CHAPTER 13

PHARMACODYNAMIC ACTIVITY (PART TWO)

ANTI-FATTY ACID GROUP

PARALLEL TO INVESTIGATION OF AGENTS capable of correcting offbalances of type A, attention was directed to agents that might influence the opposite offbalance, type D. Since fatty acids are involved in the pathogenesis of type D offbalance, agents with anti-fatty acid properties had to be sought as correctives. Some of these are natural constituents used by the body to control normal and abnormal intervention of fatty acids. They were consequently isolated and studied. Synthetic agents also were obtained and studied, their choice largely inspired by the control mechanism used by the body.

Anti-Fatty Acids Constituents

We have seen that a free fatty acid loses most of its biological activity when its polar group is bound to another radical. This led us to investigate substances which naturally are bound to fatty acids. It could be shown further that each major group of fatty acids is bound in the organism to specific constituents. The saturated fatty acids are principally bound to glycerol, the low unsaturated acids to glycerophosphoric acids as lecithins, and the high unsaturated members to sterols. The conjugated fatty acids, found in abnormal conditions, appeared to be opposed by neoglucogenic corticoids. The constituents were conceived of as being naturally occurring anti-fatty acid substances and our first effort was to study how they intervene to balance the activity of fatty acids, especially when the latter act as pathogenic factors.

In this study, two types of influences were investigated: one, a relatively direct effect induced through a neutralization of the energetic centers resulting in a more or less advanced degree of inactivation of the fatty acid; the second, an indirect effect achieved through changes in the metabolic processes in which fatty acids intervene. In a different kind of intervention, the anti-fatty acid to which a fatty acid is bound governs its ultimate biological fate. For example, the bond to glycerol favors caloric metabolism. The bond to glycerophosphoric ion converts a fatty acid, saturated as well as unsaturated, into an organizational constituent. The bond to sterols favors a functional role, even for monoethenoids.

We started the study of the naturally occurring anti-fatty acids with those agents known to be bound to fatty acids in the organism. The simplest such agent is glycerol.

Glycerol

Glycerol is the most ubiquitous fatty acid-binding substance in nature. We attempt to explain this fact on the basis of glycerol's structure and the special biological role it confers. We have seen that fatty acid molecules take reciprocal parallel positions when they form monomolecular layers. In their bond to glycerol, these fatty acid molecules conserve this reciprocal relationship. *(Fig. 129)* This could explain why, in the body, the bond of fatty acid and glycerol always is a triglyceride, mono and diglycerides being

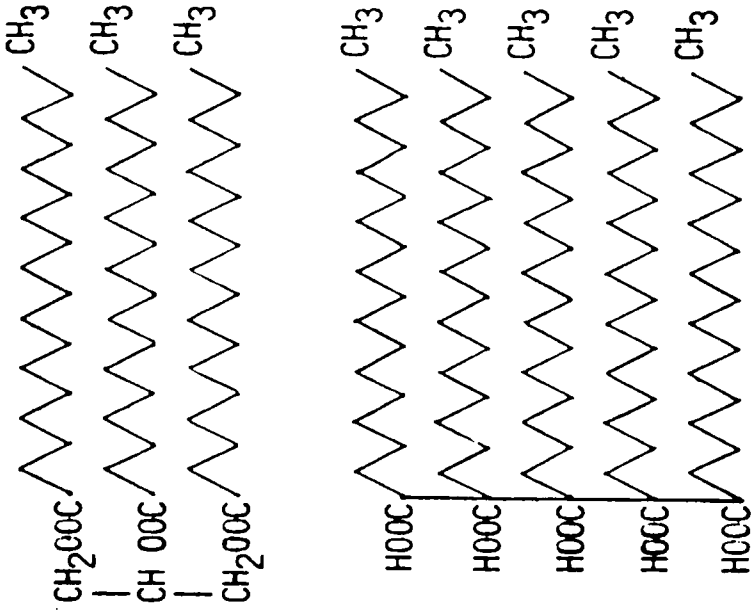


FIG. 129. The fatty acid radicals take in the triglyceride molecules a similar parallel position as when they form a monomolecular layer at the surface of water.

only intermediate steps. The same fatty acid has different biological activities if administered as free acid or as triglyceride. The combination seems to serve as an energy-furnishing metabolite. Bound to glycerol, fatty acids with long or short chains, saturated, monounsaturated, polyunsaturated, and even conjugated, seem to represent energetic reserves which are utilized as caloric metabolites, especially in those species which are able to store them.

With this relationship in mind, we administered glycerol with two objectives: 1) to obtain, as an immediate effect, the inactivation of the free fatty acids present in abnormal conditions through the neutralization of their polar groups, and 2) to eliminate these fatty acids by turning them into caloric metabolites.

Studying the activity of glycerol at different levels, we could see no influence upon phages. However, an indirect effect was observed upon viruses. Glycerol is widely used as a special medium for the preservation of viruses in tissues. Its preservative value can be correlated, at least in part, with its influence upon fatty acids. We have seen that fatty acids have a noxious effect upon viruses, leading to their disappearance in various organs. The treatment with pure glycerol reduces autolysis of organs through a dehydration effect. Curtailing the lytic activity of the enzymes active in autolysis, glycerol reduces the amount of fatty acids liberated through such autolysis, and thus prevents the destruction of the virus. Glycerol may also preserve viruses by acting antagonistically to any fatty acids which still manage to appear.

Glycerol has a bacteriostatic effect upon only a few species of microbes and only when applied in high concentrations.

A minimal influence upon cells was seen for glycerol in Tetrahymena pyriformis and ascites tumor cells. To study its action at higher levels, glycerol was administered orally or parenterally to animals or humans. Solutions of 20% glycerol were well tolerated when injected subcutaneously or intramuscularly. It should be noted that when glycerol was administered to complex organisms, it was largely absorbed and circulated without alteration, a fact which would explain the effectiveness of relatively small amounts. At the tissue level, glycerol induced a change of the local pH of a lesion toward the acid side, as seen in the second day wound crust. Figure 130 illustrates this. The change explains glycerol's action in increasing intensity of acid pattern pain and decreasing intensity of the alkaline. This influence upon pain was obtained constantly with very small amounts, permitting the use of glycerol even as a test for diagnosis of pain pattern. Intramuscular injection of ½ cc. of a 20% solution or oral ad-

ministration of ½ cc. of a 50% solution in water has been used for this purpose. However, later, when other agents were found to produce even more overt responses, we stopped using glycerol as a routine test.

Glycerol has almost no beneficial influence upon the healing of wounds or radiation lesions. Healing was even retarded in some experiments. Various changes in the evolution of tumors occur when host or transplant are treated with glycerol. In some, these changes are minimal; in others an obvious reduction in growth occurs. In a high proportion (12/20), a marked involution has been noted for Walker tumors in rats. Ascites Sa 180 in mice, after repeated intraperitoneal injections of a solution of 2%

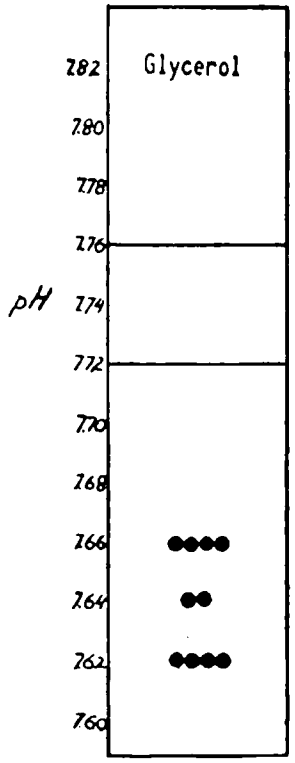


FIG. 130. Glycerol induces a lowering of the second day wound crust pH.

glycerol, disappeared in 70% of the cases. A lesser effect was seen in Ehrlich and Krebs ascites tumors and still less in the solid tumors obtained with these ascites cells. *(Fig. 131)* One of the most interesting effects of glycerol was that seen upon the tumors in humans where a manifest involution was obtained in cases in which an offbalance of type D was present. This important effect will be discussed below with the therapeutic use of glycerol. Glycerol administration had an interesting effect upon the amount of cholesterol in the blood in a few subjects. When ten drops of glycerol were given orally three times a day for a month or more, cholesterol values

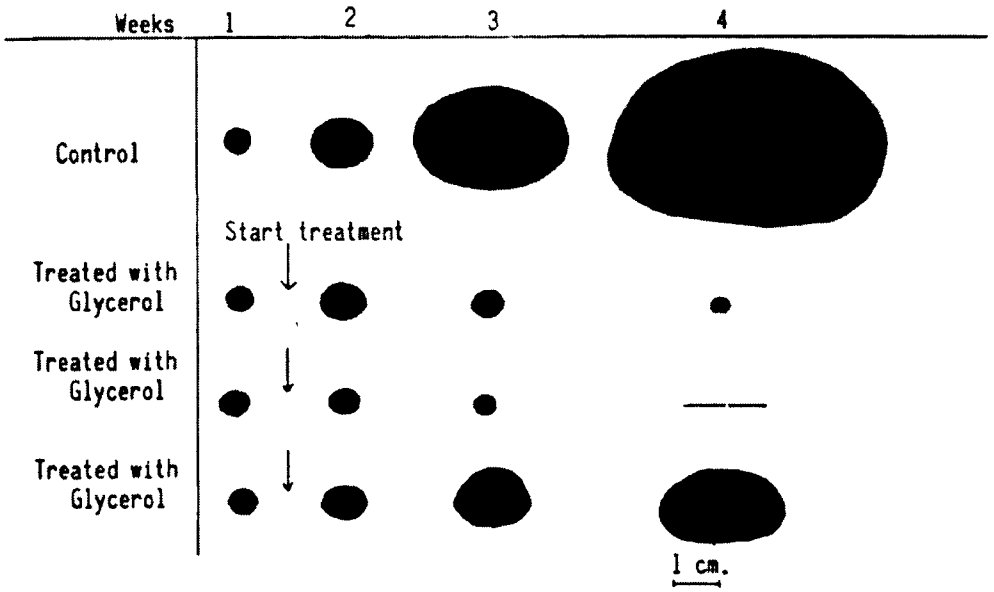


FIG. 131. Changes induced in Walker tumors in rats by the treatments of the animals with glycerol (daily subcutaneous injections with ½ cc of 5% glycerol solution in saline isotonic solution).

decreased. In some patients, with no change in diet and no medication other than glycerol, values originally above 300 mgr./100 cc. serum fell to below 170 mgr. If these patients also were hypertensive, long-term administration of glycerol produced a reduction of blood pressure.

An impressive hemorrhagipar effect was noted, frequently ulcerated lesions starting to bleed shortly after administration of even a few drops of glycerol. The relationship of hemorrhaging to glycerol was clear when in the same subjects, repeated administration of this agent invariably was followed by bleeding. The bleeding usually was arterial; only occasionally was an oozing hemorrhage seen.

Many years ago we became interested in studying, in a group of severely burned subjects, the role of fatty acids in the pathogenesis of burn complications. Glycerol was administered to these patients with good effects upon pain. Before the use of antibiotics, one of the principal manifestations in burn patients with widely infected wounds was repeated chills. These chills also were influenced by glycerol. *(Note 1)* A direct action upon the parasympathetic system could be attributed to glycerol and could explain the effect upon chills. This view has been confirmed by studying the effects upon cardiac rhythm produced by intravenous administration of glycerol in rabbits. *(Note 2)*

Convulsions could be induced by glycerol in animals and also were seen to occur in humans. *(Note 3)* They could be induced, with much

smaller doses in animals when, along with glycerol, an otherwise harmless dose of deoxycorticosterone acetate was administered. Injection of 0.1 mg. of this hormone in mice weighing 25 to 30 grams, followed by an injection of ¼ cc. of a 5% solution of glycerol, induced convulsions which were usually lethal. In terminal cancer patients, too, concomitant administration of the cortical hormone and glycerol for a few days has produced convulsive seizures, which proved to be lethal in one subject in whom no

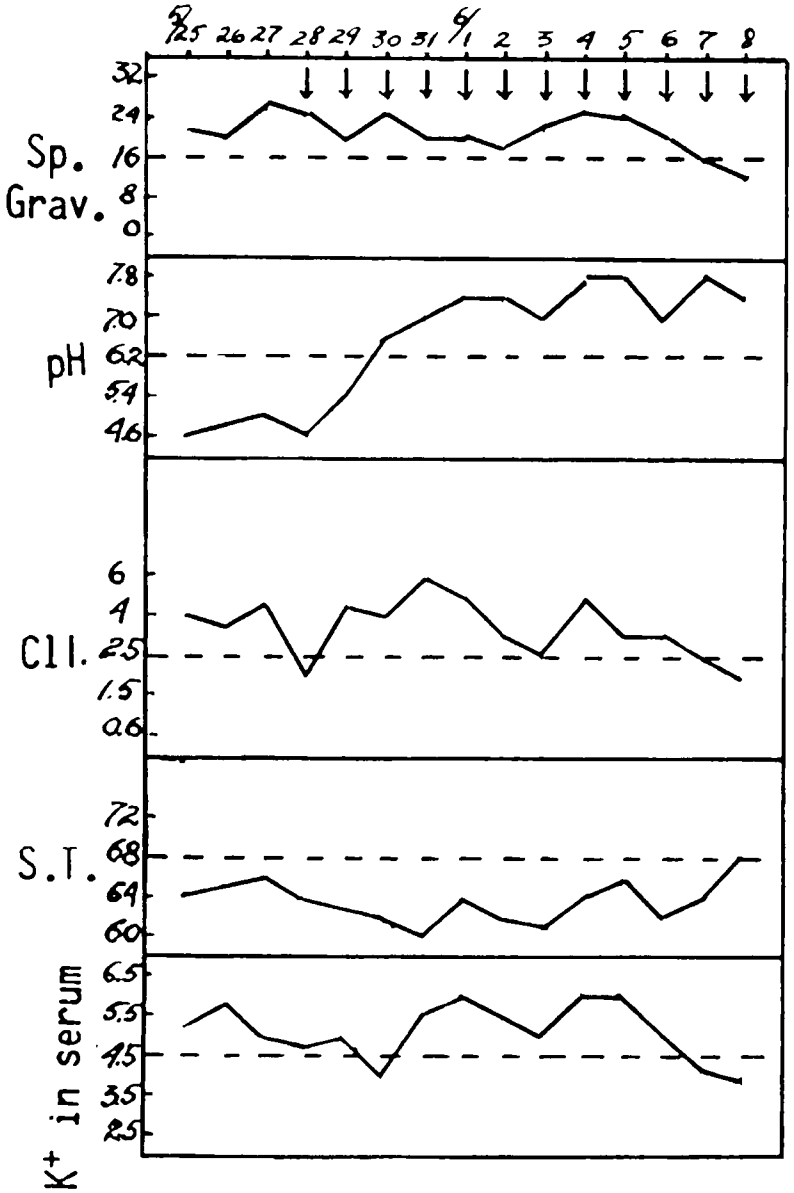


FIG. 132. The administration of 1 cc glycerol daily induces a change of the urinary pH toward the type A, before changes in the other analyses take place.

previous abnormal cerebral manifestations had been observed, and who had received these two medications for only a few days.

