CHAPTER 12

PHARMACODYNAMIC ACTIVITY

IN OUR RESEARCH, we have investigated the pharmacodynamic activity of a series of substances in terms of the physiopathological concepts discussed above: the level of organization at which they act, the dualistic nature of their activity, their relationship to body constituents, especially to lipids, and the changes they induce in the defense mechanism. We will limit ourselves in this presentation only to those of the substances investigated which are of therapeutic interest.

The dualistic concept has permitted didactic separation of agents into two groups with antagonistic properties. In the last analysis, this separation could be related to the two fundamental tendencies in nature, homotropy or heterotropy. For inorganic agents, this criterion appears to be directly related to the elements present. However, for organic agents, especially for lipids and lipid-like substances, this simple criterion is less valid. Another, the positive and negative electrical character of their active polar groups could be used. It must be emphasized, however, that some of these polar groups show either positive or negative properties depending upon the medium in which they work. An alcohol, for example, can act as an acid under special circumstances, in which case it forms metal alcoholates; or it can act as an alcohol forming esters with acids. For this reason, in considering these substances, we have limited the sphere of changes studied to those corresponding to the conditions present in biological entities.

Since, as seen above, substances with negative polar groups actively intervene in various processes, while those with positive polar groups control the activity of the first, we will start this presentation with the former.

AGENTS WITH ACTIVE NEGATIVE POLAR GROUPS

Among agents which have negative polar groups, a further division can be made according to the nature of these groups. Under biological conditions, the most important of negative polar groups is the carboxyl. Losing a proton when dissociated, the carboxyl confers acidic property to the molecule into whose structure it enters. We will consider, first, such carboxylic acids. Among such agents, the individual differences seen in biological activities have to be related to their nonpolar parts.

In the frame of our research, with organic acids, we were especially interested in those with a predominance of the nonpolar group; that is, those having lipoidic properties. They are, principally, fatty acids, which, according to the presence of one or more double or triple bonds in the nonpolar part, can be separated into saturated and unsaturated.

Fatty Acids

Saturated Fatty Acids

The lipoidic character in this homologous series starts with the five carbon valeric acid, although caproic acid is the first lipoidic member found in animals. The role of the saturated fatty acids in organization is largely related to the activity, as caloric metabolites, of the members with even carbon numbers. These saturated fatty acids are absorbed, circulated and stored as triglycerides, and it is also this bond to glycerol which apparently favors their biological role as caloric metabolites.

As previously noted, only the members with a relatively short chain, less than 12 carbons, can be directly metabolized through Knoop beta oxidation. Longer chain saturated fatty acids undergo a breakdown before further metabolic degradation. Members with 16 and more carbons also play a limited constitutional role, usually together with an unsaturated member, when bound to glycerophosphoric acid to form phospholipids. Even when free—that is, when their polar group is not neutralized—they do not have a manifest functional role and, consequently, show no important pharmacodynamic activity.

The higher fatty acids exert no demonstrable influence upon microbes nor upon viruses such as bacteriophages. No influence has been observed upon monocellular organisms, cells or tissues in vitro. No changes were seen in the respiration of liver slices or of Sa 180 ascites cancerous cells in the Warburg microrespirometer. Similarly, no influence can be seen on red blood cells nor on leucocytes treated in vitro. No changes in the chloride

content of tumors or wounds are induced by administration of these acids. The fact that no change could be induced in the second day wound crust pH explains the lack of any effect upon pain.

Even large doses, such as 20 cc. of a 10% solution of these acids in sesame oil, do not change the intensity of pain of alkaline or acid pattern in humans. No influence has been noted upon tumor evolution, even with the direct technique of dipping tumor transplants in the agent, repeated for successive transplants. Existing edema or ulceration is not influenced. At the organic level, no changes can be seen in the function of various organs or upon liver regeneration rate. These fatty acids do not influence convulsions induced by thiamine in rats even when administered in very large doses, such as 5 cc. or more of 10% solutions for 100 gr. of body weight. The same lack of influence is seen on systemic metabolic manifestations, as recognized through various analyses. Except for palmitic acid, which shows an adrenal defense index of 12, the index for the other members of this series is below 6, indicating only a certain participation of the adrenals in the defense mechanism against these acids.

No changes can be found in the number or character of leucocytes in animals treated for a few days with these acids. No change in the sodium or potassium content of the blood or in urinary analyses is induced by these substances even in subjects whose analyses reveal abnormal patterns. No action upon body temperature and no influence upon the evolution of the defense mechanism can be observed.

It is interesting however, to note the effect of the sodium salts of these acids, especially on in vitro lysis of different cells. *(Note 1)*

Unsaturated Fatty Acids

Of the unsaturated fatty acids, oleic acid is the most widely encountered in nature. As previously noted, *monoethenoids* have a group of 9 carbons either toward the carboxyl or methyl end of the molecule. With the double bond between C_9 and C_{10} in a molecule of 18 carbons, oleic acid appears to satisfy both tendencies, which could account for the ubiquity of this acid.

Oleic acid circulates, is deposited as reserve, and is used as caloric metabolite especially when bound to glycerol. In considerably lesser amounts, when bound to glycerophosphoric acid, it takes part as a phospholipid in the formation of the lipidic system of the organism. It is only slightly active in oxidation processes. For this reason, large doses are necessary to influence abnormal processes at different levels. Even then, only limited changes are induced. In in vitro studies, oleic acid produces no changes in bacteriophages. A certain influence is noted for receptivity of

dermotrope viruses. Injected subcutaneously in rabbits, oleic acid induces in the skin, at the site of injection a zone of low receptivity for smallpox virus. Oleic acid has a reduced effect upon microbes, causing the appearance of some gram negative individual forms of Bac. subtilis, for instance. Mixed directly with blood, oleic acid induces hemolysis. When plasma is treated with this acid, and then mixed with red cells, the influence exerted upon red cells because of the small amount of acid fixed upon plasma, is reduced. Although oleic acid may influence the pH of the second day wound crust, causing an increased local alkalosis *(Fig. 119),* its influence

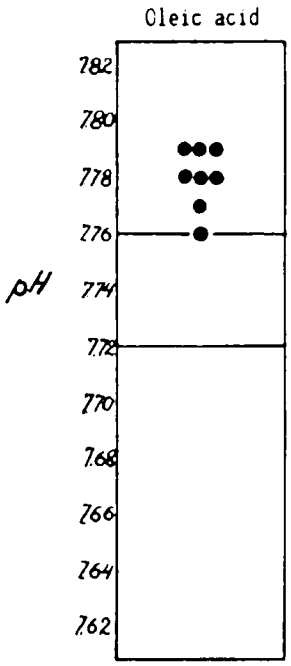


FIG. 119. The second day wound crust pH for oleic acid shows the constant presence of a change toward more alkaline values.

upon pain is almost nil. Little or no change is seen in standardized radiation wounds in animals treated with the acid. A limited influence can be observed on tumor growth by using the technique of dipping transplants for successive generations. Use of a 10% solution of oleic acid in tricaprylin before grafting, repeated for successive generations, impairs growth of Ehrlich mouse adenocarcinoma after the sixth or seventh generation in some experiments, even later in others. Negative passage takes place between the eighth and tenth graft. Under the same condition, very little or no changes are noted in other tumors, and no changes are seen in tumors in animals treated with this acid even though changes have been reported by some authors. (124) Suspensions of cells of different organs, treated in vitro with colloidal suspension of oleic acid and injected in animals of

the same species, induced no changes with a single injection. Repeated injections after 3 weeks induced lesions in the respective organs.

At the organic level, some effects can be obtained by using high doses of oleic acid. For example, to prevent convulsions induced by thiamine chloride, doses as high as several hundred milligrams per 100 gr. of body weight are required and even then this effect is not constantly seen. Systemic changes, recognized through blood and urine analyses, are almost nil, even with large doses of oleic acid. The compound however, does prolong liver regeneration time.

