CHAPTER 6

LIPIDS AND LIPOIDS

TWO DIFFERENT PATHWAYS, one theoretical and the other experimental, have led us to consider lipids as possibly the most important constituents involved in the dualistic patterns of physiopathological manifestations. The study of all the constituents of the organism—electrolytes, proteins, carbohydrates and lipids—has shown that for each of them, a rough division into two classes with antagonistic reactivity can be made according to the positive or negative electrostatic character of their polar groups—nucleophilic or electrophilic for some, anionic or cationic for others. However, these fundamental differences which can explain their intervention in processes in which dualism is apparent, do not represent the reason for their role in the induction of patterns.

The reactions in which some of these constituents take part are carried out as rapid changes while others are completed only slowly. It is these slow reactions, once accomplished, which tend to be stable for long periods of time. Since such stability is characteristic of clinical and analytical manifestations which have dual patterns, it appeared logical to consider the constituents with slow reactivity which are related to these manifestations. Because of their hydrosolubility, and the rapidity of the reactions in which they take part, most electrolytes, proteins and even carbohydrates probably play a lesser role in these long lasting processes.

The lipids, on the contrary, seem to be especially suited for this role. Many of the reactions in which the lipids participate are slow. As we will see later, this is primarily because of their insolubility in water. They form in the organism a group "apart" from all the water soluble constituents, a fact which permits them to function through proper reactions largely without continuous interference from the other constituents. For these reasons, the lipids appeared to be the most likely of all constituents to be

of major importance in physiopathological manifestations with long-lasting patterns. The study of the lipids has substantiated this.

However, before discussing these substances and their properties, a nosological problem must be considered: What are lipids? How can they be defined?

DEFINITION OF LIPIDS

The literature fails to furnish an adequate definition for the group of substances that show those properties which biochemistry and experimental biology attribute to the lipids. A definition on a chemical basis, such as one which considers lipids to be fatty acids and fatty acid derivatives, appears to be insufficient. It excludes substances such as those forming insaponifiable fractions which not only have properties attributed to lipids, but continuously intervene in the processes related to them.

Physical characteristics such as "greasiness" and solubility come nearer to the real situation without providing a satisfactory definition. Bloor's definition *(Note 1),* widely accepted today in spite of having been found inadequate, has introduced—in addition to the important solubility characteristics—certain less acceptable criteria such as the origin of these substances and the direct relationship to fatty acids. Without these criteria, lipids would have to include the group of hydrocarbons which have the same solubility property but usually are not encountered in organisms. However, with these criteria, Bloor's definition, besides limiting the field too much through the requirement for a relationship to fatty acids, excludes the entire important group of synthetic agents with similar properties. To be complete, a definition would have to include these artificial substances.

Thus confronted by the need for a satisfactory general definition, we proposed one in 1940 (23) which has since been of great help to us in all our research: *a lipoid is a polar-nonpolar substance in which the nonpolar part is predominant. It is thus formed by one or more polar groups bound to one or more nonpolar groups, the last being energetically predominant.* In terms of intervening forces, this definition considers the cohesion forces of the nonpolar part, and especially those related to its surface and known as the constant "b" of van der Waals forces, which in lipoids are predominant upon the electrostatic forces of the polar part. The definition has provided the key for the study of the multiple problems in which these substances appear to be involved. This definition appears acceptable since it explains all the known properties of the lipids. Further-

more, the study of the specific relationship between the forces involved could even predict new properties, as will be shown later.

The distinction between natural and synthetic substances as a basis for a definition has been obsolete in biochemistry for a long time. In our study however, it appeared to be didactically useful to indicate whether or not a substance is encountered naturally in the organism. Therefore, while adhering to our general definition, we have employed the term "lipoids" for the entire group of polar-nonpolar substances with a predominance of the nonpolar part, and have conserved the term "lipids" to designate the naturally occurring members. With this separation, we have also avoided

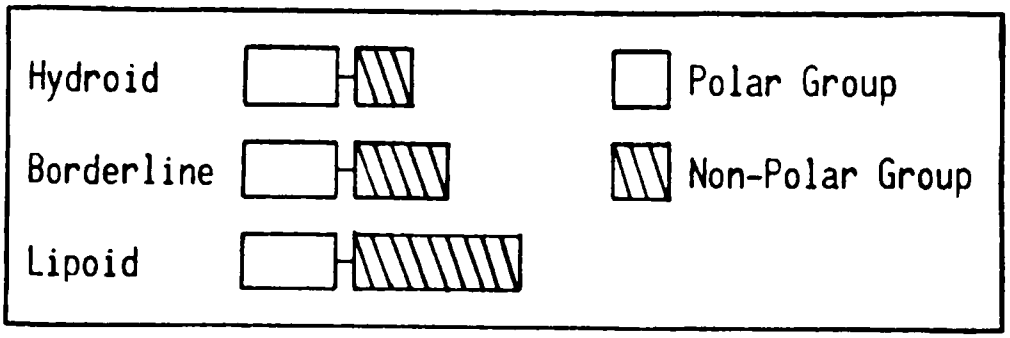


FIG. 61bis. Schematic representation of the predominant relationship of the polar and nonpolar parts in hydroids, borderline substances and lipoids.

a certain apprehension felt by many workers about incorporating indistinctly, in the same group of agents, substances with vastly different chemical constitutions, which until now have not been associated with lipids. The fact that, beyond physicochemical constitution, biological properties characterizing the lipids are common to the entire group of lipoids, will in time, we hope, help to reduce the importance of this separation between lipoids and lipids.

The structure of the lipoids—with a large variety of polar and nonpolar groups but always with the same characteristic energetic relationship between them—has led to a logical systematization of these substances, using the nature of the polar and nonpolar groups as criteria.

CLASSIFICATION OF LIPOIDS

Lipoids may be subdivided according to different criteria.

I. *According to the polar group.*
   A. *Lipoids classified according to the nature of their polar group.*
      1. Lipo-carboxylic acids (−COOH)
      2. Lipo-thiols (−SH)
      3. Lipo-sulfonic acids ($-SO_3H$)

4. Lipo-amines ($-NH_2$)
5. Lipo-amides ($-CONH_2$)
6. Lipo-alcohols ($-OH$)
7. Lipo-aldehydes ($-CHO$)
8. Lipo-ketones ($=CO$)
9. Lipo-halogens ($-Cl$, etc.)
10. Lipo-metals ($-Na$, etc.)

etc.

B. *Lipoids classified according to the predominant element of the polar group.*

1. Lipo-sulfur compounds
   a. Lipo-thiols ($-SH$)
   b. Lipo-sulfonic acids ($-SO_3H$)
   c. Lipo-sulfides ($=S$)
   d. Lipo-sulfoxides ($-SO$)
   e. Lipo-sulfones ($=SO_2$)
   f. Lipo-sulfites ($=SO_3$)

   etc.

2. Lipo-nitrogen derivatives
   a. Lipo-amines ($-NH_2$)
   b. Lipo-amides ($-CONH_2$)
   c. Lipo-nitriles ($-CN$)
   d. Lipo-isocyanides ($-NC$)
   e. Lipo-nitro derivatives ($-NO_2$)

   etc.

C. *Lipoids classified according to the energetic character of their polar group.*

A. Lipoids with negative polar groups

1. Lipoacids
   a. Lipo-carboxylic acids
   b. Lipo-thiols
   c. Lipo-sulfonic acids, etc.
2. Lipo-aldehydes

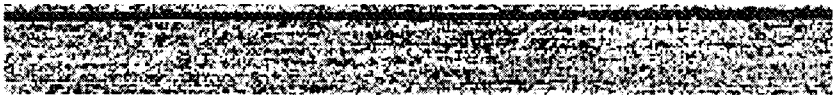
B. Lipoids with positive polar groups

3. Lipobases
   a. Lipo-amines
   b. Lipo-guanidines
   c. Lipo-imines, etc.
4. Lipo-alcohols

II. *According to the nonpolar group.*

A. *Lipoids classified according to the structure of their hydrocarbon chain.*

1. Aliphatic
2. Alicyclic
3. Aromatic
4. Heterocyclic, etc.



B. *Lipoids classified according to their carbon bonds.*

1. Saturated
2. Unsaturated
   a. Ethenic (mono-, di-, poly-)
   b. Ethynic

Some aspects of this classification require discussion.

The generic term lipoacid has been employed to describe simple lipoids having polar groups with acid functions. While the principal lipoacids are the fatty acids, other members have other acid polar groups, such as SO_2, SH, NO_2, etc. The significance of this grouping together of lipoids with negative polar groups has become evident especially in studying the similarities in the biological effects of these substances. In certain aspects of our research, this correlation has permitted us to substitute one category of lipoids (lipo-thiols or lipo-aldehydes) for another (lipo-carboxylic acids), thereby avoiding certain undesirable effects of the latter group of substances.

Lipoids having polar groups energetically opposite to those of the acids have been grouped together. Of these, the members with a polar group with alkaline functions have been classified as lipobases. The term base is generally applied to ionizable compounds which influence the pH of solutions and combine readily with acids by losing an OH^- and gaining a proton. Another group is formed by the lipoidic alcohols. Recent evidence indicates that, in many circumstances, the differences in the reactions of alcohols and common bases are quantitative rather than qualitative. Quite often the reaction of an alcohol with an acid is analogous to the reaction between an acid and sodium hydroxide, the H^+ of the acid combining with the OH^- of the alcohol. The differences between reactions are considered to be matters of time-rate. Whereas the reaction of the base is almost instantaneous, that of alcohol is a slow reaction and is less complete. This behavior of alcoholic substances is particularly clear when the hydroxyl group of an alcohol is replaced by a halogen to prepare the alkyl halides. For instance, according to Karrer (24): "This can be done by the action of the concentrated halogen acids on the alcohol:

$$CH_3OH + HCl \longrightarrow CH_3Cl + H_2O$$

The reaction corresponds superficially to the formation of a salt from an acid and a base:

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

There is, however, a difference between the two processes. Bases and acids are largely dissociated; when they come together, hydrogen ions and hy-

droxyl ions combine almost at once to give the very little electrostatically dissociated water, so that the reaction which really occurs is:

$$Na^+ + OH^- + H^+ + Cl^- \longrightarrow Na^+ + Cl^- + H_2O$$

Ionic reactions always occur instantaneously. Reaction between alcohol and hydrogen halides is governed by other laws. Alcohol is only very slightly ionized. For the removal of the hydroxyl group a certain time is required. The reaction between alcohol and acid with elimination of water, known as esterification, is therefore a time reaction." No essential difference exists between the reaction of lipo-alcohols or lipo-amines, for instance, with organic acids.

These considerations would have been sufficient to allow lipobases and lipo-alcohols to be grouped together. There are other considerations as well. Their common biological activity and mutual interchangeability also appear to justify grouping them together. Furthermore, the recognition of the existence of a general mutual antagonism between lipoacids on the one hand and lipobases and lipo-alcohols on the other hand, chemically, physically and biologically, has proven of considerable value in explaining a variety of experimentally observed facts in many aspects of our research.

Following this through, we have found it advantageous to define the two groups of lipoids by a more general character, the electrical aspect of the polar part, negative for the lipoacids and lipo-aldehydes and positive for the group of lipo-alcohols and lipobases. The terms, "positive and negative lipoids," serve also to emphasize the nature of their antagonism.

The structure of the nonpolar group as it confers physical, chemical and biological properties on the lipoids permits further subdivisions. Lipoids may be classified on the basis of the aliphatic, alicyclic, aromatic or heterocyclic character of the nonpolar group. While the negative lipids are principally formed by fatty acids, the positive are made up principally of sterols. The presence or absence of double bonds defining saturated and unsaturated carbon chains has been one subject of our study and considerable biological importance has been found to be related to this character as well as to the positional relationship of the double bond to the polar group and the polarity induced by the double bond.

The study of lipoids has shown that, besides properties contributed by the elements and groups which compose them, they have additional physico-chemical and even biological properties which are characteristic. We have termed these "lipoidic properties" to indicate that they are considered to result directly from the particular constitution of the lipoids.

Physical Lipoidic Properties

Solubility

Solubility represents the first and most important of these lipoidic properties. Characteristically, a lipoid has a greater solubility in neutral solvents than in water. This is explained by the fact that the two constituent groups, polar and nonpolar, induce different solubility properties.

As is well known, solubility corresponds to a free movement between molecules of the solvent and the solute. (25) Solubility is greater when the physical properties of the groups forming the solvent and those forming the solute are similar; it is impaired when they are different. Consequently, polar groups in a solute will tend to favor solubility in solvents with polar groups, such as water. At the same time, they will oppose solubility in neutral solvents formed by nonpolar groups. On the other hand, nonpolar groups in a substance will favor solubility in nonpolar neutral solvents but will oppose it in polar solvents such as water. Polar groups thus are hydrophilic and lipophobic, while nonpolar are lipophilic and hydrophobic. (26)

While the solubility characteristics of substances composed only of polar or nonpolar groups are readily apparent, the problem is more complex when a substance contains both polar and nonpolar groups. Since such a compound possesses groups with antagonistic solubility tendencies, its solubility "in toto" will depend upon the relationship between the opposing forces. For a borderline group of polar-nonpolar substances with approximately equal forces, there will be equal solubility in polar and nonpolar solvents. For other substances, the predominance of one or the other group will determine solubility characteristics. If the electrical forces of the polar group predominate, the substance will be hydrosoluble but insoluble or only partly soluble in nonpolar solvents. If, on the contrary, the cohesion—*i.e.,* the van der Waals forces—of the nonpolar group predominate, the substance will be soluble in nonpolar solvents and less, or even not at all, soluble in water. (TABLE VI)

Polar and nonpolar forces can be calculated and their study can indicate the place of a substance in this systematization. The importance of solubility for defining and systematizing lipoids became apparent in a physicomathematical study of these substances carried out by Jean Mariani in our laboratories. *(Note 2)* We have defined as "hydroids" those substances with predominant polar groups which are more soluble in polar solvents such as water. The "borderline substances," with no predominance of either group, show the same solubility in polar and neutral solvents.

TABLE VI

CLASSIFICATION OF CHEMICAL COMPOUNDS

Composition	Predominance	Name	Example
Polar groups only			Water
Polar-nonpolar groups	Polar group predominant	Hydroides	Glycerin
	No predominance	Borderline substances	n–Propyl alcohol
	Nonpolar group predominant	Lipoides	Oleic acid n–Butyl alcohol
Nonpolar groups only			Paraffin

The "lipoids," in which the nonpolar groups predominate, are more soluble in neutral solvents than in water.

As we have mentioned above, from a practical point of view, a substance could be judged to be a hydroid, borderline substance, or lipoid by considering the differences in its solubility in water and in a nonpolar solvent, such as petroleum ether, which corresponds to a mixture of the first aliphatic saturated hydrocarbons liquid at normal temperature and pressure. *A polar-nonpolar substance more soluble in water than in neutral solvent is considered a hydroid; one equally soluble in both solvents is classified as a borderline substance; while a substance more soluble in the neutral solvent than in water is a lipoid.*

Different polar groups such as COOH, OH, NH_2, CO, SO_2, SH, etc., enter into the constitution of various lipoids. They differ considerably in their electrostatic forces. As a result, the forces of the nonpolar groups required for predominance, if a lipoid is to be formed, also will differ. A different nonpolar group thus is necessary for each different polar group. For aliphatic molecules, it is principally the length of the chain which determines cohesion forces and a different number of carbons in the nonpolar group appears to be necessary, depending upon the polar group, in order to form a lipoid. The study of homologous series from this point of view is interesting.