Of the different systemic manifestations influenced by glycerol, the effect upon acid-base balance is most striking. Even before any other effect upon systemic analyses becomes apparent, an immediate change of urinary pH from acid to alkaline values is induced by glycerol administered in sufficient quantity. *(Fig. 132)*

Glucose

The pharmacological activity of glycerol has raised the question of the relationship between this substance and glucose since some of the manifestations induced by glycerol could be obtained with glucose. The same effect is seen on local pH in the second day wound crust. Glucose administered for a few days prior to, and immediately following, wound induction shifts local pH toward more acid values. A similar effect is obtained on the pH of tumors. (173) Glucose decreases intensity of pain of the alkaline pattern and increases pain of acid pattern. We were able to induce convulsions in rats by injecting 20 to 25 cc. of an isotonic solution of glucose subcutaneously twice daily. After 4 to 6 days, convulsions appeared and often led to death. In some terminal cancer patients with brain metastases, who had had previous convulsions, intravenous administration of glucose as a therapeutic procedure produced convulsions. We have even seen lethal convulsions in a patient after a few days of intravenous administration of glucose in saline solution in conjunction with intramuscular doses of 1 mg. twice daily of deoxycorticosterone.

Although rarer than for glycerol, hemorrhages occurred after each glucose administration in some patients with previously bleeding ulcerated lesions. Bleeding stopped when glucose administration was discontinued. Renewed administration was followed each time by renewed bleeding. We want to emphasize this relationship of glucose to hemorrhage because of its clinical importance.

It seems possible that glyceric aldehyde and glyceric acid, which appear during glucose metabolism, play a role in the manifestations mentioned above.

Glycerophosphoric Acid

The ability to relate the pharmacological activity of glycerol to its bond with fatty acids, has led us to consider another substance able to bind fatty acids, glycerophosphoric acid. The bond of this acid with quaternary bases such as choline or ethanolamine, and the fatty acids results in phospho-

lipids which take part in the formation of boundaries and separating membranes. Although various fatty acids, both saturated and especially unsaturated, enter into these phospholipids, resulting in a variety of compounds, their general biological behavior appears to be the same. Certain fatty acids, the di- and tri-ethenic, however, are preferentially bound as phospholipids when they pass from the intestine into the circulation.

In investigating its influence as an anti-fatty acid agent, we administered glycerophosphoric acid, 50 cc. of a n/10 solution diluted in 1000 cc. of isotonic saline, glucose or other solutions, intravenously or subcutaneously. It had salutary effects upon pain of an alkaline pattern and upon corresponding lesions. A manifest influence was exerted upon the systemic acid-base balance, especially in cases with high urinary pH and with all other analyses showing an offbalance of type D. No influence was observed upon evolution of tumors in animals or humans.

The increased basal metabolism and a marked increase in work capacity, which are observed in subjects taking sodium glycerophosphate for long periods, has made us suspect a possible effect upon thyroid secretion. The appearance of thyrotoxicosis in a subject who had inadvertently taken a large dose of sodium glycerophosphate seemed to confirm this view.

Sterols

A third group of natural constituents, which act as anti-fatty acid agents is composed of sterols, which are absorbed and circulated bound to polyunsaturated fatty acids. Except in brain and red cells where only free sterols are encountered, the sterols are found both as esters and free substances in all cells, tissues and organs.

Cholesterol, phytosterol and a few sterols were utilized in pure form in our research. In addition, sterols were obtained and used as mixtures, as in the insaponifiable fractions of tissues, organs, organisms and biological products. In some studies, these fractions were further separated into constituents, largely through column chromatography. We used cholesterol in different preparations. *(Note 4)* Watery or gum cellulose suspensions were used for in vitro and in vivo studies.

Cholesterol was seen to induce a change in the shape of some bacilli, such as B. subtillis, B. megatherium and B. anthracis, turning them into irregular round formations. At the same time, their Gram positive staining became abnormally intense. The agar cultures had a creamy aspect. The influence upon Gram positivity explains the fact that Gram positive individuals could be obtained in cultures of various Gram negative microbes such as Esch. coli or Eb. typhy after repeated treatment with colloidal

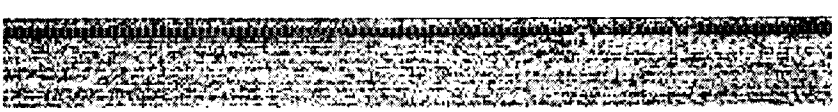
cholesterol preparations added to broth. The Gram positive forms, however, could not be isolated.

Cholesterol was seen to influence red cells in form, shape, volume, sedimentation, velocity, and oxygen-combining capacity. A vermillion color, which persisted for a long time, was obtained through in vitro treatment of blood with cholesterol or through intravenous injections in animals. Although such injections were lethal, they induced the abnormal vermillion color. Cholesterol produced a manifest change toward less alkaline values in the second day wound crust pH. A favorable effect upon rabbit skin wounds was obtained, with abnormally intensive proliferation of the epithelium, this healing effect, however, was less manifest for irradiated wounds. In subjects with ulcerated lesions, prolonged administration of cholesterol was frequently observed to induce hemorrhages, especially of an arterial character. However, in patients with coronary occlusion or endarterial obliterations, the administration of cholesterol was followed by an increase of symptoms apparently related to exaggeration of the degree of occlusion. This effect upon blood vessels also could be seen in animal tumors. Administration of cholesterol induced zones of necrosis in the tumors which could be related to proliferation of the endarterial cells leading to thrombosis and ischemic infarct. The portions of tumor corresponding to these ischemic infarcts showed characteristic necrosis with unaltered structure but without the normal staining.

In animals injected with cholesterol and then submitted to trauma in the Noble-Collip drum, shock was prevented. Injection of cholesterol prior to the experiment reduced mortality to zero while in untreated controls mortality was high. A similar but less constant effect was obtained when cholesterol was administered immediately after trauma. It is noteworthy that in animals injected with cholesterol before being placed in the drum, the blood not only did not become abnormally black but the usual bleeding from the nose, mouth and paws (if not taped during the trauma) was abnormally bright red.

The effect upon experimental tumors was investigated through the dipping technique repeated in successive generations. Changes in the evolution of certain tumors, such as mammary adenocarcinoma in mice, were induced. The effect of cholesterol was similar to that of insaponifiable fraction but was less manifest and will be discussed in more detail later.

Cholesterol's effect upon the central nervous system was interesting. Often, immediately after administration, both in animals and humans, transitory somnolence was observed. However, repeated administration induced convulsions. *(Note 5)* Exophthalmia was seen in mice after injec-



tion of cholesterol and was most manifest 24 hours thereafter. It contrasted with normals and especially with animals injected with fatty acids or similar lipoids, who showed enophthalmia.

An ether-oil cholesterol solution induced paraplegia with early foot ulcerations in rats and rabbits. This occurred particularly in females and was related to a predominance of sterols in this sex. Castration or administration of sex hormones did not change this special susceptibility of the nervous system of females to cholesterol. However, the administration of fatty acids or of acid lipid preparations of organs or tissues did suppress it. *(Note 21, Chapter VI)* Changes in systemic analyses were generally not obvious and, when present, were slow in appearing. They corresponded to changes toward offbalance A.

Unsaponifiable Fractions (Insaponifiable or Non-saponifiable)

When insaponifiable fractions were prepared from various tissues and organs, big differences could be seen in the quantity and the number of sterol compounds naturally present. However, a certain specificity related to the origin of these insaponifiable fractions appeared most interesting. The insaponifiable fractions of various materials were prepared by the usual methods. Most of the fractions are soluble in oil in higher proportion than cholesterol, with some of them even miscible with oil. More concentrated solutions in sesame oil could be prepared than for cholesterol. In most of the experiments, 5 or 10% solutions were used. Colloidal suspensions also were prepared in the same manner as for cholesterol.

In spite of the extreme variations in sources of the insaponifiable fractions, almost all have some properties in common. Some are similar to cholesterol in their effects particularly at the lower levels. The marked differences appear at higher levels. They induce hemorrhages in the adrenals between the fascicular and reticular zones.

On the healing process, especially of radiation wounds, insaponifiable fractions of placenta, embryos and butter—materials related to growth—show impressively greater activity than cholesterol or preparations of insaponifiable fraction of other origin. They induce healing processes even in standardized radiation lesions where cholesterol has a weak effect.

In their influence upon tumors, the preparations of insaponifiable fractions of different organs differ markedly. No changes at all were obtained with some preparations such as from pig intestine, for example, while interesting results were obtained with others. The differences appeared especially evident in experiments in which a direct influence upon the tumor

was exerted. Transplants of Ehrlich mammary adenocarcinoma in mice were dipped in insaponifiable preparations and grafted. In general, no immediate visible effects were seen with this technique for the first transplant generation. By repeating the same procedure for following transplant generations, changes were obtained which varied with the preparations used. The insaponifiable fraction of human placenta, for instance, produced a marked increase in malignancy, together with morphological changes, the tumor changing from an adenocarcinoma to an encephaloid. Further treatment of the transplants led to still greater malignancy with a sarcomatoid transformation. Thereafter, negative results were obtained with new treatment of the transplants. *(Note 6)* With this procedure, plancenta preparations showed a manifest influence even at the third transplant generation, and they were negative passages for the fifth to sixth transplant generations. With pig intestine preparations, even after ten successive passages, malignancy was unchanged.

The specificity according to origin was also seen in other experiments, such as in the influence exerted by these preparations upon the development of specific lesions produced by smallpox virus in low-reacting species such as mice or rats. Preparations from receptive animals, and especially from organs sensitive to the virus, were more capable of inducing local receptivity than were preparations from refractory animals. For instance, positive effects were obtained with vaccinia virus in mice and rats previously injected subcutaneously with the insaponifiable fraction of rabbit skin or brain, while no such effects were seen when the insaponifiable fractions of pig or hen intestines were used.

Differences were observed between the insaponifiable fractions of different organs for conditions principally manifested at the organ level. Conditions affecting mainly one organ were treated with the insaponifiable fraction corresponding to that organ. These preparations often appeared much more active than those from other organs. We investigated the effects of a heart insaponifiable fraction on patients with myocardial insufficiency, especially when responses to other therapeutic agents could no longer be obtained. In like manner, we used the insaponifiable fraction of liver for manifest liver insufficiency. The rate of liver regeneration in rats after subtotal resection was found most accelerated by liver insaponifiable fractions. The good effects obtained in the treatment of intractable diarrhea with insaponifiable fractions from pig and hen intestines will be discussed below. We investigated preparations obtained from lymph nodes and spleen for the treatment of shock, particularly in its acute form. Similarly we also used adrenal insaponifiable fraction to influence induced adrenal insuffi-

ciency, and brain insaponifiable fraction in an attempt to influence insomnia. The results of these studies will be discussed in the section dealing with therapy. The changes obtained with the respective preparations indicate that they have a specificity which represents an important factor in normal and abnormal physiology.

In a second group of researches, constituents of the rough preparations of insaponifiable fractions from various sources were separated by different methods. The ketonic and nonketonic constituents were obtained and, when tested in animals, showed several differences in biological properties.

Further research of specificity was made using separations through the chromatographic column method. Most of these preparations are still under laboratory investigation. Experiments are being conducted with different organ preparations, some fractions obtained being identified as common to all organs, while others are specific to one organ or to a group of organs. These experiments already have revealed a marked plurality of constituents for the insaponifiable fractions of organs which has to be related to the plurality of constituents found in the acid lipid fractions of the same organs and which was discussed above. The specificity seen for organs would thus greatly concern their lipidic constituents which form the acid and the insaponifiable fractions. It is especially in terms of specificity that the acid lipidic and insaponifiable fractions of various organs are being investigated in research now in progress. *(Note 7)*

Corticoids

The study of the defense of the organism against fatty acids focusses attention once again on the adrenals whose constituents appear to be part of the natural defense mechanism. To date, around 30 different crystallizable compounds have been isolated from less than a third of the total cortical extract. The amorphous part, biologically more active than the crystallized part, would contain other important compounds. Even if some of them are intermediary compounds or artefacts, adrenal intervention still is characterized by plurality of its active agents. Furthermore, several opposite tendencies are recognized between groups of adrenal compounds. While all the corticoids show a certain antagonistic action toward fatty acids, mineralocorticoids are, from several points of view, antagonistic to neoglucogenics.

With these considerations in mind, and recognizing the adrenals as one of the principal means for relatively rapid defense against noxious agents, we have investigated the relationship of the adrenals to lipids.

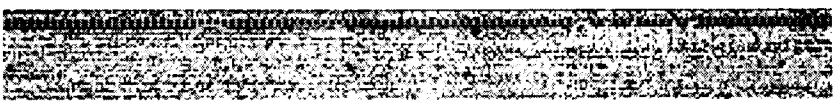
We have already noted the striking richness of the adrenals in arachi-

donic acid. About 25% of the total fatty acid content of the glands is made up of this acid which is found only in small quantities in other organs. We also found pentaenes and hexaenes present in greater amounts than in other organs. These fatty acids must have a biological purpose and two hypotheses can be advanced. According to one mentioned previously, *(Note 11, Chapter VI)*, the corticoids would be synthesized from arachidonic acid through cyclization. According to the second hypothesis, arachidonic acid, as well as other high fatty acids present in the adrenals, would be used as active functional fatty acids. Secreted by the adrenals, they would pass into the circulation and intervene as needed by the organism, especially for immediate defense purposes.

We have seen that an intervention of polyunsaturated fatty acids occurs in the first defense response of an organism to a noxious agent. These acids are responsible for the exaggerated oxidation processes through which the organism attacks the noxious agents themselves or the heterogenized constituents resulting from their action. We consider that some of the fatty acids intervening in this defense mechanism are liberated locally, especially if they appear in response to a condition limited to a lower entity. In this case, they would come from changes induced in the constituents of the entity itself. The local intervention of lipolytic enzymes would lead to a liberation of free fatty acids. In a defense response for the organism as the highest entity, the actively intervening fatty acids appear in the general circulation in the first phase of the diphasic phenomenon. Some of these fatty acids would be of adrenal origin, liberated at these moments. In the second phase of the defense mechanism, a further liberation of steroids by the adrenals would occur, aimed at counteracting the effects of fatty acids. The diphasic systemic process which results can be considered to represent an exaggeration of the processes which occur alternately and which, through normal oscillations, insure the dynamic systemic balance.

The adrenals conceivably control abnormal fatty acid activity by their quantitatively exaggerated intervention and by release of qualitatively abnormal products that would pass into the circulation and result in off-balances. The activity of the adrenals in counteracting the influence exercised by fatty acids has been made the subject of a special investigation in our laboratories by E. F. Taskier.