Among the *diethenoics,* we studied linoleic acid. The caloric activity of the uncombined fatty acid is reduced while constitutional and functional activities are increased. Linoleic and linolenic acid appear to be organizational rather than functional fatty acids because of their preferred bond to glycerophosphoric acid. They are absorbed from the intestines, mainly as phospholipids. No effects upon phage can be seen. The refractivity to smallpox virus induced on rabbit skin is more manifest than for oleic acid. However, the effect upon microbes, such as subtilis, is less evident than for oleic acid. Crenelated red cells, with increased tendency to conglutinate and increased sedimentation rate, are found after linoleic treatment of the blood in vitro. To avoid hemolysis, the acid is not added directly to the blood but to the plasma which is then reunited with the cells. Crenelated cells also appear in vivo in rats injected intraperitoneally with large amounts of this acid, such as 10 cc. of 10% in oil. A definite shift toward alkalosis is found in the second day wound crust pH, which explains the influence seen upon pain. Linoleic acid increases pain of an alkaline pattern and decreases pain of an acid pattern, though only slightly. Only in relatively large doses (2-400 mg./100 gr. animal), does it prevent the convulsions induced by thiamine.

In lesions induced by radiation, the administration of small doses of this fatty acid often produces a favorable healing effect. This effect can be related to the growth-stimulating action of essential fatty acids in small quantities. The effect is just the reverse of the unfavorable influence on healing observed when larger doses are administered. An effect upon tumors in animals can be achieved only through repeated treatment of successive transplants or through the treatment of successive generations of the host. The effect of repeated injections of tissue cells treated in vitro with a suspension of linoleic acid was more manifest than that obtained with oleic acid. The effect upon the systemic level, although more manifest than for oleic acid, still is limited, even under abnormal conditions. Blood and urinary analyses are only slightly and briefly changed toward the D type of

offbalance even when large amounts are administered. Blood eosinophiles are decreased and potassium content increased, both only slightly. Body temperature is slightly depressed.

Preparations especially rich in triethenoid *linolenic acid* were used and no differences between their biological activity and that of linoleic acid could be noted.

We obtained preparations especially rich in *arachidonic acid* from salmon oil. The caloric contribution of this acid can be considered almost nil compared to its functional role. It is absorbed, circulated and stored, principally as esterifying sterols. Although this acid is present in the body in relatively small amounts, it represents more than 25% of the fatty acids of the adrenals. In view of the highly functional role of the adrenals, it is logical to suppose that arachidonic acid's abundance in the glands is not merely coincidental. A liberation of this acid, together with other higher polyunsaturated fatty acids from the adrenals appears to take place during the first phase of the diphasic defense phenomenon. In the first minutes following a noxious intervention inducing shock, a depletion of fatty acids in the adrenals occurs, coinciding with increased amounts in blood.

Besides their role in the defense mechanism, the adrenal fatty acids seem to intervene in normal physiology. Successive liberations seem to occur, alternating with liberations of sterols, as well as corticosterones. Together, these produce the diphasic oscillations which characterize the physiologic dynamic balance.

Preparations rich in arachidonic acid seem to act at different levels. No manifest influence upon phages is observed. The influence upon smallpox viruses and upon microbes is similar to that of the linoleic preparations. The in vitro and in vivo effects upon red blood cells, such as crenelation, conglutination and increased sedimentation rate, are more manifest than for linoleic acid. Leucopenia also is seen. The effects upon the pH of the second day wound crust and upon pain are, however, the same as for linoleic acid. The intensity of acid pain is reduced while that of alkaline pain is increased. In doses much smaller than for linoleic acid, arachidonic acid preparations are able to prevent convulsions induced by thiamine. But they do not seem to change the evolution of radiation lesions. The influence upon tumors is very similar to that of linoleic acid, and only limited changes are observed after treatment of successive generations, either through the dipping of transplants or through treatment of successive hosts. Organ cells treated in vitro with suspension of this fatty acid were seen to induce changes in the respective organs, if injected twice at 3 weeks interval. Systemic influence is not manifest even under abnormal conditions.

Blood and urine analyses are slightly and temporarily changed toward the pattern of fatty acid predominance.

Continuing these studies, we have investigated *polyunsaturated fatty acids* with more than four double bonds, particularly clupanodonic acids from cod liver and sardine oils. Most of the studies were made by using the fractions which when brominated are soluble in acetone at a low temperature, *i.e.*, around $-63°C$. The different fractions obtained were identified through iodine number, neutralization value and spectral analysis after conjugation through treatment with KOH

All the biological effects upon viruses, microbes, cells, etc., are more accentuated than for linoleic or even arachidonic acid. Changes in red cells and leucocytes are much more apparent. At this point we must emphasize the preference of these fatty acids for red blood cells over plasma both in vitro and in vivo treatments. *(Note 2)* They have greater effects upon pain than do linoleic and arachidonic acids, reducing pain of an acid pattern and exacerbating pain of an alkaline pattern. The local pH, as determined by second day wound crust measurements, also is shifted toward increased alkalinity. Convulsions induced by thiamine are influenced by much smaller doses than those required with other unsaturated fatty acids. For some preparations with iodine indices around 350, doses as low as 35 mg./100 gr. of body weight are sufficient to prevent convulsions. Changes in the evolution of tumors are more striking with these preparations, especially when the transplants of successive generations are dipped in the preparation. With this technique, negative results are obtained even at the third transplant for Ehrlich mammary adenocarcinoma. An obvious effect is noted on radiation-induced lesions, with ulceration increased and healing slowed. The influence upon cholesterol levels in the blood and upon hypertension is greater than for arachidonic acid. The effects upon systemic changes, observed through analyses, are temporary and no greater than for linoleic and arachidonic acids.

Acid Lipidic Fractions

Bearing in mind the role of acid lipidic constituents in the physiology of various biological entities, preparations containing these fractions were obtained. Tissues, organs, organisms, and organic products were saponified and acid fractions soluble in ether were isolated. We called them "acid lipidic fractions," or "lipoacidic fractions." Their analyses revealed, in addition to various fatty acids, other substances with lipidic and acid character. Some were identified as porphyrinic acids.

Significant differences related to the sources of these preparations could

be recognized in biological effects at different levels. Preparations obtained from intestine, for instance, had no obvious effects on any of the manifestations; there were no systemic or organic changes, no effects upon pain, red cells or leucocytes in vitro, and no influence on tissue respiration. Treatment of successive transplants showed no apparent effects, even after ten passage generations. No effects upon organs through repeated injections of cells treated in vivo by these preparations were seen.

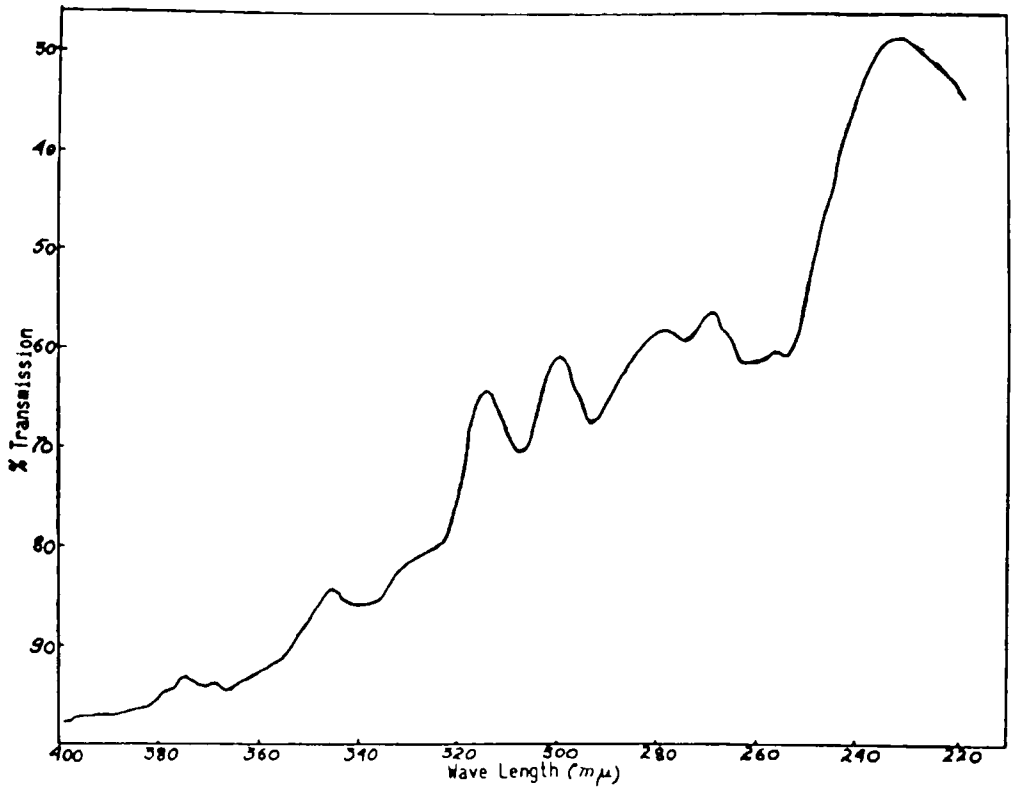


FIG. 120. Spectral analysis of rat liver fatty acids after chemical isomerization shows the presence of fatty acids with 3 and 4 double bonds. (0.002% in ethyl alcohol)

On the other hand, other preparations from placenta, liver, blood, etc. did show activity upon all manifestations, including pain. They induced negative results on tumor growth after transplant dipping for just two or three generations.