Since the value of the electrostatic forces varies greatly from one polar group to another, the first members of the various homologous series, which are also lipoids, will differ from series to series, depending upon

the nature of the polar group. The length of the carbon chain of the nonpolar group will thus indicate in what member of a series the lipoidic character appears. By comparing mathematically the value of the electrostatic forces of each polar group and the cohesion forces of the nonpolar group in the respective series, it is possible to determine which member of each homologous series of substances will first show the properties of the lipoids. This also can be determined experimentally, as seen above, using the solubility characteristics of the lipoids. For the different members of the series, degrees of solubility in a polar solvent such as water, and in a nonpolar solvent such as petroleum ether, were determined. The first member of an homologous series to be considered a lipoid was the one found to be more soluble in the nonpolar than in the polar solvent. All members with a large number of carbon atoms show lipoidic properties; those with fewer carbon atoms lack those properties.

Thus, lipoidic properties first become manifest, among the carboxylic acid series, in valeric acid, *i.e.*, the five-carbon member. The shorter carbon chain members are soluble to an equal or greater degree in water than in petroleum ether, while those having a carbon chain longer than four show a higher degree of solubility in the nonpolar solvents than in water. (TABLE VII)

TABLE VII

SOLUBILITIES OF CARBOXYLIC ACID HOMOLOGUES

Substance *	Common Name	No. of Carbon Atoms	% of solubility in	
			Polar Solvent (Water at 20°)	Nonpolar Solvent (Petroleum Ether)
Methanoic acid	Formic acid	1	∞	insol.
Ethanoic acid	Acetic acid	2	∞	∞
Propanoic acid	Propanoic acid	3	∞	∞
Butanoic acid	Butyric acid	4	∞	∞
Pentanoic acid	Valeric acid	5	3.7 (at 16°)	∞
Hexanoic acid	Caproic acid	6	0.4	∞
Heptanoic acid	Enanthic acid	7	0.24	∞
Octanoic acid	Caprylic acid	8	0.25 (at 100°)	∞

* Names approved by International Union of Chemistry.

The same is true for the alkyl alcohols. n-Propyl alcohol and the members below it are either miscible with both water and petroleum ether or more soluble in water, indicating that the nonpolar forces do not predomi-

nate in their molecules. Therefore, they are not lipoids. n-Butyl alcohol, more soluble in neutral solvent than in water, thus is the first lipoidic member of this homologous series. However, this is not true for all its isomers. The primary, secondary and iso butanol are the first in their respective series to possess the solubility properties characteristic of lipoids. In the tertiary alcohol series, however, the four-carbon member, the tert.-butanol, does not show the same solubility properties. Tert.-butanol is miscible with water and neutral solvent and as such, is not a lipoid. For this tertiary alcohol series, it is the five-carbon member, the tert.-amyl alcohol, which first shows the solubility properties of a lipoid, being only 12.5% soluble in water and infinitely soluble in petroleum ether. Thus, of the four isomers of butyl alcohol, three are lipoids, while one, tert.-butyl alcohol, is not. (TABLE VIII)

TABLE VIII

SOLUBILITIES OF THE ALKYL ALCOHOLS

Substance *	Common Name	No. of Carbon Atoms	% of solubility in	
			Polar Solvent (Water at 20°)	Non-polar Solvent (Petroleum Ether)
Methanol	Methyl alcohol	1	∞	∞
Ethanol	Ethyl alcohol	2	∞	∞
1-Propanol	Propyl alcohol	3	∞	∞
2-Propanol	Isopropyl alcohol	3	∞	∞
1-Butanol	n-Butyl alcohol	4	7.9	∞
2-Butanol	sec.-Butyl alcohol	4	12.5	∞
2-Methyl, 2-propanol	tert.-Butyl alcohol	4	∞	∞
2-Methyl, 1-propanol	Isobutyl alcohol	4	9.5	∞
1-Pentanol	n-Amyl alcohol	5	2.7	∞
2-Pentanol	sec. act. Amyl alcohol	5	5.3	∞
3-Pentanol	Diethyl carbinol	5	insol.	∞
2-Methyl, 2-butanol	tert.-Amyl alcohol	5	12.5	∞
2-Methyl, 1-butanol	n-act. Amyl alcohol	5	insol.	∞
3-Methyl, 2-butanol	Isoamyl sec. alcohol	5	sl. sol.	∞
1-Hexanol	n-Hexyl alcohol	6	very sl. sol.	∞
2-Hexanol	sec.-Hexyl alcohol	6	very sl. sol.	∞
3-Hexanol	Ethyl propyl alcohol	6	0.9	∞
1-Heptanol	n-Heptyl alcohol	7	insol.	∞
1-Octanol	n-Octyl alcohol	8	insol.	∞

* Names approved by International Union of Chemistry.

The same methods were used to recognize the first lipoidic members of various alkane derivatives studied. TABLE IX shows the first lipoid members of several homologous series.

TABLE IX

FIRST LIPOIDIC MEMBERS IN VARIOUS ALKANE DERIVATIVE HOMOLOGOUS SERIES

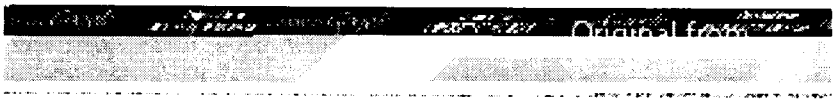
Substance *	Common Name	Polar Group	No. of Carbon Atoms
Methanethiol	Methyl mercaptan	—SH	1
Propanal	Propionaldehyde	—CHO	3
Propylcarbylamine	Propyl isocyanide	—NC	3
1-Butanol	n-Butyl alcohol	—OH	4
2-Butanone	Butyl ketone	=CO	4
Butanamide	Butylamide	$-CONH_2$	4
2-Methyl, 2-butanol	tert.-Amyl alcohol	—OH	5
Pentanoic acid (n)	Valeric acid	—COOH	5
Hexylamine (n)	Hexylamine	$-NH_2$	6
1, 8 Octandiol	1, 8 Octandiol	—OH	8

* Names approved by International Union of Chemistry.

Molecular Layer Formation

Other fundamental characteristics result from the different solubility properties of the two parts, polar and nonpolar, forming a lipoid. Introduced in a diphasic medium in which one phase is water and the other oil or even air, the polar group, with the tendency to be soluble in water, will penetrate the water. Since the nonpolar group, which is hydrophobic, is predominant, not only will it not enter the water but it will also prevent the entire molecule from moving freely in water. Consequently, the lipoid molecule will remain at the surface of the water with only its polar group penetrating. Because of this, the molecule will assume an oriented position toward the surface of water. If the second phase is a neutral solvent, the lipoid molecules, which will accumulate at the interphase with the polar group in water, will have the nonpolar group penetrating the neutral solvent.

In both cases, the molecules form oriented molecular layers which, if present at the limit between two phases, would appear as organized formations. This property which appears as a direct consequence of the characteristic constitution of the lipoids has further consequences. In such a layer, the polar groups penetrating in water will influence the properties of its surface, and thus reduce its surface tension.



Through the coulombian character of their electrostatic forces, the polar groups will thus confer, according to their nature, a positive or negative electrical character to the layer.

In a mixture of water and oil, the presence of a lipoid layer will lower the intersurface tension and will favor the breaking down of the phases, facilitating the formation of an emulsion. The presence of the same electrical charge at the surface of these resulting emulsion droplets will act as a repellent force between them and increase the stability of the emulsion. *(Fig. 62)* This is another important characteristic of lipoids which results from their peculiar constitution.

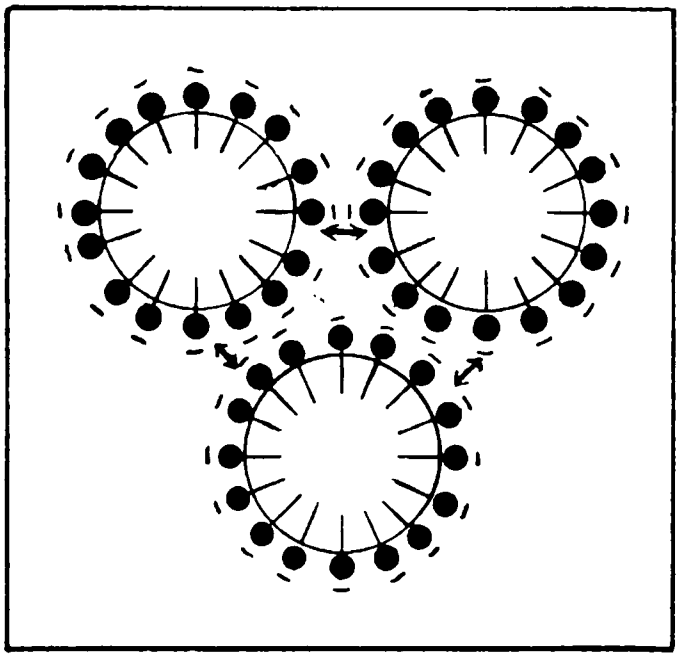


FIG. 62. The presence of the same electrical charge at the surface of droplets of an emulsion insures, through the repellent forces, the stability of the emulsion.

Chemical Properties

Lipoids have two groups of chemical properties which can be related to their two principal parts, polar and nonpolar. The polar groups with their electrostatic forces give to the lipoids one group of characteristic reactivities. A carboxylic lipoid will act like any other organic acid, while the lipo-alcohols will act like other alcohols, a thio-lipoid like a mercaptan, and so on. A characteristic of chemical reactions induced by the polar groups is that, while they are occurring in a water medium, they are largely limited to the site where the lipoid is localized due to the insolubility of

the entire molecule in water. Through this localization, the polar reactivity of the lipoids becomes largely a "surface reactivity." It is interesting that even minute amounts of lipids are able, through this localization at separating surfaces, to induce important changes.

A second group of reactions take place at the nonpolar group and especially at the different formations present in it, such as double bonds, cycles, etc. The hydrophobic and lipophilic character of the nonpolar groups confers a special character on these reactions. Most of them take place in nonionic nonpolar media. Many occur at the semipolar double bonds with nucleophilic or electrophilic carbons which appear to be especially suitable for this reactivity. This would explain the fact that nondissociated molecules may take part in these reactions. Most of these reactions are relatively slow. This double reactivity, ionic through the polar group and rather nonionic through the nonpolar group, makes the study of these lipoids one of great interest and it will be discussed below in more detail.

Biological Properties

The biological properties of lipoids in general also can be related directly to their physiochemical characteristics and, thus, to their peculiar constitution.

Lipidic System

The relative insolubility of the lipids in water and their solubility in neutral solvents has permitted us to separate these substances as a group from the other constituents of organisms. For more than just didactic purposes, we consider lipids to constitute a separate system in the organism. The part played by lipids in the organization and the functioning of various entities supports this concept. For example, when a lipoid is introduced into the organism, it will be selectively dissolved in, circulated through, retained by and metabolized as part of the lipidic system. Overtone's "Index of Repartition" of anesthetics in the organism can be seen to be a direct corollary of the existence of such a system although the anesthetic agent can be a lipoid or a nonpolar substance.

A great degree of independence of this system is morphologically evident as in adipous cells, when fats circulate as chylomicrons or when they form oriented layers. We have seen above how the orientation of lipoids at the surface of water results from the relationship between the solubilities of the two constituent groups, polar and nonpolar. Along with

their insolubility in water, the orientation of lipoids has allowed them to play a very important role in biology.

The very existence of biological entities appears to depend upon the ability of lipids to build up boundary formations separating and thus assuring the individuality of biological entities.

Through peculiar, reciprocally opposed orientations, two or more layers of lipids can form a membrane with two polar faces which has the ability to separate two aqueous media. In its simplest form, such a membrane appears in mitochondria. *(Note 3)* Similar boundary formations identify nuclei and cells and appear in higher entities, as in the membranes and intercellular cements of lymphatic and blood vessel endothelia. It is this peculiar orientation which allows lipids to establish the necessary boundary formations resulting in complex hierarchic organisms. The existence of biological entities, at least from the chromosome level up (and probably even below that level), can be seen to result directly from the intervention of lipids as a separate system, particularly in the formation of the dipolar lipidic boundaries.

However, boundary formations which separate the biological entities would not have been efficient if they did not fulfill another capital role: that of allowing selective passage of metabolites. A totally impermeable membrane would isolate the respective entities and result in their death. On the other hand, a totally permeable membrane would have no usefulness. The boundary formation has to act selectively, permitting the passage of some, but not all, substances. But even this does not seem to be sufficient to insure an efficient boundary. Most important, such a membrane must be able to alter its permeability, quantitatively and qualitatively, according to variations in circumstances. Such capacity for altering permeability can be related to the presence of the two groups of lipids, fatty acids and sterols, with their antagonistic properties relating to permeability.

The fatty acids appear to induce permeability in the membrane they form, especially permeability for anions. The perpendicular position to the surface of water assumed by the nonpolar aliphatic groups when the fatty acids form this boundary membrane appears to be favorable for the passage of a substance through the membrane. The fatty acid molecules thus can be separated, permitting other molecules to pass between them; that is, to pass through the membrane formed by the fatty acids. The negative electrical character of the polar groups of these fatty acids explains why they represent a kind of barrier to the free passage of cations. These cations are attracted and retained by the acid polar group. This would explain the manifest changes in permeability under the influence of calcium ion. The

removal of calcium from cellular membranes, through treatment with oxalates, increases permeability, while treatment with calcium salts reduces permeability. The bivalent calcium ion, when it binds the polar groups of two adjacent fatty acid molecules in the membrane, prevents the passage of other molecules between these parts of the membrane, a fact which explains the manifest decrease in permeability induced by this cation.

The other group of lipids, the sterols, have an effect on permeability opposite to that of fatty acids. This can be related in part to the bond which these sterols make with the fatty acids. Consequently, they block any passage through the part of the membrane formed by fatty acids. The impermeability is due, to some extent, to a peculiarity of the layers formed by these sterols themselves. The polycyclic molecules of sterols do not take the same perpendicular position toward the surface of water as fatty acids do. (27) *(Fig. 63)* Since sterol molecules assume a position almost parallel

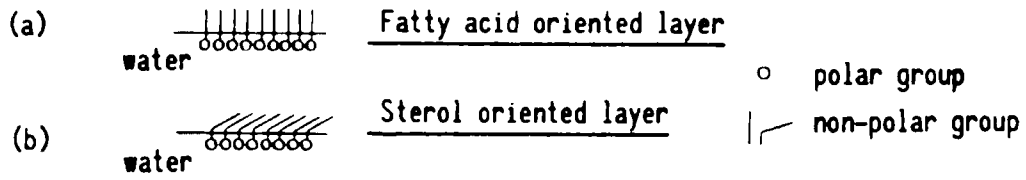


FIG. 63. *Schematic aspect of oriented interface layers.* The perpendicular position to the surface of water of fatty acid molecules (a) favors the passage of other molecules through the separating membranes they form. Oppositely, the almost parallel position to the surface of water of the polycyclic molecules of sterols (b) prevents the passage of other molecules through the membrane they form.

to this surface, the layer which they form exhibits no permeability properties. It even opposes any passage through it. It seems that fatty acids and sterols make separate "spots" in the cellular membranes so that, through their quantitative relationships, they confer different degrees of permeability to different regions of the membrane. The changes in permeability which result from the antagonistic intervention of the two groups of lipids seem to play an important role in normal and abnormal physiology.

With fatty acids inducing permeability, and sterols opposing it, the fundamental character of their biological relationship can be recognized. It would seem that part of the function of sterols is to oppose the activity of fatty acids. Conceptually, sterols would appear, in this specific activity, to be "anti-fatty" acid agents, with a capacity to control the activity of fatty acids rather than be active by themselves. Partly for this reason as well as for greater general understanding, it is necessary first to investigate fatty acid activity.

