation energy*) = Amount of energy that reactant molecules must absorb to start a reaction (E_A).

Transition state = Unstable condition of reactant molecules that have absorbed sufficient free energy to react.

Energy profile of an exergonic reaction:

1. Reactants must absorb enough energy (E_A) to reach the transition state (uphill portion of the curve). Usually the absorption of thermal energy from the surroundings is enough to break chemical bonds.
2. Reaction occurs and energy is released as new bonds form (downhill portion of the curve).
3. ΔG for the overall reaction is the difference in free energy between products and reactants. In an exergonic reaction the free energy of the products is less than reactants.



Even though a reaction is energetically favorable, there must be an initial investment of activation energy (E_A).

The breakdown of biological macromolecules is exergonic. However, these molecules react *very slowly* at cellular temperatures because they cannot absorb enough thermal energy to reach transition state.

In order to make these molecules reactive when necessary, cells use biological catalysts called *enzymes*, which:

- Are proteins.
- Lower E_A, so the transition state can be reached at cellular temperatures.
- Do *not change* the nature of a reaction (ΔG), but only speed up a reaction that would have occurred anyway.
- Are very selective for which reaction they will catalyze.

B. Enzymes are substrate-specific

Enzymes are specific for a particular *substrate*, and that specificity depends upon the enzyme's three-dimensional shape.

Substrate = The substance an enzyme acts on and makes more reactive.

- An enzyme binds to its substrate and catalyzes its conversion to product. The enzyme is released in original form.

  Substrate + enzyme ⟶ enzyme-substrate complex ⟶ product + enzyme

- The substrate binds to the enzyme's *active site*.

Active site = Restricted region of an enzyme molecule which binds to the substrate.

- Is usually a pocket or groove on the protein's surface.
- Formed with only a few of the enzyme's amino acids.
- Determines enzyme specificity which is based upon a compatible fit between the shape of an enzyme's active site and the shape of the substrate.
- Changes its shape in response to the substrate.
  - As substrate binds to the active site, it *induces* the enzyme to change its shape.
  - This brings its chemical groups into positions that enhance their ability to interact with the substrate and catalyze the reaction.

Induced fit = Change in the shape of an enzyme's active site, which is induced by the substrate (see Campbell, Figure 6.11).

C. The active site is an enzyme's catalytic center

The entire enzymatic cycle is quite rapid (see Campbell, Figure 6.12).

Steps in the catalytic cycle of enzymes:

1. Substrate binds to the active site forming an *enzyme-substrate complex*. Substrate is held in the active site by weak interactions (e.g., hydrogen bonds and ionic bonds).
2. *Induced fit* of the active site around the substrate. Side chains of a few amino acids in the active site catalyze the conversion of substrate to product.
3. Product departs active site and the enzyme emerges in its original form. Since enzymes are used over and over, they can be effective in very small amounts.

Enzymes lower activation energy and speed up reactions by several mechanisms:

- Active site can hold two or more reactants in the proper position so they may react.
- Induced fit of the enzyme's active site may distort the substrate's chemical bonds, so less thermal energy (lower ΔG) is needed to break them during the reaction.
- Active site might provide a micro-environment conducive to a particular type of reaction (e.g., localized regions of low pH caused by acidic side chains on amino acids at the active site).
- Side chains of amino acids in the active site may participate directly in the reaction.

The initial substrate concentration partly determines the rate of an enzyme controlled reaction.

- The higher the substrate concentration, the faster the reaction - up to a limit.
- If substrate concentration is high enough, the enzyme becomes *saturated* with substrate. (The active sites of all enzymes molecules are engaged.)
- When an enzyme is saturated, the reaction rate depends upon how fast the active sites can convert substrate to product.
- When enzyme is saturated, reaction rate may be increased by adding more enzyme.

D. A cell's physical and chemical environment affects enzyme activity

Each enzyme has optimal environmental conditions that favor the most active enzyme conformation.

1. Effects of temperature and pH

Optimal temperature allows the greatest number of molecular collisions without denaturing the enzyme.

- Enzyme reaction rate increases with increasing temperature. Kinetic energy of reactant molecules increases with rising temperature, which increases substrate collisions with active sites.
- Beyond the optimal temperature, reaction rate slows. The enzyme denatures when increased thermal agitation of molecules disrupts weak bonds that stabilize the active conformation.
- Optimal temperature range of most human enzymes is 35°– 40°C.

Optimal pH range for most enzymes is pH 6 – 8.