The differences in activity could be related to the richness of these preparations in polyunsaturated fatty acids. It could be shown that it was not the total number of double bonds present, as determined by the iodine number, that was significant but rather the relative abundance of higher

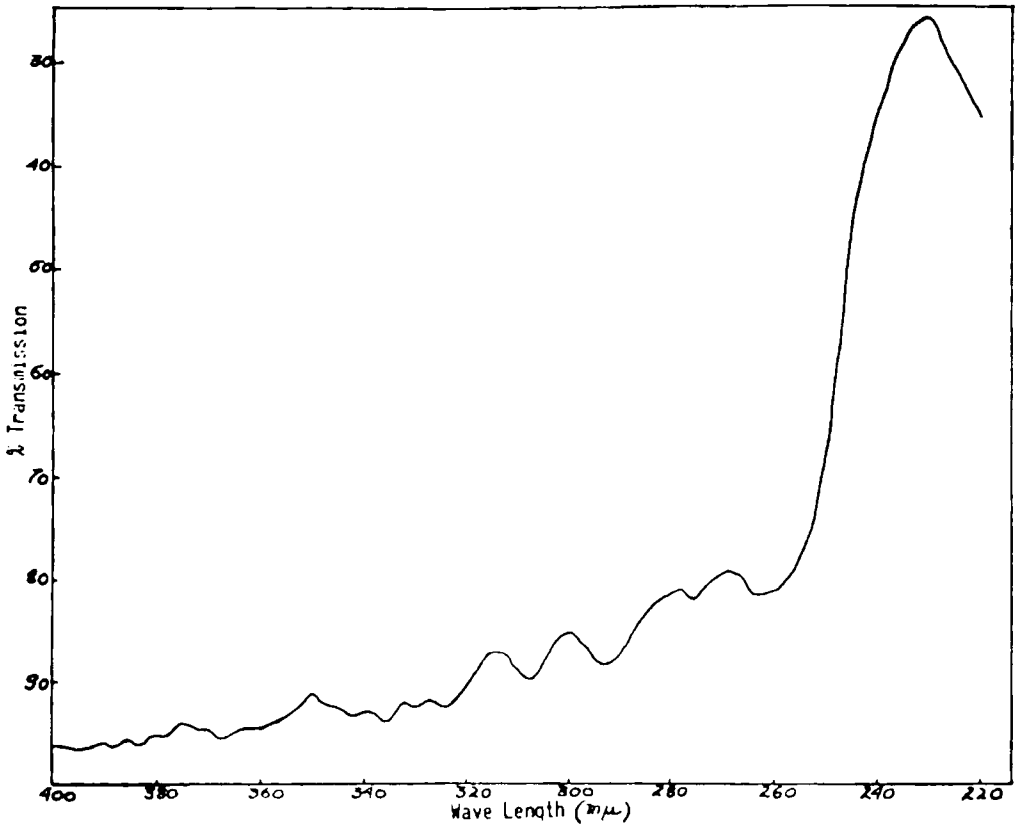


FIG. 121. Spectral analysis of the intestinal fatty acids of rats after chemical isomerization shows minimal amounts of members with 3, 4 or more double bonds. (0.002% in ethyl alcohol)

unsaturated members, as recognized by special analysis after adequate chemical conjugation. Figures 120, 121 and 122 show such analyses.

Abnormal Fatty Acids

Because of the role of abnormal fatty acids in the pathogenesis of the offbalance type D, particularly related to radiation, a study was made of preparations of acid lipids obtained from abnormal tissues, organs and organisms. In an initial group of researches, animals that had died of radiation sickness, shock, acute infections or after adrenalectomy were used. Guinea pigs infected with B. anthracis and mice infected with strep hemolyticus were used as sources of abnormal lipids in a large number of experiments. Acid lipids obtained from autolysates of tissues were employed. We also used the fatty acids of cod and sardine oil, which may be considered to correspond not to natural but to altered fatty acids since they were obtained after autolysis of cod liver and whole sardine bodies.

After having determined that conjugation of double bonds is the basis

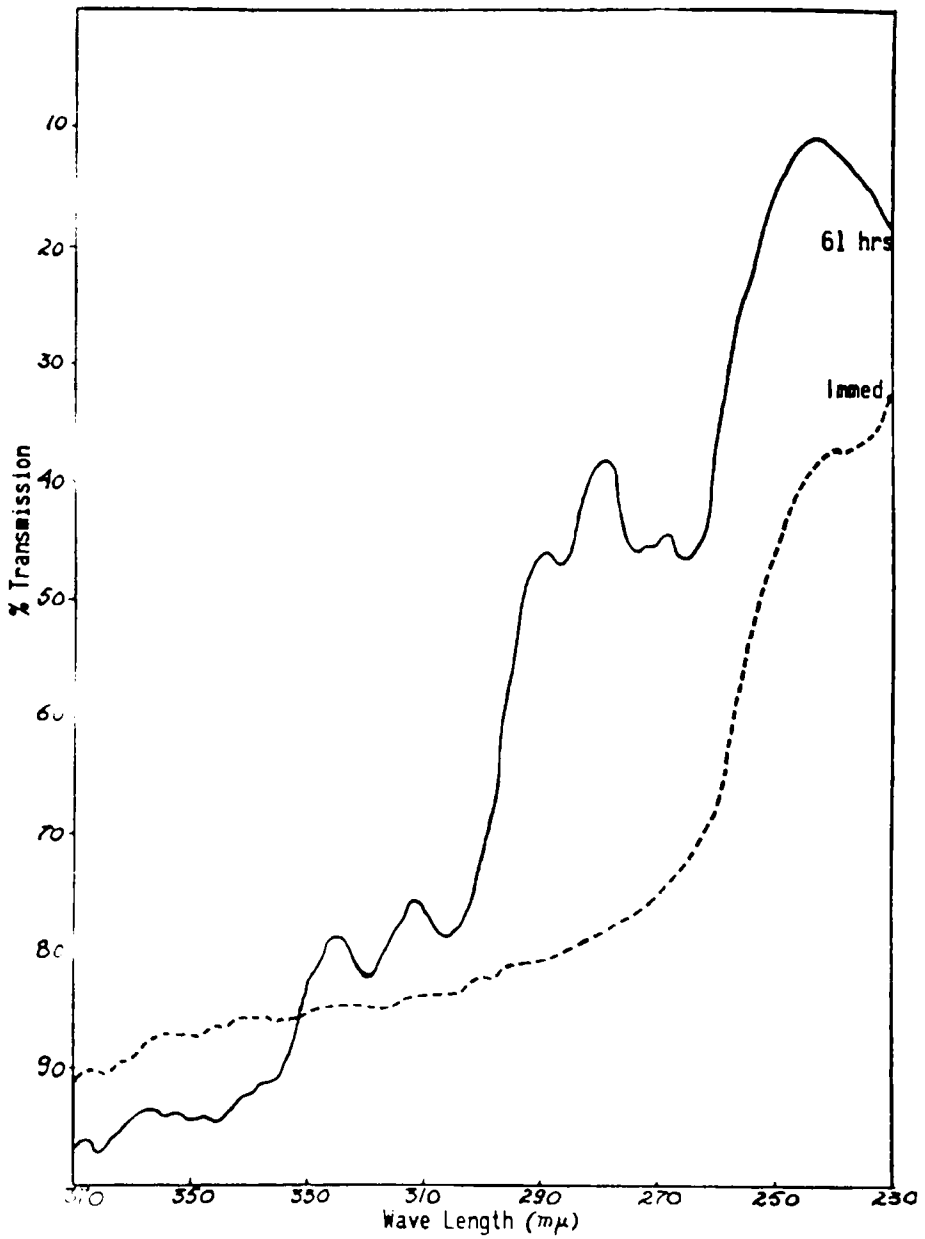


FIG. 122. Spectral analysis of the fatty acids of the entire body of rats, after chemical isomerization shows the presence of di- and triethenic members, and little of members with more double bonds. (0.002% in ethyl alcohol)