By comparing the doses of an agent required to kill normal and adrenalectomized animals, it has been possible not only to identify this intervention but to judge the degree of this specific defense mechanism. The "Adrenal Defense Index" for an agent—the ratio between the minimal lethal dose in normal animals and in adrenalectomized animals represents



a numerical estimate of this response. It could be shown that for certain fatty acids, such as conjugated trienes, which are related to trauma, or alpha hydroxy fatty acids which are related to microbial invasion, a highly effective intervention of the adrenals occurs, through release of neoglucogenic corticoids. The administration of neoglucogenic corticoids manifestly increases the resistance of the organism to the noxious effects of fatty acids. This influence is reduced for the mineralocorticoids and is nil for sodium chloride, otherwise an important factor related to adrenal intervention. *(Note 17, Chapter VI)*

We will discuss later an important difference, even an antagonism, between these two groups of corticoids when their influence is exerted concomitantly with that of other agents.

Synthetic Anti-Fatty Acids

Analysis of the natural anti-fatty acid agents has revealed the importance of their positive polar groups. And this has guided us in attempts to obtain synthetic agents with anti-fatty acid effects.

An important step was a study of alcohols with lipoidic properties, the lipoalcohols, starting with the primary mono-alcohol homologous series. This study also has permitted us to recognize the importance of the lipoidic properties for their biological activity. We started with butanol which is the first member of the homologous series of aliphatic alcohols with lipoid characteristics.

Butanol

Butanol has a special place among the alcohols that have been utilized as anti-fatty acid agents, not only by virtue of its physico-chemical and biological properties but also because of interesting therapeutic results obtained in animals and humans.

Extensive studies with butanol have helped considerably in defining the physico-chemical and biological differences between lipoids and nonlipoids. According to the concept advanced previously, lipoids and nonlipoids can be distinguished by solubility characteristics which are determined by the energetic relationship between their polar and nonpolar groups. The nonpolar group is predominant in a lipoid; the polar group is predominant in a hydroid. Lipoids have greater solubility in neutral solvents than in water, and this provides a simple criterion for their recognition.

Methyl, ethyl and propyl alcohols are all equally more soluble in water than in neutral solvents and therefore are recognized as nonlipoids. Butanol,

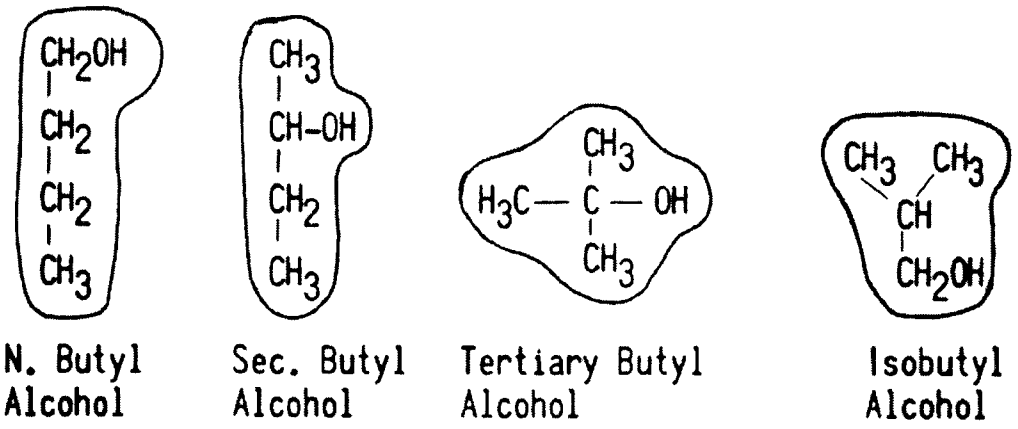


FIG. 133. Schematic representation of the molecular surfaces of the 4 isomers of butanol. The constant b of the van der Waals forces related to their surfaces are unequal. A minimum value is seen for almost spheric molecule of tertiary butanol, a fact which explains the nonpredominance of the polar group in this molecule, respectively its non-lipoidic character.

however, differs from the lower members of the aliphatic alcohol series by being a lipoid, more soluble in neutral solvents than in water. This, however, is true only for three of the four isomers of butanol. n-Butanol, sec-butanol and iso-butanol are all more soluble in neutral solvents than in water, whereas tert-butanol is equally soluble in both. According to our criterion therefore, while the first three are lipoids, tert-butanol is not.

These considerations have enabled us to correlate lipoidal properties

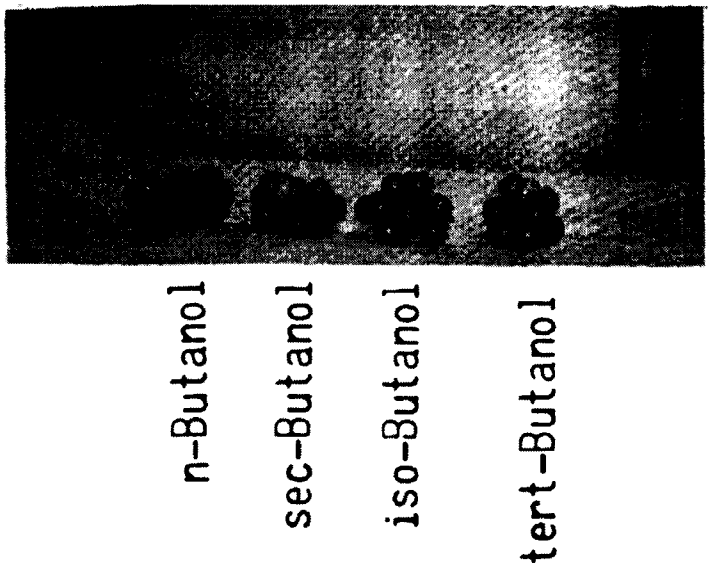


FIG. 134. The differences between the round shape of tertiary butanol and the longer form of the other isomers is evident with models of molecules.

with one more precise intermolecular factor, the predominance of one of the van der Waals cohesion forces. Comparative analysis of the structural formula of the four isomers of butanol *(Fig. 133)*, reveals the importance of forces related to the surface of the molecules in determining differences in their solubility. In contrast to the three lipoid isomers, the molecule of tertiary butanol is rounder and hence has a smaller surface. The difference between tert-butanol and the other three isomers is apparently due to the cohesion forces related to the surface area of the molecule. Of the van der Waals forces, those described as related to the surface of the molecules, or

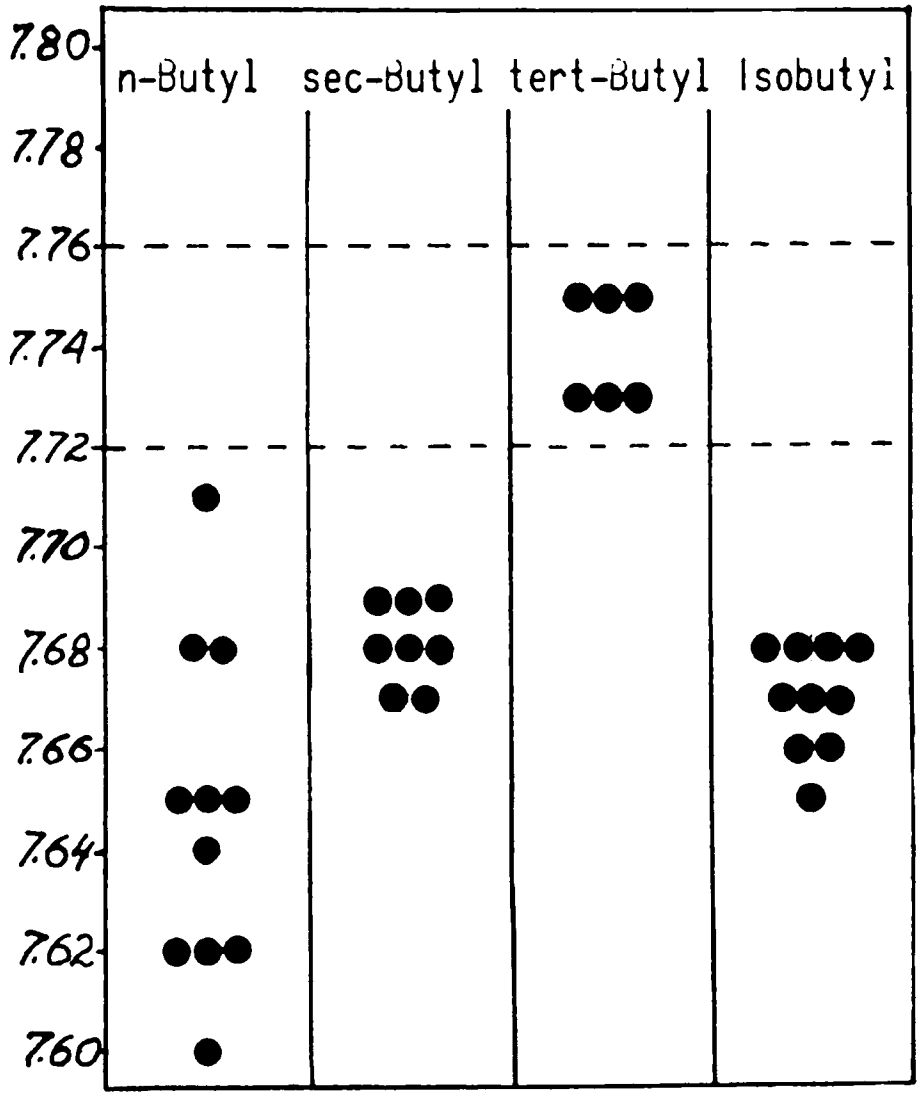


FIG. 135. While the 3 isomers of butanol which are lipoids influence the second day wound crust pH, lowering its values, tertiary butanol which is not a lipoid, does not influence it.

as the constant b of the cohesion forces, thus appeared to be most important in determining lipoidic properties. *(Fig. 134)*

Study of the four isomers of butanol has confirmed the importance of lipoidic properties for biological activity. Like the lower members of the homologous series of aliphatic alcohols which are not lipoids, tertiary butanol does not influence pH of the second day crust of a wound, while the three other isomers, all with lipoidic characters, lower the pH as the higher members of this series do. *(Fig. 135)*

FIG. 136. Effect of 0.5% solution of *n-butanol* administered instead of drinking water upon the increase in weight of young rats. The values represent the average for 20 females (. . . .). No differences are seen from nontreated controls (——).

The fact that a saturated water solution at 20°C still contains 7.9% n-butanol is of great practical importance. Because of its degree of solubility in water, n-butanol could be utilized in aqueous solutions in sufficiently high concentration for pharmacological studies and could be used as a therapeutic agent in this form without need for an oily solvent vehicle.

The acute toxicity dose for butanol corresponds to the narcotic dose for the respiratory centers which is related to interference with the aerobiotic life of these cells.

The minimal lethal dose of n-butanol administered subcutaneously was found to be 4.6-6.4 gm./Kgm. for mice, 3.7-5.9 gm./Kgm. for rats and 3.3-5.6 gm./Kgm. for rabbits, guinea pigs and hamsters. These values closely approximate the findings of other workers. The minimal lethal dose of n-butanol injected intraperitoneally is very close to that for subcutaneous

and intramuscular administration, indicating that absorption from the tissues is almost as rapid as from serous cavities.

We have administered butanol in large doses to human subjects, and these clinical studies have confirmed the laboratory findings that the toxic effect is especially manifest through the narcotic effect and is attained only with the use of very large doses. *(Note 8)*

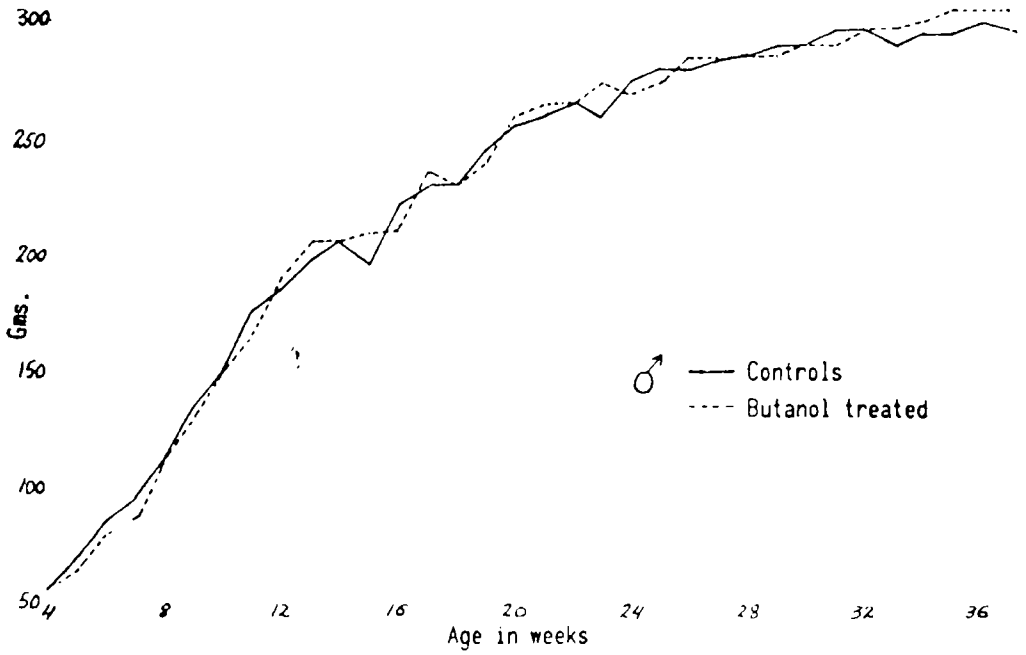


FIG. 137. The same daily change seen in male rats.

Long term use of n-butanol has virtually no influence upon normal physiology in animals. Administered continuously in the drinking water of young animals, it had no effect on growth. *(Fig. 136)* It also did not affect reproduction capabilities of mature animals or influence their offsprings.

n-Butanol shows a definite influence on white blood cells in rats. The leucocyte count is increased in adult rats receiving daily injections of a saturated solution of n-butanol. *(Note 9)*

The influence exerted by small doses of n-butanol, as for other lipoids, appears to be almost entirely confined to abnormal tissues and cells. This is evident in the influence upon the pH of experimentally induced wounds in animals. Administered before wound inductions, n-butanol showed no influence upon normal tissue, no differences were observed between pH of their normal tissues in treated and untreated animals. During the first day

following wound inductions, pH of the lesion in treated animals was no different from pH in untreated control animals. However, by the second day, pH of the wound crust was lowered by butanol, as seen in Figure 135.

n-Butanol accelerated the healing rate of wounds, although the differences between treated animals and controls was not striking. n-Butanol enhanced healing of radiation burns to some extent but the effect was not constant in different groups of animals. In several animals treated with n-butanol, radiation wounds healed within two or three weeks, while in controls healing took more than four weeks.

Butanol, when administered to patients with pain of alkaline pattern, has repeatedly provided relief within a very short time—in some cases within three to five minutes. In pain of an acid pattern, exacerbation occurs, also within a few minutes. Its quick effect has led to use of butanol as a diagnostic means for determining the pain pattern.

The anti-fatty acid action of n-butanol has led to the investigation of its effect upon shock since, as previously noted, shock appears to be related to intervention of abnormal fatty acids. Administration of butanol subcutaneously, even together with large amounts of saline, is only slightly beneficial for shock in mice with caloric burns. The addition of sodium lactate has markedly prolonged survival time in these animals. *(Note 10) (Fig. 138)* Still better effects upon traumatic conditions are obtained by associating butanol with glycerophosphoric acid in saline or in glucose saline solution. Especially effective and well tolerated is a solution containing 0.3-0.5 gm.% butanol with n/300 to n/200 glycerophosphoric acid and with 5% glucose in saline, used for intravenous infusions, as well as for subcutaneous clysis.