FATTY ACIDS

Besides their constructive role in establishing boundary formations, fatty acids appear to serve various other purposes in the organism. They can be used as caloric metabolites, and they play an active functional role in a biological change. While all fatty acids may exhibit these three activities—caloric, constructive and functional—there are important individual differences. With the carboxyl as common polar group, the differences between the various fatty acids can be related to the nonpolar groups. We will discuss this aspect of fatty acids, emphasizing only what can be considered to be new contributions to understanding the biological role of the substances.

Rancidity

The study of changes which take place in vitro, on lipids and especially on fatty acids after they have been separated from the organisms, led us to consider a possible parallelism between them and the changes which take place in the organism. We tried thus to utilize especially the knowledge furnished by the study of the chemical deterioration of natural fats generally known as rancidity (28), to better understand and also to systematize many of the processes occurring in vivo.

Three types of rancidity are described. In one—hydrolytic rancidity—fats are separated in free fatty acids and glycerol (or mono- or di-glycerides), through the intervention of lipolytic enzymes. These are often produced by molds (Penicillium, Aspergillus, etc.) or by microbes rich in such lipolytic enzymes or even the lipase present in the tissues from which the lipids are obtained. The characteristic of this type of rancidity is the intervention of enzymes and the appearance of free fatty acids as a result.

In a second type of rancidity, also occurring under the intervention of enzymes, an oxidative process is involved. The characteristic of this type of rancidity is that it affects almost, if not exclusively, saturated fatty acids, converting them into methyl-ketones by a beta oxidation process. This "perfume rancidity" called so because of the odor of the methyl-ketones with seven, nine or eleven carbons which result—takes place apparently through the intervention of a peroxidase present in certain molds (such as penicillium glaucum). One of its characteristics is that it occurs especially on saturated fatty acids with a low number of carbons (8 to 12).

The third type of rancidity groups together the oxidative changes which take place at the unsaturated nonpolar group of the lipids. As they result from the intervention of double bonds, the reactions differ according to

the energetic center present. In one which occurs at room temperature only for the conjugated fatty acids, such as eleostearic acid, and at 100°C only to some extent for oleic, linoleic and linolenic acid, the oxidation leads to the appearance of peroxides. (29) In another form of this oxidation, taking place for oleic, linoleic and linolenic fatty acids at room temperature or below 50°C, hydroperoxides result, as it has been shown by Farmer and coworkers, first for rubber (30) and after for fats (31). Another important fact seen in rancidity changes is that the atmospheric oxidation of polyethenoid fatty acids can result in a displacement of the double bonds with the appearance of conjugated isomers. (32)

The study of natural rancidity has represented the basic guide for our study and systematization of the processes encountered in normal and abnormal physiology. We searched and found this similarity not only in general outlines, but also for most of their details. By referring to the processes found in rancidity, we were able to identify, besides enzymatic lipolysis and enzymatic Knoop beta oxidation, known to occur in the organism, also the intervention of hydroperoxides, peroxides and the conjugation of double bonds. Not only the processes themselves but also the conditions under which they take place and their inter-relationship have been found to parallel in vivo those which can be seen in vitro.

We will see all along in the study of fatty acids how far the biological intervention of this parallelism goes.

Caloric Metabolism

Although all the fatty acids are ultimately used by different organisms as caloric metabolites, the saturated and monoethenic members are most important from this point of view. Among the saturated and monoethenic fatty acids, the members with long chains appear to be those which are kept in reserve for caloric purposes. We could show that the principal form of caloric desmolysis, the Knoop beta oxidation, takes place directly, almost exclusively, on members with relatively short chains, that is, with a maximum of 10 or 12 carbons.

While fatty acids with short chains take part directly in these caloric metabolic changes, those with longer carbon chains must undergo preliminary changes before entering into caloric metabolism. A desaturation, changing a saturated fatty acid into a monoethenic, appears to be a first step in caloric metabolism of the long chain members. The monoethenoids thus can be seen to be intermediary forms between the saturated reserve and the short-chain, easily metabolized fatty acids.

The double bond in these monoethenic acids would thus appear to

have two uses: one, to reduce the melting point below body temperature and thus permit easy mobilization, and two, to induce changes which lead to the breaking up of the long molecule into two shorter ones which can be metabolized through the Knoop oxidation.

All the data indicate that this fission would not take place at the double bond but through a more complex process. A first change consists of oxygen fixation at the carbon near the double bond. This leads to the appearance of a hydroperoxide group. It is only in a subsequent step that the molecule breaks at a place between this carbon near the double bond and the double bond itself, resulting in the appearance of short chains which have an even number of carbons capable of being directly metabolized through the beta oxidation. *(Note 4)*

The position of the double bond in the naturally occurring monoethenic fatty acids, separating almost always a group of nine carbons toward the carboxyl or the methyl end, *(Note 5)* acquires a special significance for the breaking down of the molecules for caloric purposes.

The desaturation of the saturated fatty acids, which would represent a first step toward allowing them to participate in metabolic caloric changes, would usually take place in the liver, apparently through the same processes by which polyunsaturated fatty acids are partially saturated. *(Note 6)*

An interesting part of the caloric metabolism of the saturated and monoethenic fatty acids, which will be shown below, is their combination with glycerol to form triglycerides.

Constitutional Role

Although saturated and monoethenoid fatty acids enter into the formation of boundary membranes, the di-, tri- and tetraenic members seem to have a particularly important role in the constructive function of fatty acids. Some of them enter directly into the formation of the membrane; some form complex lipoids such as lecithine with the glycerophosphoric radical and nitrogen containing bases. As a rule, these last represent a lipoidic substrate which would act as a neutral natural solvent present in membranes, and as such, intervene in the realization of a diphasic medium at the level of the boundary formation. This medium would largely insure the orientation of the fatty acids at the separation surface and the formation of permeable lipidic layers.

Functional Role

The third role of fatty acids is as functional agents taking part in certain reactions. This activity appears to be strongly related to two factors:

the presence of an uncombined carboxyl group and the energetic intervention of the double bonds of the nonpolar part of the polyunsaturated members.

Free fatty acids appear to be functionally active while the combined ones usually are inactive. The activity is related only partially to the direct capacity of the carboxyl to realize new combinations. It results from the induction exerted by the carboxyl upon the nonpolar group. The so-called free fatty acids of the organism are probably bound in a labile form to proteins, but this bond will not influence the induction effect exerted upon the nonpolar group. The intensive positive carbon of the carboxyl, together with the zig-zag disposition of the fatty acid molecule, causes the inductive effect to charge the successive carbons of the chain. They will thus show alternative signs. The even carbons show a negative character, while the odd ones are positive. The fact that oxygen combines with positive carbons explains not only why, as in Knoop oxidation, this bond occurs at C_3, which is strongly positive, but also explains the so-called alternate oxidation (33) where the other following odd carbons are binding oxygens. Through the influence exerted by the carboxyl, the double bond shows a special activity which has been worth studying.

Double Bonds

There has been some tendency to regard the double bond as a weak, easily broken point of the molecule. Actually, it emerges as an important center of activity. With its capacity to become a semipolar center, and consequently, to bind or lose radicals, the double bond is an energetic center in the molecule. Its important characteristic is the ability to effect such changes without altering the chain of the molecule itself. Since this type of reaction is reversible and can be repeated for the same molecule, the double bond appears to represent a functional entity. Because the reaction principally involves nonmetallic elements, the unsaturated fatty acid takes an active part in the metabolism in which these elements appear.

The study of rancidity has helped us, by analogy, to systematize oxidation processes as they take place in vivo. In addition to Knoop beta, several other types of oxidation could be recognized in which double bonds intervene more directly. The double bond, with its semipolar character, influences nearby carbons, rendering them highly reactive. In one form of oxidation, a molecular oxygen is bound to a nearby carbon to produce a hydroperoxide formation, as was shown to occur in vitro by Farmer. (31) When, under certain circumstances, this oxygen fixation becomes reversible, the fatty acid will liberate the oxygen. It appears highly probable that in

such a process, the oxygen is liberated as a free radical, the entire process thus corresponding to an activation of oxygen. The change of a molecular oxygen into a free radical would represent the physiological role of unsaturated fatty acids in oxidation processes.

The presence of two double bonds in non-parallel position, common to most of the naturally occurring polyunsaturated fatty acids, is even more important; the two double bonds exert a particularly strong influence on the special carbon which is in the intermediary position between them. Because of the alternate induction produced by the strongly positive carbon of the carboxyl, the carbons of the chain have alternate characters, positive and negative. When an intermediary carbon also has a strong positive character, it appears to be especially able to fix oxygen. This strongly positive intermediary carbon, occurring in natural polyunsaturated fatty acids with more than two double bonds, may be the reason for the important role played by these acids when they act as essential fatty acids in the organism. *(Note 7)*

The study of rancidity has further shown that, while the in vitro oxidation of an unsaturated fatty acid under mild conditions such as room temperature leads to the appearance of hydroperoxides, oxidation at a higher temperature will result in another fixation of oxygen, this time at the double bond itself. Epoxides or peroxides will appear according to the ionic or molecular character of the oxygen. This extremely important process also occurs in rancidity under the influence of an enzyme. It is highly probable that a similar process takes place in vivo in those pathological conditions in which peroxides appear in the urine. Radiation, certain inflammations (especially those due to streptococci), administration of selenium preparations or of highly polyunsaturated fatty acids are followed by the appearance of these oxidizing substances in urine. As mentioned above, when these substances appear, there also are increases in indoxyl and glucuronic acid, which can be considered, up to a certain point, to result from abnormally intensive oxidation taking place on tryptophane and glucose. (See below.) While activation of oxygen is a physiological process, peroxides appear under abnormal conditions.

ABNORMAL FATTY ACIDS

The study of the relationship between abnormal conditions and lipids has progressively led us to consider the existence of qualitative changes in these lipids, besides the quantitative ones. The existence of abnormal metabolic processes, and especially the fact that such abnormalities are often of long

duration, could hardly be attributed to variations in the quantity of the intervening lipids alone. More probably they would result from changes in the nature of the intervening lipids themselves. We have investigated this aspect of the fatty acids present under abnormal conditions.

As a guide for the direction to be followed in these investigations, we used the information furnished by the study of rancidity. We believed rancidity would be able to indicate broadly the nature of the qualitative changes which the lipids may undergo under abnormal conditions. Conceptually, the abnormal can be considered to result from a loss of the capacity of the organism to sufficiently control occurring processes and keep them in the frame of the constants which characterize the entity. Due to this lack of effective control, the in vivo occurring changes under abnormal conditions would closely approach those which take place in vitro where such a control does not exist. These considerations led us to search for changes similar to those seen in rancidity, or occurring in vivo in lipids, under abnormal conditions.

As mentioned above, in rancidity a first group of changes concerns the polar group. Some of them result in the appearance of free fatty acids, others correspond to changes in the carboxyls themselves, while still others are represented by processes of oxidation which occur in the chain near the polar group. A second group of rancidity changes concerns the nonpolar group and especially the energetic centers present in it, the double bonds.

The study of this last group of changes led us to consider, the changes appearing in vitro under the direct influence of heat and oxygen. As part of these changes, we considered of special importance the conjugation of the double bonds seen to occur in vitro as a step in the oxidation of polyunsaturated fatty acids. This conjugation corresponds to a characteristic displacement in the molecule of two or more of the double bonds present, so as to result in parallel reciprocal positions.

While in the simple bond two tetrahedric carbons are bound through their peaks, in the double bond they are bound by one edge, and in the triple bond by a surface. In the conjugated formation, the common edges of two double bonds, being separated by one simple bond, are consequently parallel. The planes in which the electrons of these double bonds are moving for each double bond and which are perpendicular to that of the bond itself, become parallel. Through the resulting reciprocal induction their energetic value is enhanced.

We have studied systematically the different qualitative abnormalities concerning the fatty acids, guided mainly by the information obtained through the study of rancidity.

Methods of Investigation Used

Following this line, we first investigated the forms under which the lipids in general are present in the organism. We utilized the differences in solubility between these different forms, separating them into free lipids, lipids kept in a labile bond with other constituents, as in cenapse, lipids bound through their polar group as in fats, or in the still stronger form as lipids in combinations so firm that they cannot be separated except through saponification. The method devised for this study and some examples are in Note 8A. This research showed that under abnormal conditions, very important variations occur in the amounts of the different forms. This study pointed out that the free lipids are greatly responsible for the important manifestations in which lipids appear as active agents.

In the study concerning the abnormal metabolism of the carboxyl and nearby carbons, we investigated the appearance of fatty aldehydes or ketones in blood, urine and in the cells.

One of the major problems encountered was the appearance in vivo of conjugated fatty acids, as abnormal fatty acids. In order to ascertain their presence and to measure their amounts, we had utilized three different methods of investigation: spectral analysis in ultra-violet and in the first portion of the visible spectrum *(Note 8B)*; the study of the place of the double bond in the fatty acids molecule through the fission of these molecules and the analyses of the resulting fractions. *(See Note 1, Chapter 10)* More recently we have tried the vapor fractionation method (gas chromatography) *(Note 8C)*.

The first, and especially the second method, gave us valuable data permitting us to recognize the intervention of conjugated fatty acids in abnormal conditions. These studies revealed the appearance of conjugated fatty acids, especially as trienes, the increase of their amount with the progress of the conditions and especially the fact that death occurs when their concentration in the bodies has reached a critical value. This has marked the importance of these substances in physiopathology. The fact that gas chromatography did not reveal the presence of conjugated fatty acids appears due to the conditions under which the method actually works.

Later, we will frequently return to the various problems related to intervention of conjugated fatty acids. We could thus directly correlate the intervention of these abnormal fatty acids with the pathogenesis of the manifestations of many conditions such as trauma, shock, adrenalectomy, and especially with the noxious manifestations following irradiation. *(Chapter 10)*

In abnormal metabolic changes, an important factor is the intervention of abnormal fatty acids in the metabolism of chloride ions, producing an especially strong fixation of the chloride ion to the carbons at the double bonds. The conjugated double bonds in a fatty acid molecule appear to be especially suitable for this since an abnormal, irreversible fixation of chlorides occurs in two steps. First, the halogen is fixed at the extreme carbons of conjugated formations with a displacement of the double bond in the intermediary position. In the second phase, the fixation takes place in the intermediary carbons, too. *(Note 8D)*

Functionally, fatty acids induce activation of oxygen as a normal process, but the appearance of peroxides or irreversible fixation of chloride ions is an abnormal event.

It is the abnormal fixation of chlorides by the conjugated fatty acids which leads to a more complex group of processes involving sodium chloride metabolism. With the chloride ion fixed, the sodium ion of sodium chloride remains free to enter into other combinations, especially with a carbonate ion, producing strongly alkaline compounds. This process explains the appearance of local alkalosis as a result of the intervention of conjugated abnormal fatty acids, corresponding to the chloride phase of "D."

The division of fatty acids into four groups—1) saturated and monounsaturated, 2) di-, tri- and possibly also tetra-unsaturated, 3) tetra- and higher polyunsaturated, and 4) conjugated—corresponds schematically to the four principal roles—caloric, organizational, functional and pathogenic —which fatty acids play in the organism. These roles are seen to be dictated both by the different structures of the fatty acids and the different substances to which they are preferentially bound. The fate of a fatty acid in the organism seems to be greatly influenced by its bond to other substances. As already noted, we have called these other substances "anti-fatty acids."

