changes were seen in the normal tissues. Definite and opposite effects were thus obtained with acids corresponding to the elements of the VIIth series and with those of the VIth series. The first group of acids, especially HCl, induced a frank acidifying effect while the second produced an alkalizing effect. The phosphate and nitric ions showed strong acidification, the iodide and bromide ions were weaker than the chloride ion. The bicarbonate ion clearly alkalized, as did sulfate and selenate. The thiosulfate ion had an obvious alkalizing effect, with pH values as high as 7.85. Among the organic acids, citric acid produced one of the strongest effects, even stronger than that of the inorganic acids. The fact that citric acid is only slightly metabolized could explain its strength. The gluconate ion induced a slight acidification. The cacodylate ion seemed to induce no changes although not enough animals were utilized to judge its effect thoroughly.

In studying the effects of salts, the roles of both cation and anion were considered. Using different anions for the same cation, it was possible to judge the effect of the cation.

Sodium and lithium produced relatively slight acidification. Potassium manifestly acidified as did ammonium, the latter, however, to a less marked extent. Marked acidifying effects were seen for iron, mercury and bismuth. A lesser effect was obtained with molybdenum and aluminum. On the other hand, a manifest alkalization was found for bivalent calcium, strontium, copper, barium and cobalt. Manganese and silver cations seemed to influence the second day wound pH only slightly toward alkalization. It seems that there is an additive effect for the different elements in their acidifying or alkalizing influence. This has permitted us to judge the effects of the different ions. Potassium induces greater acidification than sodium, and still greater acidification than ammonium. Potassium chloride, in which the two ions have an additive effect, is thus more frankly acidifying. The same is true for the acid phosphate. The alkalizing tendency of sulfate ion opposes the acidifying effect of potassium and explains the slight acidifying influence of potassium sulfate. We must arrive at the relatively strong alkalizing tendency of the carbonate ion to find an anion able to counteract the acidifying effect of potassium. The s.d.c. pH effect of potassium carbonate is in the normal range. The data obtained through this study led to the research on the intervention of the elements in biology, which is the subject of Chapter 5. Information concerning the effect upon the s.d.c. pH of these elements as well as the relationship between this effect and the structure of the elements is discussed in this chapter.

Calcium ion has an alkalizing influence strong enough to counteract the acidifying tendencies of such anions as chloride and phosphate. Weaker acidifying anions, such as lactate and gluconate, are not sufficiently strong to counteract the alkalizing tendency of calcium, and calcium salts of these acids have a strong alkalizing effect.

This analysis indicates that the effect of a salt upon the local pH of abnormal tissues can be judged by considering the additive influence exerted by anion and cation, the effect increasing if both have tendencies in the same direction, and decreasing if the tendencies are opposed. We will not

emphasize here the other antagonistic biological effects of cations and anions, as they pass from acidifying (citric acid and potassium) to alkalizing (calcium and thiosulfate). We will discuss these effects in connection with the pharmacological studies of these agents. For the moment, we want only to emphasize the value of this investigational method.

Acid Lipoids

The next step in the use of the s.d.c. pH was the study of the effects of a special group of acids in which we were interested, the fatty acids. Analysis of the results obtained clearly shows the importance of the nonpolar group. A carboxyl, when bound to a long chain as in the fatty acids, does not by itself seem able to induce a change in the second day wound crust pH, the values remaining in the normal range. The substance appears inactive when the nonpolar group does not have its own energetic center or formation. The saturated fatty acids with 10-16 carbons do not influence the s.d.c. pH. The presence of double bonds in the nonpolar group changes the influence exerted. All the nonsaturated fatty acids studied show an alkalizing effect, with relatively slight differences for the higher desaturated members such as linolenic or arachidonic fatty acids, or for the fatty acid mixture obtained from cod liver oil. However, the conjugated fatty acids studied, such as eleostearic acid, or the mixture of acids starting from cod liver oil, showed the highest values in this group, even for so small an amount as 5 mgr. per animal per day. These data indicate the role of energetic formations in the nonpolar groups of fatty acids.

We must emphasize the difference between the hydrosoluble organic acids and the group of fatty acids mentioned above. In the former, the action seems due to the intervention of the carboxyl, probably explaining the strong activity of tricarboxylic citric acid. With an important nonpolar group, this carboxyl seems to be unable to carry the molecule, and therefore cannot act. The intervention of a double bond could serve in two ways: 1) by bending the molecule, thus increasing its mobility as it also reduces the melting point; 2) as an energetic center where reactions take place. The fact that the 10 carbon lauric acid is inactive would indicate the slight influence to be expected from the bending of the molecule alone, as in oleic acid. The influence exerted by metabolic changes in which fatty acids intervene through the energetic centers in the nonpolar group explains the fact that they have a local alkalizing effect instead of the acidifying effect of many other organic acids. *(Fig. 235)*

Alcohols

The role of the relationship between polar and nonpolar groups appeared very clear in the study of the series of aliphatic alcohols.

We have considered separately a group of agents extensively used in our research. These are organic substances that have a radical with a bivalent sulfur as the polar group. We have mentioned above that an inorganic substance with similar constitution, sodium thiosulfate, has a strong alkalizing effect upon the s.d.c. pH. Such an effect upon the s.d.c.

pH has been obtained with all preparations containing a polar thiol group when administered orally or parenterally. We must emphasize the unusual uniformity of results, rarely encountered in other biological experiments. It appears that the alkalizing effect of these preparations is sufficient to override the individual differences in subjects receiving them. The same alkalizing effect has been apparent for sodium thiosulfate, and seems to be a common characteristic for substances having a bivalent sulfur in their polar group.

These alcohols exert no influence upon the pH of normal tissues, as seen in measurements taken immediately after the skin is cut. The first members of the series, the methyl to propyl alcohols, do not influence the

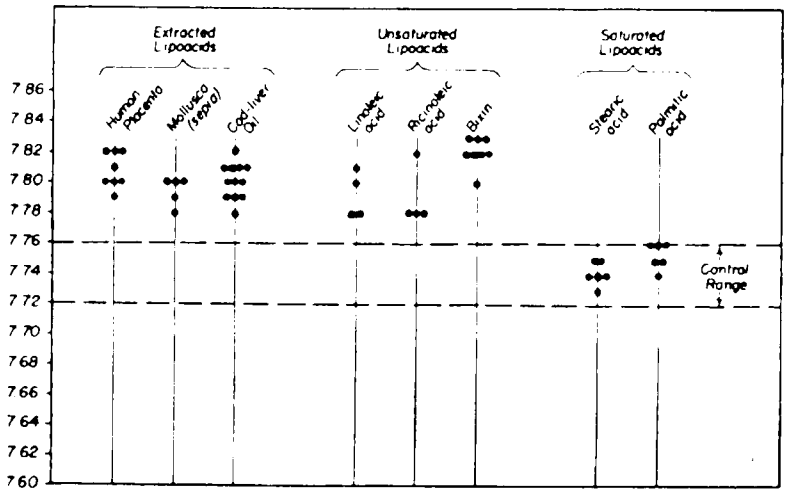


FIG. 235. The administration of various lipoacids upon the s.d.c. pH shows that while the saturated fatty acids do not influence it, the non-saturated fatty acids as well as the lipoacid preparations obtained from different sources induce an elevation of the local pH.

s.d.c. pH. From butyl alcohol to nonyl alcohol, the members of the series show a consistent acidifying influence. However, a very important observation was made when the four isomers of butyl alcohol were studied. Three showed the acidifying effect while one, the tertiary isomer, like the lower alcohols, did not influence the second day wound crust pH. This could be correlated to a special characteristic of these substances, their relative solubility in water and in neutral solvents. Just as do the lower alcohols, tertiary butyl alcohol mixes with water and neutral solvents, while the other three butyl alcohols, like the higher members of the series, are more soluble in neutral solvents than in water. *(Fig. 236)* This characteristic, which was used in systematizing the polar-nonpolar substances, appears to determine the activity of the aliphatic alcohols upon the s.d.c. pH. We must again emphasize that this activity is not direct but influences certain metabolic processes, since the active alcohol induces local acidification rather than the alkalization to be expected with a direct effect.

Another factor which seems to influence the activity of this alcohol series is the length of the carbon chain. While heptanol induces the characteristic acidification, octyl alcohol does so in only some animals. Nonyl and decyl alcohols appear inactive.

The possibility of a biological competition between these agents and fatty acids has led to research with other alcohols capable of combining with fatty acids, particularly in vivo. We studied glycerol, glycerophosphoric ion and sterols, which are frequently found combined with fatty acids. We added glucose to this group because of its metabolic relationship to glycerol derivatives, although it is apparently not related to fatty acids.

It is interesting to note that glycerol produced only minimal influence upon the immediate pH. The highest values were still in the normal range. The acidifying effect of glycerol upon the s.d.c. pH, seemed to be subject

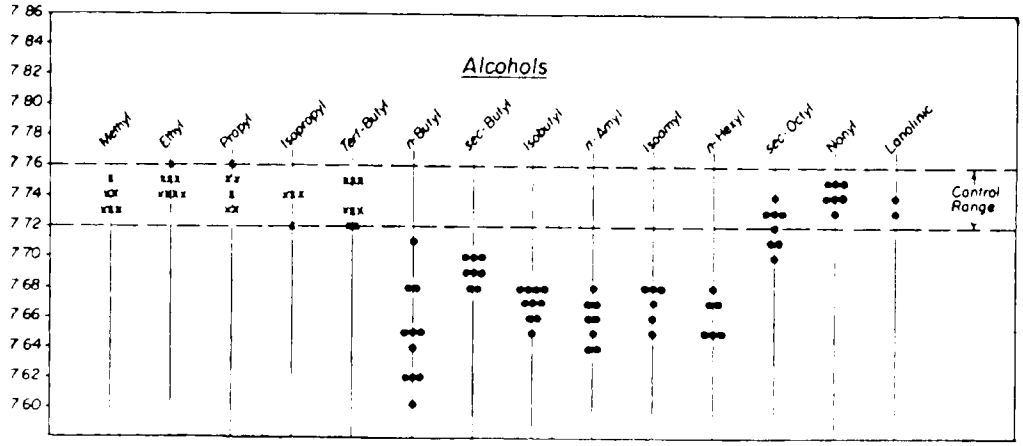


FIG. 236. Second day wound crust pH values for various alcohols shows the relationship between activity and lipoidic property. All the alcohols below butanol and tert. butanol, which are not lipoids, are inactive. This also holds true for alcohols with chains longer than eight carbons.

to individual variations but also with values at the lower limit of normal, such as 7.72. Glucose showed a slight acidifying effect, while cholesterol showed definite acidifying activity upon the s.d.c. pH, as did all the preparations of the insaponifiable fraction of various organs or tissues. The acidification produced by the insaponifiable fraction preparations is more intense than for cholesterol alone, indicating that the other constituents of these preparations also have an acidifying influence.

Other Agents

We investigated some of the hormones that are often used clinically and found that the biological antagonism between male and female sex hormones is also apparent in their influence upon the s.d.c. pH, the male hormone having an alkalizing effect, the female hormone, an acidifying one. We must note here that the acidifying effect is also apparent for pro-

gesterol. This effect is opposite to that of the male hormone and similar to that of stilbestrol, contrary to what we expected. There is similar antagonism between desoxycorticosterol and suprarenin, the former being acidifying and the latter slightly alkalizing. The liver antianemic extract also has an alkalizing effect.

Several vitamins also were investigated. We were able to see that while vitamins B_1, B_2, E and K have an acidifying effect, vitamins A, D and B_6 have an alkalizing action. Ascorbic acid seems to have no effect upon the s.d.c. pH.

A few alkaloids and glucosides, important for their pharmacodynamic activity, were studied. The two opium alkaloids and a similar synthetic agent are moderately acidifying. Atropine, caffeine, and quinine have a slight alkalizing effect. The different effects of digitaline and saponine were unexpected; both show slight but opposed action, the first acidifying and the second alkalizing.

Because of their effect upon the central nervous system, narcotics and hypnotics were studied. While ether and chloroform are slight alkalizing, the two barbiturates which we tested showed an acidifying effect. This is interesting, especially when related to the opium alkaloids and demerol which also induce acidification, although to a lesser extent than the barbiturates.

Various other agents were studied. Among pyretogenics and antipyretics, there is an obvious antagonism in influence upon the s.d.c. pH. While the pyretogenic, methylene blue, induces a frank acidification, the three antipyretics we examined produced alkalization. However, acetylsalicyclic acid does not follow this rule—it has an acidifying effect.

Among antimicrobial agents, a parallel action was apparent for the three antibiotics of fungal origin and the two sulfa drugs studied. All have an alkalizing effect, like the antipyretics. The acidifying effect noted for benzedrine does not accord with a similar effect observed for substances with hypnotic and sedative activity. This discordance between principal pharmacological activity and effect upon the s.d.c. pH indicates that the latter must, on many occasions, be considered to be due to a secondary influence exerted at the interstitial level.

The antagonistic biological activity of anti-anemic liver extract and iron preparation, the former favorably influencing the hyperchromic anemias, the latter favorably influencing the hypochromic forms, is reflected in their opposite effect upon the s.d.c. pH. The anti-anemic liver extract induces alkalization, while iron induces acidification. The same antagonism is seen with two agents having an opposite action on blood coagulation. While vitamin K induces acidification, dicumarol is alkalizing. Although rutin acts upon other factors when it intervenes in bleeding, it has the same effect as vitamin K on the s.d.c. pH.

Aminophyllin induces acidification in contrast to caffeine, which produces slight alkalization. On the other hand, procaine's effect is like that of the opium alkaloids and barbiturates, all inducing acidification. This can be

related to the effect of higher alcohols which also have narcotic activity and, as seen above, also induce acidification.

TABLE XXVII shows the effects upon the s.d.c. pH of all of the substances examined.

TABLE XXVII

THE EFFECT OF VARIOUS SUBSTANCES UPON THE S.D.C. pH.

	Elevate	Reduce	No Effect
Vitamins	A, D, B_6	B_1, B_2, K, E	C
Hormones	Testosterone, Epinephrine	Stilbestrol Progesterone, Desoxycorticosterone	
Alkaloids	Atropine, Quinine	Codeine, Morphine	
Antibiotics	Penicillin, Streptomycin, Aureomycin		
Sulfonamides ..	Sulfathiazole, Sulfamerazine		
Antipyretics and analgesics	Acetophenetidin, Aminopyrine Antipyrine	Acetyl-salicylic acid	
Narcotics	Chloroform	Phenobarbital, Pentobarbital	
Anti-anemics ..	Liver extract	Iron (reduced)	
Xanthines	Caffeine	Aminophylline	
Miscellaneous ..	Dicoumerol, Benzene, Toluol, Saponin, Pteryl-glutamic acid Teropterin)	Procaine, Benadryl, Coramine, Glucose, Glycerine, Sodium gluconate, Rutin, Methylene blue, Mercuhydrin, Benzedrine, Demerol	

Based upon the findings for such different groups of agents, the s.d.c. pH method appears to be an interesting tool for the study of pharmacological activity. We must emphasize that not one of these substances, at least in the dosage given, has had any influence in changing the pH of normal tissues from normal range, as indicated by pH values obtained immediately after wound production. As our research concerns only ab-

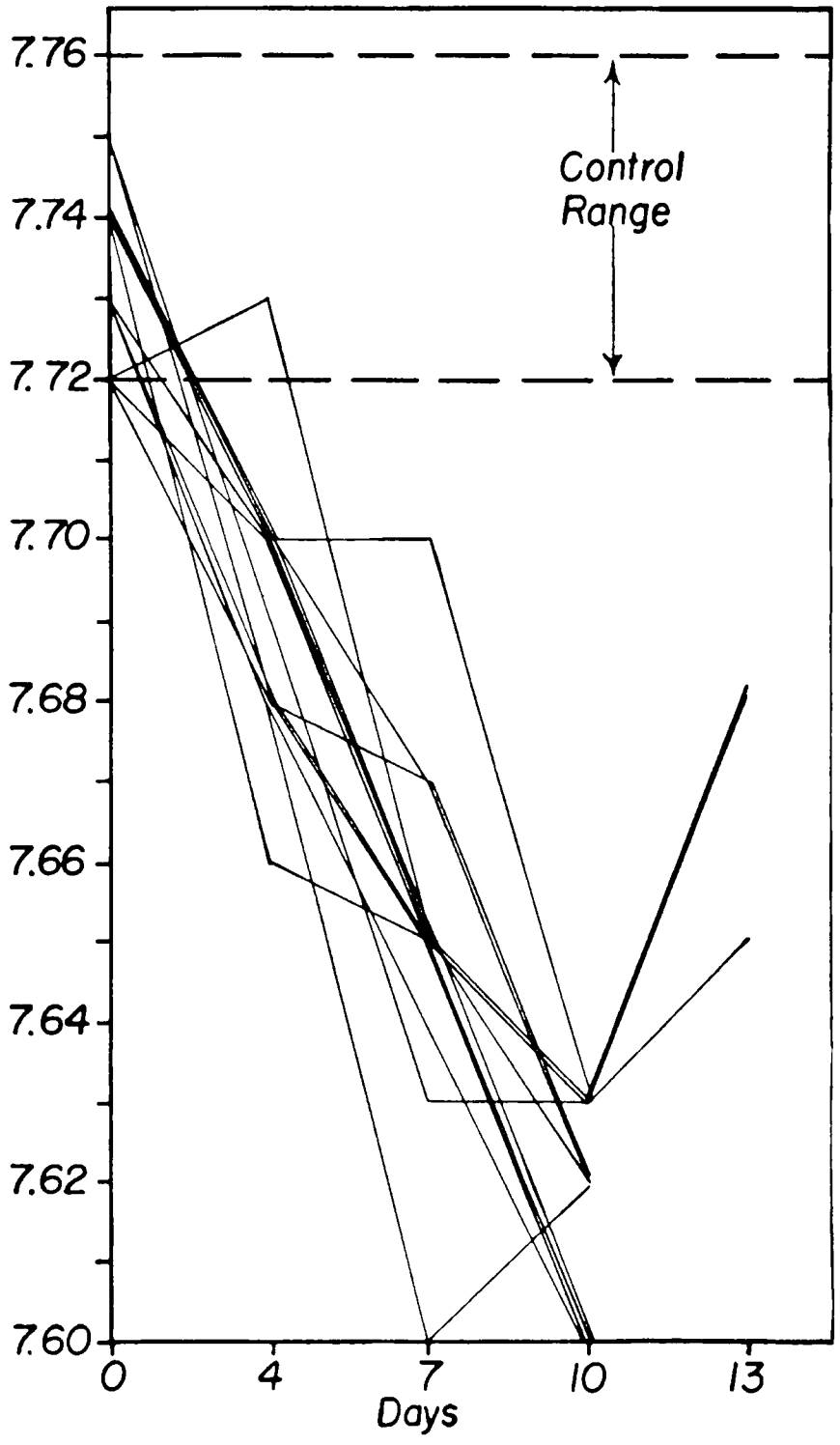


FIG. 238. The effect of a growing transplanted Walker tumor on the s.d.c. pH determinations in surgical wounds produced serially on the day of transplant and every three days thereafter. A marked reduction in the s.d.c. pH was observed in all animals.

normal tissues, the s.d.c. pH is of use. However, in integrating the pH values into the general picture of the offbalances, it must not be forgotten that they represent changes in the interstitial fluids, which correspond to the secondary part of the tissue level.

PHYSICAL AGENTS

Animals kept in an incubator at 38° C. showed a definite tendency toward a lowering of the s.d.c. pH. The effect of cold upon animals maintained in a refrigerator for forty-eight hours before operation was less apparent.