The administration of butanol in sufficient amounts to many patients having massive hemorrhages has clearly demonstrated that this substance has a hemostatic effect which will be discussed below.

After butanol studies, the effects of other aliphatic alcohols were investigated and revealed the importance of the nonpolar group in their biological activity.

Higher Alcohols

The study of aliphatic alcohols has shown that only few members of this homologous series, beginning with butanol and ending with octanol, have an effect upon the s.d.c. pH. For octanol, only half of the test animals showed changes in second day wound crust pH. *(Fig. 139)* We thought it worthwhile to study the biological effects exerted by these members of the series, including those which demonstrated no effect on the second day

wound crust pH. Comparative studies indicate definite differences between the two groups with odd or even number of carbons in their ability to act upon an existing offbalance and reduce the abnormal metabolism. Along with differences, many common properties were recognized through effects

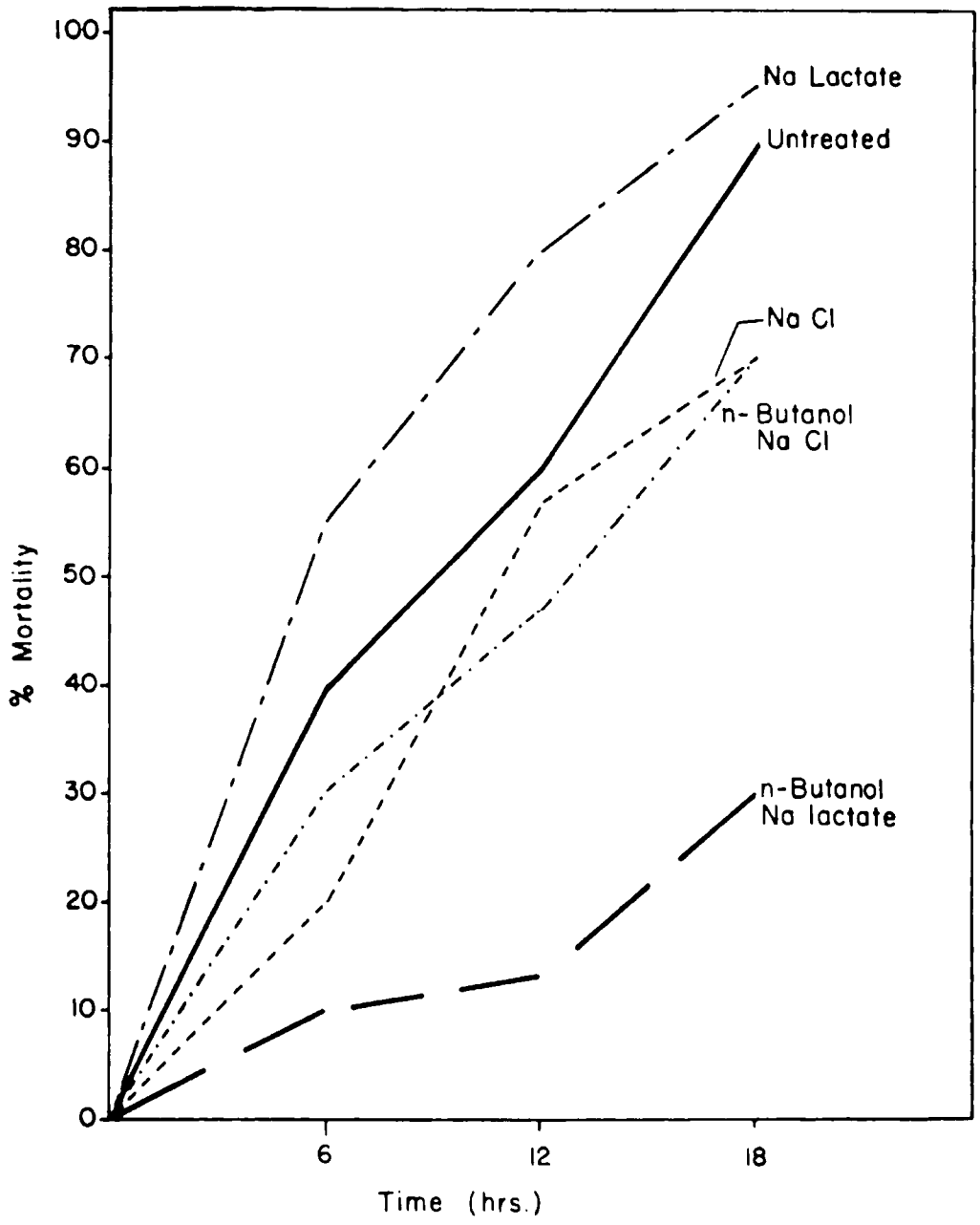


FIG. 138. Influence exerted by different agents upon the mortality of mice scalded for 3 seconds in water at 90°C. While sodium lactate seems even to increase the mortality and NaCl and butanol in saline have little influence, a marked prolongation of the survival time is induced by the mixture of n-butanol and Na lactate.

induced at various levels. In general, the effects are more profound for members with longer chains. This is true for butanol compared to hexanol in the even carbon series and for pentanol compared to heptanol in the odd carbon series. For octanol, most of the effects are diminished. In the group with odd carbon numbers, nonanol has very little or no influence.

On viruses, a protective effect against external influences such as heat or fatty acids is evident. It is more striking for the even carbon group. In microbes, little except an antibacterial effect is produced by members in the even carbon group. The odd carbon group induces Gram positivity, irregularities in form with a tendency toward roundness, and vacuolization.

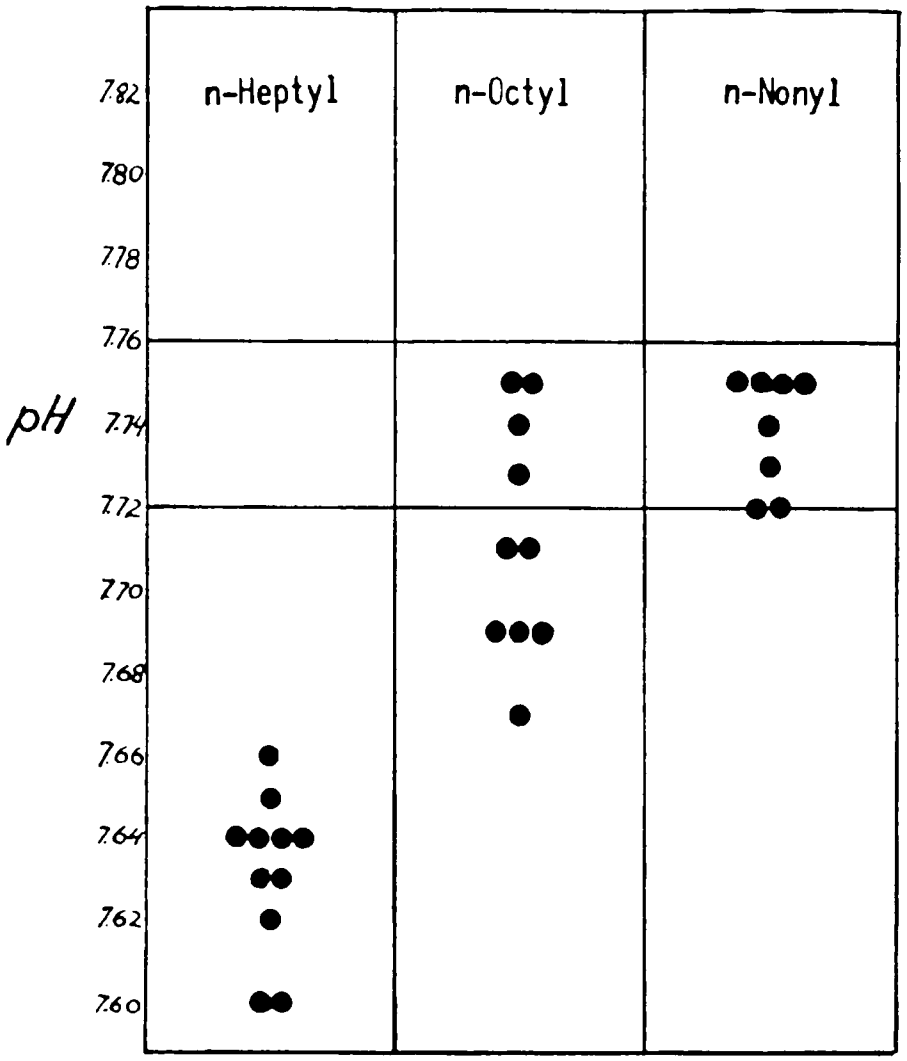


FIG. 139. The influence exerted by heptanol, octanol and nonanol upon the s.d.c. pH. While heptanol induces constantly a lowering of the alkalinity of the second day wound crust, this effect is less constant for octanol and nil for nonanol.

No important changes occur at the cellular level. We have already mentioned the different effects upon wound crust pH for these alcohols. On pain, nonanol has no effect and hexanol and octanol relatively little. But pentanol and especially heptanol show a very marked influence, both immediate and prolonged. It is interesting to note another striking effect at the tissular level, observed only for heptanol. It corresponds to an abnormal accumulation of fluid in certain abnormal tissues. This was first seen in surgical scars even several months old. Edema of the entire scar occurred followed by blistering at the surface, or even by formation of fluid-filled cavities in the scar itself. The same phenomenon appeared in other lesions such as tumors, especially when they were infected, although there had been no clinical indication of infection before the administration of heptanol. The effect sometimes was very intense, transforming an entire lesion, scar or tumor into a cavity with septic fluid exudate, but few leucocytes.

The influence of high doses of heptanol upon inflamatory processes could be judged experimentally in the gas pouch induced subcutaneously in rats or mice by injecting nitrogen and subsequently injected with a low pathogenic microbe. In controls, no unfavorable effects were noted. In animals injected with heptanol subcutaneously, fluid exudate accumulated in the pouch in a few days. *(Note 11)* Subcutaneous administration of heptanol also induced an exudate in the peritoneal cavity in mice and rats injected with the same microbial suspension. This did not occur in the controls. It must be emphasized that these effects were seen only with relatively high doses of heptanol.

At the organic level, while nonanol again showed no activity, the two higher alcohols, heptanol and octanol, had an influence upon the central nervous system. In humans, even in larger doses, such as 200 mgr. six times a day (2 cc. of a 10% solution in oil every four hours) repeated for ten days or more, the two higher alcohols produced no abnormal central nervous system manifestations. In some subjects who had previously had convulsive attacks, administration even in small doses, such as 25-50 milligrams once a day, did induce convulsive seizures. If these substances were given along with desoxycorticosterone, the latter even in doses of 1 mgr. a day, severe and even fatal convulsions were produced. Nonanol had no such effect. Somnolence followed by coma was observed with concomitant administration of cortisone and heptanol or octanol, but nonanol did not produce this effect either.

Of interest was the influence exerted by heptanol upon the different analyses. Fig. 140 shows how these values change toward the offbalance A

under the influence of heptanol. It is to be noted that of all the analyses, the urinary pH and the blood serum potassium are the first to be changed. They are followed by specific gravity, while the urinary surface tension seems to be influenced last.

M. Bier, in our laboratories, has shown that alcohols, when added in vitro to freshly obtained blood, reduced blood clot retractibility. It is interesting to note here the relationship Bier has shown between this effect in

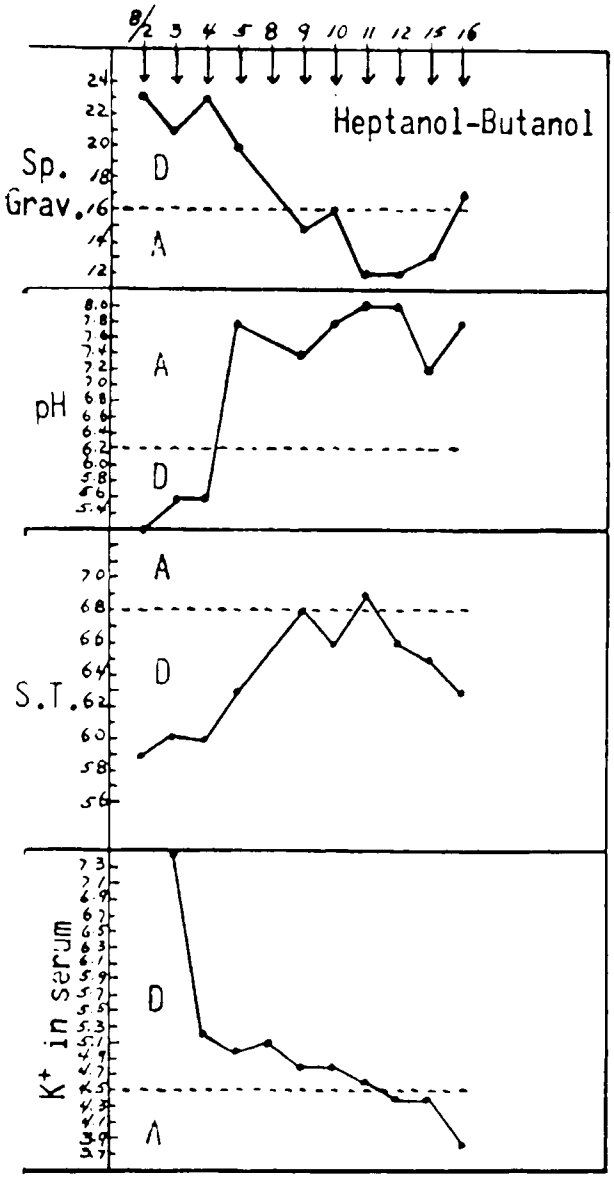


FIG. 140. In a patient with bone metastatic lesions the administration of heptanol and butanol shows a progressive decrease in the values of serum potassium and increase in the urinary pH, toward offbalance type A.

retractibility and other properties of the alcohol series members. Thus, he could demonstrate that there is a critical value for the concentration of each alcohol, when mixed with fresh blood: blood clot retraction is prevented only when this value is exceeded. The critical value varies with the length of the chain, decreasing for the higher members. *(Fig. 143)* Bier also

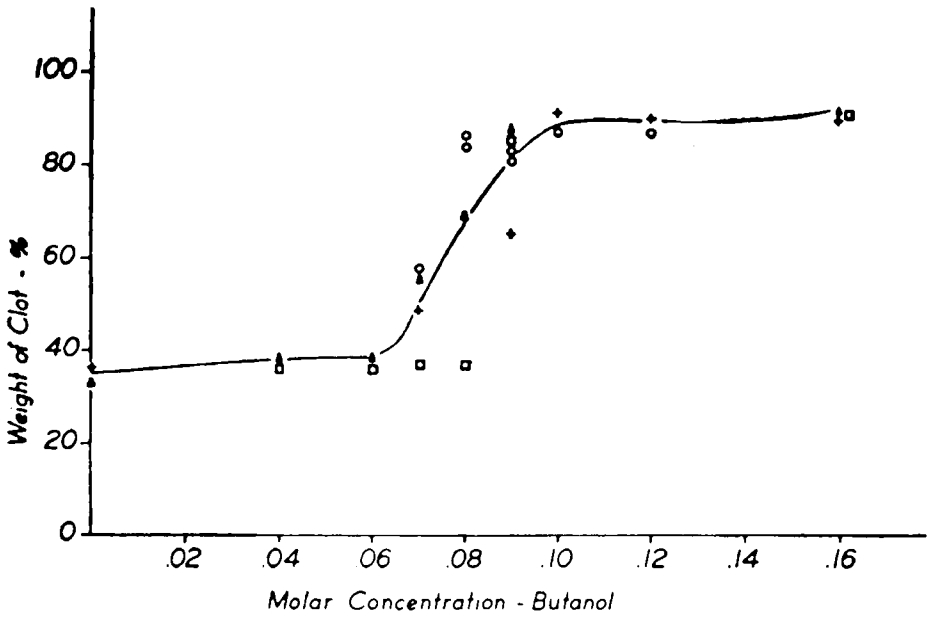


FIG. 141. Clot retraction, measured as percent weight of clot/total weight of blood, plotted against molar concentration of butanol in blood. Different symbols used for blood samples of each animal.

has shown that, since the toxicity of these alcohols seems to be related to the same factor, a correlation can be established between critical concentration values and lethal toxic doses.