THE ANTI-FATTY ACIDS

Glycerol and Glycerophosphoric Acid

It is classically accepted that the intestinal absorption and circulation of fatty acids is made through bonding to various substances. The analysis of this absorption shows, however, that different fatty acids have preferential bonds. For saturated and monoethenic fatty acids, the bond is principally with glycerol. Although mono- and di-glycerides can be identified in the cells of the intestinal mucosa, these fatty acids leave the intestine as triglycerides, forming the largest part of the chylomicrons. They are

also found in reserve in adipous cells as triglycerides. The di-, tri- and even tetraenic fatty acids usually enter the circulation as phospholipids, that is, in direct combination with glycerophosphoric ions. The polyunsaturated acids are bound to sterols when they enter the blood, circulate and are stored. While the structures of the various fatty acids determine their different roles in the organism, it is the anti-fatty-acid constituents to which they are bound which enhance these roles. The study of the anti-fatty acids has shown that these substances can even dictate, by themselves, different fates for the various fatty acids they bind.

The combination of glycerol with any fatty acid seems to establish a caloric metabolic character. This is true for the very different fatty acids found in plants and animals as triglycerides. Even the ricinoleic triglyceride, if fresh, is used as comestible oil, *castor oil*. The same is true of the oil of triglycerides of polyunsaturated fatty acids found in marine animal oils. In the seeds, all the triglycerides of fatty acids, even the conjugated ones such as eleostearic and parinaric, represent energetic sources. It seems that it is their combination with glycerol which has given all these fatty acids value as caloric metabolites. The same is true for the bond to glycerophosphoric ion.

Combination with glycerophosphoric acid endows various fatty acids with the ability to participate in the construction of membranes. The bond to sterols, on the contrary, induces an ultimate functional activity provided the fatty acid itself is so constituted as to be able to fulfill this function.

The influence exerted by anti-fatty acids can be understood in terms of the changes they induce in the activity of the fatty acids. Since the activity of the last is largely related to their presence as free substances, it is principally through their combination with fatty acids that the anti-fatty acids intervene. By inactivating those free fatty acids which form a membrane and insure its permeability, an anti-fatty-acid agent can cause the membrane to change its permeability and even to become completely impermeable. Similarly, an anti-fatty acid, by combining with a polyunsaturated fatty acid, can reduce or even suppress its functional activity. It is to be noted that, by both changing permeability and suppressing functional activity, the anti-fatty acids exert their influence ultimately by altering oxygen metabolism. From this point of view, metabolism becomes predominantly anoxybiotic in contrast to normal oxybiotic metabolism. For glucose, for instance, suppression of the oxidative phase arrests metabolism at pyruvic acid which passes into lactic acid. The appearance of acid substances as a biological effect of the action of anti-fatty acids results, in fact, from the

reduction of the fatty acid's activity, affecting oxidative processes directly, or indirectly through reduction of membrane permeability.

While one group of anti-fatty acids can be directly related to hydroids, and especially to glycerol or to glycerol bound to phosphoric acid as in the glycerophosphoric ion, a second group is represented by lipoids, principally formed by derivatives of a characteristic ring system, the cyclopentanophenanthrene. As anti-fatty acid lipoids, these compounds, the steroids, were of special interest in lipoid research. Only some aspects of the biological activity of steroids—mainly, those which represent new views in the study of these substances—will be discussed here.

Steroids

A fundamental role of these substances in biology is determined by the fact that they are polycyclic. This leads us to consider the role of the ring itself in reactivity, as shown by a study of the steroids in opposition to the fatty acids. In the fatty acids, the bonds between carbons as present in the aliphatic chain, insure a high reciprocal mobility between these carbons. As a result, the entire aliphatic chain is highly flexible. On the other hand, rigidity is characteristic for all the rings, and is increased by the polycycling of the molecules. The constituents of the molecules are kept in fixed reciprocal positions. While, in the fatty acids, the flexibility of the chain permits the energetic centers to take different relative positions among themselves toward other molecules, the rigidity of the polycyclic molecules maintains the energetic centers of the cycle, or those attached to it, in the same relative position. This fundamental characteristic of the cyclic molecules appears to be an important factor in determining the biological role of the various agents which have such cycles in their molecules.

In the case of steroids, this attribute acquires special importance. An understanding of the different biological activities of steroids can be obtained by an analysis of the forces resulting from this characteristic composition. Besides the energetic centers or formations attached to it, two energetic centers appear as part of the steroid nucleus itself. One is at C_3 and the other center is represented by the cyclopentanic group. The fact that these centers are maintained in fixed relative position through the rigidity of this polycyclic nucleus has resulted in an important property of the nucleus itself which becomes translated into a dipolarity of the molecule. The study of these two energetic centers has advanced our knowledge of the role of steroids.

The study of the polar groups bound to C_3 of the polycycle skeleton of

steroids has permitted us to recognize the conditions which induce stronger activity for these polar groups, conditions which are usually fulfilled in the naturally occurring members. It could thus be seen that the reactivity of an oxygen bound to C_3 is increased if another double bond present in the cycle is parallel to the double bond through which the oxygen is bound to C_3. A double bond between C_4 and C_5, as shown in Figure 64 (a), fulfills

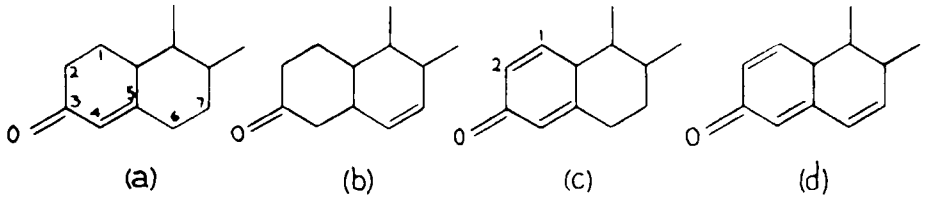


FIG. 64. Influence exerted upon the oxygen bond at C_3 by the position of the *double bond* in the cycles 1 and 2 of the cyclopentanephenanthrene molecule. A parallelism between the double bond of oxygen and that present between C_4 and C_5 increases the energetic character of the carbonyl (a). A similar influence but less active, is exerted by the double bond between C_6 and C_7. A double bond added between C_1 and C_2 (c) increases the activity. Still more activity would result from a third double bond added between C_6 and C_7 (d).

such a condition. A similar influence is exerted indirectly by a double bond between C_6 and C_7 (b) which, through induction, will influence the parallel C_4 and C_5 bond and further the double bond of the oxygen. This explains the influence exerted by the double bond present between C_1 and C_2 (c), as in the synthetic, prednisolone. Further enhancement of reactivity would be obtained with a third double bond added between C_6 and C_7. The parallelism between three double bonds (d) would produce an increased reactivity.

For the hydroxyl, a similar enhanced reactivity is induced by double bonding of the carbon to which the hydroxyl is attached with a double bond for the $C_3 - C_4$ or $C_2 - C_3$, as shown in Figure 65 (a and b). A similar condition is fulfilled if a double bond is present in the molecule parallel to any of these bonds, as seen in Figure 65 (c) where the double

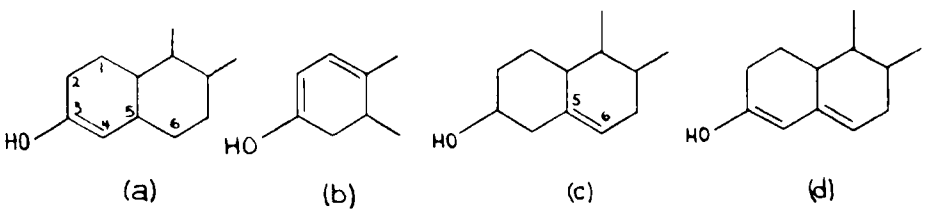


FIG. 65. The influence exerted upon the hydroxyl bond at C_3 by a double bond in the cycle 1 and 2 of the phenanthrene is increased if the double bond is adjacent or parallel to the bonds of C_3, bearing the hydroxyl.

bond is between C_5 and C_6. An enhanced reactivity of these compounds would be obtained with one double bond between C_3 and C_4 and another between C_5 and C_6 (d).

We will come back to this important intervention of the double bonds in cyclic molecules.

The energetic property of the cyclopentane group appears to be correlated with its odd number of carbons. The alternate succession of carbons with positive and negative characters resulting from the induction effect causes two carbons of this cycle to have the same sign. This "twin formation" induces a special molecular reactivity related to the pentanic cycle of the steroid molecule. *(Note 9)*

The special reactivity seen for C_3 of the cyclopentanophenanthrene molecule can be explained through a hypothesis covering the origin of these substances. Although the origin of a cholesterol molecule through a cyclization of squalene (35) appears plausible, this seems less probable for the corticoids. We have tried to connect their origin to arachidonic acid.

Several considerations such as the high levels of arachidonic acid and corticoids in the adrenals, and the reduction of the former when an important amount of the latter is excreted *(Note 10)*, seem to establish a correlation between these substances. According to our hypothesis, the steroids with a two carbon chain at C_{17}, as seen present in the corticoids and luteoids, would result from a cyclization of the arachidonic molecule. *(Note 11)* This would explain the special reactivity of C_3, which would correspond to C_9 of the arachidonic acid bound in this molecule by a double bond.

A study of the different steroids under this energetic aspect has permitted us to understand their physiologic properties.

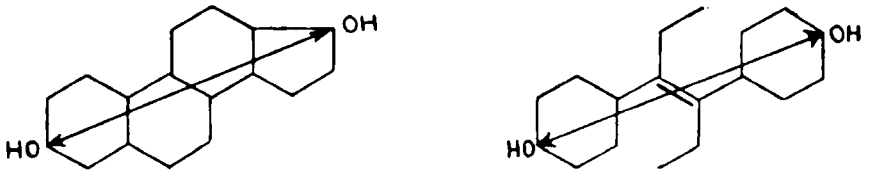
With the C_3 having a hydroxyl or an oxygen as polar group in almost all the steroids, the variety of the biological properties would be related to the different conditions at the other extremity of the molecule, principally at C_{17}, which result from the special energetic conditions prevalent at this region of the molecule. The simplest steroids are those having a polar group represented by an OH or O fixed at C_{17}. Such naturally occurring steroids have properties related to secondary sex characteristics. We will discuss them briefly here.

Sex Hormones

This group of steroids has two polar groups, one at C_3 and one at C_{17}. The energetic center at C_3 can have negative or positive polar characters, according to the presence of oxygen or hydroxyl. The energetic center at

C_{17} also can have an oxygen or hydroxyl group and thus be negative or positive. An important factor for the properties of the substance is the relationship between the two polar groups in the same molecule. It is apparent that the polarity of the molecule will vary according to what polar groups are present at C_3 and C_{17}. In a very simplified concept, which we consider only partially accurate, we have tried to associate female and male hormonal characteristics with this polarity.

In the simple steroid molecules, a folliculinoid or estrogenic biological property seems to be conferred if the two polar groups—at C_3 and C_{17}—are formed by hydroxyls. The molecule appears to have a dipositive polarity. It seems to be important that the two hydroxyls be kept in the relatively fixed reciprocal positions—corresponding to C_3 and C_{17}—as part of the solid skeleton of the steroids. Estrogenic properties are present in various steroids that fulfill this condition. Furthermore, substances far removed from the steroids have folliculinoid properties if they have this relationship between the two hydroxyls. As shown in Figure 66 diethylstilbestrol, which has its two hydroxyls maintained in a fixed relative position similar to that of steroid estrogens, also shows potent estrogenic activity.

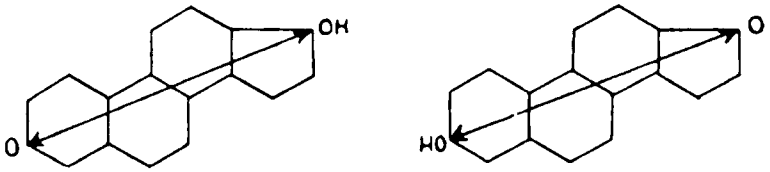


Estradiol Diethylstilbestrol

FIG. 66. *The folliculinoid* activity appears to be related to the existence of two hydroxyls kept at the same relative position, as it appears in estradiol and in diethylstilbestrol.

In the same way, we tried to correlate testoid activity with the presence of positive-negative polarity; that is, with two polar centers energetically different, one corresponding to an oxygen and the other to a hydroxyl, maintained in the same fixed relative position. The importance of this relative steric position of the two polar groups for testoid activity becomes evident when it is found in substances other than testosterone, the principal male hormone, with an oxygen at C_3 and a hydroxyl at C_{17}. Testoid activity is present in androsterone, which has an oxygen and an hydroxyl maintained in the same reciprocal positions, although here the oxygen is at C_{17}

and the hydroxyl at C_3, the reverse of testosterone. In both substances, testosterone and androsterone, the two conditions for testoid activity, positive-negative polarity and the same relative position between the polar groups, are fulfilled. *(Fig. 67)* The differences which exist between these substances in their specific hormonal activity can be explained through the different influence exerted upon the two polar groups in these substances by the rest of the molecules.



Testosterone Androsterone

FIG. 67. The *testoid* activity seems to be related to the presence of a hydroxyl and a carbonyl in the same fixed relative position which is insured by the rigidity of the steroid molecule. The same positional reciprocal relationship is seen to exist between these two polar groups in testosterone and androsterone.

The testoid activity seen for cortisone, hydrocortisone and other hormones also can be explained by the presence in these molecules of oxygen and hydroxyl at C_3 and C_{17}, and maintenance of the fixed position between these two polar groups.

Conceptually, the antagonism between estrogenic and testoid biological activities can be considered to be ultimately related to the differences in polarity, which in one form or another can be found in other factors differing for the sexes. We will mention here only that a similar difference between male and female character is seen in the sexual chromosomes, where the female character is related to the XX chromosome, and the male to an X and a Y chromosome. As we will see below, a relationship exists between lipids in general and sex.

Besides the sex hormones, fatty acids appear to be connected with male sex characteristics, while female characteristics are related to another group of steroids, the sterols.

Sterols

Characteristic of the structure of this group of steroids is the presence of a hydroxyl at C_3 and a long chain bond at C_{17}. Through the hydroxyl,

the center at C_3 has a nucleophilic character. This is reinforced by the presence of a double bond between C_5 and C_6 which, by paralleling the bond between C_3 and C_4, increases its ionic character and consequently the reactivity of the hydroxyl bond to C_3. Through this hydroxyl, sterols combine in general with substances having a negative polar group to form esters.

Besides the capacity to combine with fatty acids in general, one of the most important characteristics of the principal sterol of animals, cholesterol, is its selective affinity for certain fatty acid members, the polyunsaturated. We tried to explain the specificity of this bond through an interesting process which could be called "steric coupling."

In this process, two molecules, usually lipoids, are kept together not only by the combination of their polar groups but also through a bond between their nonpolar parts. The two molecules are reciprocally attracted through the multiple forces present in the nonpolar groups. Some are related to attached centers, while some, such as those corresponding to cohesion forces, are related to the rings themselves. An important factor is the rigidity of the sterol molecule which permits another molecule, if it is flexible, to make the steric coupling. The rigid skeleton not only keeps the energetic centers of one molecule in a fixed position but permits the flexible aliphatic chain to cover over the polycyclic molecule and thus bring the energetic centers of one molecule in contact with those of the other. Through this, steric coupling completes the bonding of the polar groups. The greater the concordance between energetic centers in both molecules, the more perfect the coupling is, for the more complete is the reciprocal neutralization of the energetic centers of the two molecules. Steric coupling explains why, of all the fatty acids present in the organism, cholesterol seems to prefer to bind those with polyunsaturated chains. It is these fatty acids which have several energetic centers in the nonpolar group as represented by double bonds. The long chains of these fatty acid molecules, having a certain degree of flexibility, will then complete the steric coupling. *(Note 12)*

Steric coupling, in addition to its general importance in biology, where it represents a kind of molecular reactivity, seems to explain the antagonistic influence exercised by different constituents, especially the sterols and polyunsaturated fatty acids. Through steric coupling, cholesterol could influence the activity of these fatty acids more directly related to the nonpolar group. It has to be emphasized, however, that the neutralization resulting from steric coupling is not irreversible. On the contrary, through the intervention of various factors, such as the breaking down of the bond

between the polar groups, the two coupled molecules can regain their independence. This would explain the relative lability of the combinations between fatty acids and sterols. The antagonism between fatty acids and sterols is an important aspect of biological dualism which will be discussed in more detail later when these substances are studied in terms of their influence at the different levels of organization.