of abnormality in pathogenic fatty acids, conjugated isomers of different fatty acids were prepared and studied. Eleostearic acid obtained from tung oil was used extensively in animal and clinical research. Parinaric acid was obtained from nuts of parinarium laurinum and was used to a lesser degree. Various conjugated fatty acid isomers, recognized through spectral analysis and oxalic indices, were obtained in a higher proportion from

different lipoacidic preparations by using a modification of classical methods of conjugation. *(Note 3)* Many mixtures of naturally occurring fatty acids found in saponifiable fractions were conjugated by the same method. Conjugated di, tri, tetra, penta—and hexaenic fatty acids were further separated from these mixtures and studied. The unexpected relationship of these conjugated fatty acids to oxygen was of interest. While the treatment of linoleic acid at 37°C with oxygen leads to progressive increase in the amount of peroxides present, this does not occur for the conjugated isomer *(Fig. 123)* apparently because of repeated destructions of the peroxides formed.

In general, the effects produced by conjugated fatty acids at different levels of the organization are more intensive and persist longer than those

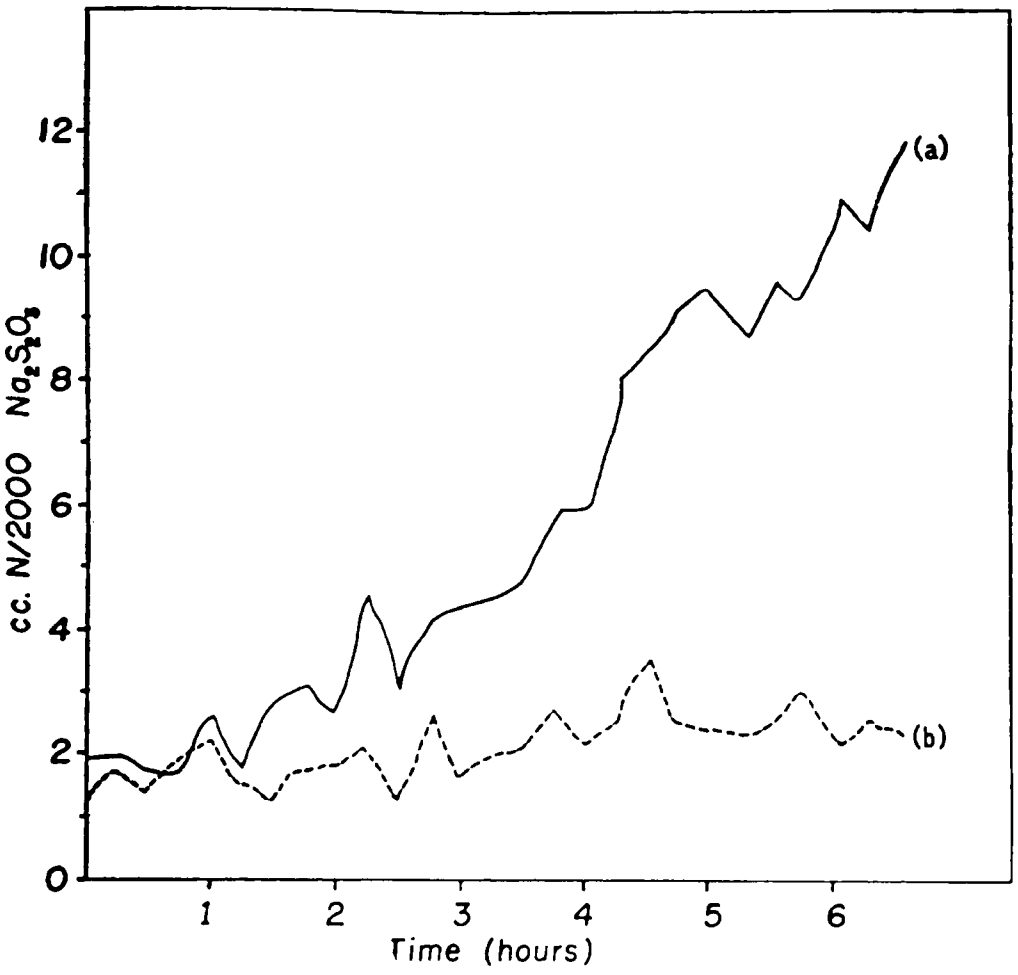


FIG. 123. Curves of peroxides of samples of linoleic acid (a) and its conjugated isomer (b) induced through the passage of oxygen (100 ml per minute for 30 cc of sample) at 37°C. While peroxides are progressively increasing in the linoleic acid preparation, they do not change in the conjugated isomer.

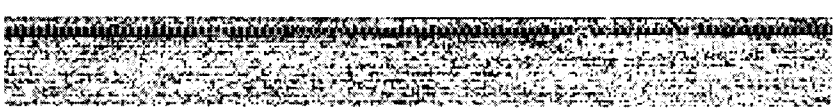
of the nonconjugated isomers. It is interesting to mention here the influence exerted by these preparations upon viruses. Although there are no changes for bacteriophages, a marked influence is seen in vivo upon receptivity of the organism to viruses. Subcutaneous injection of conjugated fatty acids in rabbits establishes a zone of refractivity toward inoculation of smallpox vaccine in the skin, which is greater and more persistent than that observed for nonconjugated isomers. The effect upon microbes also is clear. Gram negative strains with manifest morphological changes were obtained from B. anthracis, and persisted as such for 6-15 passages before the old morphological and tinctorial characters returned. The effect upon red cells and leucocytes in vitro is more apparent than for corresponding nonconjugated isomers. This is also true for the influence upon the respiration in vitro of tissues or ascites cells.

The difference in the effects of conjugated and nonconjugated fatty acids is particularly striking in certain manifestations. For example, pain with an acid pattern is more easily changed to alkaline by treatment with conjugated fatty acids than with nonconjugated isomers. Once the alkaline pattern appears, it persists for a long time. A manifest effect is seen upon lesions induced by radiation. Standardized radiated wounds in rats, which heal in about four weeks in control animals and require six to eight weeks to heal when treated with nonconjugated fatty acid preparations, fail to heal at all when treated with corresponding doses of conjugated isomers of the same fatty acids.

The isomers of fatty acids also differ in their effect upon animals in shock. When anaesthetized animals are scalded by immersion up to the level of the xiphoid in water at 90°C, immediate fatal shock occurs if the immersion lasts for more than four seconds. With a three second scalding the animals die after several hours. The administration of fatty acid preparations markedly reduces the survival time, and this effect is more manifest for conjugated members, especially for eleostearic acid. In general, animals in shock induced by any means, such as by the Noble-Collip drum, show a special sensitivity toward conjugated fatty acid preparations.

The effect of conjugated fatty acids at the systemic level, as recognized by blood and urine analyses, is in the same direction as that for the nonconjugated but is more manifest.

There are significant differences between the effects of conjugated and nonconjugated fatty acids upon the evolution of transplanted and spontaneous tumors. In a small proportion of mice, a mixture of nonconjugated fatty acids prepared from cod liver oil or sardine oil prevents the appearance of methylcholanthrene-induced tumors. The conjugated fatty acids



obtained through the treatment of these preparations show this preventive effect in a large proportion of mice. These experiments are interesting from several standpoints and are presented in some detail in Note 4.