This relationship, as shown in Fig. 142, applies to the members of this series of saturated alcohols, but not to alcohols of another series also studied. For the latter, the toxic dose is higher than the critical dilution at which the clot retraction is influenced, and this can be explained by the intervention of the double bond in the molecules.

Systemic effects were seen for these alcohols if administered in sufficient doses. Some special effects also were seen. Heptanol decreased the sulfhydryl index in urine analyses, especially if it had been high previously. Octanol's action was mainly to increase surface tension if it had been low. Nonanol did not show any such activity at all.

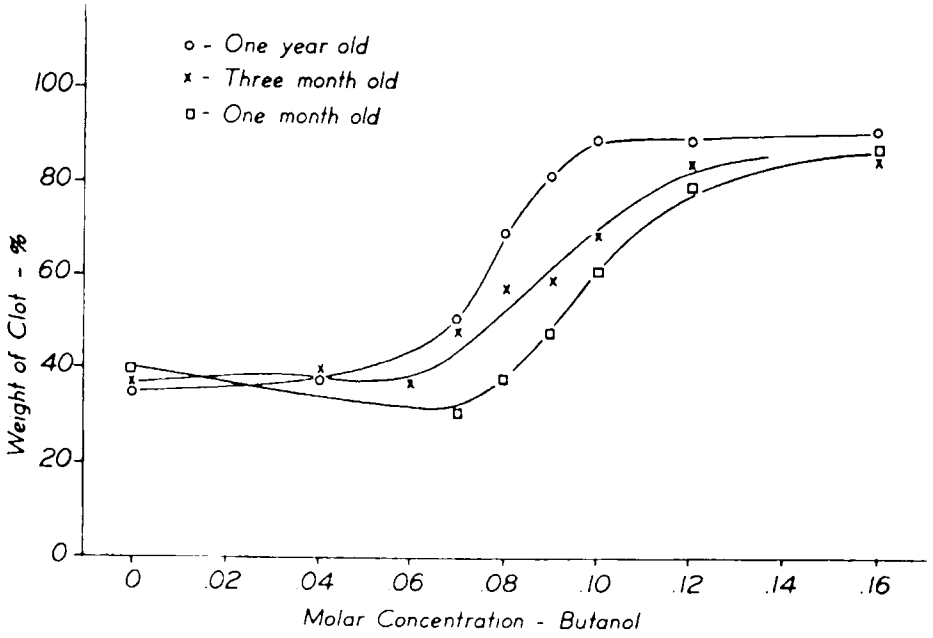


FIG. 142. Clot retraction, measured as percent weight of clot/total weight of blood, plotted against similar concentration of butanol in blood. Averages of different age group animals were studied.

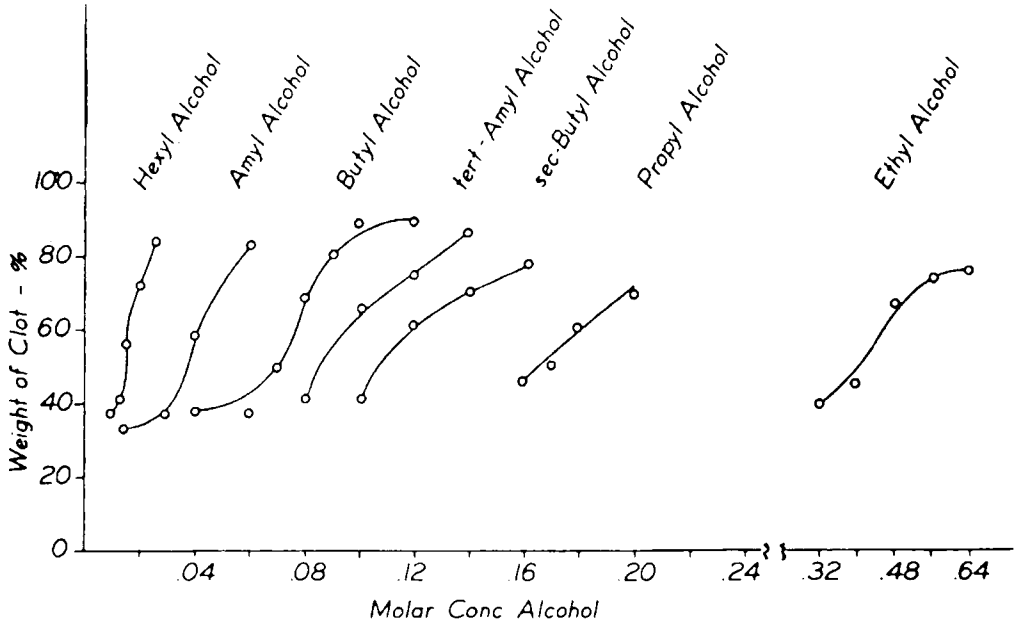


FIG. 143. Clot retraction, measured as percent weight of clot/total weight of blood, plotted against similar concentration of various alcohols in blood.

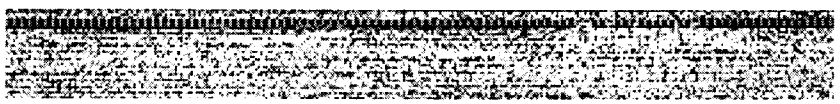
Polyols

In another study, we considered the polyalcohols, bearing in mind the important role played by glycerol in the biological activity of lipids. In animals, ethylene-glycol and diethylene-glycol proved to be too toxic for parenteral administration. However, near toxic doses produced interesting results especially in tumors. Even in relatively small subcutaneous Walker rat tumors, 2.5 cm. in diameter, for instance, necrosis was constantly induced and followed by skin ulceration. The characteristic influence of these alcohols was to induce a necrotic process not limited to the tumor alone but affecting surrounding tissues.

1.2 Diols

By following the influence exerted by more than one hydroxyl in the molecule, we tried to relate properties of glycerol to those of aliphatic lipoids. We prepared lipoids having a polar formation of 2 or 3 hydroxyls bound to the first carbons of an aliphatic chain. The lipoidic character was induced by the length of the chain. To prepare these substances, we started with corresponding alph-hydroxy fatty acids in which the carboxyl was reduced to a primary alcohol by treatment with lithium aluminum hydride. As a prototype, we studied 1.2 octanediol. The lipoidic character was recognized by its high solubility in neutral solvents and a limited solubility in water.

There were no marked differences between the effects of octanol and 1.2 octanediol in systemic analyses. Both raised surface tension values in particular. However, the new component had an effect upon the central nervous system different from most other higher alcohols. As mentioned above, these aliphatic mono-alcohols do not induce convulsions without the concomitant intervention of another factor. The second factor can be a local condition in the nervous system itself, as in subjects with cerebral tumors, or others who have had previous convulsions. It can also be another substance; desoxycorticosterol, coramine, glycerol or glucose, when administered with octanol, for example, induced convulsions in some subjects. However, 1.2 octanediol, in repeated doses of around 200 mgr. daily, induced convulsions by itself. This could be explained by the fact that 1.2 octanediol contains in its molecule a group which energetically resembles glycerol. Beyond this effect, there were no manifest differences between this substance and corresponding mono-alcohols in influence upon pain, tumor growth or systemic manifestations.



Lipoalcohols with Energetic Centers in the Nonpolar Group

We also studied other alcohols with energetic centers in their nonpolar group. From various ethenic fatty acids, we prepared the corresponding alcohols by reducing the carboxyls to primary alcohols through treatment with lithium aluminum hydride. Thus we obtained, in addition to oleic alcohol, linoleic and ricinoleic alcohols for the nonconjugated fatty acids and eleostearic alcohol for the conjugated members, as well as the corresponding alcohols of an entire series of mixtures of fatty acids from safflower oil, cotton seed oil, cod liver oil, acid lipids of organs, etc., and of conjugated fatty acids derived from them. Having two series of substances with the same common nonpolar group, but with different polar groups, COOH and OH, we could relate the effect of these alcohols to their respective fatty acids and thus ascertain once more the fundamental role played by the polar and nonpolar groups in determining the biological effects of lipoids.

Study of the effects induced upon skin viral infection has largely helped to define the differences. The polar group determines the direction of the intervention—increased receptivity or refractivity—but the extent of intervention is determined by the nonpolar group. For example, the effect is very much reduced for oleic and even for linoleic alcohols and it was similarly reduced, in the opposite direction, for the respective acids. Effects were more apparent for polyunsaturated alcohols, ricinoleic and eleostearic alcohols, polyconjugated members and corresponding acids.

The same antagonism between the corresponding acids and alcohols was very clear for systemic analyses, pain, healing of wounds, and effects upon tumors. The extent of the effects in either direction is generally determined by the nature of the nonpolar group and its energetic centers.

This comparative study of acids and alcoholic lipoids has permitted us to arrive at an important conclusion concerning the general behavior of lipoids. Thus, while the nonpolar group is extremely important for the extent of the changes induced, it appears to be secondary in importance to the polar group which determines the direction of the changes. And this explains the role attributed to the polar group in the biological activity of these agents and their separation into two fundamental groups with antagonistic biological properties, which is the basis of our approach to lipoids.

We studied the effects of alcohols and mixtures of polyunsaturated nonconjugated and polyconjugated fatty alcohols on a larger scale. These preparations were obtained from safflower and cod liver oil. The immediate effect upon alkaline pain was nearly complete relief. With prolonged treat-

ment, the relief persisted in most cases. No influence upon tumors was noted in most experiments in animals. In humans, tumor arrest in a few cases was obtained. The effect was more accentuated than with isolated members such as eleostearic, linoleic or oleic alcohols. The convulsant effect was much lower than for any other lipoalcohol of this group; even large doses did not produce convulsions except in patients who previously had had convulsions. Effects upon systemic analyses were the same as for most of the higher alcohols, manifest especially upon urinary surface tension and sulfhydryl index. The polyconjugated alcohol mixture, as an agent acting at the systemic level, produced euphoria but had very little effect upon growth and evolution of tumors.

With the intention of lowering the level at which alcohols would act, we studied a special group characterized by having a double bond between C_2 and C_3. We were particularly interested in two members of this series, allyl and crotyl alcohols.

Other Alcohols

Crotyl alcohol is a lipoid since it is miscible with neutral solvents and only slightly soluble in water. Allyl alcohol, soluble both in water and neutral solvents, appears to be an intermediary substance. Since the 1% solution of crotyl alcohol in saline was painful when given by intramuscular injection, an oily 2% solution was used. No marked differences from the effects of previously discussed lipoalcohols were seen.

We studied polyalcohols having, in addition to a primary alcohol, one or more OH attached to the molecule, such as 9.10-dihydroxy-stearic alcohol. However, this substance did not show any properties other than those noted for oleic alcohol. The alcohol obtained from ricinoleic acid, in which the carboxyl was reduced to a primary alcohol, showed a limited systemic effect. Even in larger doses, the changes were slow and not intensive, although passages from one pattern to the other could be seen more often than with other higher alcohols. A state of euphoria appeared in some subjects. The immediate effect upon pain was less than that obtained with other alcohols but, in many instances, was satisfactory. There was no favorable effect upon growth or persistence of tumors in animals or humans. In several cases, on the contrary, rapid growth of the tumor occurred despite lack of pain and even sensations of well being. In general, ricinoleic alcohol seems to act at the interstitial level and above, but not below.

Lipamines

Theoretically, it was to be expected that lipids with an amine as polar group would have a marked anti-fatty acid action. We studied several of these substances from the standpoint of their influence upon physiopathological changes considered related to lipoidic predominance. The first lipoid of the aliphatic amine series is hexylamine. A nonpolar group of at least 6 carbons is required for predominance over the potent amino radical. From commercial sources, we obtained amines corresponding to the usual saturated fatty acids with even numbers of carbons, ranging from 6 to 18; a few unsaturated with 18 carbons; and heptylamine with an odd number of carbons. All these compounds, when injected in mice and rats produced severe local reactions, often followed by skin ulcerations, even when administered in oily solutions. For this reason, we tried salts of these amines usually obtained with acetic or hydrochloric acid. Salts of the lower members of the series, no longer had lipoidic character. However, we could use hexylamine in an oily solution for intramuscular injection. It appeared to be relatively well tolerated locally even in humans. No apparent changes were seen, however, in systemic analyses, and the immediate and long range effects upon pain were minimal. No changes were obtained in experimental tumors except by local treatment, as in ascites tumor, or by injecting the product at the level of the transplant itself, in which case the growth of the tumor was slowed or even halted. A similar effect was seen when the transplant was dipped in the oily solution of the product and the procedure was repeated in successive generations.

On a larger scale, we utilized, both in animals and humans, the salt obtained from hexylamine with nicotinic acid. It showed favorable influence on pain of alkaline pattern, and exacerbated pain of acid pattern, but had no other effects. Heptylamine has been used by others as a hypertensive agent. In our studies, its hypertensive activity appeared to be weak and transitory.

The study of lipamines was the starting point for an entire series of researches into the biological role of the amino group, especially as it intervenes in a complex molecule. We have seen that, like all other polar groups, the amino group will act as a fixing group in a molecule. Its characteristics appear to be related to its capacity to bind the molecule to other molecules in a relatively stronger and more specific way than other polar groups.

The realization of more complex chemical polymers as biological entities evidently is related to this capacity of the amino group. This appears clear when the amino group is bound to various acids in the alpha position

to form the alpha amino acids that enter into the formation of complex proteins. In amino sugars, the amino group shows the same property, producing the polymer formations characteristic of connective tissues. Furthermore, it is the amino group, acting as a second polar group, which gives to alkaline amino acids their fundamental role in the biological realm, as mentioned above. The alkaline amino acids, like other amino acids, form polymers through their amino acid group. However, as these polymers, histones or protamines remain reactive through the terminal alkaline nitrogen-containing groups and it is through these groups that they realize new bonds, such as to nucleic acids.