Steroids with a Two-Carbon Chain

Among the most important steroids are those having a two-carbon chain fixed at C_{17}.

Two groups, the luteoids and corticoids, appear directly related to allopregnane hydrocarbon, the steroid polycycle with a two-carbon lateral chain fixed at C_{17}. As we have already seen in the hypothesis concerning the origin of the steroids *(Note 11)*, this hydrocarbon could have been directly derived from arachidonic acid, the two-carbon lateral chain corresponding to the tail chain of this acid, a tail which remains after cyclization.

The Luteoids

The prototype of the luteoids is progesterone. Two polar groups C = O are present, one at C_3 of the polycycle and the other at C_{20} of the tail chain. A parallel double bond between C_4 and C_5 completes the formula. Energetically, progesterone presents a first center at C_3 which appears strongly nucleophilic for two reasons: first, because it corresponds to the potent electronegative C_3 and second, because it is reinforced by a double bond present between C_4 and C_5, and which is hence parallel with the double bond of the carbonyl. The second = O is attached to the C_{20} of the tail chain. This also appears reinforced, the double bond of this carbonyl being parallel to the bond between the C_{13} and C_{17}, which in the cyclopentane, according to the hypothesis of twin carbons, binds two negative charged carbons. Through its constitution progesterone is also a lipoid, the complex hydrocarbon group being predominant over the polar groups. With its polar nucleophilic centers, progesterone has the fundamental character of acid lipoids. Progesterone's luteoid activity corresponds to the presence of two relatively strong neutrophilic centers kept in the characteristic positions, one at C_3 and the other at C_{20}.

We can see that any disturbance in this energetic picture, any change from the dinucleophilic at any center, decreases the luteoid properties of the substance. With more profound changes, the luteoid activity is even suppressed. *(Note 13)*

Corticoids

The corticoids represent the group of hormones upon which the attention of scientists recently has been intensively focused because of their new therapeutic applications.

Chemically, they appear to be the same as luteoids, derivatives of the same parent hydrocarbon, allopregnane. Structurally, all these adrenocorticoid hormones have: a) a C_3 binding an O group; b) a double bond between C_4 and C_5 in the first cycle; c) a two-carbon tail chain with an O attached in ketone form to C_{20}; d) an OH as primary alcohol present at C_{21}. This structure, common to all corticoids, seems to be responsible for the principal properties of these substances. Corticoids have been separated into subgroups based upon the presence of attached groups OH or = O at C_{11} or OH at C_{17}. The presence or absence of attached radicals at C_{11} appears to be most important. Corticoids without attached radicals at the C_{11} have a major influence on the metabolism of electrolytes. The second group of corticoids, having the radical, are known as neoglucogenic corticoids, the name indicating their principal biological characteristics.

Energetically, the corticoids present a nucleophilic center at C_3, reinforced by the presence of the double bond in the cycle between C_4 and C_5. The double bond is parallel to the double bond of the carboxyl, and thus inductively increases the ionic character of the latter.

A second energetic group of the tail chain appears in toto as a strong tripolar center with a nucleophilic center at C_{20} of this chain and an electrophilic center at C_{21}. *(Note 14)* To this basic pattern is added, in the neoglucogenic corticoid, a separate energetic center at C_{11}, which can be either electrophilic, formed by a hydroxyl, or nucleophilic, formed by an oxygen.

Corticoids appear, in general, to act as positive lipoids. *(Note 15)*

Because of their importance in relation to anti-fatty acid activity, we will discuss first the neoglucogenic corticoids, the members with a polar group also at C_{11}. According to our hypothesis, these steroids have a special biological activity, a role in the process of synthesis in the organism. The part of the molecule between C_{11} and C_{21} constitutes an energetic formation with a peculiar property. It represents a kind of energetic mold or template, in which each carbon has its specific energetic character. Different radicals would be attracted by the energetic centers of this template formation according to their own energetic nature. Kept in their respective positions, they would be induced to bind together in order to form new substances. In this manner this template formation would promote new

syntheses. In different corticoids the constitution of the $C_{11} = C_{21}$ formation will differ and this will determine which substance is to be synthesized by the respective mold or template formation. *(Note 16)*

Using the template hypothesis, we studied an entire series of body constituents forming the "gluco group." Glucose, galactose, glucosamine and galactosamine, with their respective acids, as well as ascorbic acid, are among theses substances. According to our hypothesis, these neoglucogenic corticoids would have the important role of producing, possibly along with other mechanisms, the entire series of "gluco" constituents. The existence of different template formations would result in a variety of synthesized constituents.

The intervention of the template formation in synthesis can occur again and again without affecting the molecule of the corticoid as such. It is interesting to note here a structural curiosity which could be interpreted as being related to template activity. In this template, the group of successive C_{11}, C_{12}, C_{13} and C_{17} are part of the rigid skeleton of the cyclic molecule, while C_{20} and C_{21} are forming the lateral chain attached to C_{17}. This can be regarded as conferring a certain proper mobility to this lateral chain as related to the polycycle. It is conceivable that this lateral chain would become a closed formation when synthesis takes place. A movement of the chain at C_{17} would permit the mold to open and thus liberate the synthesized molecule. It is interesting to note here the importance of the structure of the template for the constitution of the substances synthesized. Besides the polar group at C_{17}, that at C_{11} is also important for neoglucogenic activity since it insures a six-carbon chain in the synthesized molecules. A hydroxyl or carboxyl at the C_6 of the synthesized substance will appear, according to the nature of the polar group at C_{11} of the steroid. The respective characters and positions of C_{21} and C_{12} will permit the appearance of a cycle formed by five carbons and an oxygen, characteristic for the pyranic form of newly synthesized substances.

An interesting confirmation of the template hypothesis was obtained when glucosamine which, according to the hypothesis, is synthesized by the cortisone molecule, was found to induce in patients many of the clinical changes which are obtained by treatment with cortisone. We will consider these results later in our discussion of therapy. The capital role played by glucosamine, galactosamine and the respective uronic acids in the constitution of the connective tissue represents the "missing link" for the explanation of the relationship between cortisone, the other neoglucogenic corticoids, and this tissue. Some part of the therapeutic effect obtained

with these neoglucogenic corticoids in diseases of the connective tissue has to be attributed to the intervention of the amino sugars.

In the study of anti-fatty acid activity, glycerol and glycerophosphoric ion were found to control the absorption and circulation of saturated mono-, di-, or tri-unsaturated free fatty acids. The sterols appear to counterbalance the normal polyunsaturated members while adrenal corticoids, and especially the neoglucogenic corticoids, counteract the toxicity of fatty acids in general and of the abnormal conjugated members in particular. Research done in our laboratories by E. F. Taskier indicates that the adrenals intervene in the defense mechanism against fatty acids, and especially against the conjugated members which appear to be related to abnormal conditions. *(Note 17)*

The part of our research concerned with the role of lipoids in normal and abnormal physiology has been almost entirely guided by the concept of an antagonism between the two groups, one with a positive and the other with a negative polar character. This specific aspect has led us to study, together with the fatty acids and the anti-fatty acids, other substances related to this antagonism. In the group of lipoacids or acidic lipoids, as obtained from tissues, organs or organisms, we recognized the group of porphyrinic acids, related to various hemes present in the organisms. In the group of anti-fatty acids obtained from the same sources, different constituents form the insaponifiable fractions.

As related to this dualistic aspect we have studied another group of substances, which appear to act in the organism against the anti-fatty acids themselves. These other substances would represent a kind of biological brake to counteract an exaggerated intervention of anti-fatty acid constituents.

We have made a special investigation of two substances of this group, glucuronic and sulfuric acid anions, which characteristically seem to oppose certain anti-fatty acid substances. These substances appear as a result of an exaggerated oxidation of normal metabolites. Under abnormal conditions, the oxygen resulting from the intervention of peroxide may be fixed to carbohydrates even before they have undergone the preliminary fermentative transformations seen in normal metabolism. With the aldehyde group bound to phosphoric acid, the oxidation takes place at C_6, the second most reactive carbon in the molecule. This direct oxidation would represent, according to our view, one of the sources of glucuronic acid. Similarly the sulfuric anion would result from the oxidation of sulfur present in the organism. They correspond to the oxygen phase of offbalance D.

Glucuronic and Sulfuric Anions

Urine specimens that contain abnormal oxidizing substances show significant amounts of glucuronic and sulfuric acid compounds. *(Note 18)*

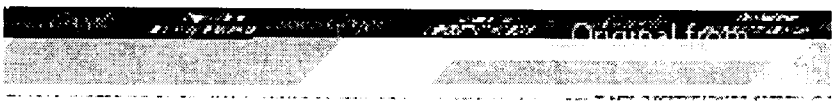
The analysis of the conditions under which these two substances exert anti-toxic activity permits a better understanding of their role in general biology. A certain parallelism exists, and has always been emphasized, between a detoxifying and an eliminating function exerted by these two radicals. Not only do sulfo- and glucurono-derivatives appear in the urine, but it often has been noted that glucuronic acid intervenes when large amounts of certain substances, such as menthol and phenol, are present and there are insufficient sulfuric acid radicals to insure detoxification and elimination. When mineral sulfates are administered, the proportion of sulfo-derivatives increases.

This parellelism appears especially interesting when we recognize that sulfuric acid represents the end result of the oxidation of sulfur introduced into the organism in combinations in which it is a bivalent negative element. Only a smaller amount of sulfur is introduced as a hexavalent positive element: that is, as sulfate. Sulfur is introduced mostly in bivalent negative form, as in methionine, cystine, etc. Both sulfuric and glucuronic acid result from oxidative processes, acting in one case upon the thiol group and in the other upon glucose.

The relationship between sulfuric and glucuronic acid goes still further. It has been noted that glucuronic acid appears when enough sulfuric radicals necessary for detoxifying action are not available. However, this is not entirely true since one process does not duplicate the other. Qualitative differences intervene. *(Note 19)*

The significance of glucuronic acid in the defense mechanism seems clearer when we recognize that, with but few exceptions such as benzoic acid, all the substances with which glucuronic acid combines are lipids or lipoids having one or more *positive* polar groups. The combination with glucuronic acid takes place through these positive polar groups. *(Note 20)*

In our view, glucuronic acid like sulfuric acid, has a specific role in the defense of the organism and this seems to be directed especially against lipids or lipoids with a positive polar group. Bound by glucuronic acids, the latter are eliminated as excremental substances. Glucuronic acid thus would act against many anti-fatty acid agents. We can conceive of sulfuric and glucuronic acids as means by which organisms are protected against an exaggerated activity of anti-fatty acid agents. Along the same lines, when lipoids with positive polar groups are predominant and able to act



in an exaggerated manner to oppose the fatty acids physically and chemically, the same means can be utilized to reduce this exaggeration. Thus, the intervention of glucuronic acid as a result of an abnormal oxidation of glucose induced by fatty acids appears to be biologically sound. This is also true for the sulfuric radical.

The importance of these substances does not reside only in the fact that the organism can easily produce them in larger quantities than fatty acids. The fact that they combine to form excremental substances is important too, for in this way, they help in materially eliminating the anti-fatty acid substances from the organism. This would not take place if only a combination with fatty acids were possible, since the esters of fatty acids are usually retained in the organism and, under certain circumstances, can again liberate their constituents. The intervention of glucuronic acid and sulfuric acid appears to be more effective than that of the fatty acids which have their own activity and are more toxic in exaggerated amounts. This appears to be especially true in the case of glucuronic acid because the amount of glucose available is practically unlimited as compared with other metabolites. Glucuronic and sulfuric acid would thus intervene in the biological antagonism between fatty acids and anti-fatty acid substances, inactivating and eliminating agents from the last group, especially when in excess. Teleologically speaking, their intervention appears to be still more interesting since the body has, as part of its defense mechanism, a tendency to manufacture anti-fatty acids in excess. The intervention of agents other than fatty acids would prevent a vicious circle and permit an excess of anti-fatty acids to be removed by excretion.

FATTY ACIDS VS ANTI-FATTY ACIDS

This study of the relationship between fatty acids and anti-fatty acids has been guided by the dualistic concept. It must be recognized, however, that the direct activity of these substances could be largely reduced to that of one group, the fatty acids. The action of anti-fatty acids is largely indirect. They control and thus limit the activity of the fatty acids. It is within this framework that the different anti-fatty acids selectively influence different specific functions of the free fatty acids.

The lipids and associated constituents with their multiple activities create for each entity a balance responsible for many of the manifestations of the entity. Variations in manifestations can be attributed in large part to qualitative and quantitative variations in the intervening lipids. A sys-

tematization of these variations would help us understand many of the processes encountered in normal and abnormal physiology.

As we have mentioned before, the balance between two antagonistic forces, especially for normal conditions, is not static. Instead, there is alternating predominance of the forces, which results in an oscillatory movement. Several groups of such coupled forces, each group with its proper rhythm, are at work. Operating simultaneously, they make for a series of very complicated variations. Yet analysis is possible since each of the variations follows a dualistic pattern. The variations, as they occur at different levels and with different intensities, have been identified through various tests. In a second step they have been tentatively correlated with changes in lipids. And next, didactically, lipid changes have been related to various etiologic factors, some intrinsic and some extrinsic.

Sex

The influence exerted by the sex of the organism upon lipidic balance was brought to our attention by a curious effect seen when cholesterol was administered in an ether-oil solution to rats. Only the females showed paraplegia and ulcerations of the hind legs. While castration or administration of sex hormones did not alter this response, it was influenced by the administration of two groups of lipids. The insaponifiable fraction of human placenta, for instance, was seen to induce a high sensitivity to this preparation of cholesterol even for males, while the acid lipidic fraction of placenta prevented paraplegia in females. *(Note 21)*

Similarly, the fact that in females alone, adipous cells appeared quickly in the skin of the ear after the application of sulfur mustard could be related to the intervention of the insaponifiable fractions. *(Note 22)*

Starting with these observations, it could be seen that, in general, a higher proportion of positive lipids exists in females than in males. This could be shown by direct analyses and by analyses of manifestations related to such lipids. While many differences are to be seen in various manifestations between females and males, only some could be related to the direct or indirect intervention of sex hormones. In such instances, castration with or without the administration of sex hormones was able to change, and even to reverse, the differences in manifestations seen between sexes. However, in instances in which these measures were without effect, the differences could be related to the intervention of lipids.

Age

The changes in lipidic balance related to age have been made the object of an extensive study which also sought to determine the role of lipids in aging processes. A general predominance of positive lipids, more manifest in the cellular and tissue levels than in the blood, was seen in youth. This would be expected in view of the special metabolic influence exerted by this group of lipids. The anoxybiotic character of metabolism induced by sterols results in the intervention of dehydrogenases which lead to an abundance of hydrogen ions. This, in turn, leads to a predominance of the kind of syntheses which favor anabolism. Growth thus could be related to the predominance of lipids with positive polar groups, especially sterols.