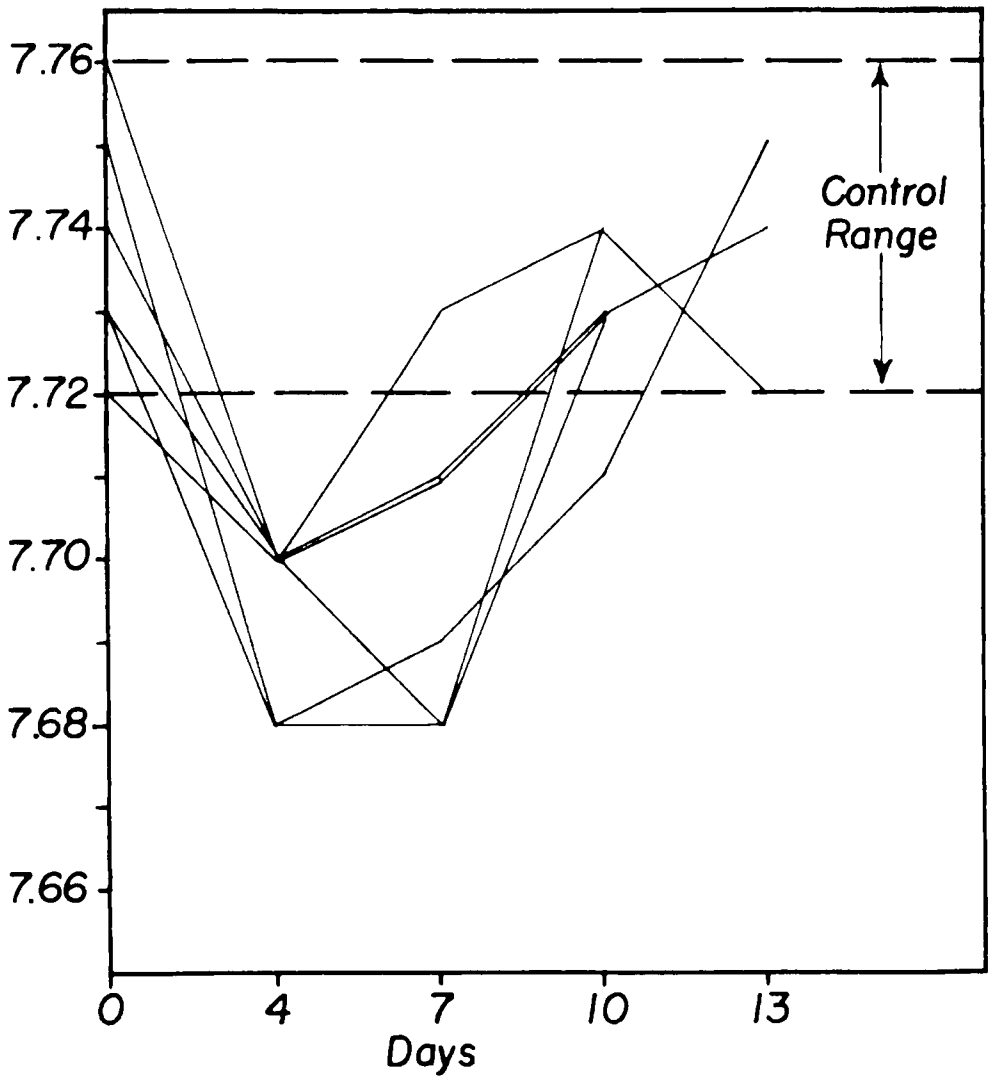


FIG. 239. Serial s.d.c. pH changes in animals with transplanted tumors that regressed rapidly or failed to grow. A slight fall is seen to occur in the fourth and seventh post-transplant day determinations, with a return to the control range in all the animals by the tenth day.

BIOLOGICAL FACTORS

Transplanted Tumors. Preliminary studies regarding the effect of a transplanted Walker tumor upon the s.d.c. pH of an experimental wound have been carried out. Prior to transplantation, the control s.d.c. pH was determined for each animal. Tumors were then transplanted subcutaneously to the left flank region by the trocar method. After the tumor had been transplanted to the animals, experimental operative wounds were produced at intervals of three days and the serial s.d.c pH values on the

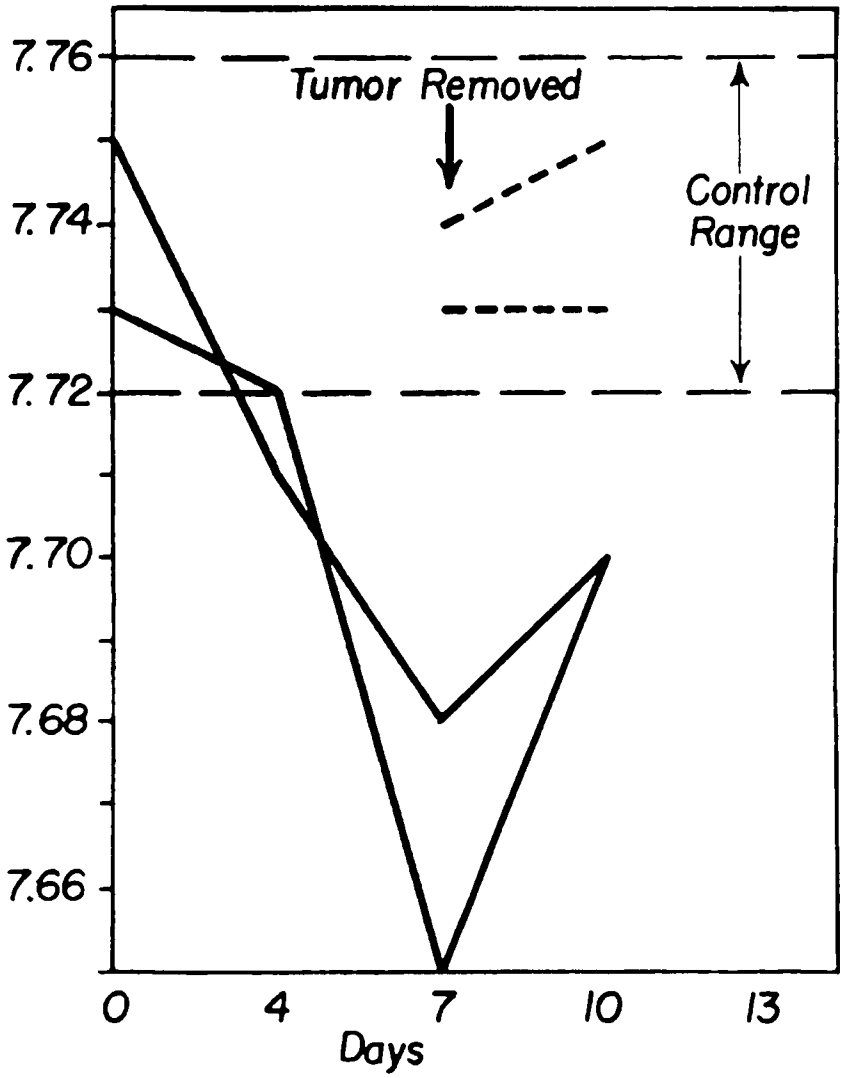


FIG. 240. The s.d.c. pH changes in two rats with growing Walker tumors (——). from which the tumor was removed on the seventh post-transplant day. Following the removal, the s.d.c. pH began to return towards the control range. A similar large incision was made in two animals without tumors and no effect was observed upon the s.d.c. pH in these animals (. . . .).

fourth, seventh, tenth and thirteenth post-transplant days determined. The pH values determined each time immediately after the wound was produced, did not differ from the values found in untreated controls. As in all other animals, the s.d.c. pH only showed significant changes.

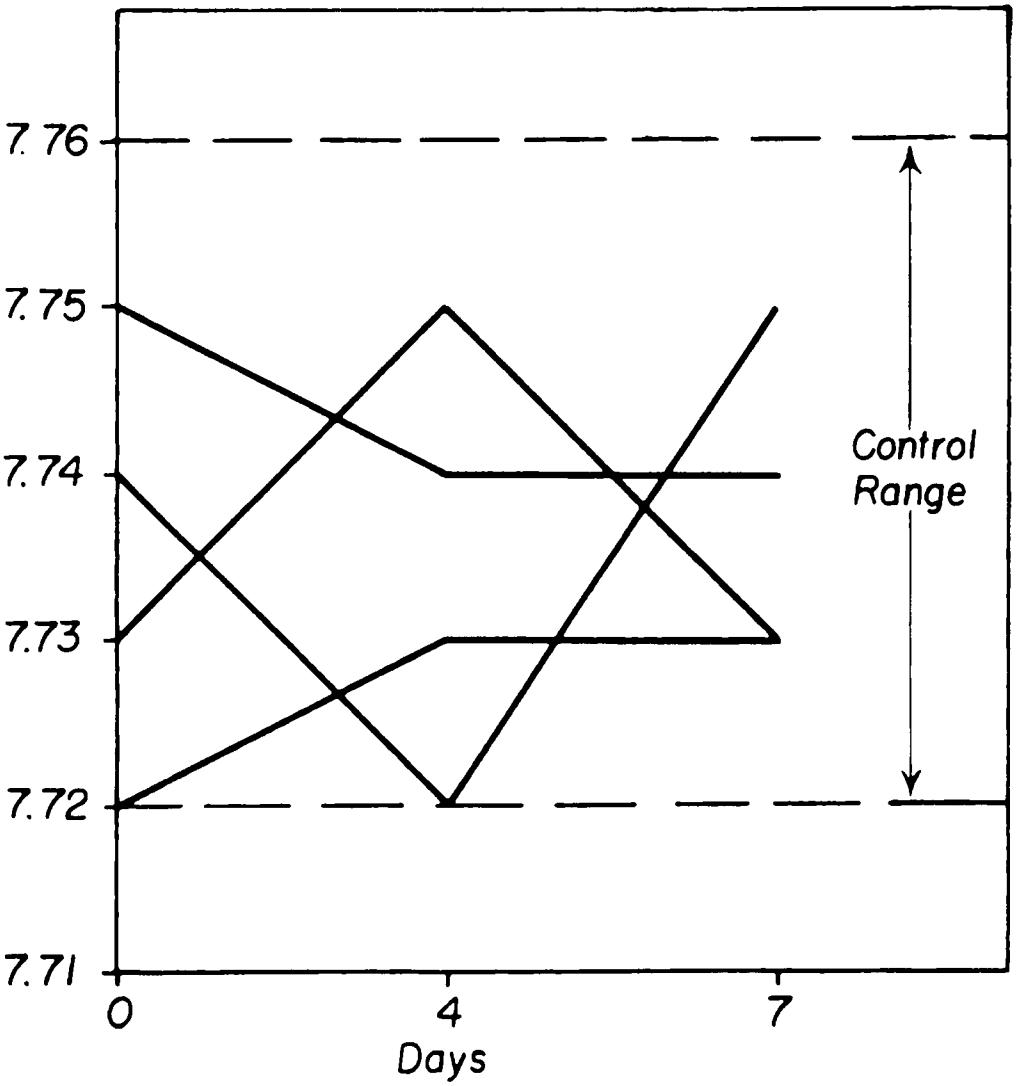


FIG. 241. Serial s.d.c. pH determinations in rats without transplanted tumors. The serial small surgical procedures did not alter the s.d.c. pH from the control range.

Figure 238 illustrates graphically the changes in the s.d.c. pH in a group of twelve animals with successful tumor transplants. A steady and maintained lowering of the s.d.c. pH was observed in each animal, with a tendency for it to rise slightly in those animals surviving thirteen days.

In some of our animals the tumor has shown a tendency to regress spontaneously or fail to take. In six animals in which the tumor failed to take or underwent early regression and disappeared, the s.d.c. pH in the

fourth and seventh post-transplant day tests fell slightly, but thereafter returned to control levels. *(Fig. 239)*

When transplanted tumors that were growing well, were surgically removed following the second post-transplant determination on the 7th day, the s.d.c. pH was seen to increase at the next test. *(Fig. 240)*

Surgical wounds. The same incision as for the removal of the tumors was produced in control animals, but the incision alone did not affect the s.d.c. pH. *(Fig. 240)*

In order to further control these experiments, repeated s.d.c. pH determinations were carried out upon wounds produced serially as in the tumor bearing animals. When no tumor was present the serial s.d.c. pH values did not vary from one examination to the next. *(Fig. 241)*

The serial small surgical procedures did not alter the s.d.c. pH from the control range.

Chapter 5, Note 2. Potassium

We have seen in Chapter 2, Note 1 how the place occupied in nature by sodium and potassium can explain their peculiar distribution in the body. Proper to the earth crust, one of the environments through which the complex individual has passed during its phylogenetic evolution, potassium appears as an element of the secondary part of the cellular compartment in the hierarchic organization. As a monovalent heterotropic element, potassium represents the principal organizational cation of this compartment. Its influence exerted in normal and abnormal physiology can be understood through its specific intervention at the cellular level, the changes in potassium content of the other compartments of the hierarchic organization being secondary to those occurring at the cellular level.

Potassium, absent at the nuclear compartment, is thus found in the nuclear or chromosomal sap, only in minimal amounts. We have no direct information about a passage of potassium from cytoplasm into the nuclear compartment under abnormal conditions. Judging by analogy, it appears probable that such a passage would occur and result in the appearance of nuclear vacuoles.

Ample data are available concerning the relationship between potassium of the cellular and of the metazoic compartments. This information receives a special interpretation when related to the above mentioned hierarchic distribution of the elements.

The cells maintain a proper amount of potassium in the cytoplasm which corresponds to a cellular primary constant. This constant insures a normal cellular metabolism and is controlled in part by the selective intervention of the cellular membrane. Under normal conditions, only a slow passage of potassium through this membrane takes place as compared to other constituents, such as of water, for example. On using radioactive potassium, Moore has shown that it takes about fifteen hours to bring it into balance with the intracellular potassium, while for heavy water, such an equilibrium was reached in less than two hours. (254) Due to the

intervention of the membrane, only minimal changes result in the cellular potassium even though rapid potassium variations take place in the extracellular compartment. Under normal circumstances, the body is insured through a regulatory mechanism against too strong or too rapid systemic changes. Normally, no potassium is stored in the body beyond that which is contained in the cell and metazoic compartment. Following a high intake, potassium is rapidly excreted. A very small amount is lost through perspiration, an additional amount, of around 10% is lost in the stool (252), and the remainder is lost through the kidney. (253) For insufficient intake, or an abnormal loss through excessive diuresis, prolonged diarrhea or vomiting, the organism tries to reduce this loss of potassium to a minimum. A prolonged systemic potassium deficiency brings about an increase in the weight of the kidney with tubular hyperplasia, which can be interpreted as a compensatory hyperthrophy, in order to insure the reabsorption of very small amounts of potassium from the glomerular filtrate, and thus save this important element for the organism. Consequently, a quantitative abnormal intake or loss of potassium will result in an abnormal amount, either too high or too low in the metazoic fluids, only in case of a concomitant deficiency of the regulatory system. Prolonged quantitative changes in the amount of potassium available to the total body, influence only up to a certain point, the potassium content of the cells. Thus when the total body potassium remains low over a period of time, the cells too lose potassium. (250) On the other hand, the potassium in the cells increases after an abundant, prolonged administration of the cation. (251)

Besides these quantitative changes we have to consider abnormal conditions as affecting the specific intervention of this element. The proper level to which potassium belongs and the characteristic changes which take place at the cellular level, could be translated in too high or too low values. We tried to further interpret its intervention through the heterotropic character of this element.

The changes seen to occur in muscles have permitted us to relate this dual occurrence observed for the potassium content of the cells to the two abnormal metabolic conditions which take place in the cells. A muscle in anabolic metabolism and characterized by a process of glycogenogenesis, shows an increase of its potassium content. On the contrary, catabolic processes such as those occurring during muscular exercise (255) or in tissues in agonic states, are related to a loss of potassium from the cells. This metabolic loss of potassium is different from that seen to result from the death of the cells.

The destruction of cells in general, results in a liberation of their potassium into the interstitial fluids. However, such a destruction explains only in part the progressive loss of potassium encountered in abnormal conditions. The erythrocytes of stored blood lose their potassium to plasma through another process than their destruction. The same is true for patients undergoing surgery. They experience a constant loss of cellular potassium into the metazoic fluids, for which the process of destruction is only partially responsible. The breakdown of cells as it occurs in starvation or dehydration

for instance, releases a proportion of 2.4 gm. of potassium for every gram of nitrogen which is liberated under these conditions. (256) However, in surgical patients, the potassium loss was found to be two or three times higher than expected, considering the nitrogen loss. (257) This indicates a passage of potassium out of the cells without cellular destruction.

The study of the red cell potassium changes in stored blood and in the diphasic biological phenomenon such as in hemo-shock, has increased our knowledge of the conditions which correspond to these changes. We found thus that in the first phase of hemo-shock there are lower amounts of potassium in the red cells, followed by a second phase where this cellular potassium content increases. These two changes were thus connected with the characteristic offbalances occurring in hemo-shock: type D in the first phase, and type A in the second. Further studies of tissues in offbalance A or D in pathological conditions have confirmed this correlation between the two fundamental offbalances and the two opposite variations in cellular potassium.

The heterotropic character of potassium as seen through the test of the second day wound crust pH, *(See Note 1, Chapter 5)*, has correlated the changes in potassium distribution to the heterotropic or homotropic character of the occurring processes. A low cellular content in potassium would correspond thus to the homotropic character of the processes characterizing the offbalance D. Those corresponding to a high cellular content concord with the group of heterotropic processes. Increased intracellular potassium results in a heterotropic anabolic effect, while loss of cellular potassium corresponds to catabolic metabolism.

The relationship between the hierarchic compartments explains the changes under abnormal conditions in the amount of potassium present at the different compartments. The study of the diphasic phenomenon of hemo-shock has permitted us to follow this relationship between cellular and plasmatic potassium. In the first phase of a hemo-shock, to the low potassium of the cells corresponds a hyperkalemia, while in the second phase, to a higher cellular potassium corresponds a hypokalemia.

With potassium, a cellular element, the changes seen in the metazoic compartment can thus be considered to be secondary to those occurring at the cellular level. An abnormal cellular condition, with an increase in the cellular potassium content, would thus have an opposite effect upon the amount of potassium present in the metazoic fluids. The fact that plasma potassium values are kept below normal, has to be interpreted as a means of compensating the high values present in the cells. These low values in plasma would result in a reduction of the cellular potassium and favor its return to normal values when possible. It is especially through changes in the urinary excretion that the respective hypokalemia are induced and maintained. On the other hand, an abnormal change in the cellular condition resulting in a low amount of potassium in the cells is seen to induce a prolonged compensatory increase in the potassium content of the metazoic fluids. Oliguria with reduced loss of urinary potassium, permits the creation and maintenance of this secondary hyperkalemia.

Potassium and Offbalances

We tried to utilize the information concerning the relationship between the two types of offbalances and the changes in potassium distribution between cells and metazoic fluids, in order to recognize the existence and nature of these offbalances. This led us to compare the amount of potassium present in red cells and in serum as an indication of this distribution between cells and metazoic fluids. From a practical point of view, we utilized the total blood instead of the separated red cells. *(Chapter 4, Note 5)* From the relationship between the two values, we could interpret the nature of the occurring changes as corresponding either to a quantitative abnormality excess of deficiency—or to a qualitative abnormality due to an offbalance A or D. Fig. 127 shows this correlation. With the values of serum potassium around 4.5 mEq and of total blood around 38 mEq, the condition is considered normal from the point of view of the potassium intervention. Low values in serum and total blood correspond to a quantitative deficiency, while high values for both, a quantitative excess. High serum with low total blood potassium correspond to the offbalance type D, while low serum potassium and high values in total blood to the offbalance type A.