In a molecule with two polar groups far apart the amino group will fix the molecule, while the other active radical will provide reactivity. The selective fixation upon certain constituents in various places in the organization accomplished by the amino group localizes the intervention of the other active groups of the molecule.

We have considered many of the biological substances containing an amino group and a second active group. Typical examples are the local anesthetics in which the amino group acts to fix the molecule, while the other energetic formations intervene more actively to induce the anaesthetic effect. Similarly, in epinephrine and ephedrine, the amino group serves to fix the molecule, while the hydroxyls later intervene more specifically.

In some molecules such as the alkaline amino acids, the second active group can be another amine or another nitrogen containing group. It was for this reason that we first became interested in investigating natural and synthetic compounds with an amino acid to serve for fixation, and another energetic center to intervene more actively. We studied amino-butanols, but no particular activity could be found. These agents, however, were not lipoids. We were consequently interested in substances having a lipoid character as well as two active polar groups of which one is an amine. A study of these agents is now in progress.

Procaine

In this group of agents we studied procaine. We became especially interested in procaine after we had seen some cases of ulcerations due to varicose veins healed in a few days with only two intra-arterial injections of procaine according to Leriche's method. With the idea in mind that an action through its lipoidic properties intervenes, we studied procaine base. Solutions of procaine base in sesame oil, or suspension in Tween or gum cellulose were prepared. Parallel with these procaine base preparations, we studied also the hydrochloride as well as several salts of procaine, such as

lactate, glycerophosphate, where a further effect was sought through the acids at which procaine was bound. We studied all these agents but only under the aspect of their activity in relationship to the dualistic offbalances.

No special effects were seen upon viruses and microbes from this point of view. Injected subcutaneously to rabbits, it was seen to increase in the skin corresponding to the place of injection, the manifestations of a subsequent smallpox inoculation. Added to the medium for cultures of tetrahymena pyriformis in higher doses, it led to the appearance of almost round forms. We studied the survival time through the capillary tubes method mentioned above. For progressive amounts of procaine hydrochloride added to a culture of tetrahymena, no immediate effect of the life-span of the culture could be seen. In another group of experiments, the procaine was added to the culture before its inoculation and the life-span of the tetrahymena grown on this medium was studied. Under these circumstances a prolongation of the life of the culture itself could be seen, but only in the cultures which were showing also a slow growth.

Procaine in general, and much more evident in the base preparations, was seen to reduce the pH values of the second day wound crust. In mice and rats, the lethal doses were seen to be manifested through the appearance of convulsions. Sublethal doses were seen to lead to convulsions after a few successive days of administration. In humans, an intramuscular injection with procaine base was seen to influence pain but this effect was apparently limited only to the cases with an alkaline pattern present. This and the fact that the effects were no more manifest with procaine base than with other lipoidic preparations with positive polar group, even when administered in relatively high doses such as of 2 cc. of the 5% solution of procaine in oil, indicates that its action other than at the place of the injection, has to be related largely to the intervention of procaine as lipoid. A special mention, however, has to be made for the angina pain which seems to be more evidently influenced by the injection with procaine base than other pains.

The immediate influence exerted upon systemic analyses, if any, was in general reduced even after the administration of 10 cc. of 2% procaine hydrochloride. It was manifested particularly upon the urinary pH, inducing a change toward more alkaline values. This effect was transitory and especially seen for the first injections. With repeated doses, this effect decreased even to the point of disappearing.

The study of the pharmacology of procaine has emphasized the character of its activity as a lipoid with a positive polar group rather than a direct one through its chemical constituents. This view is confirmed by the

fact that the same effects are not obtained with any one of the two constituents of procaine, para-aminobenzoic acid and diethylaminoethanol, administered separated or even together. The only difference in this case between procaine and its constituents resides in the properties of procaine as such, which we related largely to its lipoidic properties.

Furthermore, the study of the different effects induced by procaine can be seen to result from a nonspecific effect in which these agents intervene indirectly upon the metabolic processes which occur at the cells for instance, rather than to a direct influence exerted by procaine constituents upon specific metabolic processes. In this group would enter the effects seen upon different enzymatic processes indirectly influenced by procaine through the nonspecific changes induced.

We have investigated under this special aspect the use of procaine hydrochloride in solutions with a pH between 3 and 4, as indicated by A. Aslan, against the manifestations of old age. The low pH seems to intervene by preventing, as long as possible, the enzymatic hydrolysis of procaine and thus permitting its absorption and action as a nondissociated lipoid. We found that the protection exerted by the low pH would result from the chemotactic negative influence exercised by hydrosoluble acids upon the leucocytes, which thus will reduce the intervention of the leucocytic enzymes in the process of hydrolysis.

Furthermore, the fact that the bond of procaine, as such, takes place through the amino radical of the P A B A explains why a high acidity would prevent this local bond of procaine to occur at the place of the injection and thus would favor its action at other sites in the organism. This action is confirmed by the fact that the analgesic properties of procaine decrease through the low pH, while at the same time, its other metabolic effects increase.

The fact that good effects were observed with procaine in old age and arthritis, schizophrenia and many other conditions, indicates that a nonspecific action would take place. The fact that the effects obtained are similar to those observed in the same conditions with other lipoidic agents with a positive polar group, permits us to see as principal factor in the pharmacological action of procaine, its nonspecific intervention as positive lipoid. Besides this nonspecific lipoidic effect, some others related to its chemical constituents and which would represent specific added factors have to be considered. The relation to the folic acid would be an example.

All these considerations led us to study various preparations in which procaine was bound to different agents, with the aim to enhance its nonspecific intervention. Through procaine lactate, malate and citrate, the

chemotactic negative effect was highly increased and procaine activity enhanced. Bound to maleic or citraconic acids, the anti-fatty acid activity of procaine was further directed toward influencing more specifically, the abnormal changes occurring leading to the appearance of abnormal fatty acids.

The Elements

With the recognition that elements act biologically in two opposite directions, we became interested in those with anti-D character, capable of intervening in offbalance D. We studied the direct action of these agents upon fatty acids, as well as their indirect action upon processes and substances related to the metabolic changes characterizing the offbalance D.

We separated these inducing elements—according to their series and the compartments where they predominantly exercise their influence. The following table shows this systematization.

TABLE XIX

"A" Inducing Group or Anti-D Group

	Metals						*Non Metals*		
	Series							*Series*	
Compartments	IA	IVB	VIB	VIII	VIII′	IIB	IIIA	VA	VII A
Organism	Li						B	N	F
Metazoic	Na						Al	P	Cl
Cellular	K	Ti	Cr	Fe	Ni	Zn	Ga	As	Br
Nuclear	Rb	Zr	Mo	Ru	Pd	Cd	In	Sb	I
Submorphologic	Cs	Hf	W	Os	Pt	Hg	Tl	Bi	At
Primary	Fr								

To these elements we can add others from the lanthanum and actinium series which have anti-D characteristics. They would act in the submorphological and primary compartments.

Compartment	*Anti-D Elements*							
Submorphologic	La	Pr	Pm	Eu	Tb	Ho	Tm	Lu
Primary	Ac	Pa	Np	Am	Bk			

Many of these elements are known to have influences antagonistic to those of members of the D inducing group. We will consider them in more detail below.

Concerning their influence on fatty acids, some members such as those of the VII A series are known to affect fatty acids with an active nonpolar group by inactivating their double bonds, while other elements have anti-fatty acid action by binding their polar groups. Some exert this influence indirectly through the metabolism in which they take part.

We have studied these different aspects of the influence of the elements which will be presented here only in condensed form.

Monovalent Cations

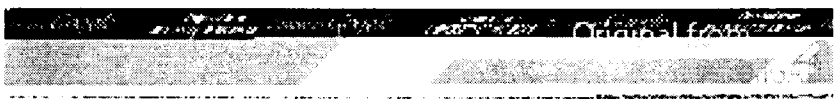
One interesting aspect of monovalent cations is their correlation with organizational hierarchic compartments. Study of this correlation was first suggested by the selective distribution of monovalent cations according to levels of organization. From the previous discussion, it seems clear that the distribution into compartments can be related to positions of these elements in different periods. Sodium is the principal cation at levels above the cell, forming the metazoic compartment. Potassium, which is the next higher element in the series, is the predominant cation in cytoplasm. According to our hypothesis, ammonium, with properties resembling those of rubidium, can be considered to be the cation of the nuclear compartment.

We have only limited evidence of direct anti-fatty acid activity for members of this I A series. However, they produce characteristic changes in conditions in which an offbalance exists. They induce the type "A," as an antagonistic to the type "D" offbalance. The changes are especially evident in terms of the function of the compartment to which the cation belongs.

Sodium

Sodium, the cation of the metazoic compartment, corresponds to the environment of the sea in which the entities of this compartment developed. We have indicated previously the relationship between the time when the metazoic compartment was formed, the degree of salinity of the sea, and the concentration of the cation established as a constant in the compartment. Any excess of sodium is eliminated through the kidneys in order to conserve the metazoic constant. If an excessive supply is retained for long, it favors the appearance of manifestations, which correspond largely to an offbalance of type A in the metazoic compartment.

We could show that the appearance of aorta atheromas in animals receiving an excess of dietary cholesterol is promoted by concomitant administration of sodium. This occurs not only in rabbits but also in rats in which a cholesterol-rich diet alone does not induce such lesions. Excess of



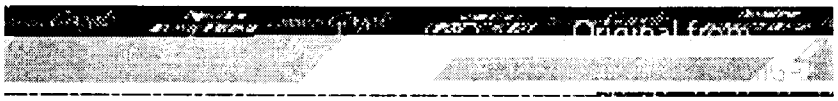
sodium also favors the appearance of thiamine-induced convulsions in animals. In rats and mice kept on high salt intake, the convulsant dose of thiamine fell from around 150 mgr./100 grams to below 100 mgr./100 grams of body weight. Administration of sodium—in the form of sodium chloride and especially as sodium lactate—favorably influences the state of shock which, as we have seen, corresponds to a change taking place principally at the metazoic level. In superacute and acute shock, we considered the excess of sodium present at still lower levels, such as cells and tissues, to be one of the pathogenic factors. The anomaly lies not alone in the excess but in the fact that the excess is at a level to which the cation does not belong.

The study of sodium metabolism in the light of organizational systematization of elements has revealed the importance of two factors; the combination which is "proper" for an element in its normal compartment and distribution of the element among compartments. In abnormal conditions, the unusual combinations occur. At the metazoic compartment, the bond of sodium to chloride can be considered to be normal. We have seen that, when this combination does not take place, the result is an anomaly characterized by accumulation of excess amounts of sodium in the immediately higher compartment.

In state of shock, for example, the pathogenic anomaly in fatty acids leads them to bind the chloride ion. This removes the normal combining factor for sodium which then passes on to the immediately superior compartment, the gastro-intestinal tract. This explains not only the excessive passage of sodium to the duodenum but also its retention there. The fact that sodium is present in reduced amounts in blood and in excessive amounts in the duodenum in the state of shock, illustrates the rule discussed above which we believe governs the distribution between levels of elements in abnormalities.

Potassium

This same rule would explain the distribution of potassium, another element of the same series. Potassium is the principal anti-D cation for the cellular compartment, as it is one of the principal constituents of the earth's crust, the environment in which the nuclei developed. Excess of potassium in the cell results in a cellular A offbalance with consequent active proliferation. Potassium in excess appears thus to be the cellular growth-inducing factor and its role in cancer has to be considered especially at the cellular level. Through the induced growth, the excess of cellular potassium would thus represent the factor immediately responsible for the invasive phase.



Following the rule of distribution of elements between the levels and compartments, an excess of cellular potassium will result in low blood potassium. This permits us to associate excess of cellular potassium and hypokalemia in cancer with active cellular proliferation. The opposite occurs in the state of shock and in offbalance D. The amount of potassium decreases in the cells. Consequently, the element accumulates and is retained in excessive amounts in the compartment immediately above the cells, the metazoic. Teleologically speaking, while the excess of sodium in the intestines and the excess of potassium in blood could be eliminated easily by means available to the organism, they are kept in high amounts in these superior compartments as reserves, disposable when and if the abnormality disappears and they can be used properly again. We have seen how this same mechanism explains the excessive amounts of copper in the blood in cancer patients.

This redistribution between compartments explains another fact about potassium. An excess of potassium is found in cancer cells: the greater the degree of malignancy, the greater the excess of the element. Along with the cellular excess, the amount of potassium in the blood drops to low values, even below 4 mEq. The low blood value cannot be related to lack of potassium, since quantities of the elements are eliminated in the urine. It has to be considered as a kind of defense through the higher level to an excessive amount of the element at its proper level. This relationship has led to the comparative study of the potassium content of red cells and serum as a means of obtaining indications concerning the intervention of this metal at its proper cellular level. The changes in the potassium content of red blood cells are considered to parallel those occurring in the cells in general. It is not the ratio between these values which is of interest, but each value by itself. Low amounts in red cells and in serum correspond to a quantitative deficiency; high amounts in both, to an excess of the metal; low amounts in cells and high in serum indicate a metabolic anomaly corresponding to a depletion of potassium in the cells as seen in offbalance D; while a high amount in cells and with low values in the serum indicate a cellular offbalance of the type A.

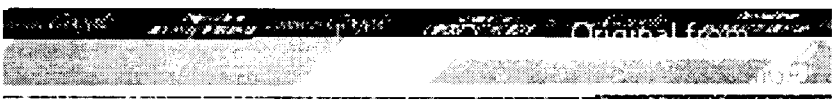
Administration of sodium and potassium as therapeutic aids has to be guided by these findings. Isotonic saline appears to be adequate as a replacement product but it is useless to administer it only because hyponatremia exists, except if this hyponatremia results from a quantitative insufficiency, as in excessive perspiration. The problem to be considered is how to restore the normal bond for the cation at its proper level. Often, what is needed is chloride ions for sodium, not more sodium. Similarly,

with hyperkalemia, if it exists in cases of type D offbalance, it is not the hyperkalemia which has to be directly attacked; efforts must be made to have potassium again normally fixed in its own cellular compartment. The use of glucose, insulin and ACTH seems to accomplish this for potassium. While administration of potassium in cases of cancer with hypokalemia possibly produces some immediate subjective improvement, it is constantly followed by an exacerbation of tumor growth as long as it does not correspond to a quantitative deficiency. In cases where this potassium quantitative deficiency can be eliminated as the cause of hypokalemia, beneficial results are obtained through administration of agents such as magnesium sulfate or calcium salts.

Searching for a cation that might compete with potassium and sodium, we first chose ammonium. Theoretically, it seemed to be a likely choice since it penetrates into the cell and nucleus with ease. In the pharmacology of ammonium, the missing link is the factor or factors—the substances and conditions—which determine the role of this cation in the nuclear compartment. Ammonium proved valueless because it was taken up by the liver and transformed into urea. Therefore, we resorted to the use of another monovalent cation of the same homotropic 1A series but with a higher atomic weight, *rubidium*. This cation is very similar to *ammonium* ion. Rubidium and ammonium, in nitrates, sulfates and especially in double sulfates of aluminum or magnesium, are isomorphic. We tried rubidium salts in animals and found them to have a very low degree of toxicity.