- Some enzymes operate best at more extremes of pH.
- For example, the digestive enzyme, pepsin, found in the acid environment of the stomach has an optimal pH of 2.

2. Cofactors

Cofactors = Small nonprotein molecules that are required for proper enzyme catalysis.

- May bind tightly to active site.
- May bind loosely to both active site and substrate.
- Some are inorganic (e.g., metal atoms of zinc, iron or copper).
- Some are organic and are called *coenzymes* (e.g., most vitamins).

3. Enzyme inhibitors

Certain chemicals can selectively inhibit enzyme activity (see Campbell, Figure 6.14).

- Inhibition may be *irreversible* if the inhibitor attaches by covalent bonds.
- Inhibition may be *reversible* if the inhibitor attaches by weak bonds.

Competitive inhibitors = Chemicals that resemble an enzyme's normal substrate and compete with it for the active site.

- Block active site from the substrate.
- If reversible, the effect of these inhibitors can be overcome by increased substrate concentration.

Noncompetitive inhibitors = Enzyme inhibitors that do not enter the enzyme's active site, but bind to another part of the enzyme molecule.

- Causes enzyme to change its shape so the active site cannot bind substrate.
- May act as metabolic poisons (e.g., DDT, many antibiotics).
- Selective enzyme inhibition is an essential mechanism in the cell for regulating metabolic reactions.

III. The Control of Metabolism

A. Metabolic pathways are regulated by controlling enzyme activity.

Metabolic control often depends on allosteric regulation

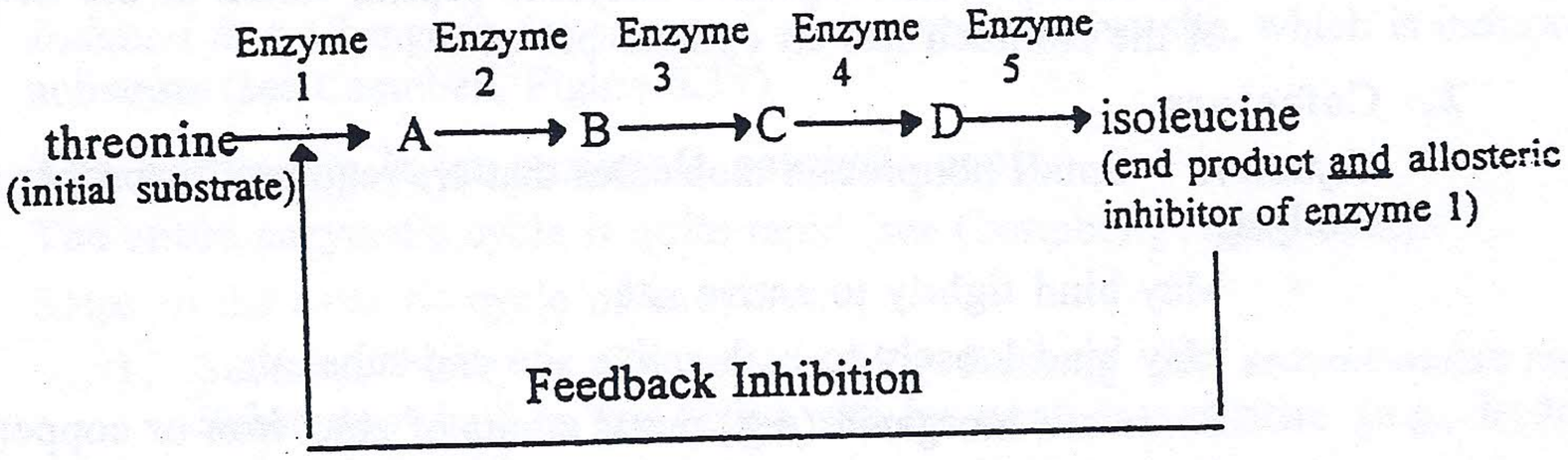
1. Allosteric regulation

Allosteric site = Specific receptor site on some part of the enzyme molecule other than the active site.

- Most enzymes with allosteric sites have two or more polypeptide chains, each with its own active site. Allosteric sites are often located where the subunits join.
- Allosteric enzymes have two conformations, one catalytically active and the other inactive (see Campbell, Figure 6.15).
- Binding of an *activator* to an allosteric site stabilizes the active conformation.
- Binding of an *inhibitor* (noncompetitive inhibitor) to an allosteric site stabilizes the inactive conformation.
- Enzyme activity changes continually in response to changes in the relative proportions of activators and inhibitors (e.g., ATP/ADP).
- Subunits may interact so that a single activator or inhibitor at one allosteric site will affect the active sites of the other subunits.