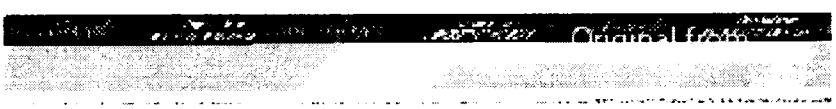
In chronic conditions, such offbalances are seen to persist over long periods of time. Fig. 214 shows an example of a typical D offbalance, with the serum potassium high and the total blood potassium low. Fig. 215 shows an offbalance type A, with low serum potassium and relatively high total blood potassium. Fig. 216 shows an example of lack of potassium, with low values in both serum and total blood.

Potassium and Lipids

The correlation between potassium distribution and the offbalances A and D, has linked this information to the lipids and lipoids. The administration of agents of one or the other of the two groups, positive or negative, has produced opposite changes in the distribution of potassium total blood and serum. These experiments were made in rabbits in collaboration with Ismail Eroglu, Patricia McLachlan and Lee Weston. Administered in large amounts, all the positive lipoids and especially heptanol were seen able to reduce potassium in blood and increase it in the red cells. The negative lipoids have an opposite effect. Administered in reduced amounts, big differences could be see between the agents of the same group, many having no influence on the potassium and only few showing manifest effects. Among the negative lipoids, the most active agents have appeared to be heptyldisenide, sulfur and selenium tetra-hydronaphthalene and epichlorohydrin.

Potassium and Sodium

The changes in the amount of potassium could be connected to its relationship to sodium, and further interpreted in the frame of the hierarchic organization. Both are members of the same series in the periodic chart,



but they correspond to different compartments of the organization. Sodium is the cation of the metazoic compartment, and potassium that of the cellular. They would consequently act differently towards cells, for instance. Under abnormal conditions, sodium is able to enter the cells. For example, following an injury, the cellular membrane which is almost impermeable to sodium, lets it pass through. A penetration of sodium into cells occurs, as demonstrated by the use of radioactive sodium. (42) As a consequence, potassium is released from the cells in order to maintain the necessary osmotic pressure constant. Many abnormal processes occur following the penetration of the sodium in the cells and others occur due to the release of potassium. The sodium which entered in the cells is partly isolated together with water, to form cellular vacuoles. The response of the metazoic compartment to those changes are interesting. Hyperkalemia, with a simultaneous hyponatremia occurs in the first phase of the diphasic phenomenon. The opposite happens in the second phase, when hypokalemia coincides with a hypernatruria.

This antagonism between sodium and potassium is seen further in the pharmacologic activity of these elements. The administration of potassium salts induces a greater elimination of sodium and water, which explains its diuretic action with dehydration of tissues and progressive alkalinization of the urine. The effect upon the different organs is also antagonistic. For instance, in their effect upon the heart, as Merrill and co-workers have shown, the characteristic electrocardiographic effects of hyperkalemia appear only when the sodium level of blood is lowered. (259) A high sodium content prevents the cardiac effects of hyperkalemia.

In opposition to the atrophy of the adrenal glomerulosa induced by an excess of sodium administration, a potassium overdose can cause an enlargement of these zones in rat adrenals.

The hyperkalemia due to external intake such as in potassium poisoning, or due to systemic response to low cellular potassium, specifically influences certain functions. The peripheral vascular collapse with lowered blood pressure, cold clammy skin, pallor, listlessness and mental confusion, are symptoms related to the offbalance D, and encountered in high plasma potassium. They appear in conditions such as shock and burns which correspond to a prolonged first phase of the diphasic phenomenon. Paresthesis and flaccid paralysis (260) (261) are also important results of hyperkalemia. The most important changes appear in cardiac physiology and can be interpreted to correspond to low cellular automatism. Hyperkalemia will thus induce dromotropic positive changes, such as the increase of the duration of the Q R S complex, or that of the P-R interval, with a delay in the ventricular contraction, or a block which can lead to the arrest of the heart in diastole. (262), (263) Characteristically appears the changes in wave T, which increased became even angular. The change seen in offbalance D, are similar to those induced by a prolonged administration of potassium. In a study of the pharmacological activity of various agents upon the heart, made in collaboration with I. Eroglu, we used these changes as an indication of the type of offbalance they induce.

Fig. 216B shows some examples of this effect on rabbits. One group comprises agents considered as able to induce an offbalance D. They induce changes in the T wave which high amplitude is characteristic for a hyperkalemia. An opposite effect is seen in the other group, formed by agents able to induce an offbalance A, and where the T waves are remarkedly depressed. (See page 574)

Potassium and Therapy

All these considerations have led to a more precise use in therapy of the information furnished by the study of potassium distribution. The quantitative deficiency—recognized by low potassium in total blood and serum—is controlled by oral and parenteral administration of potassium. A quantitative excess—with high values in serum and total blood—is treated with the administration of ionic exchange compounds, diet, laxatives and more sodium intake. For the offbalances, the treatment is especially directed to those agents which seem to influence more strongly the cellular potassium metabolism. Selenium lipoids and sulfurized tetra-hydronaphthalene are used for offbalance A, and heptanol for offbalance D.

The fact that the cellular membrane plays an important role in the metabolism through which the abnormal changes in potassium occur, has led us to use agents acting upon these membranes. Adrenaline has been seen to favor the discharge of potassium from the liver cells, simultaneously with glycogen. We thus used adrenaline for cases of offbalance A in which an especially high cellular potassium was present. On the opposite side, the administration of insulin together with an increased intake of glucose was seen to increase the cellular potassium. (258) A similar effect was seen for cortisone, and ACTH, for heptanol, cholesterol and also for the unsaponifiable fractions of organs. These agents have been used in the treatment of offbalance D at the cellular level, where abnormal high values of serum potassium are present.

Chapter 6, Note 1. **Definition of the Lipids**

According to Bloor: "Lipids may be defined as a group of naturally occurring substances consisting of the higher fatty acids, their naturally occurring compounds and substances found naturally in chemical association with them. The group is characterized in general by insolubility in water and solubility in "fat solvents," e.g., ether, chloroform, benzene, etc." (226)

Chapter 6, Note 2. **Definition of Lipoids** (227)

From a physico mathematical analysis of our definition of lipoids, J. Mariani arrives to the following conclusions:

Any substance will behave like a lipoid with respect to a polar solvent when in:

$$\varphi = \frac{1}{2} \sum_{\text{surface}} a_{11}^2 - \Sigma a \quad a_{22} + \frac{1}{2} \sum_{\text{surface}} a_{22}^2$$

φ will be positive. The electrical attractions given by:

$$\Sigma a_{11} a_{22}$$

will not be able to balance the attractive forces:

$$\frac{1}{2} \Sigma a_{11}^2 + \frac{1}{2} \Sigma a_{22}^2$$

due to van der Waals forces (and to the polar groups of A). But we can simplify this definition. Let us consider a solvent the molecules of which are dipolar and form polar groups similar to those contained in the solute. We can call those substances *lipoids* for which the dipole interaction energy in the cavity occupied by the molecule is less than the energy arising from van der Waals forces.
We get:

$$\Psi = \frac{1}{2} \sum_{\text{surface}} \left(a_{11} - \rho \right) - \Sigma \left(a_{11} a_{22} + \rho \right) = \frac{1}{2} \sigma - \xi$$

where now σ represents van der Waals forces in a cavity occupied by the molecule and ξ the dipolar forces at the surface of the cavity.

Chapter 6, Note 3. Separating Membrane Between Aqueous Media

The possibility of having a membrane with polar groups at both surfaces, as shown in mitochondria, represents one of the most important means used in biology to separate two aqueous solutions. (228) One of the characteristics of such a membrane is that often it represents an isolating boundary rather than a membrane with functional activity. This is seen in the fact that very often such a membrane breaks when the two separated aqueous media have to mix.

Chapter 6, Note 4. Fatty Acids Break Down

In a work hypothesis of the biological breakdown of a long chain molecule for caloric metabolism, we consider two factors as being of capital importance: 1) that the result be an even carbon-number chain molecule, since the Knoop oxidation leads to complete caloric utilization only for such molecules, and 2) that the length of the chain of carbons bound to the carboxyl in the new molecule be not higher than 11 carbons in order to permit direct beta oxidation. To fulfill the first condition, the breakdown process does not occur between the carbons of the double bonds themselves, since in natural fatty acids the double bonds separate portions of the chains, usually with odd number of carbons. Such a breakdown would lead to molecules resulting in further incomplete Knoop oxidation.

According to the hypothesis the breakdown of the molecule occurs at the carbon nearest the double bond. Through the energetic influence exerted by the double bond, the even numbered carbon nearby appears strongly positive. As a first step, this carbon was seen to fix a molecule of

oxygen resulting in a hydroperoxide as shown by Farmer and co-workers first for rubber (30) and later for fats in vitro. (31) It is with the passage, in a second step, of this hydroperoxide group into a carboxyl that the breakdown of the molecule occurs at this level, as shown in Figure 242.

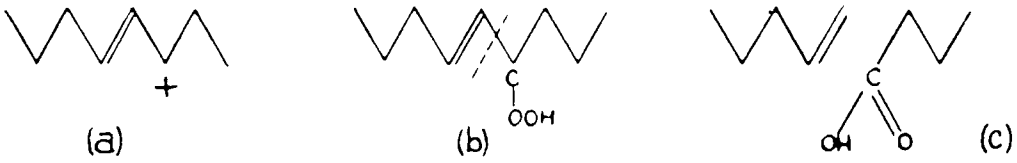


FIG. 242. The oxidative breaking down of a fatty acid molecule (a) occurs in vivo taking place through the appearance of a hydroperoxide at the carbon adjacent to the double bond (b). It leads ultimately to a carboxyl formation (c) at this adjacent carbon and results thus in chains with even number of carbons.

If the even numbered carbon near the double bond is toward the terminal methyl group, a monocarboxylic acid will result. A similar process taking place at the other carbon adjacent to the double bond toward the carboxyl will lead to a dicarboxylic molecule. The metabolic changes in vitro—and also in vivo—have shown the appearance of these two groups of even carbon mono and dibasic fatty acids. By binding two molecules of water the remaining 2-carbon chain linked by the double bond would result in acetic acid molecule. Such changes occurring in the caloric monoethenoids permitted us to explain one of the baffling peculiarities seen in the constitution of the monoethenic fatty acids.

In Note 5, we discuss the position of the double bond in the principal naturally occurring monoethenoids as it follows a characteristic pattern. In molecules with 16 or less carbons the double bond is more often placed so as to separate a group with 9 carbons toward the carboxyl end, while in molecules of 18 carbons or more, the double bond separates almost constantly a group of 9 carbons toward the methyl end. Figure 243 shows two characteristic examples.

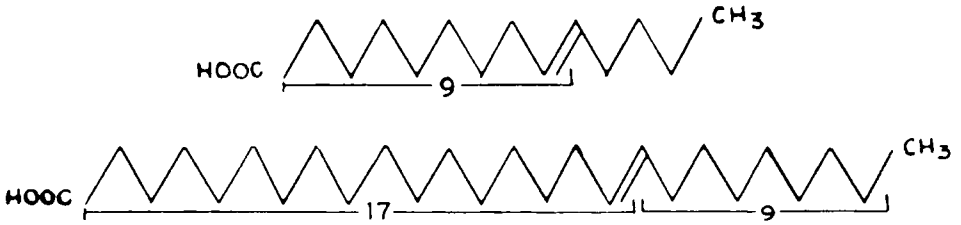


FIG. 243. Characteristic emplacements are seen for the double bond in two monoethenoids. The double bond separates two groups with odd number of carbons. For the myristoleic acid, it separates a group of 9 carbons toward the carboxylic end, and a group with a short 5 carbon chain toward the methyl end. For hexacosenoic acid (26C) a chain with 17 carbons is separated toward the carboxyl end and one of 9 carbons toward the methyl end.

The breakdown of the molecule according to the process mentioned above explains the peculiarity. For the chain with 18 or less carbons, biological fission would result in a diacid with 8 carbons and another monoacid with 8 or less carbons, both subject to Knoop oxidation. In the long chain fatty acid the double bond separating a 9 carbon fraction toward the methyl-end of the molecule will result in an 8-carbon chain as monoacid having the methyl group at the other end. The other part of the molecule, with more than 8 carbons and which corresponds to the long fraction hav-

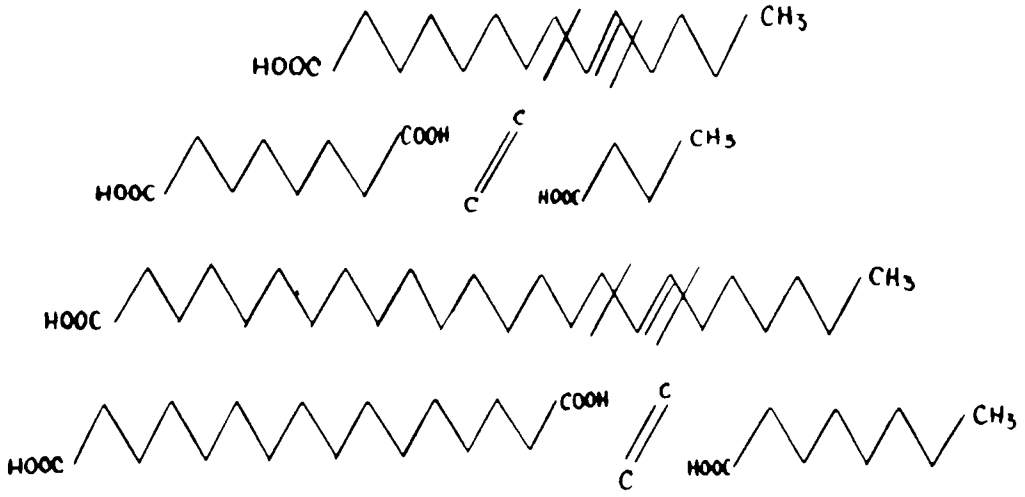


FIG. 244. Through the emplacement of the double bond in the molecules of the monoethenoids, the breaking down of the molecules of fatty acids at the carbons adjacent to the double bond leads to the appearance of a dicarboxylic acid for the part of the chain having more than 10 carbons.

ing the carboxyl at its end, results in a diacid. *(Fig. 244)* This also will be subject to Knoop oxidation, even with a long chain. In the diacid, this process, which is related to the intervention of the carboxyl, can take place at both ends where carboxyls are present.

Chapter 6, Note 5. **Position of the Double Bond in Monoethenoids**

The comparative analysis of the principal naturally occurring monoethenic fatty acids has shown a curious configuration due to the peculiar relative position of the double bond in these molecules. Figure 245 shows the position of this double bond in the principal monoethenoids. (267) Andre has shown that the double bond is more often placed so as to separate a group of 9 carbons ending with the carboxyl. We could show that this group is present toward the extremity ending with the carboxyl especially if the chain has 18 carbons or less. For the longer chain, this group of 9 carbons is present but usually toward the extremity, ending with the methyl. The importance of this configuration for the Knoop beta-oxidation of these acids is discussed in the previous Note.

Similar separation in groups of carbons totaling 9 carbons, are seen in polyunsaturated fatty acids. *(Fig. 246)*

Chapter 6, Note 6. **Saturation–Desaturation Balance in the Liver**

The total number of double bonds does not change in the simultaneous processes of saturation and desaturation occurring in the liver. To a preparation of liver cells, saturated and polyunsaturated fatty acids were added. The iodine number of the fatty acid mixture present was determined, as

Position of the Double Bond in Monoethenic Fatty Acids

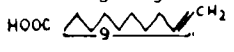
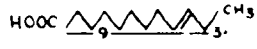
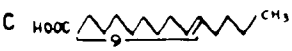
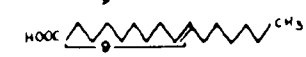
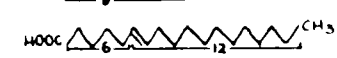
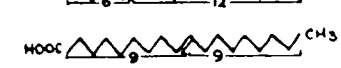
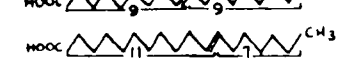
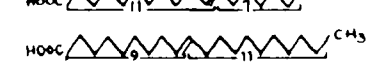
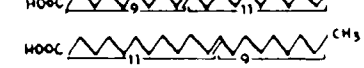
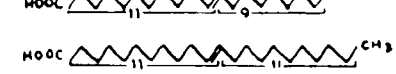
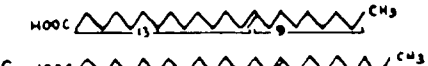
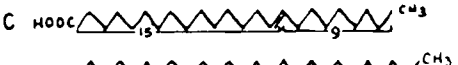
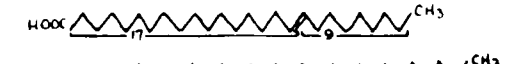
Common Name	Systematic Name	Formula
Obtusilic	Δ 4,5 Decenoic	
Caproleic	Δ 9,10 Decenoic	
Lainoleic	Δ 9,10 Dodecenoic	
-------	Δ 5,6 Tetradecenoic	
Myristoleic	Δ 9,10 Tetradecenoic	
Palmitoleic	Δ 9,10 Hexadecenoic	
Petroselinic	Δ 6,7 Octadecenoic	
Oleic	Δ 9,10 Octadecenoic	
Vaccenic	Δ 11,12 Octadecenoic	
Gadoleic	Δ 9,10 Eicosenoic	
-------	Δ 11,12 Ecosenoic	
Cetroleic	Δ 11,12 Docosenoic	
Eonicic	Δ 13,14 Docosenoic	
Selacholeic	Δ 15,16 Tetracosenoic	
-------	Δ 17,18 Hexacosenoic	
-------	Δ 21,22 Tricosenoic	

FIG. 245. Principal naturally existing monoethenoids show that for the members with a short carbon chain the double bond separates a group of 9 carbons toward the end of the molecule having the carboxyl. For fatty acids with more than 18 carbons, the group of 9 carbons separated is toward the end with the methyl group.

well as the quantity of mono-unsaturated members. After incubation at 37°C, the amount of monoethenic members was seen to have increased greatly. Analysis of the total fatty acids present in the preparation, however,

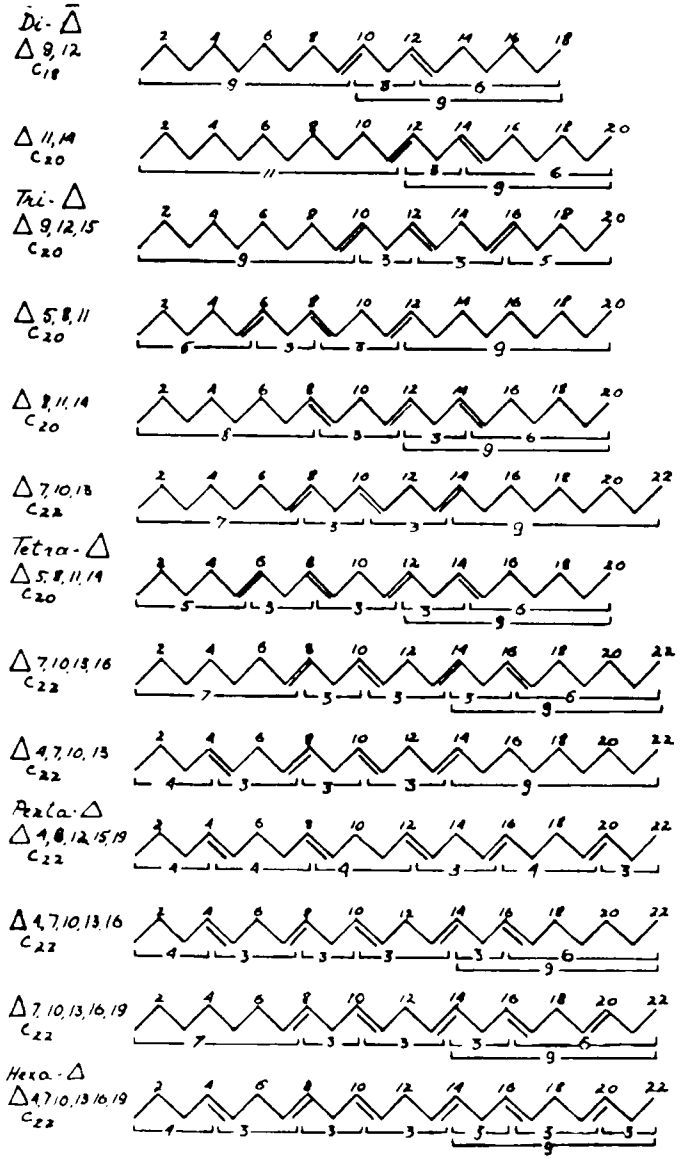


FIG. 246. In the polyethenoids, groups of 9 carbons are recognized, formed usually by the sum of two or three groups, multiple of 3 (3 or 6). The group of 9 carbons is usually placed toward the methyl end of the molecule.

showed the same iodine number, indicating that in the changes which had taken place, the saturation and desaturation processes had compensated each other through a transfer of double bonds from the polyunsaturated to the saturated members.