Conjugated fatty acids produce an increase in the chloride content of wounds in animals. Values are 40% higher than in untreated lesions. The same increase of chlorides occurs in tumors. When Dba mice with adenocarcinoma were treated for ten days with conjugated fatty acid preparations, and tumors were removed and chloride content determined, values were up to 180% higher than for controls with untreated tumors. *(Note 5)* An interesting effect was noted in two rat tumors. For years, passages of Guerin's rat tumor and of sarcoma induced in our laboratory by the injection of benzpyrene have shown a peculiar character. Although they grow to large size, the tumors have no necrotic zones. After treatment with conjugated fatty acids, large necrotic zones appeared, leading to ulceration and death. The appearance of these zones of necrosis corresponds to changes in the fundamental character of the tumor. Transplants of fragments of these treated tumors, although taken from nonulcerated regions, or young tumors, resulted in tumors showing early central ulceration. This character persisted without further treatment in succeeding generations. The ulcerating tumors appear to be a mutant of the original and the mutative change can be related to intervention of conjugated fatty acids. This was confirmed by the fact that similar changes were constantly obtained in the same tumors with the same agents. A similar but less manifest and less constant effect is obtained with preparations of cod liver oil fatty acids administered repeatedly in large amounts. The same effect was obtained with the injection of the fatty acids directly in the tumor itself.

The second day wound crust pH shows marked changes toward alkalinity under the influence of conjugated fatty acids. The effect upon regeneration time of liver is also manifest; cells full of fatty droplets do not appear at all or appear much later than in untreated controls. In rats weighing 200 grams, with a large enough dose, such as 2 cc. of 10% solution of polyconjugated fatty acids obtained from cod liver oil and repeated for two days, the adrenals show complete depletion of fats. They become small and red in color and contain no sudanophil material. The liver regenerates with small cells with compact cytoplasm and almost entirely bare of fatty droplets.

The effect of conjugated fatty acids upon the lymphatic system is manifest. A marked involution of thymus, spleen and lymphatic gland follows the injection of conjugated fatty acid preparations, particularly of a mixture of conjugated cod liver oil fatty acids. The effect upon tumors is irregu-

lar. In some animals a marked retardation occurs, in others no effect can be observed. In convulsions, no greater effect is seen than that produced by nonconjugated isomers. Blood in vitro assumes a color darker than when other fatty acids are used. Eosinophiles are markedly reduced after administration of conjugated fatty acid preparations. Important changes in organs were obtained with repeated injections of the respective cells treated in vitro with suspensions of these acids. Changes in the analytical values of urine, however, are not greatly different from those obtained with nonconjugated isomers. With sufficient amounts of conjugated fatty acids, a frank hypothermia is obtained.

Bixine

In the same group of fatty acids we can place bixine, a member of the polyterpene family with 9 conjugated double bonds which we obtain through saponification of the seeds of Bixa orellana and have studied widely. The changes produced in microbes are similar to those with other polyconjugated fatty acids. Changes in connective tissues in animals appear to be particularly interesting. The first reaction to subcutaneous injection of an oily solution of 1% bixine in rats or mice is an inflammatory process, with the injected material dividing into hundreds of tiny droplets. Some, however, melt away and the unabsorbed injected material again forms one or two big drops. The wall containing the drops is very thin and transparent and appears to be made up of very few connective cells which have extremely long fibrils, representing the highest degree of differentiation of these cells.

The effect upon pain is similar to that of conjugated fatty acids. The second day wound crust pH shows a manifest change toward alkalosis. The effect upon radiation wounds is the same as for conjugated trienes. In animals injected with convulsant doses of thiamine, only a few milligrams of the bixine preparation are required to prevent convulsions. Of all the fatty acid preparations used, bixine appears to be the most efficient in its anticonvulsivant action. The iodine number of 430, found in our preparations, confirms, thus, in this case too, the correlation seen between anticonvulsivant effect and richness in double bonds of the fatty acids.

The distribution of bixine following administration is interesting. Chromatographic study of blood constituents after hydrolysis shows that almost all the bixine is in the red cells, with minimal quantities in plasma. Lesions such as wounds or tumors, after the administration of bixine, become particularly rich in this substance in comparison to normal tissues. Changes in evolution of tumors also are manifest. The administration of this agent

often leads to rapid necrosis and edema. In animals and humans, we saw massive tumors become ulcerated in a few days after use of only a few milligrams of the substance. The ulcerating effect of conjugated fatty acids upon tumors reaches its maximum with bixine. In animal tumors which, in successive transplants, had never shown spontaneous ulcerations, the injection into the host of only a few milligrams of bixine in oily solution produced, in addition to ulceration, a change in the tumor which can be considered to correspond to a mutation. Further transplants consistently developed ulcerating tumors and the ulcerative character persisted in succeeding generations. Massive degenerating changes were obtained in organs after repeated subcutaneous injections of suspensions of cells obtained from these organs and treated in vitro with bixine.

The changes toward offbalance D induced in various systemic patterns in humans by bixine are similar to those produced by conjugated fatty acids. However, once induced, these changes are very persistent, and often remain uninfluenced by anti-fatty acids. It is this characteristic of resistance to further changes which represents a certain handicap for therapeutic use of this agent.

Fading Response

In contrast to the relatively persistent changes induced by bixine, the striking character of the effects upon pain or systemic manifestations obtained with fatty acid preparations, and especially with the conjugated isomers, is their short duration. Furthermore, at the beginning of their administration, these agents, even in relatively small doses, exert intense effects, but such effects cannot be obtained later without continuously increasing the amount used. After a certain time, even large doses have very little effect.

An explanation of the fading character of the results obtained with these and many other agents can be found in the fact that the organism defends itself against any factor able to influence its balance. In the case of conjugated fatty acids, this defense seems to be provided mainly by the adrenal glands. E. F. Taskier has studied this aspect of adrenal defense in our laboratory and this research is presented in *Note 17, Chapter 6*. An adrenalectomized animal is usually less resistant toward the administration of many agents than a normal or sham-operated animal. This drop in resistance is expressed as an adrenal defense index, as the ratio between the minimal lethal dose for the normal and for the adrenalectomized animal. For most fatty acid preparations, this index is between two and three, and becomes greater for the conjugated fatty acids. Fatty acids with three con-

jugated double bonds, however, have an adrenal defense index of 120. Eleostearic acid is 120 times less toxic for sham-operated controls than for adrenalectomized animals, indicating a specific adrenal defense against these acids. Progressively increased intervention of the adrenals would explain the fading effect mentioned above.

Alpha Hydroxy Fatty Acids

Alpha hydroxy fatty acids were obtained by fixing an OH at the carbon adjacent to the carboxyl. Some of these acids exist in nature—in significant amounts in the brain and skin, and in very small amounts in the kidneys. In our research, they were originally prepared from natural sources, such as animal brain and skin. Most of the studies however, were made with synthetic alpha-hydroxy-fatty acids. For experimental purposes in animals and humans, we principally used pure synthetic alpha hydroxy fatty acids. Mixtures of these members obtained through the treatment of acid lipidic preparations also were employed.

In animals and humans, alpha hydroxy fatty acids induce less systemic, organic, tissue and cellular changes than do the corresponding untreated acids. However, we would like to mention a striking exception: the response of lymphosarcoma 6C3HED in mice to the administration of alpha hydroxy-caprylic acid. Although this tumor uniformly kills control animals within 10-12 days, it disappears in over 80% of animals treated with alpha hydroxy caprillic acid, even if treatment is instituted late, that is, when the tumor has already grown to 1 cm. in diameter, a size usually reached 2 or 3 days before death. In the few animals in which the tumor does not disappear, its growth is so slowed down that survival time is extended to three or more weeks. *(Note 6)*

Other alpha hydroxy fatty acids close to caprylic acid, such as alpha-hydroxy-caproic and capric acids, show no influence upon evolution of this tumor. We could not obtain similar effects with any of the other saturated alpha hydroxy fatty acids that have chains with 4-20 carbons, nor with alpha hydroxy-oleic or linoleic acids. Nor did alpha hydroxy-caprylic acid or any of its homologues appear to have any influence upon other transplanted tumors in mice, the Walker tumor in rats or, spontaneous mammary tumors in mice.

Other Fatty Acids

The fading effect seen with naturally occurring fatty acids is so great a handicap for therapeutic use of these substances that we searched for heterogeneous fatty acids which the organism does not normally encounter

and against which it would not be prepared to defend itself. This brought us to the study of fatty acids having different nonpolar groups than those of the normal and abnormal constituents. While most of these were prepared synthetically in the laboratory, we utilized on a substantially large scale two natural fatty acids which exist in plants and are sufficiently heterogeneous, ricinoleic and crotonic acids.