2. Feedback inhibition

Feedback inhibition = Regulation of a metabolic pathway by its end product, which inhibits an enzyme within the pathway. For example:



Prevents the cell from wasting chemical resources by synthesizing more product than is necessary (see also Campbell, Figure 6.16).

3. Cooperativity

Substrate molecules themselves may enhance enzyme activity.

Cooperativity = The phenomenon where substrate binding to the active site of one subunit induces a conformational change that enhances substrate binding at the active sites of the other subunits (see Campbell, Figure 6.17).

B. The localization of enzymes within the cell helps order metabolism

Cellular structure orders and compartmentalizes metabolic pathways (see Campbell, Figure 6.18).

- Some enzymes and enzyme complexes have fixed locations in the cell because they are incorporated into a membrane.
- Other enzymes and their substrates may be localized within membrane-enclosed eukaryotic organelles (e.g., chloroplasts and mitochondria).

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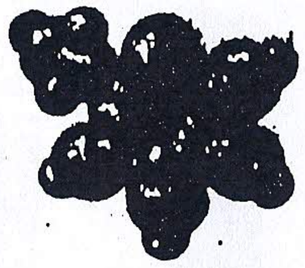
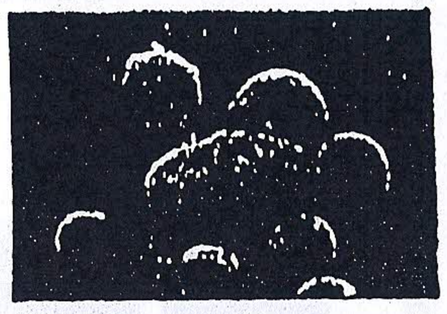
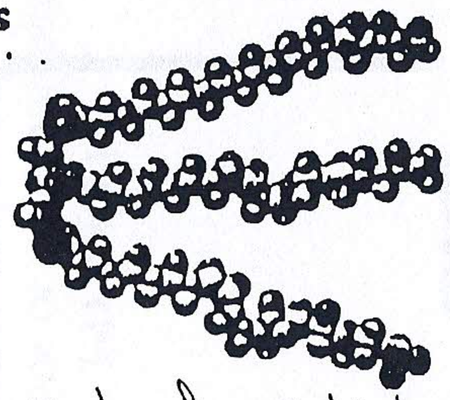
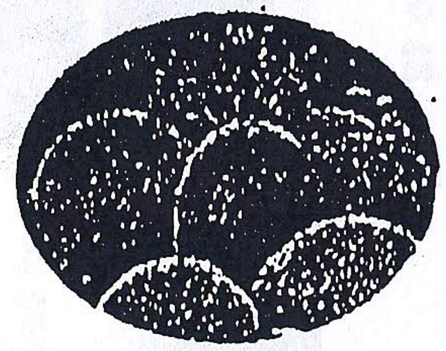
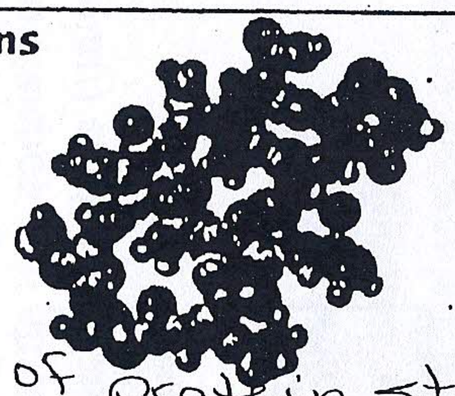
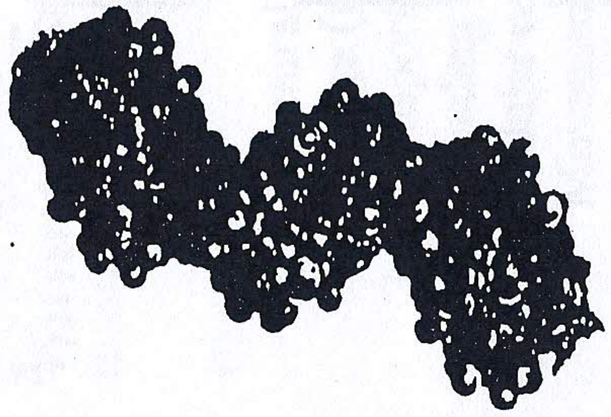
Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Lehninger, A.L., D.L. Nelson and M.M. Cox. *Principles of Biochemistry*. 2nd ed. New York: Worth, 1993.