Aging processes, on the contrary, could be related to a predominance of lipids with negative polar groups, especially fatty acids. This predominance could be found especially at the cellular level, as seen in cultures of tetrahymena. *(Note 23)* In complex organisms or in rats *(Note 24)* in which an increase in the proportion of fatty acids at the cellular level is present, an opposite change occurs at the systemic and even at the organic level. There is an excess of cholesterol, this time limited to the higher levels, as revealed through analyses of the blood, for instance. Changes in the blood vessels are related in part to this excess of sterols at the systemic level. Many manifestations have confirmed such an offbalance with sterol predominance at higher levels. For example, we found the urine surface tension abnormally high in old age. *(Note 25)* Similarly, skin wheal absorption in old people requires more than 90 minutes for completion as against approximately 20 minutes in middle-aged adults. A predominance of fatty acids at lower levels and of sterols at higher levels would thus characterize the changes in lipidic balance related to old age. *(Fig. 68)*

Other Physiological Factors

The study of the role of lipids in various physiological functions was made indirectly for the most part, using the tests previously mentioned which were interpreted in terms of dualistic patterns. These were related to the general offbalances A and D and, through them, attributed ultimately to a predominance of sterols or fatty acids.

Sleep in itself, without relation to night or day, was found to induce a marked change, comparable to a type A offbalance with predominance of sterols. Subjects with pain of an acid pattern often correlate the appearance of pain with sleep, the pain occurring uniformly at the moment they wake up. In these cases, the urine shows a low specific gravity with

a high pH and a high surface tension, corresponding to an A type offbalance. As we will see, in subjects with an intensive A type offbalance, nocturnal polyuria and pollakiuria occur.

Sexual intercourse in males was seen to induce, in analyses, transitory changes similar to an offbalance of type D, corresponding to a predominance of fatty acids. In females the change corresponds to a transitory offbalance of type A, manifested by changes at the systemic level. *Muscular exercise* was seen to induce, in a first phase during the exercise itself,

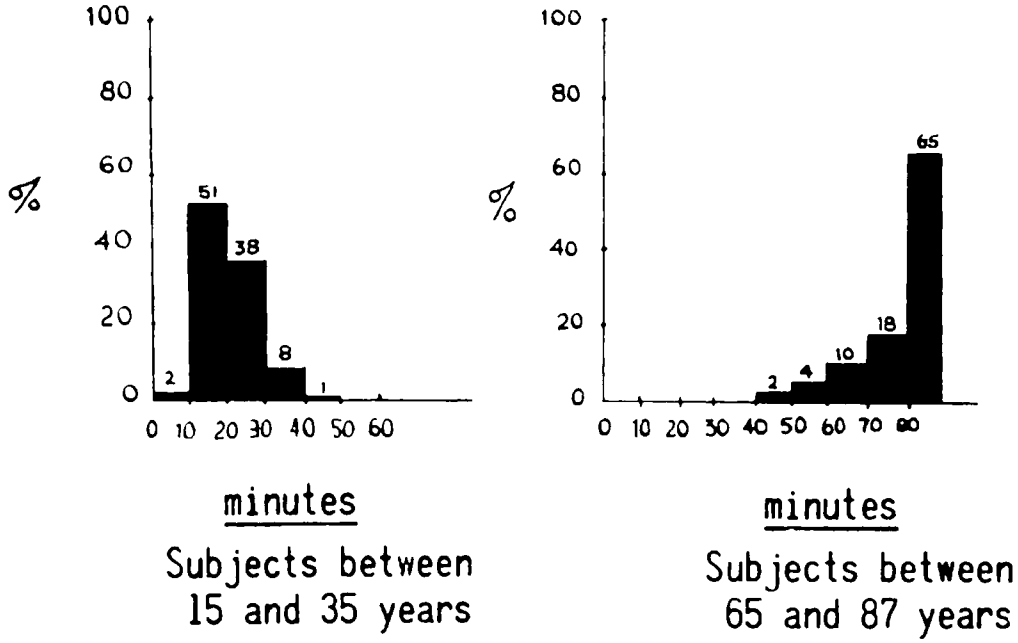


FIG. 68. The disappearance time for the wheal induced by the intradermic injection of 0.2 cc saline, varies with the age. In old age, the wheal often persists for more than 90 minutes.

changes comparable to an offbalance type D. This phase is followed by a much longer phase of type A, indicating sterol predominance. *Intensive mental exercise* produces a marked change similar to offbalance A, with all urinary tests showing the patterns found with predominance of sterols.

The responses attributed to influences exerted by *external factors* could be integrated in the same dualistic mechanism. All the data indicate the manifest influence exerted by the time of day. Two marked changes are seen, one around four o'clock in the morning and the other usually around eight or nine o'clock in the evening. The morning change corresponds to a predominance of sterols, the evening to predominance of fatty acids. These changes together with the clinical manifestations related to time of day

appear in a new light when interpreted not as being the direct results of time changes but rather of patterns of diurnal activity and nocturnal rest. This explains why in rats and mice, which are nocturnal animals, most of the analyses show variations related to the time of day opposite to those in humans. Other variations could be recognized more strongly related to time of day. Variations with a 24-hour rhythm could be seen, for instance, for urinary surface tension in mice. But, when rats and mice were maintained for a length of time under artificial conditions, with light during the night and dark during the day, the animals changed their habits, becoming active during the day and sleeping during the night. After a certain time, most of their analytical patterns such as urinary pH, blood leucocytes, eosinophiles etc. changed, acquiring the type of variation seen in humans. Urinary surface tension remained unchanged for a long time. *(Note 8 Chapter IV)* Even more interesting were other changes which could be related to changes in external temperature. The urinary surface tension measured in rats in the morning for long periods of time showed variations related to changes in the temperature of the environment. *(Note 26) (Fig. 69)*

The importance of temperature led to its more detailed investigation. Variations in lipidic balance have been found to parallel variations in body temperature. The blood of normal individuals is richer in sterols than the blood of those with hypothermia. Furthermore, in moments of high temperature, more sterols are found than in moments of low or normal temperatures. An increase of fatty acids occurs in conditions with hypothermia. These changes were confirmed also by the correlation between blood content in lipids and temperature in different abnormal conditions. In shock with hypothermia the blood is rich in fatty acids, while in infections with fever, it is rich in sterols.

The role of *temperature* was also investigated by studying the influence upon the lipidic balance by externally applied heat or cold. Characteristic variations could be seen in human analyses under the influence of hot and cold days, and of local applications of heat and cold. Manifestations corresponding to predominance of lipids with positive character were induced by heat, while others corresponding to predominance of lipids with negative character were induced by cold. Variations were seen in animals kept in an incubator or in a refrigerator. *(Note 27)* We will see below, by studying their influence at different levels, the importance of these variations produced by temperature.

The influence exerted by *barometric changes* could be seen in changes in total blood potassium, the two curves being parallel. Similar changes

could be observed related to the atmospheric humidity. Other tests as well, such as urinary pH, calcium excretion, etc., show a similar relationship but to a much lesser degree. *(Note 28)* The influence exerted by the environment could explain the changes seen from one day to the other in various analyses. *(Note 28)*

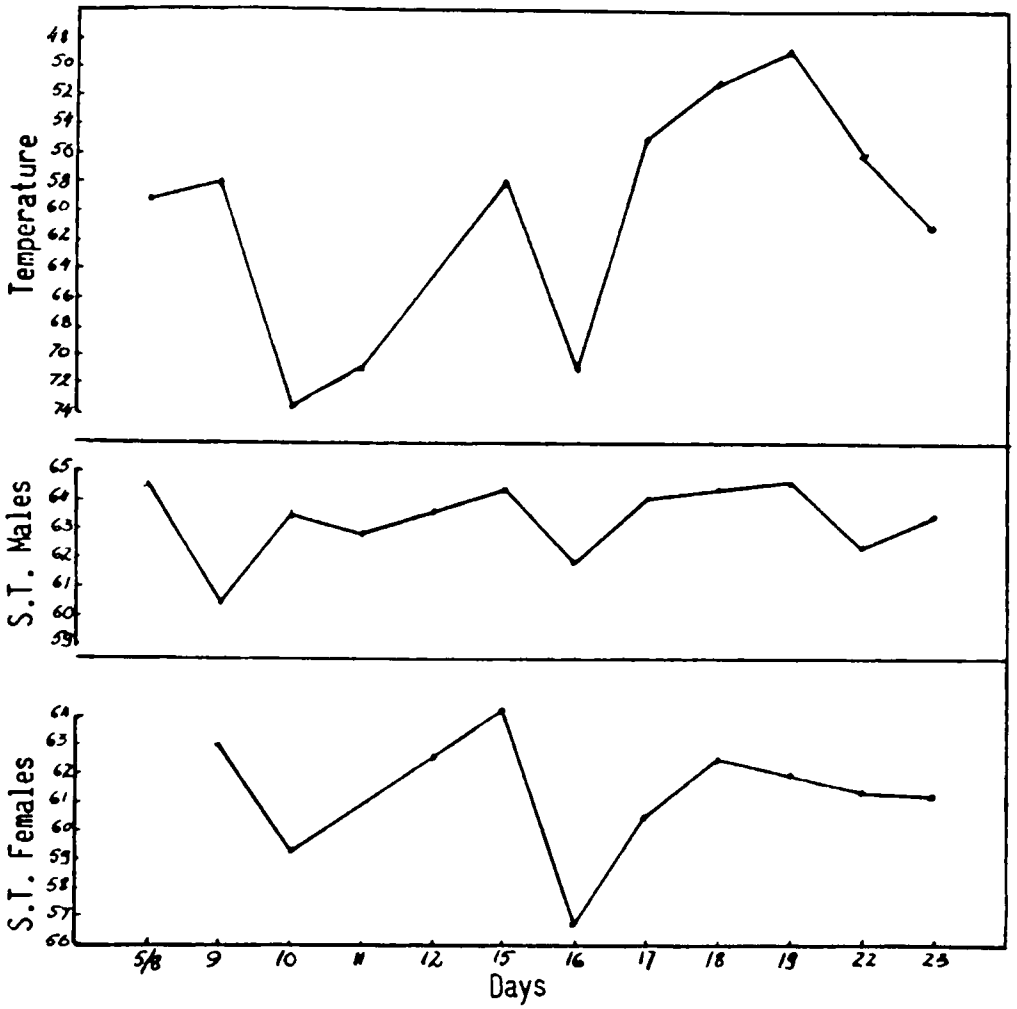


FIG. 69. The curves of the average values of surface tension of two groups of 20 male rats each, and two groups of female rats each, show parallel changes with the inverse curve of the temperature of the environment.

The influence exerted by changes in seasons was studied. An increase in fatty acids in winter and of sterols in summer could be noted. These increases also could be largely related to the seasonal variations in temperature. Very hot days were marked by analyses indicating intervention of lipids with a positive character. The influence exerted by the seasons was seen even in the responses of organisms to pathological conditions such as

tumors; variations in character and growth of experimental cancer could be noted. *(Note 29)* The relationship of many viral infectious diseases to seasonal changes which has been noted in many epidemiological studies could be related to the changes in lipids. *(Note 30)*

Effect of Antagonistic Lipids at Different Levels

A more complete study of lipids under the dualistic concept was made by considering their activity at different levels of hierarchic organization. This research was greatly facilitated by the degree of individuality which different biological levels exhibit when they are part of the hierarchic organization of complex organisms. It was also aided by the availability of lower organisms in nature which correspond to various hierarchic levels. Through this double approach, the information obtained showed the importance of the relationship between the levels of the complex organism and the influence exerted by lipoids. If high doses of the agents are applied, the influence is exerted upon all the levels. A preferential influence is exerted upon a single level if reduced doses are used. When medium size doses are administered, to the preferential effect upon one level a reactional response at other levels is added. This results often in concomitant opposite effect at these levels.

Effects of Lipoids on Viruses

The antagonistic effects of positive and negative lipids were evident in the study of their action upon viruses. Generally, agents with a positive polar group appeared to create favorable conditions for the development of viruses, while those with a negative polar group had an opposite effect. This influence, which was first seen in phages in vitro, became still more evident in viral infections.

Subcutaneous administration of positive lipids, such as sterols or insaponifiable fractions of organs, induced greater local receptivity to viruses. In experiments with smallpox virus in rabbits, for instance, virus inoculation of the skin induced an exaggerated response in those areas where positive lipids previously had been injected subcutaneously compared with the response in other previously untreated areas. In less sensitive species such as mice and rats, positive lipid injections induced abnormally high local receptivity to virus inoculation. Intracerebral injection of sterols followed by subcutaneous inoculation with smallpox virus invariably produced nervous system localization of the virus. Intraperitoneal administration of sterols in very high doses in mice prior to smallpox inoculation produced a great degree of central nervous system localization. Intra-

cerebral virus inoculation, after subcutaneous administration of high doses of anti-fatty acids, brought death earlier in test animals than in controls given intracerebral virus alone.

A striking opposite effect was noted for lipids with a negative polar character. In rabbits, subcutaneous injection of a polyunsaturated fatty acid set up a local skin area refractory to smallpox virus inoculation, although inoculation was positive in other areas of the body. Death also occurred later, following intracerebral inoculation with a neurotropic virus in test animals given subcutaneous or intraperitoneal injections of fatty acids, than in controls. This partially protective effect was opposite to the increased receptivity seen in animals injected subcutaneously or intraperitoneally with insaponifiable fractions and intracerebrally with the same virus, where death appeared earlier than in controls.

The antagonistic effects of the two groups of lipids for viral infection appeared interesting from several points of view. The effects were local, at the cellular level, where viruses themselves act. Subcutaneous injection of lipids induced manifest changes in response toward the virus in the skin at the site of injection, and little or no change at all elsewhere. We have utilized this fact, as we will see below, to obtain information regarding the level at which various agents act. A change induced in receptivity to viruses, limited to the skin at the site of injection, would indicate activity of the agent at the cellular level. Tests based upon the skin response to smallpox virus infection have shown that, among the lipids with a negative polar character, a maximum of influence is exerted by the insaponifiable fraction of organs of exodermic origin from species sensitive to the virus. The insaponifiable fractions of rabbit skin and rabbit brain were the most active of the lipids tested. Among the fatty acids, the preventive effect was seen to increase with the degree of desaturation. It was almost entirely absent in saturated fatty acids, notably present in polyunsaturated fatty acids.

The increase and decrease in receptivity of the skin to smallpox virus following injection of lipids also furnished information about the roles of the polar and nonpolar parts of lipids in this specific activity. An opposite effect was seen between two groups of substances having the same nonpolar group but differing in their polar groups. While the polyunsaturated fatty acids of safflower oil, for instance, greatly reduced receptivity, the same polyunsaturated members having alcohols as polar groups increased receptivity. The polar group—negative or positive—appears to be the factor inducing the opposite effect.

The role of the nonpolar group was studied by comparing saturated

and unsaturated acids and alcohols. Almost no activity was seen for the saturated. The unsaturated members were active in general, with activity in any direction increasing with the degree of desaturation of the nonpolar group. Thus, it appears that the nonpolar group determines whether a substance is active or inactive, but the nature of the activity—that is, increasing or decreasing receptivity—is determined by the polar group.

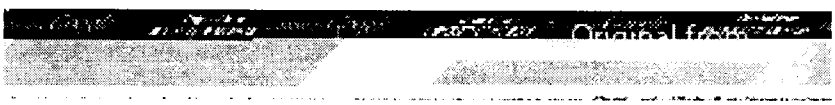
The influence exerted by agents with a positive character upon viral infection would explain the seasonal changes in clinical manifestations which are especially interesting for the paralytic form of poliomyelitis.

We could show experimentally that when mice, after being inoculated subcutaneously with smallpox vaccine virus, are kept in an incubator at 37°C, all develop cerebral involvement, while such involvement appears in only a small proportion of other animals kept at room temperature, and does not appear at all in those kept in a cool place. As we could also show that one of the effects of exposure of an animal to a higher temperature is an increase in the body of the amount of free lipids with positive character, this could explain the increase in the virus sensitivity of cells in the central nervous system which are especially sensitive to these lipids. This relationship would also explain the increased incidence of paralytic polio cases during hot weather.