In order to study its influence upon sodium and water retention in lesions in which fatty acids predominate, we administered 1-2 cc. of a 5% rubidium chloride solution in water, two or three times daily. In several cases, this caused diuresis and significant reduction in edema. With the idea of having rubidium act at the nuclear levels, we considered the use of rubidium compounds with anions that seem to intervene at these levels. Rubidium nucleinate was prepared and 1-2 cc. of a 10% solution in water administered to subjects two to three times daily. Although these studies are not yet sufficiently advanced to permit any conclusions to be drawn, it seems that the rubidium salts may be useful in cases of intractable edema related to a local condition.

These studies of the distribution of cations at different levels of organization appear to be extremely important if we want to reach cellular and especially nuclear levels with cations, particularly as radio-active isotopes. We tried to go still further and utilize heavier cations. Experiments with cesium salts seem to present more difficulties, at least for the present, be-



cause of the insolubility resulting from the high atomic weight of this element.

In the VI B series, we know little about chromium. *Molybdenum* appears to be an active agent. The influence exerted by molybdenum is neutralized by the action of methionine, with its active thiol group. Excess of molybdenum found in some pastures induces a deficiency in copper and calcium in animals followed by osteomalacy and bone fractures, just as it induces low fertility. (174) The antagonism between these elements is shown by the inhibitory effect of ammonium molybdate upon oxidase activity of ceruloplasmin, the form in which copper is bound to protein in blood serum. (175)

The anti-D effect of molybdenum is especially marked in microbes, in which it induces morphological and tinctorial changes. Bac. anthracis treated with ammonium molybdate shows a cocciform change and abnormally intensive Gram positive staining.

Iron

Iron, a member of the VIII series and an anti-D agent belonging also to the cellular level, is of special interest. Its form of activity as cytochrome oxidase or hemoglobin has facilitated the understanding of its intervention. We could thus relate the high or low amount of the red cells in hemoglobin which corresponds to the amount of iron ions to the respective offbalance. Hypochromia corresponds to a type D offbalance and hyperchromia to a type A. These patterns, which were first recognized through clinical investigations are in accord with the anti-D character of iron at the cellular level where it belongs. The existence of offbalances of A and D types in cancer explains the high and low values of Fe in the immediately higher level, the blood serum. Fever, as we have noted, corresponds to an offbalance of type A at the metazoic compartment. The fact that serum iron is low during fever would indicate increased iron activity at the cellular level, which accords with its A-inducing role. We administered iron compounds to cancer patients with hypochromic anemia to correct the anemia and also because of iron's anti-D effect which might act antagonistically on sulfhydryl groups. In several cases, with very high sulfhydryl index values, ferrous sulfate has been administered in doses as large as several grams daily. In addition to producing an increase in hemoglobin and color index, ferrous sulfate in large doses has been observed to have another effect in some cases, reducing pain of an alkaline pattern. The salutary effect upon pain was noted more often with reduced iron than with ferrous sulfate. No other influence upon systemic changes was observed.

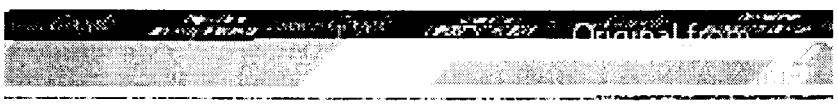
In tumors, iron produced an increase in rate of growth as expected. This peculiar effect of iron administration could be clearly seen in a case of lymphatic leukemia.

B.V., 4 years old, came under our care with the diagnosis of subacute lymphatic leukemia. He had a count of 145,000 leucocytes, of which 96% were lymphoblasts. Butanol administered in small doses reduced the number of leucocytes considerably, even after a few days of treatment. After two weeks, the count was below 5,000 leucocytes, with the proportion of lymphoblasts decreased to 6%. A count of 3,200 leucocytes made us discontinue the administration of butanol. In three weeks the count rose progressively to previous values. Butanol was again administered and again the blood count showed the same marked decrease in leucocytes.

All through this "remission," intensive hypochromic anemia persisted and led us to administer ferrous sulfate in addition to butanol. Within a few days after iron was added, the white cell count increased to 110,000 leucocytes/cmm., and the proportion of lymphoblasts rose to 90%. When the iron was stopped and butanol continued alone, the leucocyte count fell again, to 8,000/cmm. Iron was again administered and the total white count rose a second time in three days, from 8,000 to 80,000, with 96% lymphoblasts. When the iron was discontinued, the count again fell back within a week to between 6,000 and 7,000, this time with less than 30% lymphoblasts. This continued for another two weeks, when iron therapy was instituted for the third time, the count went up again to 38,000 within a single day. When butanol was administered alone, it went back to 5,600 in another five days. The boy died a few weeks later in acute shock during a blood transfusion.

While generalizations cannot be made from these findings, it could be definitely established in this particular case that the administration of iron was followed by a marked increase in the number of leucocytes and in the proportion of lymphoblasts. We saw this repeated, although not so spectacularly, in another case of lymphatic leukemia.

In several cancer patients who received iron in large doses over a prolonged period of time, tumor growth seemed to be stimulated. In animal experiments, tumor transplants grew only slightly faster in animals fed iron than in controls. Mice and rats used in these studies received approximately 0.05 gm. daily of reduced iron per 100 grams of body weight, mixed in powdered Purina chow, or were given a corresponding amount of ferrous sulfate in drinking water. Iron was added to the diet of the animals two or three weeks before tumor transplant, and was fed after transplant. Tumor growth was slightly enhanced and survival time shortened.



Similar studies were carried out using *nickel* and *zinc* alone or mixtures of them with iron. The metals reduced by hydrogen were obtained and a powder preparation was incorporated in powdered Purina chow in amounts calculated to provide approximately 0.05 gr. metal/100 gr. of body weight daily. Significant changes occurred in the evolution of Walker tumor transplants in rats receiving 0.05 gr. daily of zinc or nickel/100 gr. of body weight. In most experiments, two different results were noted. Tumor growth was retarded in a significant proportion in one group of animals, with the tumor disappearing in some cases. In another group with the same Walker tumors, tumor growth was stimulated. It is interesting to note that retardation occurred only in tumors with a necrotic, ulcerative character, while stimulation was noted in those with white masses. The correlation of necrotic and ulcerative tumors to an offbalance of type D and of massive non-ulcerated white ones to offbalance of type A explains this paradoxical result. This would confirm the anti-D or A-inducing character of these metals.

Zinc

Zinc intervenes with a certain specificity in one group of metabolic changes, those related to carbohydrates. Both pancreas "A" cells, which manufacture glucagon, and B cells which produce insulin, contain large amounts of zinc. Zinc regulates glucose metabolism through its influence on insulin. Zinc and insulin give insoluble combinations, Zinc chloride retarding the action of insulin. (176) A diet rich in glucose depletes the Langerhaus islets of zinc, (177) while protein and lipids, or even fat, increase the zinc content of the pancreas. (178) With alloxan, the islets lose their physiological capacity to store zinc. (179) Indirectly zinc appears to have anti-D activity.

A similar anti-D activity for zinc can be seen in the prostate, which is particularly rich in this element. Conceivably, zinc's role would be to favor the persistence of spermatozoids. The capacity to utilize zinc is lost in the abnormal prostate. In adenomatous hypertrophy of the prostate, zinc values decrease. (180) They decrease still more with cancer of the prostate. (181) Although cancer has been induced by excessive administration of zinc, the element's role in pancreas and prostate seems to be indirect, through the metabolic changes it influences. Calcium is antagonistic to zinc, which is to be expected considering the opposite fundamental biological groups to which they belong.

It is interesting to note the influence exerted by the oral administration of zinc powder upon radiation effects in mice of C_3H strain receiving lethal

doses of 1500 r. In different experiments in which 85 to 100% of the controls died in less than twelve days, the mortality rate for animals given zinc ranged from 25 to 50%. In one group, a mortality rate of 15% was observed. There was a much weaker effect when nickel was incorporated in food. No effect at all could be obtained with iron.

Mercury

Mercury, a member of the IIB series was studied. Theoretically, it should act upon the sulfhydryl groups, and thus limit the processes in which these groups take part.

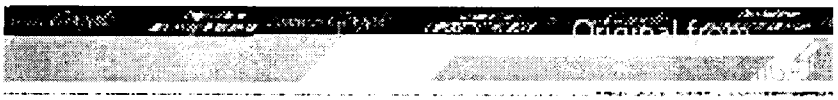
A series of compounds in which mercury is present in the anion, and which are routinely utilized as therapeutic agents because of their diuretic action and their effects on electrolyte metabolism, was investigated. Because of their well-known diuretic effect, we initially used them in patients with generalized anasarca. We extended their use to cases with localized edema which could be related to tumors with local alkalosis. It was noted that the mercurial diuretic, in addition to influencing water and sodium excretion, changed other systemic analyses toward type A, although only temporarily. However, when these substances were administered over long periods of time, other effects were observed which could not be attributed to diuretic action. In animals with slowly growing tumors, mercury was found fixed with a degree of selectivity within these lesions. After treatment for a certain time, peritumoral fats became rich in mercury, as recognized macroscopically through abnormal ash color and confirmed by histochemical analysis. It was interesting to note that mercury appeared largely in the necrotic part of tumors. A beneficial influence upon evolution of tumors was seen, if a type D offbalance was present.

Bismuth

Bismuth, from the VA series was studied. We utilized available antiluetic compounds. Again localization in peritumoral fats was seen, with the fats this time becoming abnormally reddish. Neither in animals nor in patients could other important effects be recognized.

Arsenic

Arsenic, from the same VA series, belongs to the cellular compartment. Its manifest A inducing or anti-D effect was noted in all compartments if the amount administered was high enough. It is interesting that Bac. anthracis, under the influence of arsenious acid, changes to cocci



highly irregular in dimension, with intensive Gram positive staining, and a creamy character of cultures. (182)

The carcinogenic effect of arsenic has been widely investigated by many authors. In our experiments, arsenic in various preparations did not show a carcinogenic effect. It did, however, enhance the effect of various carcinogens, and thus appears to be an active co-carcinogen. Its action at the cellular level would explain this effect.

Aluminum, from the IIIA series, belongs to the metazoic compartment. In minimal doses, it produces a D systemic offbalance. *Boron,* as boric acid, shows few effects other than in gastrointestinal disorders. This effect may be due to the fact that it belongs to the organism compartment. Insoluble compounds of boron appeared to be useful in the treatment of diarrhea.

The effect of members of the VIIA series appears to be largely correlated with a direct action upon the double bonds of the nonpolar group of the fatty acids.

Chlorine

We have noted previously the capacity of chloride ions to bind fatty acids. The bond represents the first step in abnormal metabolism of sodium chloride. In a second step, sodium forms alkaline compounds by binding with the carbonate anion and, in sufficient amounts, induces local alkalosis. Accumulated in cells along with water, the sodium compound leads to the appearance of vacuoles. In interstitial fluid, the same process induces edema and pain of an alkaline pattern. At the systemic level, it results in a state of shock.

In the last analysis, the influence of the alkaline sodium compounds can be considered to result from the lack of anions other than carbonate available to bind sodium. For this reason, we were interested in studying the effects of substances able to furnish the chloride anion to the organism. Through metabolism of ammonium chloride and calcium chloride, chloride ions are liberated in the body. They have little effects at lower levels. An immediate influence on local pH is seen at the tissue level. Favorable influence on alkaline pain is part of this action. The effect of ammonium chloride in shock is related to the fact that it furnishes the needed chloride ions.

The action of chloride upon alkaline sodium compounds, however, is handicapped by another aspect of its intervention. Studies of the pathogenesis of shock has shown a noxious effect produced by compounds resulting from the bond of chloride ions to fatty acids. The gastric ulcerations

seen in the state of shock with the severe liver damage produced by several chlorine compounds—could be largely related to the bond of chloride ions to fatty acids. This led to the idea of trying such combinations in vivo to destroy abnormal entities such as tumoral cells. By using lipoids rich in chlorides, we hoped to achieve this without the noxious effect of free sodium ions.

We administered trichlorethylene and chlorbutanol (the last also used as an antiseptic in many pharmaceutical preparations). No effect was seen at any level in experiments on animals and humans. In a second step, we added chlorine to various lipoids, especially those with negative polar groups. We started with 9, 10-dichlorostearic and 9, 10, 12, 13-tetrachlorostearic acid. The results with these preparations in animals and in a few humans were not encouraging. Investigation of products obtained through fixation of chlorides at the double bond of conjugated fatty acids, has shown that, in large amounts, they are able to induce gastric ulcerations in rats and rabbits. This brought us back to the use of chlorides as anti-D agents—of which sodium chloride appears to be the most effective.

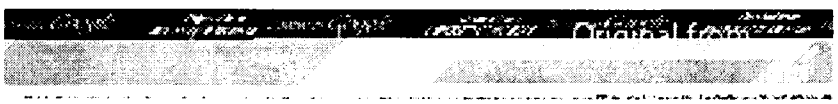
Fluorine and Bromine

In another series of experiments, we tried to replace the chloride ion with another halogen. We studied the influence of fluorine, bromine and iodine compounds, this time upon the processes that induce abnormal patterns related to predominance of abnormal fatty acids. The administration of sodium fluoride and of other compounds containing fluorine did not have any appreciable effect either in animals with tumors or other pathological conditions, or in humans with alkaline pattern of pain. The fact that fluorine belongs to the organism level led us to investigate it in terminal cases. Neither pain nor tumor evolution was changed. Bromine, too except for a sedative effect, did not influence systemic changes, pain or tumor growth.

Iodine

The influence of iodine was rather extensively studied because of its relationship to tumors and because it belongs to the nuclear compartment. Before the days of pathological diagnosis of cancer and serological methods of detecting syphilis, iodine salts were used to differentiate between gummatous and tongue cancers. This was the so-called "pierre de touche" treatment, since iodine was assumed to favorably influence luetic lesions but markedly enhance neoplastic growths.

In view of the dualistic concept of cancer, we were interested in ascer-



taining whether the effects of iodine were related to one of the two offbalances present, and if this was so, to try to take therapeutic advantage of it. Iodine was administered principally in the form of potassium iodide solutions and Lugol's solution. In most cases with alkaline pain, the intensity diminished and pain even disappeared soon after iodine administration. In several cases, doses as low as 3-10 drops of Lugol's solution were sufficient to induce such an effect for hours. However, larger doses or repeated doses produced effects that were distinctly undesirable. Edema within the neoplastic lesion was increased by the administration of iodine, sometimes to such an extent as to require discontinuing its use before any other changes in the tumor could be noted.

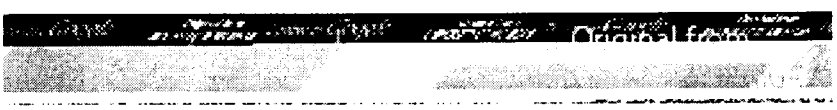
Research also was done with lipid and lipoid molecules incorporating iodine. The purpose was to determine whether these molecules would act more selectively upon abnormal cells and induce local toxic effects. 9, 10-di-iodostearic acid and 9, 10, 12, 13-tetra-iodostearic acid were prepared and tested in animals and humans. No differences were noted between these substances and inorganic iodine preparations in influence upon systemic analyses, pain or tumor growth, in both animals and humans.