Classes of Macromolecules

Class	Subunit	Function	Example	
Carbohydrates 	Monosaccharides Sugar Glycosidic linkage	Stores energy	Starch, glycogen	 Starch granules
		Structural component	Cellulose, chitin	
Lipids 	Fatty acid	Stores energy	Body fat	 Human fat cells
		Membrane bilayer	Plasma membrane	
		Sterold hormones	Testosterone	
		Pigments	Chlorophyll	
Saturated - solid at rm temp/most H		Unsaturated - "oils"		
Proteins 	20 amino acids Amino acid	Catalysis by means of enzymes	Lactase	 Hair
		Structural component	Hair, cartilage	
		Peptide hormone	Insulin	
4 Levels of protein structure ① Primary ② Secondary ③ Tertiary ④ Quaternary				
Nucleic Acids 	Nucleotide 5 C Sugar Nitrogenous Base Phosphate group	Stores genetic information	DNA	 Chromosomes
		Makes proteins	RNA	

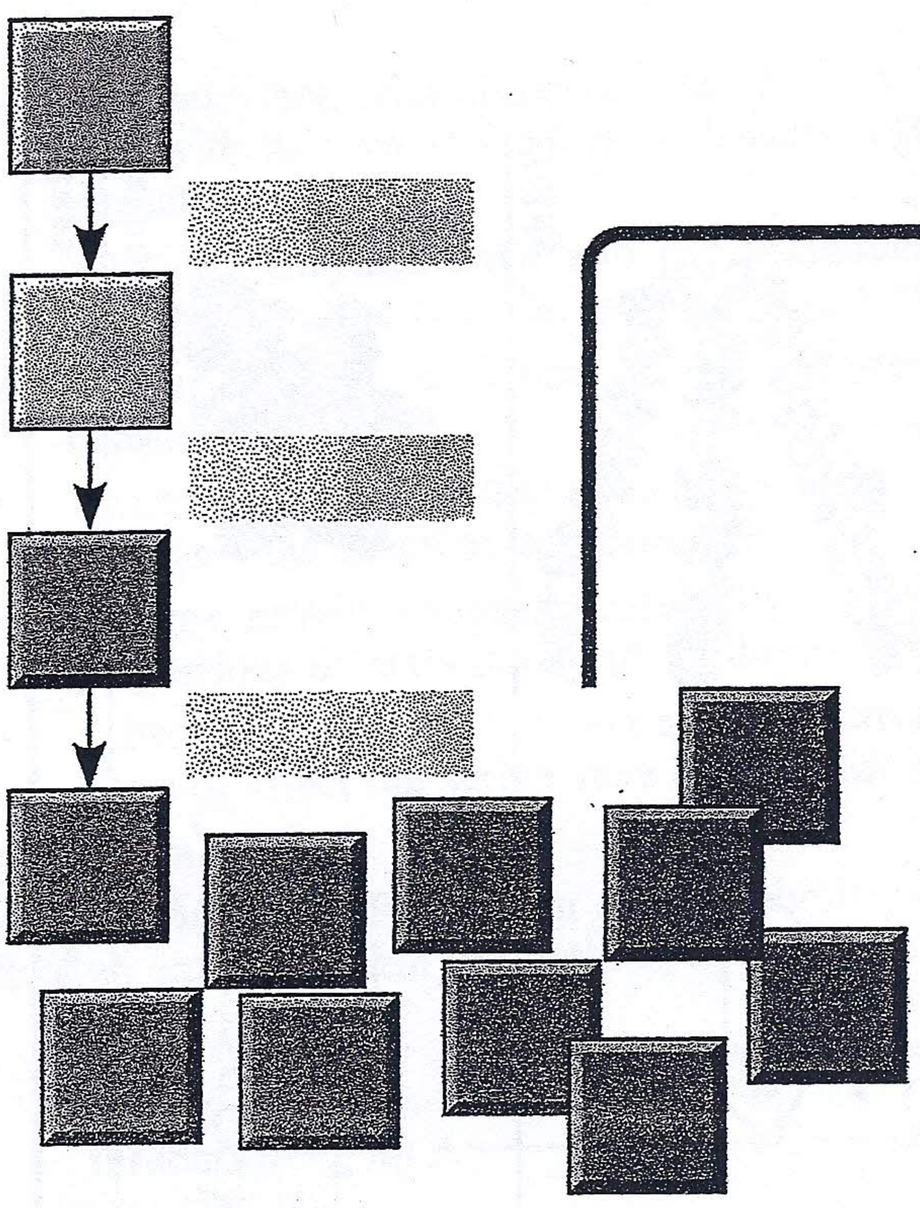


Figure 1.11 Negative feedback, page 11

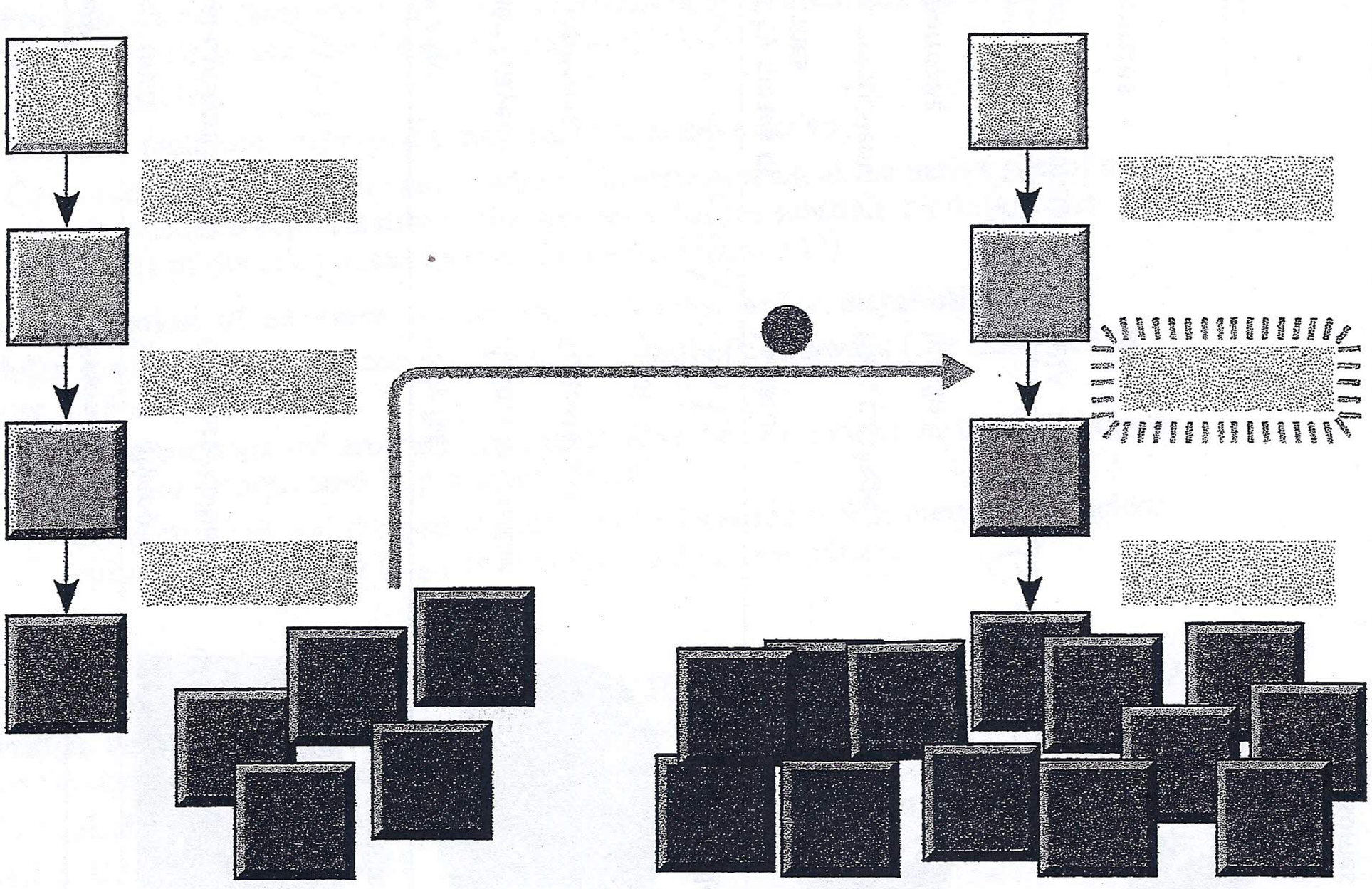


Figure 1.12 Positive feedback, page 12