Chapter 6, Note 7. Essential Fatty Acids

A strong positive character of the carbon of the carboxyl results from its bond to two oxygens. This induces an alternating polarity, with the odd carbons positive in the chain. On the other hand, the influence exerted by a double bond in the molecule corresponds to an enhancement of the proper charge of the adjacent carbons. When a carbon is situated in an intermediary position between two double bonds, the influence resulting from the two double bonds is highly increased. These two factors—positive character as an odd carbon and intermediary position between two double bonds—make C_{11} of linoleic acid a particularly strong positive carbon which appears to be especially able to bond a negative oxygen. We consider this strongly positive methylenic carbon to be the condition which determines whether a fatty acid has the character of an "essential" fatty acid.

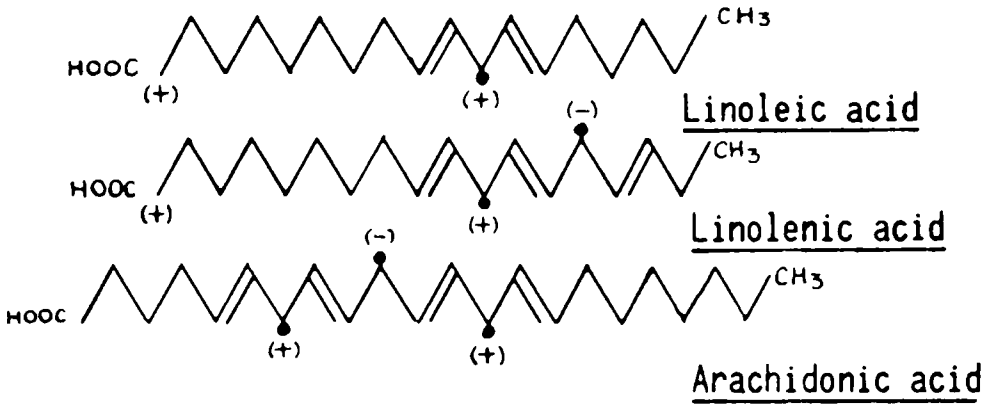


FIG. 247. Relationship between the positive charge of the methylenic carbon and the character of essential fatty acid. The similarity which exists between linoleic and linolenic fatty acids as essential fatty acids, can be explained by the fact that both have only one positive methylenic carbon. Arachidonic acid, with two such positively changed carbons, has this character of essential fatty acids markedly increased.

This explains further a peculiar relationship between the three important essential fatty acids. No differences are seen, from the point of view of activity as essential fatty acid, between linoleic and linolenic acid, although the last has 2 intermediary carbons, one at C_{11} and another at C_{14}. This can be explained by the fact that the second intermediary carbon, the C_{14}, as an even carbon, has a negative electrical character. From this point of view of strongly positive methylenic carbon, there is no difference between linoleic and linolenic acids. The relationship between the character of essential fatty acid and intermediary positive carbon is further confirmed by the fact that arachidonic acid, with two positive and one negative intermediary carbons, is also a more active essential fatty acid than linoleic and linolenic acids, each of which has only one positive intermediary carbon. *(Fig. 247)*

Chapter 6, Note 8D. Fixation of Halogens on Conjugated Double Bonds

The fact that the fixation of halogens at the conjugated double bonds occurs in two steps would explain the relative difficulty in a reverse reaction. As seen for butanediene, the halogen ions are first attached to the external carbons of the conjugated formation with the appearance of a double bond between the central carbons. It is only in a second step that two other halogens are bound to these central carbons too, thus completing the bond of halogens to all the carbons of the conjugated formation. *(Fig. 248)*

FIG. 248. Fixation of halogens at the conjugated double bonds takes place in two steps, with a displacement of the double bond in an intermediary position in the first phase, a fact which explains the nonreversibility of the process.

Chapter 6, Note 8A. Solvent Fractionation of the Lipidic Constituents

In studying the biological role of the lipids, we recognized the importance of the forms under which the different lipids are present in the body, forms which seem to determine greatly their activity. A lipid changes its reactivity when it passes from the free form to one bound to other constituents. A first step in this study was the analytical separation of these forms. We distinguished thus four fundamental forms for acid lipids as well as for unsaponifiable fractions:

I. Free lipids, or those bound in such a labile physical form as to be able to take part directly in different reactions through their polar groups.

II. Lipids bound in cenapse with other constituents, that is, in a relatively labile form.

III. Lipids in combinations through their polar groups as esters or fats. This form represents usually a reserve or an inactive circulating form which can become active through hydrolysis.

IV. Lipids bound so firmly to other constituents as to be inseparable through solvents and to need saponification of the material in order to be liberated.

The first form would represent the functional form, the second a rapid available functional reserve, and the third, a reserve. The last would represent a stable combined form as part of the building of entities themselves.

We have utilized the differences in solubility of these various forms of lipids in order to separate them from the material to be studied, and thus to study their intervention in different normal and abnormal conditions. It must be emphasized that this separation concerns only the form under which the lipids are present in the organism and not their chemical constitution.

In spite of only a relative degree of accuracy in some separations, the

differences noted from one sample to another are so manifest and so consistent that this method can be considered as an interesting and reliable source of information. We have, therefore, used this technique of separation for thousands of samples through the years.

According to the technique we devised, the material to be analyzed—tissues, organs, entire organisms or only biological products—is finely divided in a blender. It is then extracted several times with ether under stirring, or in a Soxhlet apparatus. Under these conditions, ether removes the lipids present in the form of free lipids and neutral fats. This represents a mixture of the fractions I + II. The residue is again extracted, this time with a mixture of 10% ethyl alcohol in ether which breaks the cenapses and separates the lipids previously bound in cenapse. The result represents fraction III. The residue is saponified with 10% KOH and extracted with ether. This represents fraction IV US or the unsaponifiable lipids of fraction IV. After acidification with tartaric acid, a new extraction is made which represents fraction IV LA or the acid lipids of this fraction.

The ether of fraction I + II is distilled and the residue treated with 85-90% alcohol concentration which dissolves selectively the free lipids as fraction I, and leaves the part formed by neutral fats as fraction II.

Fractions I, II and III are then saponified separately. The unsaponifiable fractions are extracted with ether, giving respectively the fractions I US, II US and III US. After acidification with tartaric acid, other extractions with ether are made which respectively represent fractions I LA, II LA and III LA. Each of these fractions is washed with distilled water, dried with anhydrous sodium sulfate and, after the ether is distilled off, the fractions are weighed.

For each material, we obtained thus four different fractions for the unsaponifiable part which we called the US fractions, and four for the saponifiable called the LA fractions. They correspond respectively to: I, free lipids; II, lipids present as esters or fats; III, lipids bound in cenapse; IV, lipids fixed in combinations which are liberated only through saponification. In the following examples, we chose different materials to illustrate the kind of information obtained through this method. Fig. 249 represents the results obtained in normal rats, in rats under abnormal conditions, as well as in tumors, all expressed as the 8 lipidic fractions.

In order to facilitate the comparison between normal and abnormal conditions, we chose for this example, rats between 180 and 200 gms. of weight, all males with one exception (case b). The data obtained were thus compared with that obtained for case (a) which corresponds to a normal male rat killed by ether. The analysis for the lipoacid fractions of this case shows a fraction (IV) at 3.5 mg./per gram of animal, the fraction III in cenapse at .2 mg./per gram, II corresponding to fats at 1.2 mg./per gram and the fraction I as free lipids at 8.1 mg./per gram of animal. For the unsaponifiable fraction, the fixed part IV is at 0.8 mg./per gram of animal, the III in cenapse at 2 mg., the II as fats at 0.8 mg., and the free part I at 7 mg./gram of animal.

The female rat (b) shows in general, lower values for the acid frac-

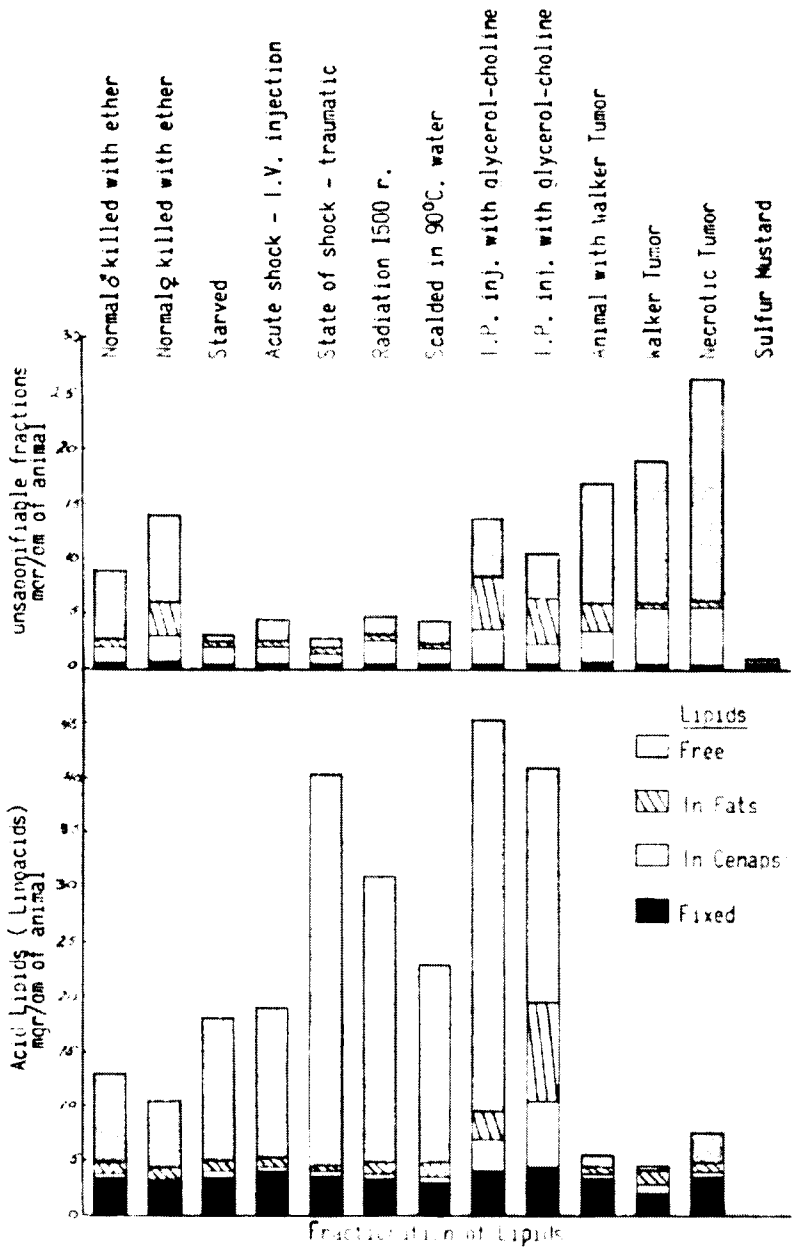


FIG. 249. Solvent separation between different lipoacids and unsaponifiable fractions in various animals and tumors. The values expressed in mgr/gr of animal show big variations concerning especially the free lipids. (Top fraction)

tions and higher for each fraction of the unsaponifiable part. It is interesting to note that the fixed part (IV) in acid lipids is lower than in any male, a fact confirmed by other analyses. The increase in unsaponifiable part concerns especially the cenapse, fats and free lipid fractions. These differences are in agreement with the data concerning the relationship between lipids in males and females, as discussed in Chapter 6.

It is interesting to note the changes in starved animals as shown in case

(c) where a marked decrease in the unsaponifiable fractions is seen, but with an increase however, in the acid part, especially in the free fraction (which reaches values of 13 mg./1 gm.). Similar changes are seen in acute shock (d) induced by the intravenous injection of a rich culture of Esch. coli in broth. Although death occurred in less than 40 minutes, a marked increase in free fatty acids is seen with a corresponding decrease in the unsaponifiable fractions. The deviation from normal in the same direction appears still more manifest for the animal in a traumatic state of shock (induced by 700 falls in the Collip-Noble drum), (e). The dying animal showed a marked increase in free fatty acid (37mg./1 gm.) with a notable reduction in the unsaponifiable fractions. Cases with over 70 mg./1 gm. of free fatty acid were also found. A lethal dose of radiation (1500r) (f), and caloric burns (g), were seen to induce similar changes.

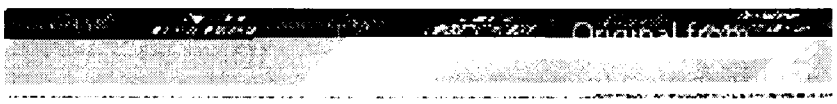
Also of interest was the influence exerted by the intraperitoneal injection of glycerol and choline, with an impressing increase in all the fractions of the acid and unsaponifiable lipids, and of those in cenapse and fat fractions in (h). We chose example (i) of another animal submitted to the same treatment to show the extent of the concordance of the information furnished by this method.

It can be seen that almost under all conditions, the amount of fraction IV as obtained through saponification, changes very little. The amount present in cenapse and as neutral fats show more variations. Varying considerably from one case to the other, are the free lipids as fraction I which thus could be connected more directly with a pathogenic intervention of the lipids.

Another conclusion can be drawn from the analysis of these results. The high amount of free lipids often obtained under abnormal conditions cannot be considered to result only from a liberation of these lipids from the pre-existing reserve as neutral fats or from the more labile cenapse form, since the total amount of the free lipids found is much greater than the sum of these forms. An appearance of new lipids, through synthesis, has become evident in these conditions.

Example (j) is the analysis of an animal with a 12-day old Walker tumor. Comparison with the normal controls shows a reduction of the acid lipidic part, with an increase in all the four fractions. The study of the tumor itself (k) shows still greater differences. The fixed fraction is smaller than in the total body of the animals, while the cenapse part is greatly increased. The free lipoacidic fraction is almost nil. For the unsaponifiable fractions, that in cenapse, and especially that corresponding to free lipids, is greatly increased. In the necrotic tumor all four lipoacid and unsaponifiable fractions are increased. The latter however, to a greater degree. Though the cenapse amount is high, an increase is most evident in the free fraction. Case (m) of an animal treated with sulfur mustard applied on the skin, appears particularly interesting. The animal, dying the 14th day, has almost no unsaponifiable fractions left (less than 1 mg./1 gm.).

We used this method extensively throughout the years, in spite of its one limitation—the imperfect separation of neutral fats from free lipids.



The great concordance for all variations however, has practically overruled any objection of a failure of the separation of these free lipidic fractions.

Chapter 6, Note 8B. **Spectral Analysis**

In collaboration with Carlos Huesca-Mejia and Priscilla Teitelbaum, we studied several thousand samples of lipid preparations through spectral analysis, in ultraviolet and first portion of visible light, and more rarely in infrared, using the Beckman spectrophotometer. We will limit ourselves to note here only some of the principal conclusions reached.

1) Concerning the chemical isomerization procedures, we could show the importance of the temperature used when a mixture of fatty acids is treated. The conjugation in vitro as it is usually carried out, with ethyleneglycol or glycerol as solvents, was seen to result in preparations with too low amounts of tetra-, penta- and hexaenic conjugated members. A relatively rapid disappearance of the conjugated formations with 4, 5 and 6 double bonds was seen to be induced by the high temperature used. This led us to utilize a new method of conjugation, at lower temperatures. Using ethyl alcohol as solvent, preparations with high conjugated formations were obtained.

2) We utilized the spectral analysis for quantitative determination not only for di-, tri- and tetraenes as usually employed, but also for pentaenes and hexaenes. For this purpose we determined the extinction coefficient corresponding to these pentaene and hexaene formations. This was made possible by isolating the respective pentaenic and hexaenic conjugated members through appropriate solvents.

3) We studied various materials and especially different organs in order to correlate their richness in different fatty acids to their biological activity, by using the spectral analysis of the in-vitro conjugated fatty acids, as mentioned above.

4) Similarly, we tried to correlate the existence of characteristic peaks in the spectral analysis curve of unsaponifiable fractions of organs to their biological activity.

5) We utilized spectral analysis for the study of the effects of various agents such as chlorine, sulfur, sulfuric acid or oxygen upon the conjugated fatty acids.

6) We showed that minimal changes are induced in the nonpolar groups of conjugated fatty acids by changing their polar group from carboxyl into a primary alcohol, by treatment with lithium-aluminum hydride.

7) We studied the influence exerted by conjugated fatty acids upon carcinogens. This can be partially revealed by the quenching action induced upon the fluorescence of these latter agents.

8) In an extensive study we investigated the influence exerted by radiation upon fatty acids in-vitro and in-vivo. This influence was characterized by the appearance of conjugated trienes, and is presented in Chapter "Radiation," and in other Notes.

Chapter 6, Note 8C. Vapor Fractionation of Fatty Acids

In a group of experiments we applied the gas chromatography method to the study of fatty acids. The principal aim was to investigate the value of the information furnished by this method concerning the presence of conjugated fatty acids. This study was made in collaboration with Ivan Bier and with Winston Dindial who prepared the samples.

Methyl esters of eleostearic acid, linoleic acid and its conjugated isomers; of linseed oil fatty acids and the conjugated preparation; of cod liver oil fatty acids and the conjugated preparations; and of samples of fatty acids obtained from animals and tissues under normal and abnormal conditions were obtained. We analyzed through vapor fractionation, these different preparations as such, the preparations obtained through condensation on cold fingers during distillation in vacuum at different temperatures, and the different fractions obtained through distillation in vacuum. For all these tests we used the Perkin-Elmer vapor fractioner with a column of succinyl polymers heated up to 235°C. Under these conditions, no differences could be seen between the respective conjugated and nonconjugated samples. Figs. 250 and 251 show examples of such analyses of a cod liver oil fatty acid preparation and of a preparation obtained after treatment with KOH in butyl alcohol. Fig. 252 shows the spectral analysis of this last product.

Under the condition of analysis used, the gas chromotography method does not permit the identification of the conjugated isomers present. This is the reason why the analytical method could not indicate the presence of such members in materials obtained during abnormal conditions. The conjugated isomers can be identified by other methods—such as spectral analysis and especially oxalic index—after oxidative fission.

We are now trying to obtain columns that would permit working at much higher temperatures and would permit us to identify these conjugated fatty acids. In view of the minimal amount of material needed for analyses and the precision of the results usually obtained, an adaptation of this method for the identification of conjugated members would be of especial great value.

Chapter 6, Note 9. Twin Formation

The odd number of carbons in a cyclic molecule represents one of the conditions which always would result in the appearance of a twin formation, since the alternation between positive and negative signs gives the same electrical charge to two nearby carbons. *(Fig. 254)* The correlation of the positive and negative charges of nearby carbons to the fact that acetic acid molecules have been utilized in the synthesis of the molecule represents an additional factor for inducing twin formations even in cycles with an even number of carbons.