The presence of greater amount of lipids with positive character in youth helps also to explain the frequency and intensity of viral infections in children. *(Note 31)*

The study of the effects of temperature and lipids upon viruses has shown that those effects are not limited to the host but also are exerted upon the viruses. *(Note 32)* The influence of heat and cold upon virus activity was studied in bacteriophages, where effects for virus and host could be separated. The direct influence upon the virus appeared relatively small and secondary to the changes which appear in the host itself. Bacteriophage, separated from microbes by filtration and kept in an incubator at temperatures 2-3 degrees C higher or lower than controls, showed no change in virulence. This was true as long as microbes were not present. Microbes kept at higher temperature were more sensitive to phages; when kept at lower temperature, they were less sensitive. This influence went so far as to change a sensitive strain to a refractory one, and vice-versa.

The fact that microbes grown at higher temperatures favor the development of bacteriophage while those grown at lower temperature hinder it could be correlated with the change in the richness of lipids in the microbes themselves. Similar results were obtained when microbes were grown for a time in media containing fatty acids or insaponifiable fractions and were



then removed and exposed to phages. These experiments *(Note 33)* indicate the direct role played by the lipids of the hosts in the activity of phages, and would explain the influence exerted by temperature. Through the change in the lipids of the host, the virus changes too, becoming more active if grown in microbes at a higher temperature and less aggressive if passed through microbes kept at lower temperature.

Effects of Lipids on Microbes

The antagonistic effects of the two groups of lipids upon microbes were investigated. As an example, we will mention here the characteristic changes in Bac. anthracis treated with polyunsaturated fatty acids and insaponifiable fraction preparations. *(Fig. 70)* We investigated the microbes for their morphological, tinctorial, cultural and virulence characteristics. With the fatty acids added to media, changes which can be considered to be mutational were induced, leading to tiny Gram negative microbes growing on agar as transparent small colonies. The changes, however, were reversible. Usually several passages in normal media were sufficient to produce reversal. First small and separate, then larger and more confluent Gram positive granules were seen to appear in the microbes which, themselves, also became progressively plumper. Ultimately, all the characteristics,—morphological, tinctorial and cultural—of the normal microbes reappeared. *(Fig. 71)*

Microbes showed opposite changes when treated with insaponifiable fractions, *(Fig. 70)* losing their bacillus form. Abnormally intensive Gram

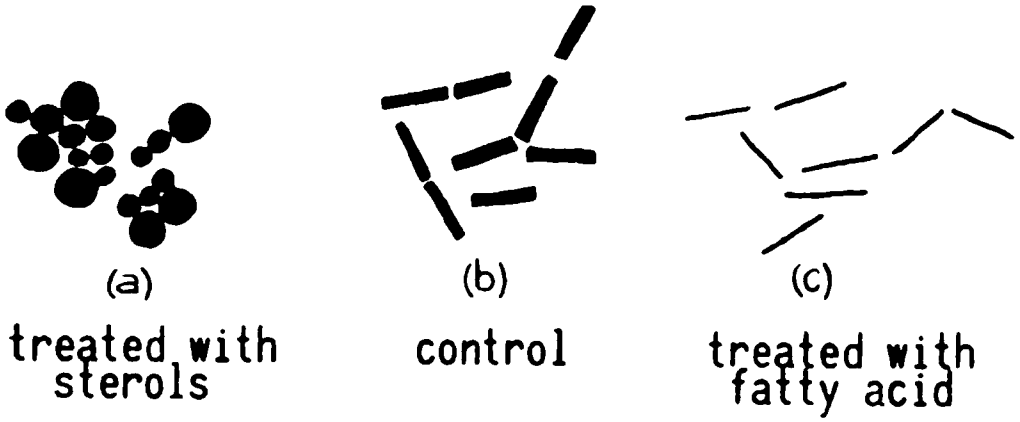


FIG. 70. *Influence of lipids upon microbes.* Schematic drawing of the changes induced in Bacillus Anthracis by the influence exerted by the two groups of lipids. Treated with sterols (a) as in the unsaponifiable fraction of placenta, the microbes change into cocci irregularly shaped and intensely retaining the gram stain. Treated with fatty acids (c) from cod liver oil, the bacilli change into very tiny gram negative microbes. (b) shows untreated microbes.

FIG. 71. *Lipids and microbes.* Drawing of the progressive passage toward normal bacilli of the tiny gram negative microbes obtained through the treatment of Bac. Anthracis with fatty acids. The passage takes place usually in successive steps. The gram positive formations appear first as fine granules; they later become clumps and finally give the microbes their normal aspect.

positive cocci appeared. They grew on agar as very thick creamy white colonies. These changes were seen to persist for a long time and seldom were spontaneously reversed. Treatment with fatty acids induced reversal although inconsistently. We attempted to correlate the differences in changes induced by different lipids to the different levels of the microbe at which they work. The change to cocci can be regarded as corresponding to an influence exerted upon the membrane and the change to Gram positive to an influence upon differentiated formations present in the body. (313)

Effects of Lipids on Protozoa

The effects of lipids upon monocellular organisms, especially tetrahymena pyriformis, were studied and an effort made to relate the nature of the main changes induced in these protozoa to changes observed at the cellular level of complex organisms. An initial effect was noted on the polarity in protozoa which seemed to be oppositely influenced by long chain polyunsaturated fatty acids and sterols. Lipids with a positive character were seen to induce a change in the form of protozoa causing them to become almost round, a change considered to correspond to reduced polarity. Lipids with a negative character had an opposite effect; the tetrahymena became abnormally elongated.

The administration of higher amounts of polyunsaturated fatty acids was seen to induce immediate changes localized at the anterior pole of the organism, changes which ultimately lead to the breakdown of the membrane particularly at this point. This effect parallels in intensity the degree of desaturation of the fatty acids. Other changes were seen in growth rate and survival time and, thus, in the aging process. *(Note 34) (Fig. 74)*

At the same time, resistance to heat was seen to increase as the result of treatment with negative lipids, while it decreased after treatment with

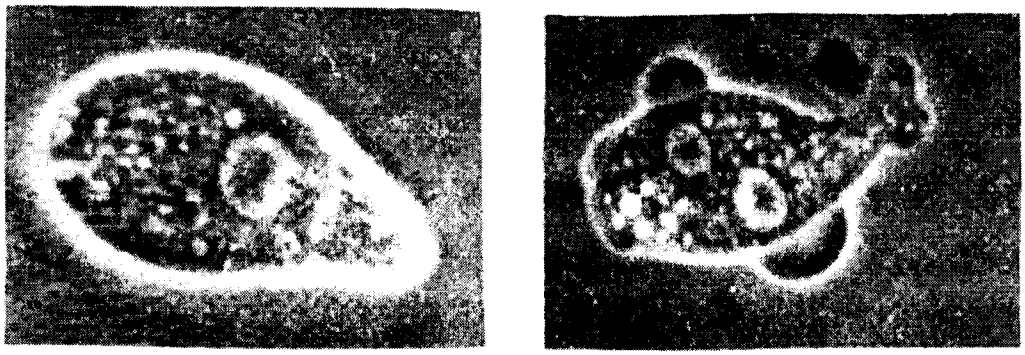


FIG. 74. In a direct action of fatty acids on tetrahymena, a passage of fluid occurs at the surface with a break of the membrane especially manifest at the anterior pole (a), control untreated (b), (1200x).

the positive sterols. *(Note 35)* The same influence upon the aging processes, as manifested in a prolongation of the life-span, was noted for polyunsaturated fatty acids with a long chain and even for some members of the saturated series but with a shorter chain.

Effects of Lipids on Complex Organisms

Morphological Changes—The same level separation was used in the study of the effects of lipids on complex organisms. Acting at chromosomal levels, lipids led to the appearance of monstrosities. Various lipids, especially insaponifiable fractions of organs, were injected into larvae of flies. While an immediate change in the cells of the larvae could be traced to the subnuclear level as seen in chromosomes, monstrosities were seen to be induced in the resulting flies. A similar effect became evident when lipids were injected into hens' eggs before or during incubation. Especially with cholesterol but also with insaponifiable fractions of organs, a high proportion of chickens were hatched with spastic paraplegia.

The same problem is being studied, in collaboration with P. Fluss, in Drosophila melanogaster, grown for many generations in media to which an entire series of different lipids from one or the other group are added. This study is in progress and the results will be published later.

We have seen that the antagonistic effects induced by the two groups of lipids could be related ultimately to opposite changes in the fundamental biological process of aging. This appeared clear for lower morphological levels of organization and was especially evident for cells. While anti-fatty

acids induce changes which can be regarded as corresponding to prolonged youth, the polyunsaturated fatty acids induce rapid aging with pyknosis and karyorrhexis and death of the cells as old entities. This could be seen clearly in tumors, in which cells with youthful character lead to non-necrotic tumoral masses, while cells that age rapidly produce necrosis in the tumors followed by ulceration if the tumor is superficial.

The effect of conjugated fatty acids was somewhat more complex, indicating an abnormality in the induced processes. Their administration was followed by the appearance of cytoplasmic and even nuclear vacuoles, corresponding ultimately to an anomaly of water metabolism.

The effect of lipids upon adipous cells appeared to be of special interest. The anti-fatty acids, especially the sterols, when injected subcutaneously in animals, induced a characteristic process in the adipous cells near the injection site. These cells became very enlarged and highly irregular, with their content changed into an emulsion only slightly stained with sudan. The fatty acids, on the contrary, imparted to adipous cells an abnormal resistance to destruction. They remained persistently unchanged even in the midst of very active processes which usually cause them to disappear. Unchanged adipous cells were found encircled by the invading cancerous cells, deep within tumors in animals treated with fatty acids.

On Pain—From the start of this research, the opposing effects of the two groups of lipids upon pain has been most impressive. For the fatty acids the degree of saturation is important. The saturated members of the fatty acid series and even oleic acid are entirely without effect. Linoleic and linolenic acids show a slight influence, while the polyunsaturated members show a marked effect. Administration of highly unsaturated fatty acids and of acid lipidic fractions of certain organs, such as placenta, liver, spleen or blood, uniformly decreased pain of an acid pattern and increased pain of an alkaline pattern. These opposite effects have, from the beginning of our study, contradicted the idea that this influence upon pain was the result of the direct action of these agents upon the nervous system. Furthermore, the opposite effects exerted upon the same pain by the other group of lipids have confirmed the hypothesis that the action takes place at the level of the painful lesion, where the differences between the two pains was found to correspond to two opposite acid-base offbalances.

In the study of the effects of lipids on the pH of the second day wound crust, made in collaboration with Carlos Huesca, we have demonstrated that lipids influence pain through changes induced on the acid-base balance present at the tissue level. The positive lipids constantly lowered this pH while the negative lipids elevated it. *(Note 1 Chapter V)* Even more im-

portant than this temporary pH effect in establishing the mechanism of lipid action in pain was the change in the actual pattern of an existing pain after administration of these agents. Polyunsaturated fatty acids in sufficient amount were found to convert an acid-pain pattern to an alkaline pattern, while sterols changed an alkaline pattern into an acid one. We will return to this important fact later.

A pathogenic role for lipids becomes evident too, when pain can be induced through the administration of lipids in previously painless lesions. Such lesions treated with large amounts of lipidic preparations became painful. An alkaline pattern of pain was seen to appear after fatty acid administration, while an acid pattern followed use of the insaponifiable fraction. *(Note 36)*

At the tissue level, lipids also affect such acid-base symptoms as vertigo, itching, dyspnea, tremor, and even mental diseases. In these conditions the same antagonism between the two groups of lipids—and the same opposite effects upon the acid and the alkaline pattern—can be noted along with the same possibility for changing the pattern to the opposite type if big doses of lipids are administered.

Wound Healing—The same manifest antagonism between the two groups of lipids was also noted in their influence upon the evolution of wounds. Changes in the sloughing and healing process were followed by measuring the size of wounds *(Note 37)* as well as by serial histological examinations. The lipids with a negative polar group were seen to retard the evolution of the processes by prolonging the first catabolic phase. Positive lipids generally had an opposite effect. However, here too it could be observed that sterols have relatively little effect on the healing of connective tissue, but manifestly favor proliferation of the epithelia. This was especially evident in the changes in scar formation of the skin of treated animals. In rabbits treated with cholesterol, the epithelial scars were found to have 8-10 layers instead of the 2-3 characteristic for the rest of the skin and for the scars in control animals.

Regeneration—In collaboration with E. F. Taskier we studied the effect of lipids upon the regeneration of liver in rats, after the resection of almost ¾ of this organ. The rate of regeneration could be estimated by correlating it to the time of appearance of fatty droplets filling up almost all the cells, as a first step in the regenerative process. In very young animals, this change in fatty liver cells was seen to take place even within the first 24 hours after resection. The change was progressively delayed as the age of operated animals increased. In old animals the change in fatty liver cells appeared only after the fourth day.

The administration of lipids had a marked effect on appearance time of fatty cells. Sterols induced precocious appearance in old animals. From this point of view, sterol-treated animals appeared to react as young individuals, with fatty cells evident even on the second day. The fatty acids and acid-lipidic fractions of organs showed an opposite effect, delaying the time of appearance of the fatty cells. Young animals treated with polyunsaturated or conjugated fatty acids showed no fatty droplets in the liver cells for as long as three to four days. With higher doses of the same agents, the fatty infiltration did not occur at all.

It is interesting to note a parallelism between fatty infiltration of liver cells and the richness of adrenals in sudanophil substances. An almost complete lipid depletion of the adrenals was seen after high doses of fatty acids and coincided with a total lack of fatty cells in liver regeneration. *(Note 38)*

Organic Level—Effects of the two antagonistic groups of lipids at the organic level have been studied in terms of manifestations clearly associated with various organs. We will review these effects briefly here, with more details to come when the therapeutic use of lipoids is discussed.

Intestines—The influence of lipids upon intestinal function is marked by the same antagonism between the two groups of agents. Oral administration of large amounts of fatty acids, especially higher unsaturated such as obtained from cod liver oil, was usually followed by diarrhea. Diarrhea also occurred after parenteral administration of these substances in large amounts. It was interesting to note that parenteral administration of the acid lipidic fraction of placenta, blood or even organs had a marked influence upon the colon and rectum in particular. High doses produced tenesmus with a mucous or even sanguinolent secretion. This localization of the effects of the lipidic fraction appeared to be especially interesting from a therapeutic point of view, as will be seen later. The oral or parenteral administration of the opposite group of lipids, sterols and insaponifiable fractions, has an opposite effect, a constipating one, which we will discuss later together with its therapeutic aspects.

Kidney—The manifest opposite effects exerted by the two groups of antagonistic lipids upon diuresis raise the question of where these effects take place. While a systemic effect can be recognized, a more direct intervention upon the kidney also must be considered. The addition of the acid lipidic fraction of organs, and especially those obtained from pork kidney, to the perfusion fluid in a dog kidney preparation produces a manifest decrease of excreted urine. The administration of insaponifiable fraction

has a marked diuretic effect which we will discuss below with its therapeutic aspect.

Nervous System—Interesting effects by the two groups of antagonistic lipids upon many manifestations of the central nervous system have been noted.