Ricinoleic acid has a double bond between 9 and 10 and a hydroxyl at carbon 12 instead of the second double bond found in linoleic and linolenic acids. As a result of the induction effect propagated from the carboxyl through the chain, the C_{11} is a positive carbon. The positive character is enhanced by the adjacent double bond between C_9 and C_{10}, and by the hydroxyl bound to C_{12}. C_{11} thus is very strongly positive. We related the intense local alkalosis with consequent water excretion corresponding to the alkaline watery diarrhea to the effect of ricinoleic acid liberated in the intestine, and have considered it to correspond to a local organic offbalance similar to that induced in tissues by unsaturated fatty acids. This would explain the intensive laxative effect of castor oil.

We utilized ricinoleic acid parenterally with the aim of obtaining a similar effect in abnormal cellular and tissue lesions. The oily solution of ricinoleic acid has low toxicity when administered parenterally. However, no manifest effect upon tumors or at different levels of organization was obtained. Crotonic acid did not show the expected influence at these levels.

Other heterogeneous agents, polyhydroxy fatty acids, were studied. They were prepared by adding one or more OH groups to the nonpolar groups at the double bonds of unsaturated fatty acids. 9, 10 dihydroxy and 9, 10, 12, 13 tetrahydroxy fatty acids were no different than the corresponding unsaturated fatty acids in their effects upon pain or systemic analyses in humans, or upon tumor growth in animals and humans.

Peroxide Fatty Acids

Peroxides and epoxides of fatty acids were prepared and studied. They showed more manifest effects upon viruses and bacteria in vitro and in vivo than the other fatty acids. Investigations of the effects of these fatty acids upon higher levels have been limited until now. It seems that the effects upon systemic and organic manifestations are somewhat different than those obtained with use of the fatty acids from which the peroxides and epoxides were derived. Influence upon pain and upon tumors was greater for the corresponding unsaturated fatty acids. This research—especially with polyepoxide fatty acids—is still in progress.

Halogenic Compounds of Fatty Acids

The study of changes in lesions where abnormal fatty acids are present has shown the importance of fixation of chloride ions in these substances. Considering its place in the periodic chart, chlorine is an element with heterotropic character. Consequently, it could be conceived of as being antagonistic to fatty acids, counterbalancing their homotropic character. This was confirmed by pharmocological study of fatty acids to which chloride ions were added at the double bonds. We were particularly interested in conjugated fatty acids in which effects of treatment with chlorine could be followed through spectral analysis.

When a mixture of conjugated fatty acids from cod liver oil was treated with chlorine, the peaks in spectral analysis progressively disappeared. *(Fig. 124)* This did not lead, as expected, to increased toxicity. Thus far, in early trials, the different preparations, from 9-10 dichloro-stearic acid to the polychlorinated mixture of fatty acids from cod liver oil, have not shown effects greatly different from those of conjugated fatty acids upon tumor evolution, systemic analyses, and pain. No difference has been noted between the derivatives and their corresponding fatty acids in animal

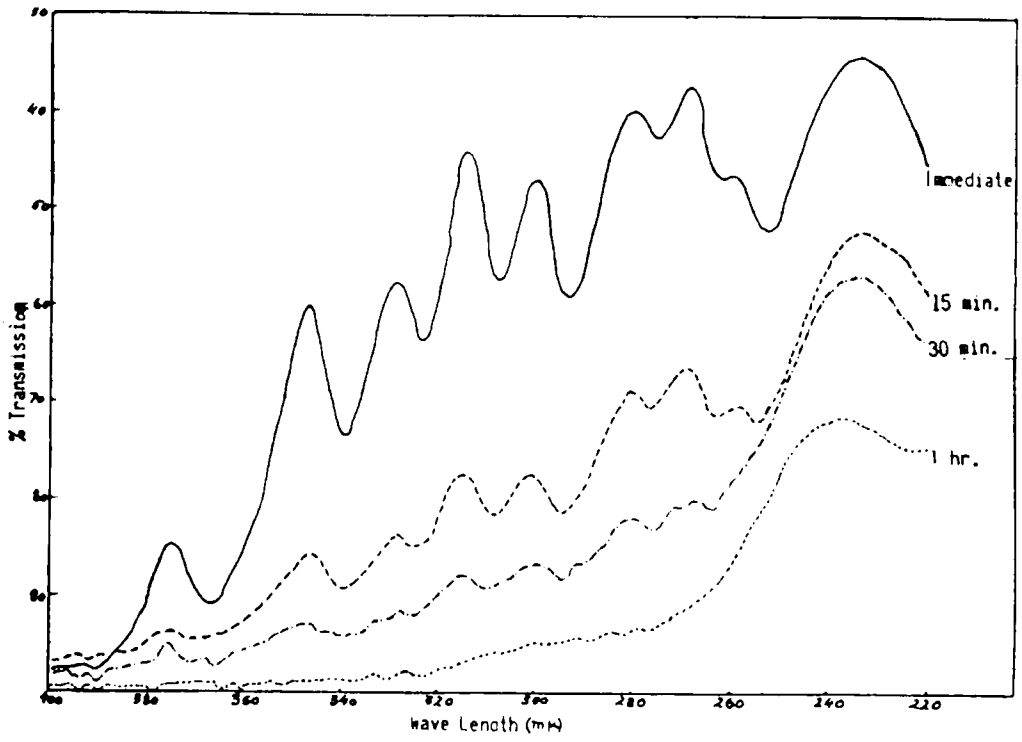


FIG. 124. Influence exerted by chlorine gas upon the conjugated fatty acids. A parallel decrease in the amount of all the members is seen. (0.002% in ethyl alcohol)

experiments or in research in humans. Experience with these products, however, indicates that they may induce milliar gastric ulcerations which we consider to result from the influence exerted by the fatty acids upon the gastric mucous membrane where they are brought by the chloride ions to which they are strongly bound. Research in this direction confirms the part which these fatty acids, solidly bound to chloride ions, take in the pathogenesis of the state of shock.

An over-all analysis of the pharmacological activity of the fatty acids mentioned above shows a similarity in fundamental effects obtained with most of these preparations. This can be interpreted as resulting principally from the fact that all have in common, their lipidic character and the same polar group with acid properties—the carboxyl. As a result, these substances are fixed in the same position in abnormal entities, a fact which seems to represent the most important factor in their pharmacodynamic activity. The further biological differences seen between the influence exerted by the various members studied would be related to a secondary effect of these substances resulting from the intervention of the nonpolar groups. This finding —that the fundamental pharmacological activity of fatty acids is connected with the site of activity which is determined by the lipoidic character and the polar group present while the proper pharmacological activity is due to the intervention of the nonpolar group—has been of capital importance not only for understanding the activity of these substances but also for determining the path of our further research. Because of this, we investigated, in a second step, lipoids with other negative polar groups.

LIPOIDS WITH OTHER NEGATIVE POLAR GROUPS

Lipoaldehydes: With a carbonyl as polar group, the lipoidic properties appear with propanal in the homologous series of the aliphatic aldehydes. With the carbonyl less dissociated than the carboxyl, the lipoaldehydes represent *negative* lipoids able to act for a longer time than the respective acids. We were especially interested in three groups of aldehydes. In one, with a nonsaturated short nonpolar chain, we searched a conjugated formation between the double bond of the oxygen of the carbonyl and the double bond of the nonpolar chain. Another group of the lipoaldehydes corresponded to long chain fatty acids, while the third was formed by saturated short chain aldehydes with an odd number of carbons. From the energetic point of view, there were the first and especially the third groups which appeared as the most interesting. In the last group the opposite influ-

ence exerted by the carbonyl and methyl groups upon the intermediary carbons of the chain gives the entire molecule an especially high reactivity. This opposite influence is seen at its maximum in propanal, where C_2 suffers the influence energetically opposite of the carbonyl and methyl group. The fact that, due to its relatively high solubility in water, propanal—which is a lipoid—can be administered in aqueous solutions and still act upon the lipidic system—makes of it an especially interesting agent.