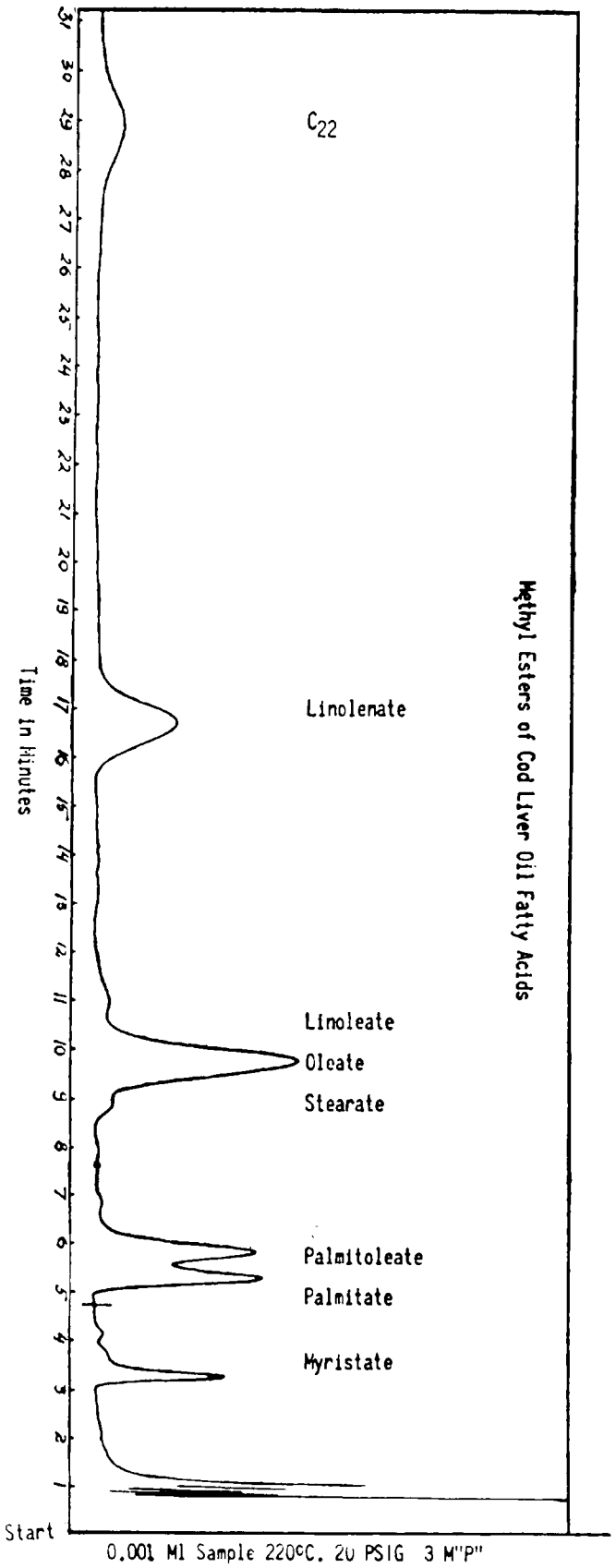


FIG. 250. Gas chromatographic analysis of a sample of cod liver oil fatty acids showing some of the different constituents.

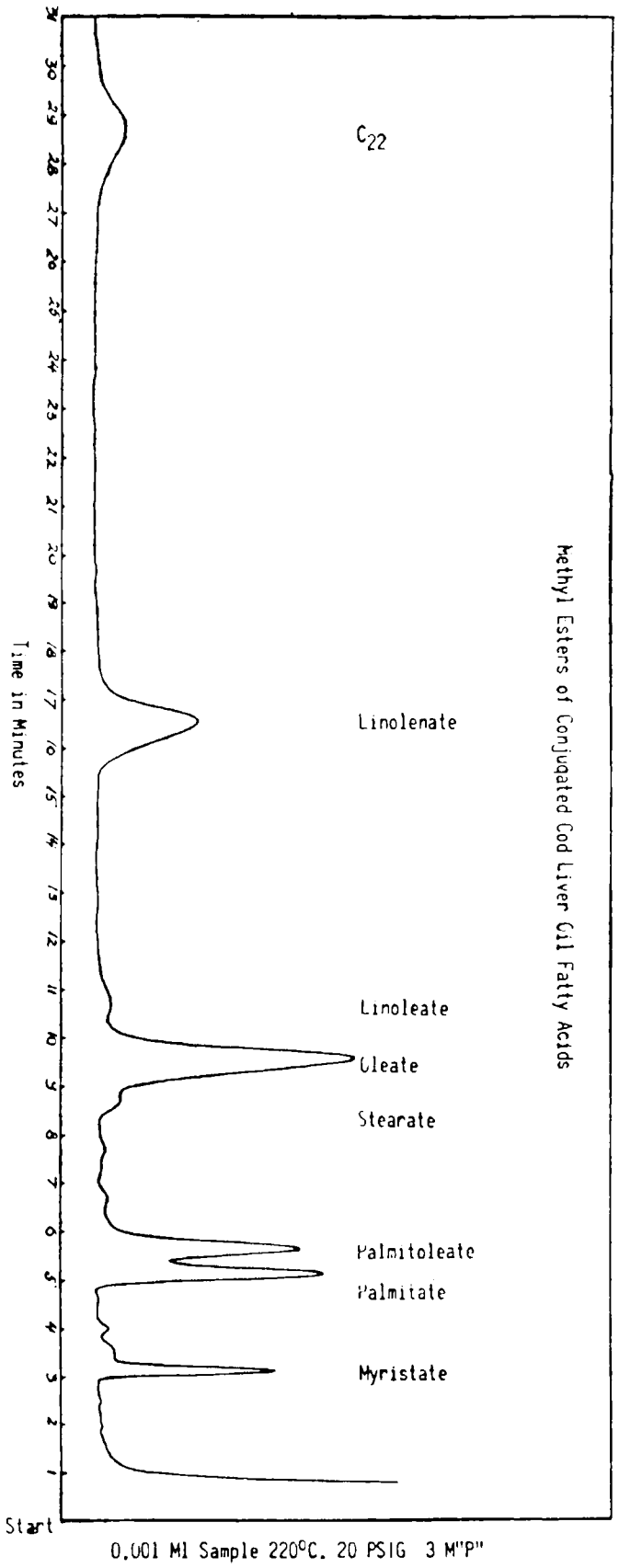


FIG. 251. Gas chromatographic analysis of a sample of the fatty acids of cod liver oil after chemical conjugation. No differences are seen between the curve of Fig. 250 and this curve, indicating that in the conditions under which the analysis was made, the conju

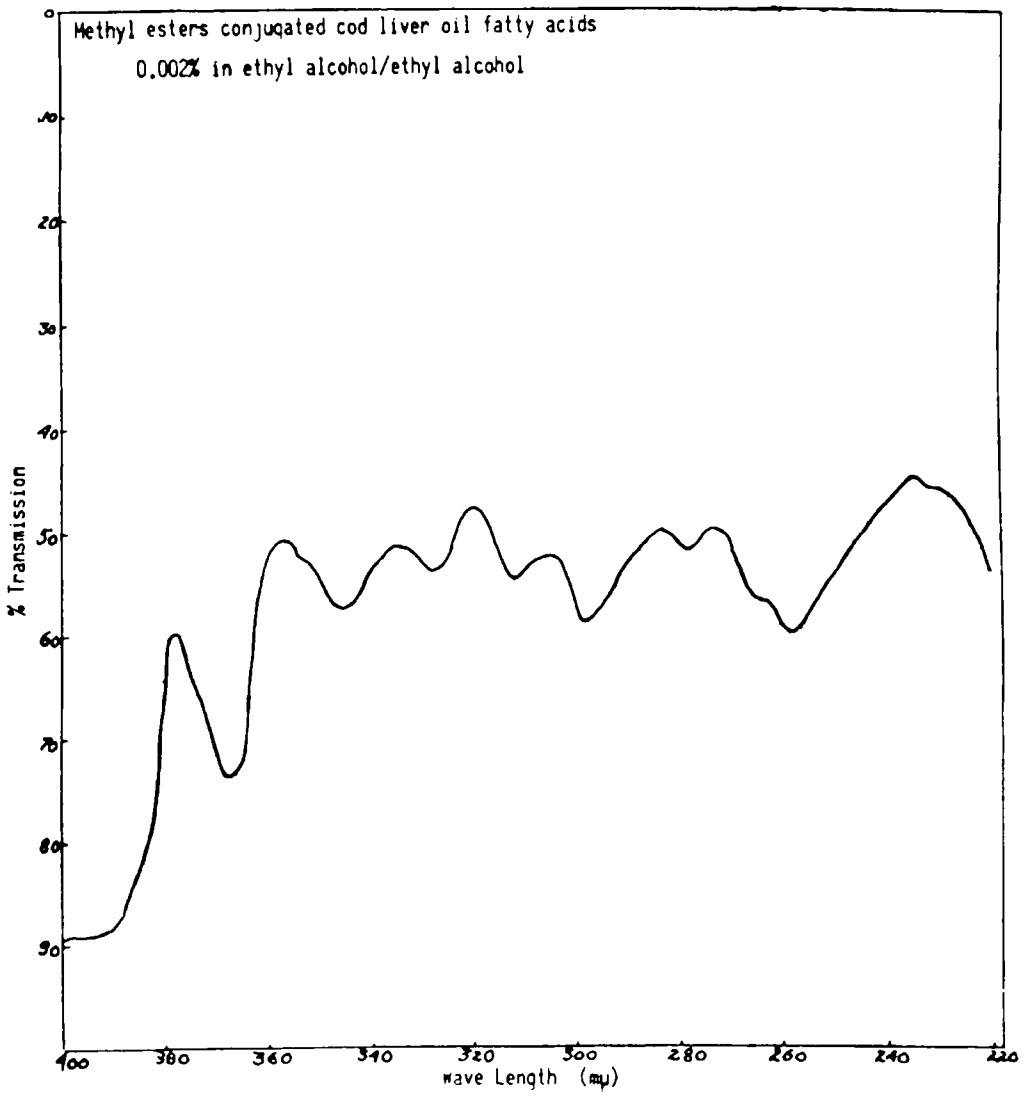


FIG. 252. Spectral analysis of the sample used for gas chromatography, shown in FIG. 251, indicating the presence of di-, tri-, tetra-, penta- and hexaenic conjugated members.

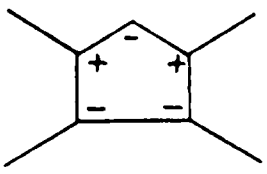


FIG. 254. A twin formation appears in cyclopentane as a result of the alternate sign of adjacent carbons.

Chapter 6, Note 10. Arachidonic Acid–Sterol Relationship

The relationship between arachidonic acid and sterols, both of which are present in the adrenals, has been followed through the changes which occur in the amount of these substances in the adrenals during certain pathological processes. Rabbits were slowly injected intravenously with a suspension of microbes until symptoms of deep acute shock appeared. The animals were sacrificed immediately by bleeding them and the adrenals and blood analyzed. The amount of polyunsaturated fatty acids of the adrenals had markedly decreased, and, in some animals, had almost disappeared from these organs. At the same time, the amount of the same fatty acids of the circulating blood had manifestly increased. The amount of insaponifiable fractions and sterols in the adrenals was unchanged as compared with controls. It seems that fatty acids have passed from the adrenals into the general circulation as a first response to the noxious intervention.

Chapter 6, Note 11. Steroids Deriving from Arachidonic Acid

The study of the relationship between steroids and certain fatty acids led us to the hypothesis, according to which some steroids are derived from fatty acids themselves. Although the synthesis of cholesterol from scalene (230) is highly plausible, it would not represent the only origin for all the steroids. The members with a two carbon chain would derive from other substances. We present this hypothesis here because it also represents an example of another important role played by the double bonds which appear to intervene in the process of cyclization in the organism.

According to the hypothesis which we have advanced, polyunsaturated fatty acids lead to allopregnane, the parent steroid with a two carbon lateral chain. Figure 255 shows the different phases of such a transformation starting from arachidonic acid. The presence of a carboxyl and four double bonds in the arachidonic acid molecule results in some of its carbons having a particularly strong energetic value. The strongly charged carbons are: 1) C_1, through its bond to = O and – OH; 2) C_2 and C_3, through an induction process since they are close to the carboxyl; and 3) C_5 and C_6, C_8 and C_9, C_{12} and C_{13}, and C_{16} and C_{17}, respectively bound by double bonds.

Due to alternate induction, all the odd-numbered carbons have a positive sign and the even-numbered have a negative one, as indicated in Figure 255a. Because of the high flexibility of the aliphatic chain, and the presence of carbons with strong positive and negative character in the same molecule, attractions between the strongly charged carbons with opposite electrical signs in the same molecule would occur. This would lead, as a first step, to a bending of the chain so that the strongly charged carbons with opposite signs would face one another. *(Fig. 255b)*

In a second step, as these carbons are bound by double bonds, the respective π electrons of the double bonds would serve to form a new

bond between the facing carbons and thus to close cycles. This would occur without any loss or gain of electrons. The three double bonds between C_5 and C_6, C_{11} and C_{12}, and C_{14} and C_{15} would serve to close the three cycles. *(Fig. 255c)* The double bond between C_8 and C_9 would serve to make C_9 highly reactive. It can be seen that C_9 of arachidonic acid corresponds to C_3 of cyclopentanophenanthrene, which explains why this carbon has a high positive character, with an oxygen fixed on it. The carbon of the carboxyl could be used either to form the methyl group at C_{13} or, more plausibly, could be lost in a process of decarboxylation, which by itself, in this case, would induce the bond between C_2 and C_{18} of the arachidonic molecule. Through the intervention of the strong energetic center, the pentanic cycle would be closed and would have two carbons with the same charge. *(Fig. 255d)* The two methyls, corresponding to C_{18} and C_{19} of steroid molecules, would result from a further process of methylation after the polycyclic molecule was formed. C_2 and C_6 of arachidonic acid would be especially likely to have a methyl group fixed on them through their electronic displacement due to the new bond.

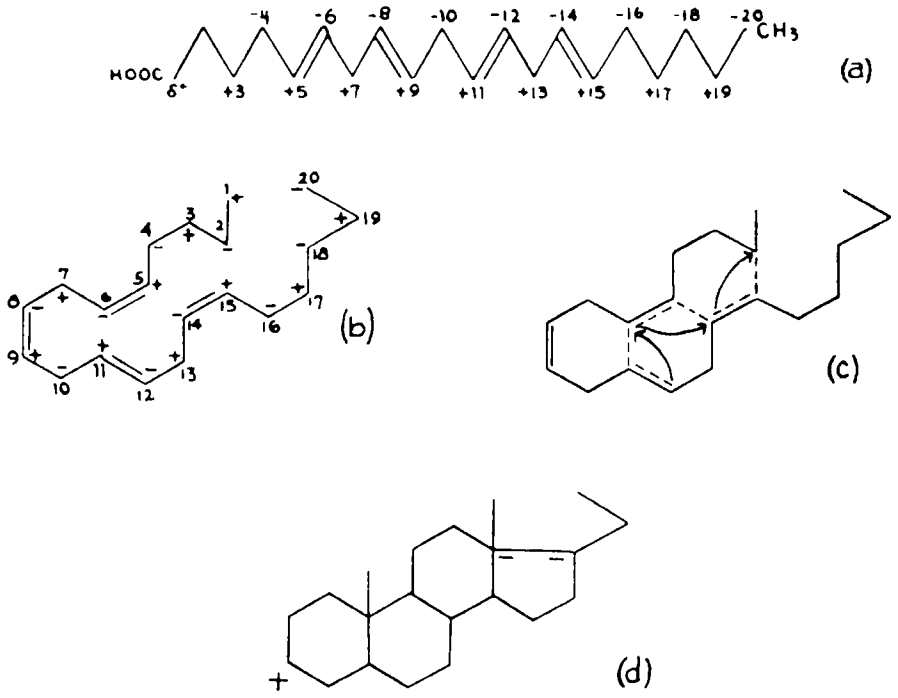


FIG. 255. Hypothesis of the synthesis of the allopregnane radical from arachidonic acid. Fig. (a) shows the relative position of the double bonds in the arachidonic acid molecule. Fig. (b) shows how the molecule bends, due to the attraction between the energetically oppositely charged C_2 and C_{15}, C_5, and C_{14}, and C_8 and C_{11}. In Fig. (c) the cycles are closed with the electrons furnished by the double bonds. (d) The closing of the cyclopentane occurs with the appearance of a twin formation. The electrons of the double bond between C_8 and C_9 become available at C_9 (C_3 of allopregnan) to realize the bond with an oxygen at this carbon.

This hypothesis explains the biological relationship between arachidonic acid and corticoids. The adrenals are especially rich in both. The synthesis, which occurs with a minimum change in the richness in electrons or atoms, explains two of the most important characteristics of these corticoids, the high energetic value of C_3 and of the cyclopentane with its twin formation. Preliminary experiments seem to show that, in a preparation of adrenal tissue, arachidonic acid can be transformed to corticoids under the influence of the adrenocorticotrophic hormone of the hypophysis (ACTH).

Chapter 6, Note 12. **Steric Coupling**

Through reciprocal influence, the energetic centers present in the nonpolar parts of two molecules which combine, largely lose their activity. Steric coupling is possible if the two opposite molecules, once bound through the combination of their polar groups, also adhere together through their nonpolar groups via the opposing energetic centers or formations present in the two molecules. In this way, steric coupling completes the partial reciprocal neutralization of the molecules obtained through the combination of polar groups. The bond between the polar groups that keeps the two molecules in a reciprocal position is thus an important condition for steric coupling.

Chapter 6, Note 13. **Luteoid Function**

It appears that the luteoid pattern can be correlated to a specific aspect of the energetic picture of this substance—the presence of two relatively strong nucleophilic centers in the characteristic opposite positions, one at C_3 and the other at C_{20}, as in progesterone. In fact, any change in the energetic picture of this steroid will decrease luteoid properties. The relationship of the luteoid property to the nucleophily at C_3 is easily seen. The lack of the nucleophilic center at the carbon 3, as in pregnane one 20, *(Fig. 256a)* leads to an inactive substance, the energetic picture being entirely changed. A single nucleophilic center thus appears to be insufficient for the luteoid property.

The substance becomes inactive if, instead of a nucleophilic center through = O at the carbon 3, an electrophilic center is present, as in the pregnane ol 3 one 20, so that the energetic picture no longer corresponds to the luteoid pattern. *(Fig. 256b)*

Any change in the nucleophily of the C - O center at C_3 will decrease the luteoid properties of the substance. The presence of a second double bond between carbons 6 and 7, as in the 4, 6 pregnandiene-dione 3-20, *(Fig. 256c)* in spite of the fact that it will increase the value of the nucleophily of the carbonyl at the carbon 3, will change the energetic pattern and thus also decrease the luteoid character. On the other hand, the reduction of the nucleophily of the center produces, through a similar influence upon the energetic pattern, a decrease in the luteoid property. In the case of pregnane-trione 3-6-20, *(Fig. 256d)* the lack of a double bond

in the cycle and the reciprocal induction realized by the parallel double bonds of the two carbonyl, will reduce the nucleophily of the C_3 center, and with it, the luteoid properties. This also is true for Δ 5 pregnene-dione-3-20-ol 6, *(Fig. 256e)* where the presence of the hydroxyl at the C_6, as well as a nonparallel double bond between C_5 and C_6, decreases the ionic character of the C = O at the carbon 3. In Δ 5 pregnene-dione 3-20, *(Fig. 256f)* where only the double bond between carbons 5 and 6 is changed, being opposite to that of the carbonyl at the carbon 3, the ionic value of C = O at C_3 is decreased instead of increased. The luteoid property seems to have almost disappeared.