Oxygen

Oxygen, an agent with a negative anti-A character, at the organism level, acts like an anti-fatty acid agent at the lower tissular level. For that reason, we will discuss it here.

At the beginning of our work, we were interested in determining the relationship between cellular membrane permeability and the pathogenesis of the two offbalances. If a change in the permeability of cell membrane was the primary mechanism involved, changing the oxygen tension in or around entities where offbalances occur might result in the correction of the abnormal manifestations present.

Clinical studies were made in which oxygen was administered to patients with acid pain pattern. The pain was not relieved as expected. Indeed, its intensity was even increased. These experiments indicate that impaired cell permeability, if it exists, is not the major factor in the pathogenic mechanism involved in the acid pain pattern. Actually, these studies indicated that another pathogenic mechanism was involved since oxygen administration increased this pain. Accordingly, it had an opposite effect on alkaline pain. The intensity of alkaline pain decreased, the pain being often entirely relieved by administration of oxygen. We found that pain produced by traumatic injuries, which was subsequently identified as invariably of the alkaline pattern, could be satisfactorily relieved by oxygen.



Such relief occurred in patients who had suffered all sorts of traumatic injuries, from superficial wounds to severe comminuted fractures.

A curious phenomenon was seen to occur which limited the practical usefulness of oxygen. After first relieving pain, continuation of oxygen administration led to appearance of a new pain. The patient was able to distinguish between original and new pain by its localization, by the different quality of the sensation, and also by the fact that instead of being relieved by oxygen, the new pain tended to increase with the continued administration of oxygen. It disappeared soon after oxygen administration was discontinued. If oxygen were administered again, the new pain returned within a short time. The new pain might become severe and even unbearable with continuation of oxygen administration. On the other hand, when administration of oxygen was stopped, the original pain again appeared within 10-20 minutes.

The appearance of a "new pain" and the resemblance between these changes and those observed with the use of lipids, suggested a change in the pain pattern itself, although the changes induced by oxygen evolved over minutes instead of days. This was confirmed by following the response to acidifying and alkalizing agents, the only adequate means to investigate the pattern in these cases. By this test the new pain was found to be of an acid pattern.

Because of the possibility of inducing a period of calm between the old alkaline and new acid pain, oxygen administration still seemed to be useful. In cases of traumatic pain, always with an alkaline pattern, successful results were obtained. A necessary condition appeared to be the physical and mental ability of the patient to guide the administration of oxygen. He had to recognize if too little or too much was being administered on the basis of the different sensations felt, and consequently, to adjust the administration to the optimum amount. Extreme pain caused by extensive traumatic injuries was controlled very successfully when the patient could be taught to utilize oxygen properly.

In addition to pain relief, an effect upon evolution of the lesion itself was manifest. In a few days with this form of oxygen treatment, guided by the subjective sensations, the healing process itself was observed to be sufficiently advanced to make the pain disappear entirely. Healing of the wounds seemed to be greatly enhanced by oxygen therapy guided by the patient. In several cases of open comminuted fractures in which amputation was considered inevitable on admission, unexpected improvement was noted. Atonic wounds were transformed, becoming rich in granulations, and healing was rapid.

However, the nature of the treatment was such that it could only be successful when properly applied. If dosages were too low, there was no sedative effect; and if doses were too large, new pain was induced. The physical and mental status of the patient thus appeared to be the determining factor for success. The use of an oxygen tent, in which the amount of oxygen can be carefully adjusted, helped in a few cases to maintain proper dosage. But even in this situation, the patient himself must furnish information not so much about intensity of pain, as about changes in the character of pain.

The relationship between the amount of oxygen administered and the clinical results, especially in pain, has suggested that this factor may be significant in other conditions in which oxygen therapy is used. The fact that, despite its general usefulness in the acute stage of myocardial infarction, oxygen does not alleviate the pain in some cases and may even increase it, suggests that the amount administered might not be adequate. In such cases, a decrease in pain intensity following temporary discontinuation of the use of oxygen would indicate that the amount utilized was too high. If the suppression of the oxygen administration is followed immediately by an increase in pain intensity, the amount previously administered has to be considered too low.

The possibility that too much oxygen can induce a proliferation of vessels and connective tissue, as seen in the fibroblastic retinopathy of premature babies kept in an atmosphere too rich in oxygen, fits in with the data mentioned above. We have noted that too much oxygen induced an anoxybiotic process with anabolic character. This explains the abnormal type of offbalance with proliferative tendencies seen in fibroblastic retinopathy. We will return later to a discussion of this important factor in oxygen therapy.

On the basis of findings in traumatic pain, we studied oxygen in cases of painful cancer. Attempts were made to employ it as a diagnostic aid to help determine the acid or alkaline character of pain present on the assumption that oxygen would intensify the first and would relieve the second pain. In a group of subjects, we compared the diagnosis of the existing pattern through concomitant variations in pain intensity and urine pH, the response to acidifying and alkalizing substances, and response to oxygen. In most of these cases, accuracy of the information furnished by the last method was confirmed. However, this method has shown great limitations. Whereas most of the patients were able to recognize an immediate change, they were less precise about a second change when it occurred. At the present stage of this research, it appears that judgment concerning the de-

velopment of changes could be improved by reducing the concentration of oxygen administered, thus increasing the length of time during which the changes would appear. In general, the results when applied as routine were insufficiently clear to be used as a practical means for the diagnosis of the pattern.

Oxygen also has been tried as a therapeutic agent for controlling alkaline pain pattern in cancer cases. Unfortunately, even in patients who are able to analyze the variations in pain character, the time between the decrease in intensity of the original pain and the appearance and increase in the intensity of the new pain is so variable that it is almost impossible to adjust the dosage of oxygen satisfactorily to obtain a long enough period of calm.

We sought a theoretical explanation for oxygen's effects upon pain. As mentioned above, the reduction of pain of an alkaline pattern and the appearance of a pain of an acid pattern, are in accord with our view of the pathogenesis of these pains through the intervention of the two groups of lipids, fatty acids and anti-fatty acids. A tentative explanation can be found in the active role of oxygen upon fatty acids. In the presence of increased oxygen tension, it is possible that oxygen is fixed in greater amounts on abnormal fatty acids, thus reducing their intervention in chloride metabolism. With less chlorides fixed by fatty acids, alkaline compounds would be reduced. This would explain the influence of oxygen upon pain with an alkaline pattern. The appearance of an acid pain pattern produced by increased anoxybiosis seems to be explained by the fact that inactivation of fatty acids, if it goes beyond certain limits, changes the balance toward a predominance of sterols. Besides this mechanism, another also can be considered. Any action upon fatty acids themselves, would reduce their availability as active agents. It seems possible that under higher tension, oxygen is bound to these fatty acids in a way different than the bond which leads to the appearance of activated oxygen. Through it, the role of the unsaturated fatty acid in activating oxygen for the cells would be to decrease intracellular activated oxygen and thus change cell metabolism to the anoxybiotic type. Since the bonds are labile, return of oxygen tension to normal allows the fatty acids to recover their function of activating oxygen. Teleologically, this process can be interpreted as a mechanism to prevent passage of excess oxygen into the cells when the external oxygen tension increases.

Studies of oxygen in normal and abnormal physiology have led us to consider also the possible utilization of various oxygenated compounds as therapeutic agents. A distinction must be made between hydroperoxides, which occur normally in organisms, and peroxides and epoxides. Peroxides

result from the binding of molecular oxygen, while the epoxides result from binding of atomic oxygen, both under abnormal conditions. Peroxides were administered as adjunct agents in cases highly refractory to therapy with lipoids having negative polar groups. In preliminary experiments, it could be seen that a certain condition was necessary to influence the desired processes. The peroxide used has to be a lipoid if it is to have influence upon the lipidic system. Some lipoidic peroxides were prepared, and their therapeutic value is still under investigation. The influence of lipoidic epoxides upon the process of carcinogenesis also is under study.

Peroxidases

It has been previously noted that hydroperoxides resulting from the oxidation of mono- and polyunsaturated fatty acids are found normally in the organism, but peroxides appear only under abnormal conditions and particularly when abnormal fatty acids intervene. The manifestations of the type D offbalance in its oxygen phase thus can be attributed to the presence of peroxides. Biologically, the intervention of peroxides would be counteracted by peroxidases and catalases. It was interesting to study a clinical curiosity which could be connected with a probable intervention of peroxidases.

In several patients with frequent headaches, whose analyses showed a typical acid pain pattern, pain was repeatedly intensified or even induced by eating pears. No other fruit had such effect; some had the opposite effect. This led us to consider that the pain intensifying action was not due to an acid-base change. The large amount of peroxidases in pears led us to isolate this enzyme in order to study its direct influence upon pain. Peroxidase could be obtained from pears in relatively small amounts, and showed reduced activity upon peroxides even in vitro. We were able to prepare much larger quantities of a highly active peroxidase from horse radish.

After being purified and tested for antiperoxide activity, preparations were administered orally to patients with acid or alkaline pain pattern. While the former was definitely intensified, there was no relief of the latter. It seemed that the effects obtained through the administration of isolated pexoridases were the same as those obtained when pears were eaten.

Antioxidants

The relationship between fixation of oxygen and chlorides has led to the study of antioxidants capable of acting in situations of abnormal oxidation. It was hoped that these substances also would be able to influence the fixation of chloride ions.

We have utilized several groups of known antioxidants, starting with manganese compounds, such as inorganic salts, and later binding these to lipids. It is too early yet to draw any definite conclusions from animal experiments, and we have not used the compounds clinically. However, our studies up to now do not show any influence that could be interpreted as sufficient to warrant hope that these compounds can control the fixation of chlorides on fatty acids.

In the same series of researches, other antioxidants—some of them used for the preservation of edible fats and others for the control of oxidation in other substances such as rubber—were tried. We investigated the influence of tocopherols, the natural antioxidants for vegetable oils. Alpha tocopherol in doses of 100 mg. was administered several times a day to patients having symptoms and signs corresponding to an intervention of abnormal fatty acids. A decrease in the intensity of pain of an alkaline pattern was observed.

Along the same lines, we investigated the influence exerted by maleic acid, used to prevent the rancidity of edible fats. In proportion of 1/10,000 this acid conserves these fats for months. Curiously enough, maleic and citraconic acid have shown an influence upon the abnormal manifestation of the type D.

For these reasons we utilized these two acids—maleic and citraconic—as anti-D agents. In one study, the acids were injected intravenously in proportion of from 0.1 to 1 mgr./100 cc. of saline. In others, the sodium salts of the acid were used, while in still others the butyl esters were prepared and administered intramuscularly in oily solutions. For the present it is difficult to judge the effects obtained.

All the above mentioned attempts were made on the basis of a direct action upon fatty acids and other lipoidic constituents with negative polar groups which intervene in inducing offbalances. For the present, it seems that no single agent can resolve the problems that result from the plural intervention of various abnormal fatty acids at the different levels. The use of various agents acting selectively at the different levels involved seems to be the only available path by which therapeutic intervention against the multiple manifestations at different levels can be accomplished.

Before going further, we thought it useful to have a synoptic view of this special part of the pharmacological activity as obtained through the study of the influence upon pain and the systemic level analyses as seen in humans. To this we added the effect seen upon tumors in humans. Tables XX and XXI which give this information in a very condensed form, were limited to the most important agents tested for each group studied. The effects are indicated as clinical results also for the facility of the presentation.

TABLE XX

CLINICAL RESULTS WITH AGENTS THAT ACT UPON THE OFFBALANCE TYPE "A"

Group	Agent	Systemic Level	Pain	Influences Exerted Upon Tumor
Fatty Acids	Saturated	None	None	None
	Polyunsaturated	Slight	Fair	Some, not consistent, not persistent
	Mixtures from organs	"	Good	" " "
	" from cod liver oil	"	Fair	Fair, not consistent, not persistent
	Irradiated	"	"	" " "
	Conjugated	"	"	" " "
	α —OH	None	None	None
	Polyhydroxy	Slight	Slight	None
	Chloro-derivatives	Fair	Fair	Some, not consistent, not persistent
	Oleic	Slight	None	None
Aldehydes	Crotonic	Slight	Slight	Slight, not consistent, not persistent
	Propionic	Good	Good	Fair
	Heptylic	Good	Slight	Fair
Sulfur Compounds	Thiosulfates	Good	Good	Fair
	S. Colloidal	None	None	None
	Mercaptans	Slight	Slight	Good, consistent, persistent
	Hydropersulfides	Fair	Fair	Fair, not consistent, not persistent
	Methyl thioglycolate	Slight	Fair	" " "
	Tetrahydronaphthalene persulfides	Fair	Fair	Good, consistent and persistent
Selenium Compounds	Alkyldiselenide	Fair	Slight	Good, consistent, persistent
	Perselenide	Fair	Good	" " "
Peracids	Perborate	Fair	Fair	Some, not consistent, not persistent
	Perchlorate	"	"	
Hormones	Testosterone	Slight	None	Seldom, not consistent, not persistent
Mustards	Sulfur mustard	Fair	Fair	Fair, " "
Hydrines	Epichlorohydrin	Fair to good	Slight	Fair, consistent, persistent

TABLE XXI

CLINICAL RESULTS WITH AGENTS THAT ACT UPON THE OFFBALANCE TYPE "D"

Group	Agent	Systemic Level	Pain	Influences Exerted Upon Tumor
Sterols	Cholesterol	Fair	Fair	Seldom, not consistent, not persistent
	Insapon. fraction			
	of organs	"	"	" " "
	of eggs	"	"	" " "
	of milk	"	"	" " "
Alcohols	*Aliphatic saturated*			
	Butanol	Good	Good	" " "
	Pentanol	Fair	Fair	Fair, " "
	Heptanol	"	Slight	Good, consistent, persistent
	Octanol	Slight	"	Fair, not consistent, not persistent
	Octanediol	"	"	Slight
	Nonanol	None	None	None
	Polyalcohol			
	Glycerol	Slight	Good	Good, consistent, persistent
	Inositol	None	None	None
	Unsaturated			
	Oleic	Slight	Slight	None
	Linoleic	"	"	None
	Polyunsaturated	"	Fair	Slight, not consistent, not persistent
	Polyconjugated	"	"	Fair, " "
	Crotonic	"	Slight	" " "
	Ricinoleic	"	Fair	Slight, " "
	Salicylic	"	Slight	Fair, " "
Hormones	Estrogens	"	"	Slight, " "
Amines	Aminobutanol	"	Fair	" " "
	Hexylamine	"	"	" " "
	Heptylamine	Good	"	Fair, " "
	Glucosamine	Good	Slight	None
Nicotinic acid deriv.	Niketamide	"	Good	None
Metals	Iron	Fair	Slight	None
	Mercury	"	None	"
	Bismuth	"	"	"
Halogen	Iodine	Slight	Fair	"
	Oxygen	None	Good	None