We have investigated these groups of lipoaldehydes from the point of view of their influence exerted upon the two offbalances. In the group with unsaturated short chains, we studied acrolein and crotonic and maleic aldehyde without seeing any special effect upon the other levels than the cellular one, where a vacuolation was obtained. Furthermore their toxicity has represented an handicap. More interesting has appeared the group of the saturated short molecules with odd number of carbons. While with heptanal we have obtained besides an influence upon pain, also a manifest inhibitory effect upon the growth of experimental tumors, it was propion aldehyde which has shown the most interesting effects upon pain.

This was seen for the group of aldehydes with aliphatic saturated chain such as *propionic* and *heptylic aldehydes* or with *cyclic,* as *salicylic aldehydes.* In adequate doses—from $\frac{1}{20}$ cc. to 2 cc. of the 10% solution of propionic aldehyde, or of the 1% solution of the heptylic or salicylic aldehydes—a manifest influence was obtained upon the systemic condition as well as upon pain. Patients in offbalance A with pain and general discomfort, were seen to have a decrease of the symptoms after the administration of propionic aldehyde. The effect upon tumors was reduced and propionic aldehyde did not change the evolution of the tumors in spite of the marked improvement of the general condition and even of the cessation of pain.

Lipoids with Thiol Groups

Mercaptans: According to the systematization of lipoids presented above, a thiol group, acting as a polar group, will form a lipoid when bound to an energetically preponderant aliphatic or cyclic nonpolar group. In the homologous mercaptan series, even the lowest members are lipoids because of the weak electrostatic forces of the thiol group.

Although methyl mercaptan is a lipoid according to our classification, this substance is too volatile to be used. Therefore, the first low member of this homologous series to be investigated was ethyl mercaptan.

The effects of ethyl mercaptan upon microbes were more limited than those seen for fatty acids. To determine the effects at the different levels

of the organization, ethyl mercaptan had to be administered parenterally. As for all other members of this series, we utilized ethyl mercaptan in 5 or 10% oily solution in cottonseed oil. In acute toxicity tests, the lethal dose was found to be 145 mgs./100 grams of body weight for mice, and 153 mgs./100 grams for rats. The immediate effects upon nuclei were similar to, but less intense than those of conjugated fatty acid preparations leading to caryorrhexis or pycnosis in abnormal cells. No abnormal mitosis was seen in organs with high mitotic activity, such as the intestinal mucosa or bone marrow, although appreciable changes were observed in mitosis in animal tumors. A secondary effect, an exaggeration of aging processes,

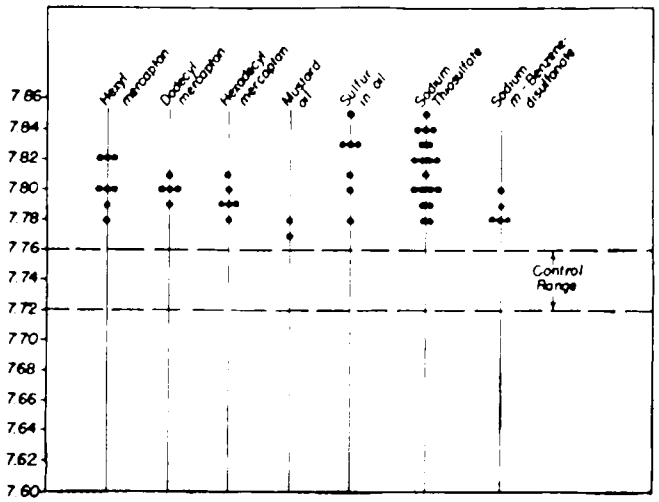


FIG. 125. Second day wound crust pH shows a constant change toward more alkaline values for all the substances having sulfur in their polar group.

was first recognized, several days after administration in the changes in granulocytes at the site of injection. The average number of nuclear lobes of the leucocytes was often very high, even above seven. This was also true for leucocytes in circulating blood when the product was injected intraperitoneally or even subcutaneously in rats. The immediate effect of such injections was a prolonged leucopenia, especially a lymphopenia.

The chloride content of tumors and wounds was especially increased through treatment with mercaptans. At the tissular level, local pH of the second day wound crust was increased, an effect characteristic for all lipoids with sulfhydryl as the polar group. *(Fig. 125)* This explains the direct effects of ethyl mercaptan upon pain and other alkaline or acid symptoms. These effects are qualitatively similar to those seen for the polyunsaturated fatty acids but much slower to appear. While placenta acid lipid prepara-

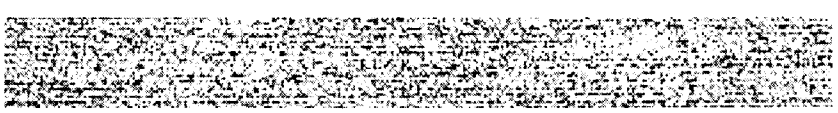
tions, for instance, produce an effect upon pain—an increase in intensity for an alkaline pattern and a decrease for an acid pattern—even within 5 to 8 minutes after parenteral administration, the effect of mercaptans is reduced and appears after half an hour, or later.

The effect upon tumors in animals was especially manifest upon a rat sarcoma originally induced by benzpyrene in our laboratories and passed through successive transplants over a period of many years. Throughout this period, this tumor showed 100% positive takes, characteristically growing to huge size, at times as large as the rest of the animal, but without ever showing either spontaneous regression or necrotic areas. When ethyl mercaptan was injected subcutaneously in daily doses of ½ cc. of a 5% solution in cottonseed oil into animals with these tumors, interesting changes were observed. If the tumors were already large, above 6 cm. in diameter for instance, only a few regressed (5/20 for tumors of 6 cm. in diameter). In tumors that did not regress, large necrotic areas developed and were followed by ulceration. Most became infected, leading to death. Tumors smaller than 6 cm. in diameter frequently regressed rapidly and then disappeared (between 9/20 and 20/20 in different experiments). No such striking results were observed in any of the other tumor strains in rats or mice treated with ethyl mercaptan, although in several, growth arrest occurred or necrotic zones appeared. Ethyl mercaptan injected in the tumors themselves induced the same necrotic changes in most animals. *(Fig. 126)*

The effect upon lymphatic organs was manifest. Spleen, thymus and lymph nodes were involuted in animals treated for a few days with mercaptans. The effect upon convulsions was irregular. Even with small doses, convulsions were prevented in some cases but, in general, the effect was less constant than with fatty acids. Eosinophiles decreased rapidly in animals or humans treated with this substance. All urinary analytical data were influenced by administration of mercaptan, with changes toward the patterns of type D offbalance.

Because of the very disagreeable odor of ethyl mercaptan, we were obliged to discontinue its use so that effects of this agent upon some analyses, such as surface tension, sulfhydryl and calcium excretion, could not be studied in humans.

Other Mercaptans: Superior homologues of the mercaptan series were used. They were divided into three groups. The first included propyl, butyl, amyl and hexyl mercaptans; the second, heptyl and allyl mercaptans; the third, members with more than 10 carbons. The first group produced much the same effects, which tended to diminish as the number of carbons in-



creased. For example, effects were considerably reduced for hexyl mercaptan as compared with ethyl mercaptan. The second group—the heptyl and allyl mercaptans—produced more intensive effects. This was especially true for allyl mercaptan. Members of the third group—with longer carbon chains such as dodecyl and hexadecyl mercaptans—produced effects so slight that they seemed almost nonexistent, except upon pH of the second day wound crust, which showed values far above the range of the controls, just as with other members of this homologous series.