The second condition for the luteoid property is the nucleophilic center at C_{20}. Any change in its character or value will influence the luteoid property of the substance. Androstandione, *(Fig. 256g)* with a nucleophilic center at carbon 3 similar to that of progesterone, but with an = O directly at-

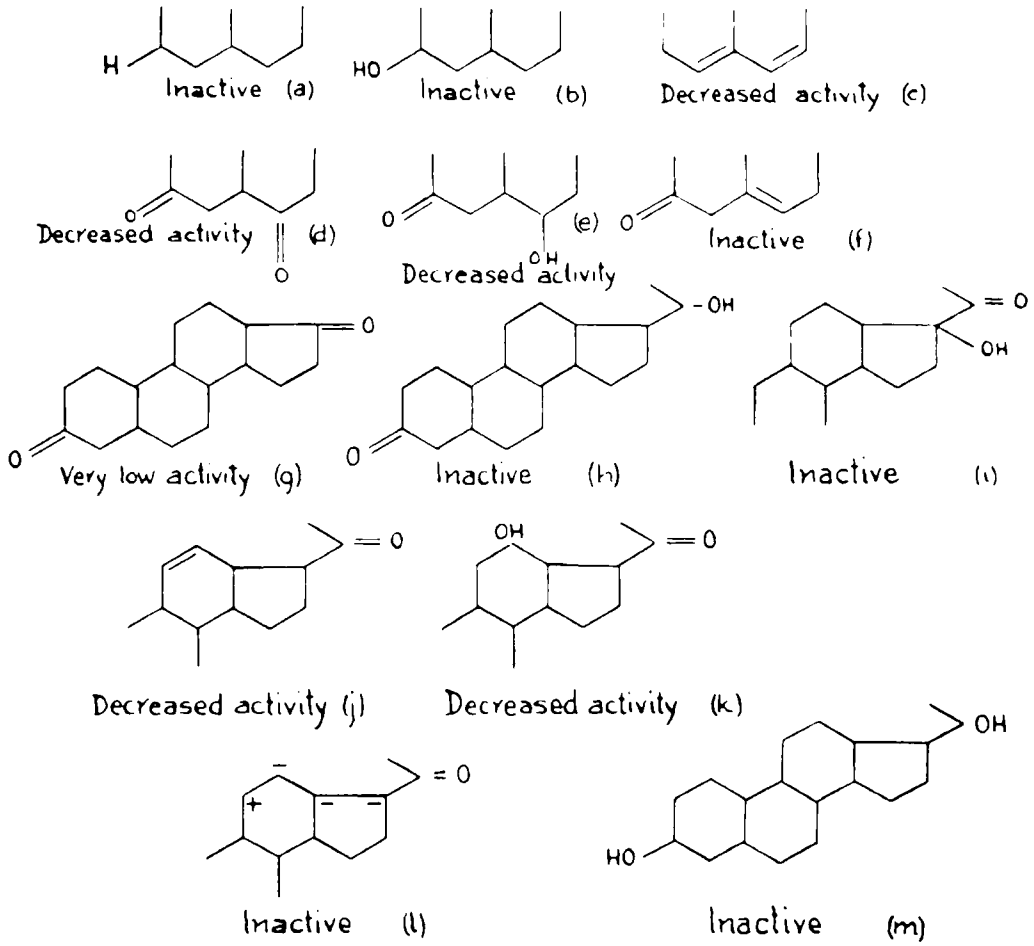


FIG. 256. Any change in the energetic characteristics of the centers which appear related to the specific luteoid activity leads to a decrease or disappearance of this activity.

tached to carbon 17 and without any side chain, presents an energetic picture with two nucleophilic centers. But the nucleophilic center = O attached directly at carbon 17, gives a different character to this part of the molecule. The substance thus has only some luteoid properties. Δ 4 pregnene-one 3-ol-20, *(Fig. 256h)* in which the carbonyl at carbon 20 is changed by a hydroxyl, is, on the other hand, inactive, the substance having a picture different from that of the dinucleophilic pattern. Also inactive is Δ 5 pregnone—dione-3-20-ol 17, *(Fig. 256i)* in which the presence of the hydroxyl at carbon 17 changes the energetic picture at this extremity of the steroid.

The presence of a double bond between carbons 11 and 12 *(Fig. 256j)* will decrease luteoid activity through an induction and field influence between the nonparallel double bonds and a decrease of the ionic character of C_{20} bound to oxygen. The influence exerted by an hydroxyl in the neighborhood of carbon 17 is interesting. Through an inductive effect, the OH attached at carbon 12 *(Fig. 256k)* changes the ionic value of the C = O attached at carbon 20 and thus decreases luteoid activity. An hydroxyl bond to carbon 11 has a much more intensive effect, changing the ionic character of the bonds between it and the = O and thus inactivating the luteoid property. *(Fig. 256l)*

For most of the steroids with = O centers, metabolism leads to a change of this center in an hydroxyl. The product of the change of both C = O into C = OH, which represents the form in which this hormone is excreted, is inactive. Pregnane-diol 3-20, *(Fig. 256m)* with a structure far removed from the pattern required for luteoid activity, has no luteoid properties.

Chapter 6, Note 14. **Energetic Center in Steroids**

Energetically, as a consequence of the presence of a nucleophilic center in this situation, there is a strong positive charge for the carbon 20 of the chain itself. Consequently, a strong negative charge appears for carbon 21. The ionic value of the bond linking OH to C_{21} is thus increased. With the reactivity of this radical increased, the electrophilic character of this OH center becomes still stronger. The respective activity of two opposed energetic centers near to each other is increased inductively.

Chapter 6, Note 15. **Relationship Between Corticoids**

Corticoids can be viewed in terms of changes taking place in the general metabolism in the organism. In the evolution of these steroids, a process of oxidation would intervene at the principal energetic centers. To the hydrocarbon, first an hydroxyl and then an oxygen would be attached. An inverse process of reduction would take place as a metabolic process, the OH being the usual form through which steroids are eliminated, often bound to glucuronic acid. This process of oxidation would occur at C_{11}.

Attaching an OH to desoxycorticosterone (Δ 4 pregnene-21-ol-3:20-

dione), would lead to the appearance of corticosterone (Δ 4-pregnene-11:21-diol-3:20-dione). A further oxidation would change this OH into an O, resulting in dehydrocorticosterone (Δ pregnene-21-ol-3:11:20-trione). All these are mineralocorticoid compounds.

With a further change, this time at C_{17}, where an hydroxyl would be attached, all three compounds—desoxycorticosterone, corticosterone and dehydrocorticosterone—display neoglucogenic properties. They represent 17 hydroxy derivatives as 17 hydroxy-desoxycorticosterone (Δ 4-pregnene-17-9 (beta):21-diol-3:20-dione); 17 hydroxycorticosterone (Δ 4-pregnene-11 (beta):17 (beta):21-triol-3:20-dione), or compound F; and 17 hydroxy-11 dehydrocorticosterone (Δ 4-pregnene-17:21-diol-3:11:20-trione), or compound E or cortisone.

The fact that the presence of an hydroxyl at C_{17} greatly changes the properties of the entire group makes it likely that the energetic formation of which C_{17} is a part intervenes in the specific activity of these substances.

The analysis of the constitution of the corticoids has further shown that they have the characters of lipoids—being polar-nonpolar substances—with the nonpolar group predominant. The members studied, mineralo- as well as neoglucogenic corticoids, have been shown to induce a change toward lower values in the second day wound crust pH, indicating thus a tendency to induce an offbalance of the type A.

Chapter 6, Note 16. The Template Hypothesis

Figure 257 shows the template formation in cortisone, which extends from C_{11} to C_{21}. Each one of these six carbons will attract a carbon from the radical in front of it. The energetic character of each of the six carbons of the template will determine the electrophilic or nucleophilic character of the carbon so attracted. This attraction is easily induced when acetic radicals, with an electrophilic and a nucleophilic carbon, form these groups. Furthermore, the value of the carbons of the template also will determine which polar radical will be bound to the respective carbon kept in front of it. In general, the carbon kept in front of a carbon of the template will have an opposite electrical sign. The polar group bound to the carbon kept in place will be opposite in sign to the polar group bound to the carbon of the template. When the first polar group takes a position parallel to that of the polar group of the template, both will have the same electrical sign.

It can be seen that C_{21} within the template has an OH, the group being electrophilic. This will cause the carbon kept in front to preferably bind an oxygen, realizing a nucleophilic center. C_{20} of the template, which corresponds to a carbonyl, represents a nucleophilic center. With an oxygen bound through a double bond, it has strong reactivity. The positivity of C_{20} also is highly enhanced through its bond to the two strongly negative carbons, C_{20} and C_{17}, each being bound respectively to a hydroxyl. With this high positivity, C_{20} will induce a strong reactivity in the carbon kept in front of it. This will be strong enough to bind a radical energetically opposite to oxygen and stronger than the hydroxyl; that is, an amino group.

The special position of the OH bound to C_{17} as related to the template will induce the carbon kept in front of it to bind another hydroxyl. The same applies to carbon 13. This is the result of the relatively strong molecular reactivity of these two carbons, due to the twin formation which they

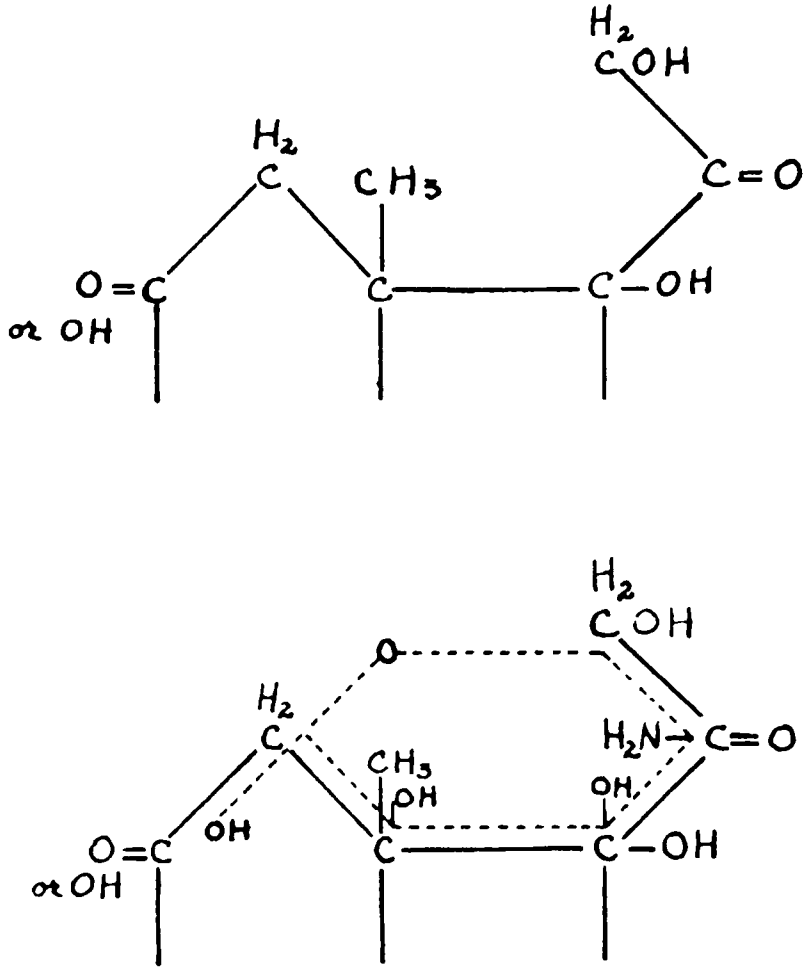


FIG. 257. Hypothetical view of the template formation between carbon 11 and carbon 21 of the corticoid molecule. The groups kept in front of the different carbons of the formation serve to synthesize new substances. In the example presented above, the template of the cortisone molecule would lead to the appearance of a glucosamine molecule.

realize. The methyl bound to C_{13} will determine the steric position of the hydroxyl bound to the respective carbon kept in front of this carbon.

The effect of carbon 12 is different. It is highly influenced by the twin formation and has an opposite energetic character to carbons 13 and 17, consequently, it will favor the binding of the carbon kept in front of it to an oxygen which is the same which binds the carbon in front of C_{21}. Positionally, the carbon in front of C_{12} also will be nearest to the C kept in

front of C_{21}, which also was induced to bind an oxygen as seen above. This will make it possible for a similar oxygen to be bound by the two carbons kept in front of C_{12} and C_{21}, thus closing an hexagonic cycle. The radical bound to carbon 11 will cause the carbon in front of it to bind an opposite radical. In the cases in which C_{11} has an oxygen bound to it, as in cortisone, the carbon kept in front of it will bind a hydroxyl. The presence of an hydroxyl bound to C_{11}, as in hydrocortisone, will cause a carboxyl to be bound to the respective carbon kept in front of it. It is thus seen that the synthesis induced by the template formation of cortisone will result in a molecule of glucosamine, while for hydrocortisone, the synthesized molecule will correspond to glucosaminic acid.

Chapter 6, Note 17. **Adrenal Defense Index**

E. F. Taskier in collaboration with the author, has studied the intervention of adrenals in the defense against various fatty acids. (231)

It is known that the adrenals have an important role in the defense of the organism against noxious agents, a normal animal being more resistant to toxic effects than an adrenalectomized one. Systematic study of adrenal intervention has shown that it occurs with a certain degree of specificity.

The method of investigation used was the following: inbred Wistar rats of the same sex and approximate body weight of 150 gr. were adrenalectomized. The surgical procedure was carefully standardized, lasting less than two minutes, and producing a minimum of trauma. This was made possible by the Noyes Fixation Forceps which is utilized in ocular surgery and is well suited to pick out the adrenal gland without damage to it or to neighboring tissue. As controls, animals of the same weight and sex were sham-operated. On the third postoperative day, the agent to be tested was injected intraperitoneally. At this time, the organism had recovered from the immediate traumatic effects of the operation and no manifest adrenal deficiency symptoms were as yet present. Only those deaths occurring up to 48 hours postchallenge were considered to be due to the direct toxic effects of the substance. Deaths occurring more than 5 days after adrenalectomy could be attributed to effects of the adrenal deficiency itself, and for this reason, were not included in the experiment.

The minimal Lethal Dose for each agent was determined by using progressively increasing doses. This was done separately for adrenalectomized and for control animals. It was the difference in the toxicity in sham-operated and in adrenalectomized animals that was considered rather than the toxicity of the substance itself.

The ratio of the Minimal Lethal Dose for the two groups of rats, sham-operated and adrenalectomized, was calculated. It furnished a numerical representation of the degree of adrenal intervention and was called the "Adrenal Defense Index" or A.D.I. We utilized this index, $\frac{\text{MLD Sham-operated}}{\text{MLD Adrenalectomized}}$, as a measure of the relative adrenal participation in the body defense against different substances. A low index points to a

general nonspecific response; a higher index indicates a more significant intervention.

We obtained the adrenal defense index for various groups of fatty acids. They included homologous series of saturated, unsaturated, alpha-OH and conjugated fatty acids. Over 900 rats were used in this study.

For the saturated fatty acids we found:

FATTY ACID	A.D.I.
Caproic Acid	2.5
Caprylic Acid	5
Capric Acid	5
Lauric Acid	1.5
Myristic Acid	2
Palmitic Acid	12
Stearic Acid	6

We interpret this to mean that the defense capacity of the organism against these fatty acids was only slightly more effective in the sham-operated than in the adrenalectomized animals. The adrenals did not seem to be especially active in the defense against these substances.

For the unsaturated fatty acids we found:

FATTY ACID	A.D.I.
Oleic	6
Linoleic	9
Linolenic	5

These values indicate more active adrenal intervention against these fatty acids.

For the saturated alpha-OH fatty acids the results were:

FATTY ACID	A.D.I.
alpha-OH Caproic Acid	4.5
alpha-OH Caprylic Acid	4
alpha-OH Capric Acid	3
alpha-OH Lauric Acid	20
alpha-OH Myristic Acid	9
alpha-OH Palmitic Acid	3
alpha-OH Stearic Acid	50

We choose this series of substances because Camien and Dunn, and others, have shown the importance of these fatty acids for bacterial growth, as well as the presence of alpha-OH lauric and alpha-OH myristic acids as part of the lipido-polysaccharide fraction of bacteria.

Through related research, we were particularly interested in the A.D.I. value of fatty acid molecules with conjugated double bonds. As conjugated diene, we administered conjugated linoleic acid. The A.D.I. of this substance was similar in value to that of its nonconjugated isomer. The index was 5. For the conjugated triene, we used eleostearic acid obtained from tung oil. The results were striking. We found an A.D.I. value of 120. The

A.D.I. of this acid thus showed a 24-fold increase over the index of its nonconjugated isomer.

The data indicate a degree of specificity for the adrenal defense mechanism. A.D.I. values of 3 or less could be interpreted to correspond to a nonspecific adrenal intervention toward fatty acids in general, while higher values indicate a larger, probably specific adrenal activity. In the case of alpha-OH lauric and alpha-OH stearic acids, the high A.D.I. values could be releated to the fact that these fatty acids have been found to be part of the constitution of bacteria.

The intensive defense evidenced by the high A.D.I. value for eleostearic acid is related to the appearance of conjugated trienes during the course of certain pathological conditions, especially trauma. The continuous increase, especially of conjugated trienes, in the adrenalectomized animals suggests that these fatty acids appear in the organism but are destroyed under normal conditions. They accumulate, however, in adrenalectomized animals and may contribute to death when they reach a certain critical concentration. It is possible that with additional administration of these conjugated trienes their critical concentration would be reached and the animals would die.

In the light of these data, we investigated the role of different adrenal factors in these responses. Cortisone, desoxycorticosterone acetate (DOCA), and sodium chloride were tested for their protective action against the toxic effects of oleic and eleostearic acids. Groups of rats were treated immediately after adrenalectomy with daily doses of 1 mg. of cortisone, two-tenths of a cc. of DOCA, or 1% NaCl drinking water ad libitum. Control adrenalectomized rats were given no sustaining therapy. Three days after adrenalectomy, a challenging intraperitoneal dose of 1 cc. of 10% oleic acid per 150 gr. of body weight was administered.

Whereas the mortality of the control rats was 90%, it was 25% in the cortisone treated animals. DOCA administration decreased the mortality only to 65% and NaCl had little effect, decreasing it only to 85%.

The protective effect against oleic acid in adrenalectomy is seen in the following table:

AGENT	% MORTALITY
Control	90
Cortisone	25
DOCA	65
NaCl	85

This suggests that the neoglucogenic hormone plays a significant protective role in the defense of the organism against the noxious effects of fatty acid. The mineralocorticoid, on the other hand, seems to play a lesser role in this mechanism, a fact which is confirmed by the ineffectiveness of sodium administration.

A similar preliminary experiment carried out for eleostearic acid shows the same protective effectiveness of cortisone, lesser effectiveness of DOCA and almost no effect at all of sodium chloride.

Chapter 6, Note 19. **Bonds of Glucuronic Acid**

The study of the detoxifying-excretion of different agents led to the following conclusions. Primary aliphatic alcohols—except methyl alcohol —are eliminated coupled by glucuronic acid; the same for the secondary aliphatic alcohols; tertiary aliphatic alcohols and glycols from propylene glycol up. Of the aliphatic aldehydes only a few are coupled and only after transformation in vivo. While most of the aliphatic ketones are coupled, phenol and more emphatically the cresols and salicylic acid are in part excreted as sulfo-coupled, and only in part as glucurono-coupled. Resorcinol, catechol, orcinol, phenolphthaleine, phloridzin are mostly eliminated as glucurono-coupled, while adrenaline almost solely as sulfate. Most of the aromatic hydrocarbons are also bound to glucuronic acid, but only after having been changed in vivo.

For the aromatic acids, the bond to glucuronic acid is conditioned by the presence of second polar groups, usually one or more hydroxyls. The aromatic nitrogen compounds are first changed into amino groups before they are coupled with the glucuronic acid. Only relatively small amounts of sulfonamides were excreted bound to glucuronic acid.