Convulsions—Administration of sterols and insaponifiable fractions of many organs such as placenta, liver, butter, eggs, etc., in large amounts induces convulsions in rats. Convulsions also were noted in humans when huge doses of these agents were administered. But even in relatively small amounts, these lipid agents sensitized animals to the administration of other convulsant agents. In rats or mice receiving such lipids, thiamine chloride induced convulsions in doses without effect in controls. *(Note 39)*

An opposite effect was observed for lipids with negative character. Saturated fatty acids showed no influence on thiamine-induced convulsions. Such convulsions were prevented by the administration of nonsaturated members. The effect was related to the degree of desaturation of the fatty acids. With increases of the iodine number, the necessary effective doses of these fatty acids became progressively smaller. While hundreds of milligrams of mono- and diethenic acids were necessary for each 100 gram of body weight, the anti-convulsant effect was obtained with only a few milligrams of clupanodonic acids, and with still less of the nonenic acid, bixine.

The study of the pathogenesis of convulsions also covered the influence exerted by these lipids of the adrenal corticoids. The administration of mineralocorticoids, especially desoxycorticosterol, even in small doses, to subjects who had received any one of the lipids with a positive polar group, such as cholesterol or insaponifiable fraction of placenta, liver or kidney, was followed almost invariably by convulsions. We will present more details on this effect later in the discussion of synthetic substances. For the moment we want only to note the relationship between mineralocorticoids and lipids with positive character in the pathogenesis of convulsions. The concomitant intervention of the two factors—an offbalance induced by lipids with positive character, and action of mineralcorticoids—seems to provide new light on the pathogenic problem of epilepsy and convulsions in general.

Coma—The role of cortical hormones in the pathogenesis of convulsions was confirmed by the opposite effect produced by neoglucogenic corticoids. We will see later that the administration of cortisone to subjects receiving higher alcohols such as heptanol, octanol or octandiol in large doses, induced a subcomatose condition at first which progressively changed into coma. *(Note 40)* Opposite properties of the mineral and neoglucogenic

corticoids, which made Seyle separate them according to their "phlogistic" and "antiphlogistic" activity, would explain the two opposite manifestations inducing convulsions and coma, produced in individuals previously treated with the same anti-fatty acid agents.

On Cardiac Rhythm—The influence exerted by the two groups of lipoids upon the cardiac rhythm was studied under the same dualistic aspect. The effects observed can easily be interpreted considering the role of the differentiation of the cardiac cells for their part in the cardiac physiology. The role of a cell in cardiac physiology is a direct function of its own automatism which can ultimately be related to its degree of differentiation. The fact that the two groups of lipids act antagonistically upon this cellular differentiation, the acid lipids exaggerating it and the insaponifiable fraction of sterols reducing it, has explained some of the effects induced by these agents upon normal and abnormal cardiac rhythm. *(Note 41)*

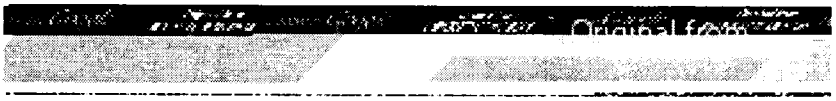
On Oestral Cycles—The action of the two groups of lipids at the organic level was also studied in the rat ovarian cycle. Daily, and even twice a day, vaginal smears were made in animals treated with these agents. When large amounts were administered, both groups suppressed the cycle. With smaller doses, only the lipids with positive polar groups, especially sterols, produced this effect.

Systemic Level—Blood has appeared especially suitable for in vitro and in vivo studies of the effects of the two groups of lipids at the systemic level. The effects on different blood constituents were analyzed and led to very conclusive results. We will outline here the principal points of this study.

Under the influence of anti-fatty acids, the erythrocytes become more turgescent, increase in volume, show a strong refringency of their crown in dark field examination, and remain isolated from one another. The sedimentation rate, if previously high, is reduced by treating blood in vitro with insaponifiable fractions of organs. Oxygen appears to be retained longer in treated red cells than in controls.

The fatty acids have an opposite effect. Under their influence, the red cells become crenelated and develop a tendency to form sludges. The sedimentation rate is increased. The color of the treated blood is dark and, even after oxidation, rapidly darkens again. In vivo, lipids with a positive character induce leucocytosis, those with a negative character leucopenia. This last effect is seen even in vitro. In Note 42, the influence exerted by lipids upon the blood is presented with more details. (36)

On Temperature—The administration of sufficient amounts of positive lipids induces a frank elevation of temperature, while hypothermia follows



the administration of negative lipids. The relationship between temperature and lipids, however, is not so simple since changes in external temperature influence the balance between the antagonistic lipids. For example, animals kept in incubators at a temperature of 35°C show an increase in lipids with a positive character. Animals kept in a cool place, such as a refrigerator, show an increase in lipids with a negative polar group. The organisms are able to combat the increase of lipids with negative character by means of the normal defense mechanism, but are less capable of dealing with an increase of lipids of positive character. Therefore, while a high proportion of animals kept in refrigerators adapt themselves to the new conditions, those in incubators die in a few days.

On Systemic Patterns—The influence exerted by lipids upon various other systemic manifestations which are reflected in abnormal patterns in urine analyses has been studied. In general, the fatty acids induce patterns corresponding to the offbalance of type D, while the sterols induce patterns of the type A offbalance. Here again we must emphasize that any lipid, if administered in large quantity, influences all analytical values. A certain specificity, however, is noted since, in relatively small doses, lipids induce changes only in certain values. Because of the inherent technical problems concerning the patterns, only a few analyses could be followed accurately over the period of time necessary for a clear recognition of changes in small laboratory animals. It is for this reason that most of our studies in this area were made on humans where pattern changes could be easily identified and followed over long periods.

It is to be emphasized that, under these conditions, the influence of lipids is exerted especially upon already existing abnormal patterns, increasing or decreasing their deviations from the normal, or changing the patterns entirely. Abnormal patterns were induced through huge amounts of lipids, which very seldom were administered to patients. TABLE X shows schematically the analytical changes induced by the two groups of lipids upon various urine and blood analyses, expressed as patterns corresponding to abnormal conditions, as well as upon the manifestations present at other levels.

We will discuss these effects in more detail when describing the pharmacodynamic properties of lipids and lipoids.

Mechanism of the Lipidic Biological Activity

The analysis of the changes induced by lipids has emphasized certain characters which appear of capital importance for the understanding of the biological intervention of these substances. In one kind of activity a lipid

acts through its *lipoidic properties*. From the data concerning its distribution in the organism it can be seen that, due to its solubility characters, a lipid introduced in an organism will be selectively retained by the existing lipidic system. When such intervention through its lipoidic properties takes place, the nonspecific character of the activity of the lipid is prevalent. A second kind of activity results from the *bond realized through the charge of the polar groups*. The positive or negative character of these polar groups deter-

TABLE X

LEVEL	EFFECTS OF STEROLS	EFFECTS OF FATTY ACIDS
Cells	Prolongs youth character	Induces rapid aging
	Increases potassium content	Decreases potassium content
	Decreases sodium content	Increases sodium content
	Reduces membrane permeability	Increases membrane permeability
	Reduces cellular oxidation	Increases cellular oxidation
	Reduces chloride content	Increases chloride content
Tissues	Lowers pH of lesions	Raises pH of lesions
	Lowers chloride content of lesions	Raises chloride content of lesions
	Lowers water content of lesions	Raises water content of lesions
Organs	Induces somnolence	Induces insomnia
	Induces diuresis	Induces oliguria
	Induces constipation	Induces diarrhea
	Induces tachycardia	Induces bradycardia
Systemic	Induces hyperthermia	Induces hypothermia
	Induces hypertension	Induces hypotension
Blood		
	Increases RC volume	Decreases RC volume
	Decreases RC sed. rate	Increases RC sed. rate
	Increases persistence of oxygen fixation	Decreases persistence of oxygen fixation
	Determines persistence of RC isolation	Determines formation of sludge
	Determines hyperleucocytosis	Determines leucopenia
	Determines eosinophilia	Determines eosinopenia
	Decreases kalemia	Increases kalemia
Urine	Induces water excretion	Induces water retention
	Induces sulfhydryl retention	Induces sulfhydryl excretion
	Induces calcium excretion	Induces calcium retention
	Induces chloride excretion	Induces chloride retention
	Induces sodium excretion	Induces sodium retention
	Induces phosphate retention	Induces phosphate excretion
	Induces retention of surface active substances	Induces excretion of surface active substances

mines thus the nature of this second kind of activity. A third kind of activity results from the *chemical constitution* of the polar group, which will induce selective combinations and consequently will have a more specific influence. A fourth group of changes are induced by the activity which takes place at the nonpolar group of the lipid and more specifically at the *energetic formations* present in it. They will have a still higher character of specificity.

With this systematization of the activity of the lipids, a further systematic analysis of the influence exerted by the lipids appears possible.

Through its selective distribution, the administration of a substance having lipoidic properties will influence those entities which have lipids in an active form in their constitution. The influence exerted will thus be proportional to the richness of the entity in these active lipids. This fact explains why the administration of a lipid or lipoid affects selectively the abnormal entities rich in free lipids and to a much lesser degree, the normal ones. It is this selective distribution which will further limit the activity of the lipoid to the lipidic system and most manifestly to the abnormal entities. In the frame of this limitation, this activity results from the charge of the polar group. Similar effects are thus obtained for all the different lipoids which have the same electric positive or negative character of their polar group. This explains why one can use different agents from the same group and still obtain similar results. Agents chemically so different as fatty acids, mercaptans, persulfides, aldehydes or epichlorohydrine, have similar activity because they all have negative polar groups. The characteristic of the effects resulting from the electrical character of the polar groups, is that they are common for the groups having the same sign and diametrically opposite for the agents with a positive or a negative polar group.

This effect was clearly seen in fatty acids in which the negative carboxylic polar group was changed into the positive primary alcohol. The biological effects of the new substance were opposite.

It is in the third kind of activity that the chemical nature of the lipoids intervene. Certain effects resulting from the bond of an amino polar group will thus be different from that of the alcohols, although both act as positive energetic centers and as such have exerted other common effects. The same is true for the carboxyl and thiol groups.

Still more specific appear the effects resulting from the intervention of the energetic factors present in the nonpolar group, such as the double bonds, and the energetic formations they realize such as conjugated, or two double bonds separated by a methylenic carbon.

The various mechanisms involved have explained further the different kinds of biological effects which result. The action of the lipid by means of

the lipoidic effect will thus influence general, nonspecific manifestations, such as those concerning the permeability of membranes. Only secondarily, will these changes in membrane permeability influence the different metabolic processes which the membrane governs.

In the second group of changes, related to the intervention of the polar groups, the antagonistic effects induced were seen to concern processes resulting from membrane permeability. It is only in a third change that a more specific action upon the different metabolic processes has to be considered. These are concerned with an intervention upon metabolites or the agents governing them. The character of this last lipidic intervention is its specific influence exerted upon a definite metabolic system.

We tried to interpret the influence exerted by a lipid or lipoid according to the above systematization. The recent development of the biochemical methods of investigation has put into limelight many biochemical processes by considering them as isolated metabolic entities. Most of them were seen to result from the intervention of enzymes upon more or less specific substrata. One of the principal objectives of the actual pharmacodynamic studies is to correlate as directly as possible, biological effects of different agents to specific metabolic processes, most of them corresponding to a change in an enzymatic process. This approach, while very interesting, would not take into consideration the important role played by the nonspecific activity of lipids and lipoids. These nonspecific influences through changes in the lipidic system induce different changes in different metabolic processes. A nonspecific change in membrane permeability will affect many enzymatic processes. It explains the existence of similar influences exerted upon these processes common to agents which have nothing more in common than their lipoidic properties and the presence of a positive or a negative character of their polar group. It is this character which binds an effect to the nonspecific intervention. This so-systematized analysis has thus permitted to separate the biological activity of the lipids and lipoids, the more specific from the lesser influences, and correlate each one to a proper or common character of the agent. This view has amply simplified the study of the pharmacodynamic intervention of these substances.

OTHER CONSTITUENTS

In addition to the chemical elements and lipids, other constituents have been studied from the dualistic point of view. Although the other constituents have received less emphasis, interesting information has been obtained.

Amino Acids

Amino acids have been separated into groups based upon their effects at different levels. The first group includes the simple amino acids. In these members the portions of the molecule which are added to the amphoteric amino acid group, are usually electrically neutral. The amino acids polymerized through the amphoteric group serve as building materials for the bigger protein molecules. They have appeared to be inert without effects upon the different levels. Beyond these simple amino acids, are two groups, energetically active, which have a second energetic center with a negative or positive character in their molecules. While the amino acid group serves to make these substances parts of higher proteins through the same bonds of amino acid groups as the simple members, it is the other energetic center, with acid or alkaline character, which confers upon these amino acids a positive or negative character.

We studied effects, at different levels, of arginine, lysine and histidine, which are members of the group with alkaline centers; of glutamic and aspartic acids which have acid centers; and of methionine with a thiolic center. Like for the lipids, the last two groups have shown similar properties, but opposite to those of the members with alkaline centers. The nature of their intervention appeared evident through the interesting opposite effects exerted upon microbes. Cultures of B. subtilis in broth containing members of one or the other of the antagonistic groups show characteristic changes. Unlike controls in which the long chains of microbes remain isolated, the microbes were seen to be kept together in media with alkaline amino acids, forming a consistent gelatinous mass separated from the medium. In broth with acidic or thiolic amino acids, the microbes remained separated or formed very small aggregates. This appeared interesting when we considered the positive character present in alkaline amino acids, as related to the heterotropic, constructive trend, while the negative, as in the acid and thiolic members, is related to the opposite trend. We saw further the same antagonism between the influence exerted by histones and nucleic acids, the first paralleling the alkaline amino acid groups and the second the opposite group. The more manifest effect of the ribonucleic acids could be seen to take place at higher levels of the organization and possibly explains the more direct action upon the genes.

We investigated the effect of the two groups of amino acids at the tissue level upon pain. Arginine, lysine and histidine displayed an analgesic effect upon alkaline pain, while glutamic acid and methionine had this effect upon acid pain. The effect could be related more to the basic tend-

ency of these substances to act through metabolic changes, than to a direct influence upon the acid-base systemic balance. The first group acts as heterotropic agents and the second as homotropic, as mentioned above.

Abnormal Amino Acids

Our research led us to several tentatives to define abnormal amino acids and the proteins they form. One concerned their rotatory capacity. The naturally occurring amino acids are all levorotatory. However, the organism constantly has enzymes able to attack dextrorotatory amino acid members as if it would have to be prepared to encounter and destroy them. Such dextrorotatory members can be conceived to appear on a statistical basis as the result of the resonance process seen to occur in all the synthesis in nature. The intervention of specific enzymes against them would have the aim to control their existence and especially to prevent their intervention in further evolution. In a work hypothesis concerning the cancerous process, we considered that their persistence and especially their participation in forming hierarchic entities would correspond to the specific abnormality characterizing this condition.

In another work hypothesis which concerns also cancerous processes and which we will discuss later, abnormal proteins are thought to appear as a result of the bond of a carbamic radical (295) to the amino acid group. The resulting cyclic formation having the characteristic NCNC group in it, would correspond to abnormal amino acids which would represent the primary characteristic formation of the cancerous condition. *(See Chapter 11, Note 1)*

Carbohydrates—Glucose acts as an anti-fatty acid agent, possibly because of the glyceryl compounds resulting from its metabolism. We have studied it in opposition to the respective acids—gluconic, glucuronic and saccharic. Glucose has an analgesic effect, although limited, upon pain of an alkaline pattern, and an opposite effect upon pain of an acid pattern.

The acid group has an opposite effect upon pain. This could be correlated with the changes toward acidosis seen in the local pH of the lesions. A manifest change toward acidosis was seen under the influence of glucose in the second day wound crust pH. We have noted previously the role played by glucuronic acid as an agent with anti-positive-lipid activity. We believe that it is largely through this mechanism that it favorably influences acid pain, having an indirect action similar to that of fatty acids.