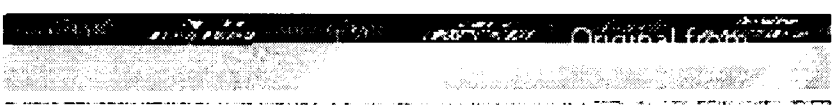
Many of the heterocyclic compounds are bound to glucuronic acid with the condition to have amino or hydroxyl groups; the same for the sex hormones, the estrogens being the group especially excreted as such.

The general characteristic of the substances excreted as coupled with glucuronic acid is the presence in their molecule of one or more positive polar groups hydroxyl or amino. As with few exceptions all these substances have also lipoidic properties, the excretion coupled to glucuronic acid appears as a means to eliminate positive lipoids.

Chapter 6, Note 20. **Glucuronic Acid—Mechanism of Coupling**

The analysis of urine specimens containing peroxides, also has revealed significant amounts of glucuronic acid compounds. We utilized a slightly changed Tollens technique for the dosification of glucuronic acid in the urine, based on the reaction of this acid with naphthoresorcine in an acid medium. To 5 cc. of urine, 0.5 cc. of a 1% solution in alcohol of naphthoresorcine (1.3 dioxynaphthalene) and 5 cc. of concentrated hydrochloric acid were added. The mixture was boiled for one minute, allowed to stand for another five minutes, then cooled, preferably in an ice water bath. When cold, a mixture of 90% ether and 10% alcohol was added, agitated, and the blue-violet color of the ether-alcohol measured, using a spectrophotometer. The values obtained in different subjects have shown a definite increase in the amount of glucuronic acid in the urines containing peroxides, indicating a probable relation between them. Based on this correlation, we investigated one of the roles of glucuronic acid in the organism, that of detoxifying agent.

Glucuronic acid could be considered to result, at least in part, from oxidation of glucose. Usually oxygen intervenes in glucose metabolism



only after the desmolyse processes * (232), corresponding to the fermentative phase has progressed to the appearance of pyruvic acid. Glucuronic acid could be considered to appear from a more direct fixation of oxygen to the glucose molecule. This fixation has to take place upon C_6 in order to lead to the appearance of glucuronic acid. Theoretically, the oxygen fixation might be expected to occur at C_1 in view of the aldehyde group present at that carbon. This takes place in vitro. It would lead to the appearance of gluconic acid. However, if a phosphoric or other radical is bound to C_1 in vivo, fixation of the oxygen at this carbon is prevented. Oxygen attaches itself then at C_6 which is the next most reactive carbon in the molecule. This reactivity at C_6 is seen when oxidation in vitro is continued beyond gluconic acid, leading to saccharic acid, a bicarboxylic acid with one carboxyl at C_1 and another at C_6.

Glucuronic acid intervenes in the physiological defense processes by combining with certain noxious products and helping to eliminate them in non-toxic forms. The resulting compound between glucuronic acid radicals and various substances—and for sulfuric acid radicals as well—has been called "conjugation," the substances being sulfo- or glucurono conjugated compounds. Because of the special attention given in this publication to the conjugation of the double bonds, we will use the term "coupled" for this bond to sulfuric or glucuronic acid.

A certain parallelism exists and has always been emphasized between the detoxifying and eliminating function exerted by the sulfuric and glucuronic radicals. Not only the two derivatives appear in the urine, but it is often noted that glucuronic acid intervenes when large amounts of certain substances, such as menthol or phenol, are present and the sulfuric acid radicals are not in sufficient quantity to insure detoxification and elimination. By administering mineral sulfates to the subject, the proportion of sulfo-derivatives is seen to increase. This parallelism appears especially interesting when we recognize that sulfuric acid represents the final stage of the oxidation of sulfur introduced into the organism in combinations in which it enters as a bivalent negative element. Both sulfuric and glucuronic acid result from oxidative processes, one involving the thiol group and the other, glucose.

While glucuronic acid often substitutes for the sulfuric radical in the excreted substances, qualitative differences exist. For example, phenol as well as indoxyl is bound to the sulfuronic radical, while the higher alcohols and especially the cyclic oxyacids do not combine with this acid. For many substances the parallelism that exists in the bond to sulfuric and glucuronic radicals extends only up to a certain point, after which the amount of the sulfuric ester no longer increases. This fact has raised the problem of the action of these two radicals and the differences between them, and can be

* The term "desmolyse," widely used by some authors abroad, corresponds to the changes occurring in metabolites under the action of hydrolases, which, when completed, result in a splitting apart of the molecules and the liberation of the energy they possess.

understood only by considering the substances that are bound by these acids and eliminated as sulfo- or glucurono-coupled derivatives.

We have seen in Note 19 that, with very few exceptions, such as benzoic acid, almost all the substances excreted or bound to glucuronic acid have an OH as polar group. However, many have more than one polar group.

The bonding with the sulfuric radical, as it occurs in the organism, follows a relatively simple pattern. *(Fig. 258a)* In a first step, the bonding of one acid function of the sulfuric radical to the substance produces an acid sulfuric ester. This is further changed by combination with a metal, usually potassium, which produces a highly hydrosoluble salt and represents an excremental derivative.

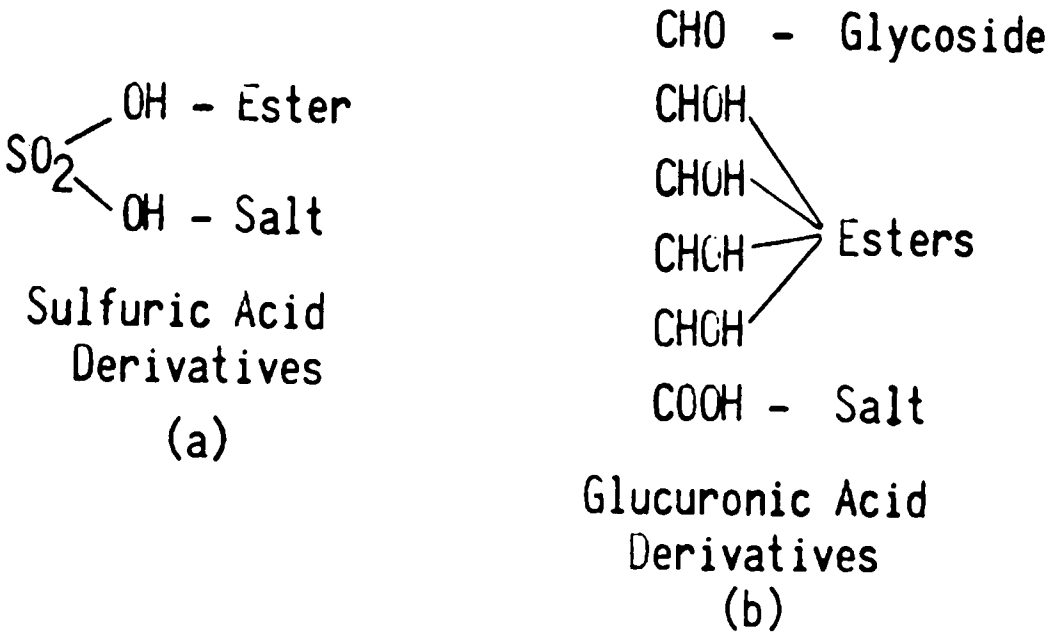


FIG. 258a. The bond of sulfuric acid in the organism can result in an ester and a salt. FIG. 258b. The bond of glucuronic acid can result in a glucoside, an ester or a salt combination.

The binding is more complicated for glucuronic acid. Glucuronic acid does not realize the bonds, since it is eliminated as such if administered. The bond occurs at the aldehyde group of glucose, forming a glucoside, which is passed further in glucuronic acid. As is seen in Figure 258b, glucose can realize different bonds. It can bind a radical either to the adlehyde group at C_1 to form a glucoside, or to its alcoholic hydroxyls to form an ester. The glucuronic acid can bind a metal to its carboxyl to form a salt. The possibility of realizing a glucosidic coupling at C_1 or an esteric at C_2 has been revealed by Quick. (233) The ability to realize concomitantly a multiple coupling, for which glucuronic acid seems to be highly suitable through its multiple and various functions, appears more interesting. The study of elimination of different oxybenzoic acids shows that it is bound to gly-

cocoll, *(Fig 259a)* while the para isomer is eliminated coupled by the glucuronic radical. *(Fig. 259b)*

The difference between sulfuric and glucuronic acid thus appears to be related to double coupling especially if the second function is acid. The presence of two opposite polar groups will thus prevent the bond to sulfuric acid and favor the bond with the glucuronic radical. Organic oxyacids are thus not bound by sulfuric radical as long as their carboxyls are free. The amides, such as salicyl amides—and, the esters such as methyl salicylates—are coupled by the sulfuric radical, while the acids are not. It is through these multiple coupling possibilities—and, in addition the possibility of forming a salt especially with potassium—that glucuronic acid corresponds to a broader activity as a detoxifying and eliminating agent than sulfuric acid.

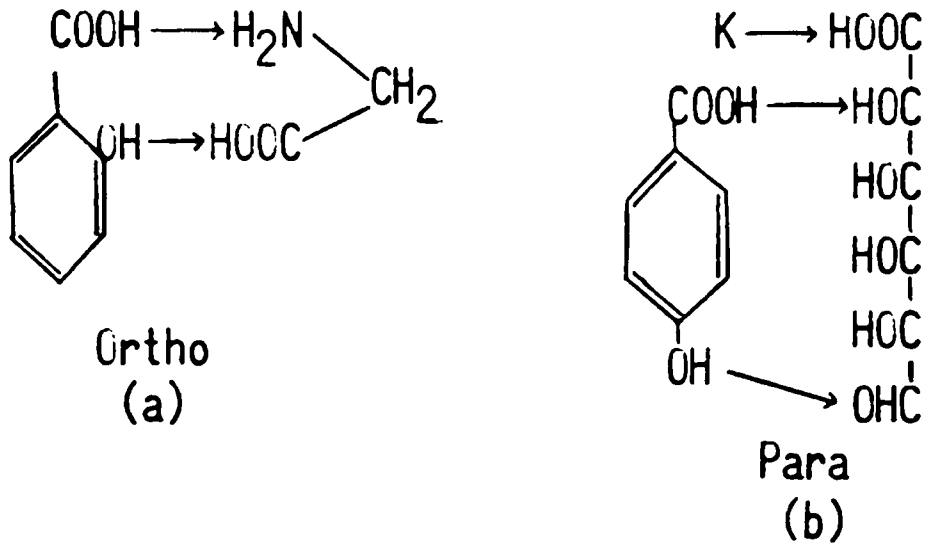


FIG. 259. The ortho-oxybenzoic acid (salicylic acid) is bound in the organism to glycocoll, (a) while the para-isomer is coupled by the glucuronic radical. (b).

The capacity to utilize glucuronic acid for excretion of noxious substances varies widely between species. Benzoic acid has been shown to appear as a glucuronic derivative in animals by Csouka, Brakefield, and Schmidt (234), but never in humans, a fact that is not confirmed by Quick. (235) Rabbits eliminate the tertiary alcohols as glucuronic derivatives more completely than dogs. While thyroidectomy in rabbits decreases the synthesis of camphoroglucuronic acid (236), the administration of thyroid extract increases it.

Chapter 6, Note 21. **Paraplegia Induced by Cholesterol**

Male and female rats, weighing approximately 250 grams, were injected with 5 cc. of 10% cholesterol only partially dissolved in a mixture of 3 parts oil and 1 part ether. The next day paraplegia with some ulcera-

tion of the hind legs was seen. Ulceration became accentuated in the following days. Curiously enough, this occurred only in the females. In repeated experiments in rats, and also in rabbits, guinea pigs and even mice, the same sex differentiation has been observed. Most of the female animals died from retention of urine as a principal complication of the paraplegia.

To investigate the nature of this sex difference, groups of males and females were castrated and spayed, then tested with the same cholesterol injection at various times from a few days to four months afterward. Castration had no influence in male rats; nor did the spaying of females reduce the incidence of paraplegia. The administration of 5 mgm. of testosterone daily for ten days to female rats, spayed or unspayed, also did not prevent the appearance of paraplegia after the injection of cholesterol in ether-oil. The administration of ½ mgm. of stilbestrol daily for 10 days to males, castrated or not, did not break down their resistance to the cholesterol injection. However, the administration of the insaponifiable fraction of placenta or of the total body of rats, in doses of 2 cc. of a 5% solution in oil daily for ten days, made the males respond with paraplegia to the injection of cholesterol in ether-oil solution. The administration to females, spayed or unspayed, of a 10% solution in oil of the acid-lipid fraction of the same origin in doses of 2 cc. daily for ten days prevented this effect.

Chapter 6, Note 22. **Adipose Cells and Sulfur Mustard**

In collaboration with the late Prof. R. Leroux, Professor of Pathology of the Faculty of Medicine in Paris, we studied the influence exerted by sulfur mustard applied on the skin. The following technique was used: One drop of the pure substance was deposited on the external side of the ear pavilion of adult white rats, left in place for 10 minutes, and wiped off. A part of their ear pavilion was excised in a V form. The fragment so obtained was immediately processed through the special technique used for the extemporaneous examination of operatory biopsies. A longitudinal section of the pavilion was made in the frozen fresh material, and this surface treated first with formalin and then stained with scarlet red or black Sudan and hematein. The section was kept in water covered with a glass cover and immediately examined under incident light, using the Zeiss "Ultropak" dispositive. A water immersion objective was used for higher amplification. From the same material, fixed in formaldehyde at 10%, frozen and paraffin imbedded sections were also obtained.

In nontreated controls, except for the small fatty droplets in the cartilage cells themselves, the only fatty cells found were at the base of the pavilion. In the mustard treated animals, two or three layers of adipose cells were seen to appear beneath the treated skin, near the cartilage, around 20 minutes after the application of the sulfur mustard.

In our group of experiments, curiously enough, this phenomenon was seen to occur only in the treated female rats and not in the males. This difference in the response between males and females was not influenced either by castration or by treatment of the animals with sex hormones. It

was induced in males however, by the administration of unsaponifiable fractions obtained from the total body of rats, or from human or cow placenta, and administered in doses of 1 cc. of a 10% solution in oil for at least one week prior to the application of sulfur mustard. In females, the appearance of adipose cells after the sulfur mustard applications was seen to be prevented, if the animals were treated daily for 10 days prior to this application with 2 cc. of a 10% solution in oil of lipidic acid fraction of human or cow placenta or of cod liver oil fatty acids.

Chapter 6, Note 23. **Fatty Acids and Old Tetrahymena**

Cultures of different ages of tetrahymena pyriformis in proteose peptone medium, were analyzed for the fatty acids present. The richness in fatty acids was seen to increase with the age of the culture, the old cultures being the richest.

Chapter 6, Note 24. **Lipids and Old Age**

In the changes related to old age, we have recognized a relationship between the amount of insaponifiable fractions and fatty acids present at different levels of the organization. A certain degree of opposite changes in two successive hierarchic levels can explain certain peculiarities found in the variations of sterols and fatty acids in old age.

In old rats we could establish that changes occur in two opposite directions between the cells and the metazoic compartment. Serum cholesterol amounts increase, as an increase of fatty acids and particularly polyunsaturated members occurs in the cells. The contrast between the two compartments becomes progressively more evident with old age. We tried to apply this data to the study of the relationship between the same compartments in humans. Instead of other cells, we analyzed red blood cells. In rats, rabbits and humans, the cholesterol in serum increases progressively with age but does not do so in the red cells. The amount of fatty acids in red cells is relatively increased with age at the same time as cholesterol content is decreased. The comparison between the cholesterol of serum and cholesterol of the red cells as well as fatty acid content of red cells seems to furnish information related to the progression of abnormalities of old age. This preliminary research, however, needs more confirmation.

Chapter 6, Note 25. **Surface Tension of Urine in Old Age** (237)

During the course of a study of the biological changes in old age, the renal excretion of surface-active substances was found to be considerably below the levels seen in younger age groups. These findings were considered important since (1) they were observed in individuals with no evidence of significant renal impairment, (2) the same decrease was observed in all subjects examined, and (3) information on this subject is lacking in the absence of a suitable analytic method.

The capillary device has made it possible to determine surface tension within a few seconds, as part of routine urinalysis, without need for complicated apparatus or involved formulas.

A group of 23 inmates of an old age institution were studied. Ages of these 14 men and 9 women ranged from 70 to 87 years. All appeared in good general condition and showed no evidence of significant renal pathology. Morning urine specimens were obtained from each patient for several weeks. These were examined within several hours after voiding.

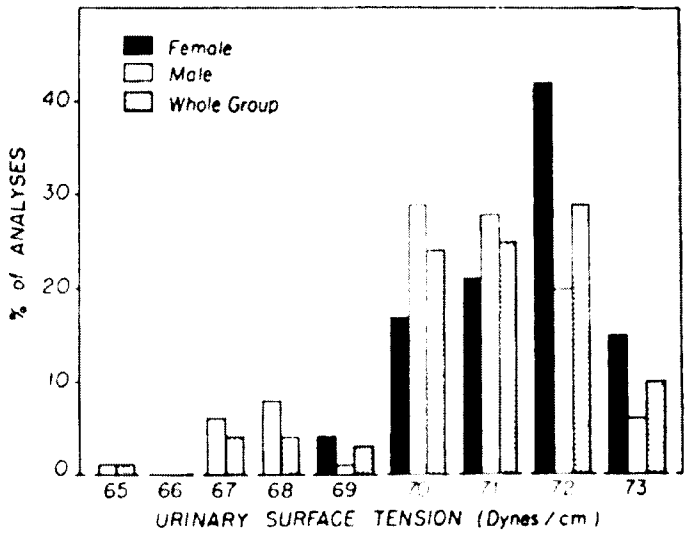


FIG. 260. Distribution of urinary surface tension values in the group of old people studied.

Surface tension values for the entire group averaged 70.9 dynes/cm. The average value for the women was 71.5 and, for the males, 70.4 dynes/cm. When surface tension values were determined in serial samples from individual subjects, variations of only 2 to 6 dynes/cm. were found. None of the samples from the women showed a surface tension below 69 dynes/cm. All but 3 of the specimens from the men had surface tension values of 68 dynes/cm. or higher. *(Fig. 260)* Specific gravity varied from 1.006 to 1.030, with an average of 1.017 for the group.

In control groups, the average surface tension is 67 to 68 dynes/cm. Diurnal and day-to-day variations of 5 to 11 dynes/cm., with values ranging between 72 to 62 dynes/cm., are found characteristically in healthy subjects.

It is evident that the pattern of excretion of surface-active material in old age is quite different. This is indicated by (1) the high average surface tension value of 71 dynes/cm. for the group, which is at least 3 dynes/cm. higher than for the controls; (2) the limited day-to-day range of variations, which in no case was greater than 6 dynes/cm.; and (3) the striking disparity in the distribution of values in healthy young adults and in old age. *(Fig. 261)* Eighty-eight percent of the urine specimens from

old individuals had surface tension values of 70 dynes/cm. or higher, and only 1 percent were below 66 dynes/cm. In contrast, 23 percent of the specimens from the control group had a surface tension of 70 dynes/cm. or higher, and 23 percent were below 66 dynes/cm. These findings indicate that the amount of surface-active substances excreted in the urine of old people of 70 to 87 years is greatly reduced as compared with that in healthy young adults. The fact that specific gravity values for the old age

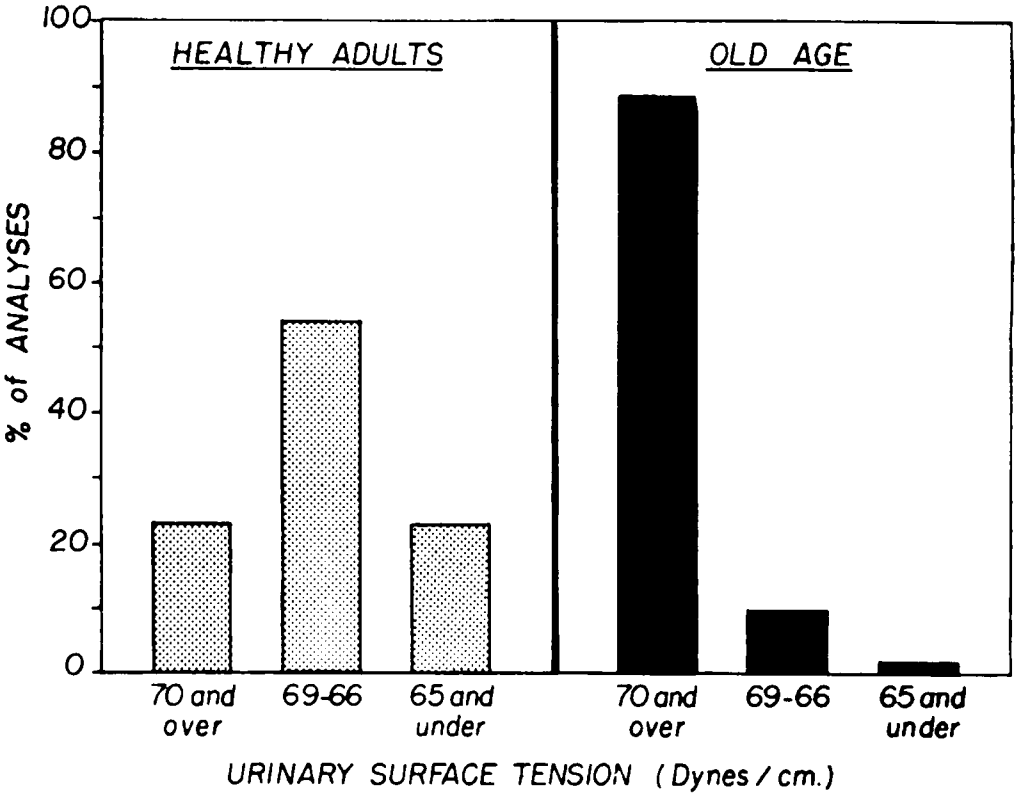


FIG. 261. Distribution of surface tension values in healthy adults and in old age. shows the high proportion of values of 70 dyne/cm and above for the last group.

group were not significantly different from the controls indicates that this change cannot be explained on the basis of a failure of the kidneys to concentrate by reabsorbing water.

The average surface tension value for the females was 1 dyne/cm. higher than for males. The same sex difference has been noted in studies of healthy young adults and in groups of patients with a variety of diseases. It is of interest to find the difference persisting in these old people.

In the light of current concepts of the possible role of surface-active "colloidal" substances in the urine, it is interesting to speculate on the possible significance of the observed decreased excretion in terms of renal physiopathology. Various authors have contended that the crystalloids of the urine are maintained in solution by the colloids, which prevent ag-

glomeration and precipitation of salts. (238, 239) Some such mechanism is undoubtedly present in the complex physiological solution finally excreted by the kidneys, since most of the urinary salts are present in proportions far exceeding their ordinary limits of solubility in aqueous solution. Low levels of colloidal activity and high surface tension values have recently been correlated with an increased tendency to stone formation in the urinary tract. (240) It is possible that this reduction in surface-active substances in the urine of old people, combined with the obstruction at the vesical neck due to prostatic hypertrophy, may lead to retention of precipitated material in the bladder, accounting for the increased incidence of vesical calculi during the later decades. In women, incidence of bladder stone is relatively low, even though the quantity of excreted surface-active substances is small, because there is no anatomical obstruction to urinary flow.

Administration of hyaluronidase raises the level of colloid activity (241) and lowers the surface tension of the urine to a slight degree. This enzyme has been utilized in the treatment of chronic recurrent stone formers with apparent effectiveness. (242) The reduced excretion of tensio-active substances in the urine may be related to the decreased enzymatic activity within the tissues.

Chapter 6, Note 26. **Environmental Influences**

In most of the experiments in animals, the analytical data followed over a certain length of time show variations which cannot be explained by the experiment itself. A direct relationship of such variations to changes occurring in the environment could be seen in the following experiment.

Six groups of 20 female white Wistar rats each, were injected on the same days, each group with a different agent. One group of animals received neutral oil as control while the others received different fatty acid preparations. The urinary surface tension was measured daily at approximately the same time of day for all the animals. The average value of these daily data was obtained for each group and used to trace the respective curves.

By comparing these curves, two characters were recognized. One was seen to concern differences from one curve to another, and consequently, would be considered as resulting from differences in the direct effect of the medications used. The other group of changes concerning variations from one day to the other, was seen to exist in all the curves, the curves having thus parallel variations. *(Fig. 262)* These variations, common to all the curves, were considered induced by a general influence. The analysis of these curves shows that the first kind of changes, related to the agents used, concerns differences in the levels of the curves themselves, when compared with that of the control. Treatment with stearic acid does not influence the level, and treatment with oleic acid has only a slight influence. A manifest change is seen for the other curves. The urinary surface values correspond to lowest values for linoleic acid (d) and for cod liver oil fatty

acid (e). Some less marked differences from the control curve is seen for the fatty acid preparation obtained from cow spleen (f).

Independent of these level differences, all the curves of the controls as well as of the treated groups show parallel daily changes. An exception

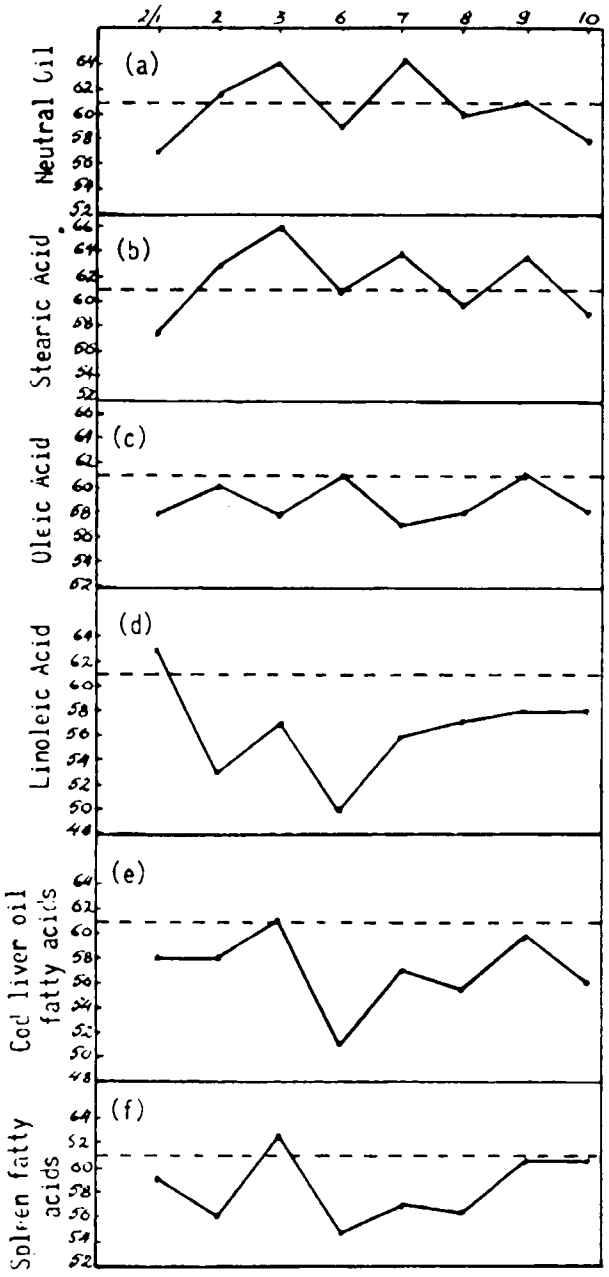


FIG. 262. Curves of the average value of the urinary surface tension in groups of rats treated with different fatty acid preparations (1 cc of 10% in oil daily). The parallel changes in the curves, except for oleic acid when the variations are opposite—indicate a common external influence. The differences in the relationship of the curves to the average value line, corresponds to the direct influence exerted by the agents.

is seen for the curve for oleic acid, which shows opposite variations. We do not have an explanation for this discrepancy. The parallel change would indicate the intervention of a common factor, independent of the experiment itself. By relating these changes to those taking place in the environment, the common variations in the curves could be recognized to follow—in an opposite sense—those of the environmental temperature.

It should also be noted that this environmental influence is progressively more accentuated for the curves where the level of the surface tension is lowered as a result of the agent administered. This correlation would suggest the possibility that the action exerted by the environment would take place largely through changes in the intervention of the fatty acids of the organisms themselves.

Chapter 6, Note 27. Environmental Influences Upon Urinary Surface Tension

The morning urinary surface tension was measured in four groups of 40 rats each: one group of 20 males, one of 20 females of the white Wistar strain, one group of 20 males and one of 20 females of a black "hooded" strain. From the data obtained on each group, the average value was calculated and its respective curve traced. The four curves were seen to be parallel, suggesting that the variations noted result from the intervention of a common external factor. We sought this factor in the changes occurring in the environment. For this reason, we compared the variations present in the surface tension curves with the meteorological data, furnished by the U. S. Weather Bureau, corresponding to the time of this experiment. Such a relationship was seen to only partially parallel the barometric changes, but appeared more closely related to the temperature changes. The observed relationship however, is inverse, that is, for higher environmental temperatures the surface tension values are low whereas for lower environmental temperatures, the surface tension values are high, as seen in Fig. 223. (Page 586)

This correlation appears still more interesting when it is compared to that induced by keeping animals at a constant temperature, as in an incubator or in a refrigerator. The effect of such an induced temperature is opposite to that caused by the environment. The high temperature of the incubator induces progressively higher surface tension values while the low temperature of the refrigerator lowers the surface tension, at least at first. Fig. 224 shows the average value of the urinary surface tension in controlled animals, while Fig. 225 that of the animals kept in an incubator at a temperature of 37°C and in a refrigerator at 8°C. (Page 588)

We tried to explain this discordant influence between the induced and natural temperatures, through the fundamental characters of these two factors. In the influence exerted by the environment, temperature with its variations, represents a factor which has acted upon organisms with the same rhythmic characters for many millions of years, while in the experiments, the constancy of the temperature represents its main character.

The influence exerted by the rhythmic environmental changes in air temperature is reflected in the parallel body temperature. The organism still tries to control this influence as exerted upon the lower levels. This is seen in rats in the opposite rhythmic changes of the urinary surface tension values. The organisms appear sufficiently sensitive to changes in temperature occurring in the environment. The body responses oppose these changes as shown in the variations in urinary surface tension. This rhythmic response, as well as that opposing the variations in the environmental

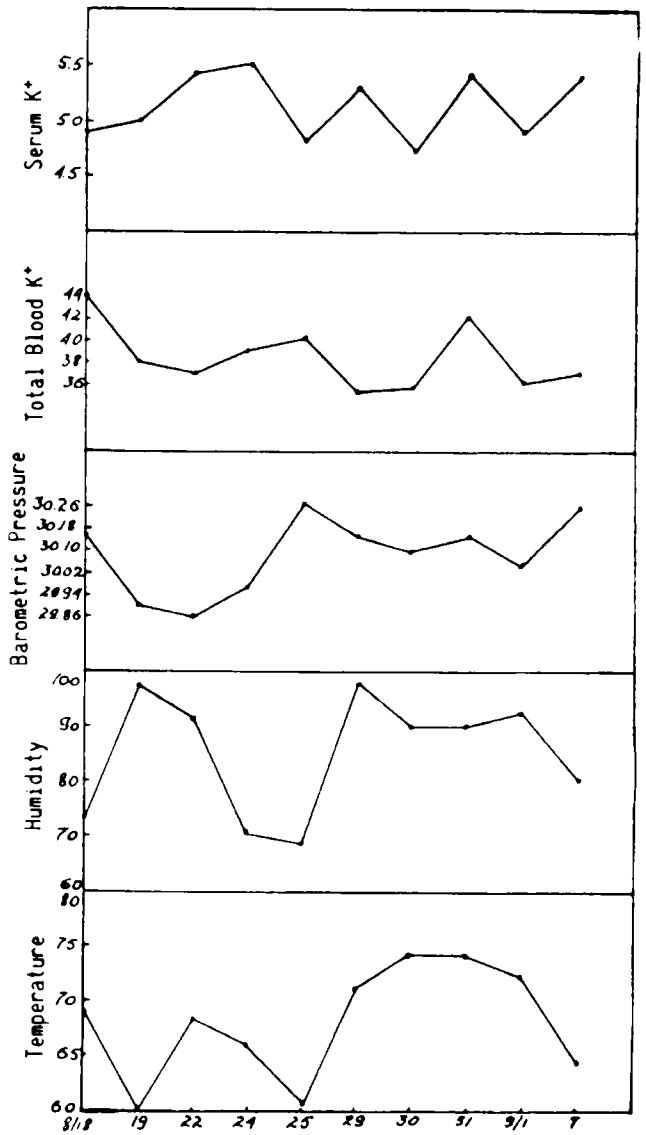


FIG. 263. Relationship between the curves of serum and total blood potassium of a group of 20 subjects and the atmospheric temperature, humidity and barometric pressure. Parallel variations are seen between the curves of total blood potassium and of barometric pressure. An inverse relationship is seen with the curve of humidity, and only partial relationship to the temperature.

temperature, are broken when a continuously unchanged high or low temperature is applied. With the reactional response exhausted after a certain time, the influence exerted appears a direct one, the high temperature inducing a high urinary surface tension and the low temperature a low one.

However, a difference is seen between the influence exerted by the high and low temperature. After some time, the animals kept in a refrigerator usually recover the capacity to fight the persistently low temperature, and the surface tension returns to normal values. This does not occur for the persistingly high temperature. The animals die with a progressively high surface tension as if the defensive response seen for low temperatures would not intervene for high temperatures.

This difference in the response of an animal toward a persistently high or low temperature can be explained by the fundamental difference which exists between these two factors from the point of view of homo- or heterotropy. While the low temperature has a homotropic character, the high temperature has a heterotropic one. The bodies are basically more prepared to react successfully toward homotropic influences as they have done it for millions of years in the past, than to an entirely unusual heterotropic influence.

Chapter 6, Note 28. **Barometric Influence**

For several years, we studied the potassium content of blood red cells in subjects with various abnormal conditions, usually following it daily for the same patient for weeks or months. Besides other informations which this study furnished and which are discussed above, we want to emphasize now the relationship with barometric pressure which we were able to establish during repeated analyses on the same group of subjects. We were impressed by the parallel variations seen in the amount of potassium in the total blood of different patients on different days regardless of medication. The variations could be correlated with changes in the barometric pressures. With lower pressures, a decrease in the amount of potassium in total blood *(Fig. 263)* was noted. Less manifest changes were seen with the opposite variations in atmospheric humidity.

Chapter 6, Note 29. **Age, Lipoids and Tumor Transplants**

The influence of age of a host upon the different manifestations through the intervention of sterols and lipoacids can be seen in the following experiments.

Transplants from the same Walker tumor were grafted at the same time in animals of different ages, such as newborn, weanlings, young animals, adults and aged. The difference between transplants was evident even from the beginning but was still more enhanced if further transplants were made in animals under similar conditions. After one transplant and especially after several transplants, the following changes could be seen. In the newborn, the tumor took on the aspect of an hemangiomatous lesion. There was

no massive tumor and the amount of blood present gave the tumor the appearance of a piece of liver. In weanlings, the character was opposite. Massive tumors without necrosis and with the aspect of fish meat were seen. In youngsters the same character was obtained. In adult animals, the tumor had a large portion of necrosis with predominance of hemorrhagic fluid. In very old animals, this character was still more accentuated, and the tumor showed big cavities with hemorrhagic fluid and very little tumor substance between.

Similar changes were obtained by changing the site of the graft. By grafting a portion of the same tumor, intramuscularly, we obtained a massive whitish, nonnecrotic tumor, while subcutaneous injection even in the same animal led to the appearance of a tumor with multiple necrotic areas and cavities filled with fluid.

We tried to correlate these changes with the nature of different lipids predominant at different ages. The administration of fatty acids tends to promote necrosis, edema, and formation of cavities filled with fluid, while administration of insaponifiable fractions, especially from placenta or liver, tends to produce a type of tumor with whitish, nonnecrotic masses.

Chapter 6, Note 30. **Temperature, Lipids and Viral Infection**

The relationship between epidemics and seasons is a well-established concept. In an attempt to explain this correlation, we considered, as one of the intervening factors, the seasonal changes in lipids, in view of the influence exerted by the two opposite groups of lipids upon the receptivity and manifestations of infectious disease.

As we have mentioned, viral infections are especially influenced by the predominance of one or the other group of lipids in the body. Among other factors, temperature changes were found related to this predominance. A relatively direct correlation was found for polio, for instance. The appearance of neurological symptoms such as paralysis was seen to increase on days with high temperature. Such a correlation could be established experimentally. Among mice injected subcutaneously with smallpox vaccine and kept in an incubator at 35° C, the incidence of encephalitis rose to more than 90% as compared to 10% in controls kept in an air-conditioned room. This correlation was further explained by the predominance of sterols in the organism under the influence exerted by high temperature. This predominance was further seen to induce a higher receptivity of the organism to viral infection, and a change in the virus virulence itself, both of which increase with temperature and with the richness of sterols.

Chapter 6, Note 31. **Youth and Viruses**

Another interesting aspect of the changes induced in viruses by lipoids with an alcoholic polar group, especially sterols, is the relationship to age of the host. The great amount of insaponifiable fraction present in youth—

which appears to be capable, in itself, of increasing virus virulence—has been discussed previously.

In an experiment, smallpox vaccine was inoculated in groups of very young, adult and old rabbits. The young animals reacted much more intensively than did the adults, with confluent pustulae; in the old animals, only a few small pustulae appeared. After several passages in animals of the same age, we tested the virus on mouse skin and found the virulence increased by each passage in young rabbits. On the other hand, virulence was decreased by passage in old animals, becoming negative with the fourth passage. After the third passage, virus obtained from young rabbits induced a strong response in mice, while no pustulae were obtained with virus from old animals. The latter was still able to induce some response in young mice, but only few small pustulae. The virus obtained from the third passage in young rabbits induced a frank response even in old mice. This indicates that opposite changes in virus virulence are induced simply by passage through young and old animals.

This experiment appears especially interesting in connection with childhood colds. It is known that children catch colds frequently and that this susceptibility disappears as they approach puberty. From a pathogenic point of view, it is an interesting fact, however, that older siblings, parents, and even grandparents are still infected if the cold comes from a child in the family. The virus itself seems to be changed by passage through the child so that it becomes virulent not only for teenagers and parents but for older people, all of whom previously may have been free of colds for years.

Chapter 6, Note 32. Changes in the Viruses Induced by Lipids

The effect of lipids upon viruses seems not to consist exclusively in an alteration of the host's response but also includes a change in the viruses' virulence. We have noted previously the big difference in the response of the skin of an animal when, prior to inoculation with smallpox virus, a lipoid with a positive or negative polar group was injected subcutaneously. The lipoid with a positive polar group induces an exaggerated response; the lipoid with a negative polar group, a reduced one.

We used viruses obtained from both types of lesions for inoculations in new animals. Viruses from lesions in which the response had been exaggerated produced again an exaggerated response, while those obtained from a small pustule induced a reduced response. The effect, in one or the other direction, was enhanced by successive passages on skin pretreated by subcutaneous injections. The changes in virulence also were confirmed in mice tests.

The importance of this experiment lies in the fact that, by treatment of the host with members of one or the other group of lipoids, we can obtain a desired increase or decrease in virus virulence. In several other experiments, we found the method applicable to other viruses, generally making the virus more or less virulent even for intracerebral inoculation. Interesting changes are produced by the polyunsaturated fatty acids and the alcohols