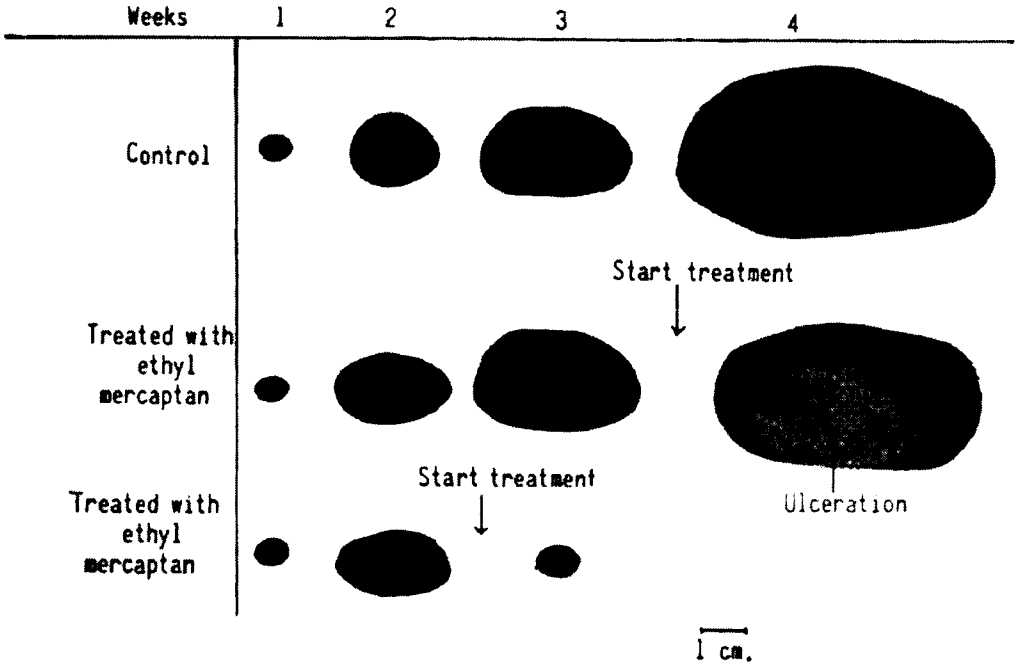


FIG. 126. Influence exerted by ethyl mercaptan upon a sarcoma induced by benzpyrene. For bigger tumors it induces constantly an ulceration, while for small tumors, their disappearance.

With the idea of using thiol as a polar group and having another center in the molecule as a secondary center, we studied a series of other substances. One was dimercapto-propanol (the B A L preparation) often used for heavy metal poisoning. It proved to be completely without effect on pain, tumor growth and systemic changes. It had less activity than the higher mercaptans, which themselves were less active than polyunsaturated and abnormal fatty acids.

The difficulties encountered in administering mercaptans, mainly related to offensive odor of lower members and inactivity of the less obnoxious members, led to a search for other chemical agents that might be active without being evil smelling. Extensive study was made of various prepara-

tions that appeared to have a bivalent sulfur bond at the nonpolar group. We investigated colloidal sulfur which, if introduced in the organism, seemed to undergo changes similar to those of bivalent sulfur. We found, thus, that the sulfur absorbed after being administered in colloidal form, was almost entirely eliminated after oxidation in the form of sulfates. In animals, no pharmacodynamic influences were observed. Only the pH of the second day wound crust was increased when these preparations were administered parenterally in suspension or were given orally mixed with food, in proportions up to 4%. There was no evident change in tumor evolution in animals or humans.

Hydro-Persulfides

Another sulfur compound, so-called "sulfurized oil," in which sulfur and fatty acids appear to form a hydropersulfide, *(Note 7)* was tested. This hydropersulfide preparation, although it exhibited no influence upon viruses in vitro, did induce a good degree of local resistance of the skin to smallpox infection. The effects upon microbes were reduced. There was little direct influence exerted upon cells. The preparations with 0.5 to 1% sulfur bound to cottonseed oil were well tolerated locally when administered intramuscularly or intraperitoneally.

The effect of parenteral and oral hydropersulfide upon pain was slow to appear, in contrast to the effect of fatty acids and even of mercaptans. However, it persisted for a long time. Pain of the acid pattern was eased; pain of alkaline pattern was exacerbated. The influence upon the second day wound crust pH was marked. The local pH increased to values even higher than 7.80. In radiation lesions, the dimension of ulceration increased and healing was retarded or even prevented. In some tumors in animals, the rate of growth was slowed. This latter effect was not uniform in the different types of tumors tested and even in the same type of tumor in different groups of animals. Systemic changes also varied. Doses corresponding to 5 mgr. of sulfur were not toxic for 30 gm. mice in a single injection. Nor were 15 mgr. doses in 200 gr. rats. Chronic toxicity studies showed that 0.2 mgr. daily injections in mice and 5 mgr. injections in rats for as long as three months did not induce pathological changes. High doses such as 1 to 2 cc. of a 1% preparation injected several times a day in humans was almost uniformly followed by a rise in temperature, usually after a few days.

Other Compounds with a Thiol Group

The results obtained with hydropersulfide preparations led us to seek other compounds with sulfur bound to fatty acids instead of triglycerides as in those mentioned above.

Sulfur compounds were prepared from various conjugated fatty acids such as conjugated linoleic acid and eleostearic acids and from mixtures of conjugated fatty acids obtained from cod liver oil, fish oil, human placenta, blood and various organs. While active in smaller amounts, they were not qualitatively different from hydropersulfide preparations obtained from cottonseed oil, producing the same pharmacological effects in most tests, especially upon pain, systemic manifestation and evolution of experimental tumors.

The fact that sulfur bound to the nonpolar group, as in hydropersulfides, produced less manifest results in animals and humans than mercaptans, which have a thiol group as a polar group, led to the study of other substances in which thiol radicals instead of sulfur were added in similar positions, and consequently were considered to act as secondary energetic groups. A series of preparations, in which one or more thiol groups were fixed at the double bonded carbons in various conjugated or nonconjugated polyunsaturated fatty acids, were obtained. These substances differ fundamentally from the fatty acids mentioned above in which sulfur atoms were fixed not at the carbons bound by double bonds but at the carbon adjacent to the double bond. 9-10, dithiostearic acid, 9, 10, 12, 13, tetrathiostearic acid, as well as polyunsaturated and conjugated fatty acids with thiol groups fixed at their double bonds, were obtained. In general, they showed no marked biological effects on animals, no influence upon pain or systemic patterns similar to those observed for the other lipidic products with bivalent sulfur.

The hydropersulfide group was added to soaps. Sodium and ammonium soaps were obtained through saponification of the triglycerides of fatty acids on which sulfur was already fixed. Effects at the different levels of organization were markedly reduced. There was no influence upon pain, organic or systemic manifestations. However, a striking effect was noted on many microbes. Growth of Bac. anthracis was prevented in some experiments, even with dilutions of 1/2,000,000. For staphylococcus aureus and streptococcus hemolyticus, a similar effect was obtained with dilutions higher than 1/200,000. In animals, even oral administration in drinking water in a dilution of 1/500 and, in some experiments even 1/1000, con-

trolled infection caused by these microbes. The antibiotic activity appeared to be similar to that of penicillin. *(Note 8)*

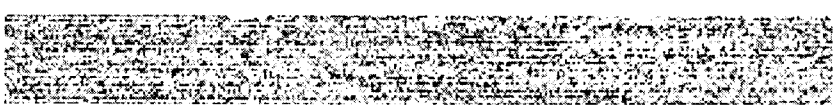
Tetrahydronaphthalene Persulfides (Sulfurized Tetraline)

We utilized the known marked tendency of tetrahydronaphthalene to fix oxygen with the resulting explosive peroxides, to fix sulfur in similar combinations. Persulfides of this substance were thus obtained and their pharmacological activity studied. While only a limited effect was noted upon viruses and microbes, the influence upon tetrahymena pyriformis approached that seen with active polyunsaturated fatty acids. The product with 5 gm. sulfur fixed for 100 grams of tetrahydronaphthalene, showed a relatively low toxicity in normal animals, 75 mgr./100 gr. in mice and 125 mgr./100 gr. in rats being well tolerated in intraperitoneal administration. The influence upon wound healing in animals, upon pain, and upon the systemic analyses in humans, was similar to that seen for the mercaptans. Although the immediate effect on pain was limited, prolonged administration was effective. The analytical changes of the urinary surface tension and blood potassium were the most manifest. The toxicity was highly increased for animals with ascites tumors (Sa 180, Ehrlich and Krebs). However, the influence exerted upon transplanted and spontaneous tumors in animals was one of the most favorable ones, compared to the effect of other tested agents.

Thiosulfates

We investigated thiosulfates with the intention of studying agents which, in addition to a manifest reduction effect, would act through bivalent sulfur liberated in the body. The elimination of part of the sulfur of thiosulfates as mercapturic acid has led to the supposition that the bivalent sulfur ion, separated from the thiosulfate ion, would act through combinations similar to those found in the metabolism of thiolipoids. It is for this indirect action that although hydrosoluble, without any direct connection to lipoids, we investigated the biological activity of thiosulfate together with and under the same specific aspect as the lipoidic sulfur compounds considered above.

There was a very limited effect upon microbes and viruses, no effect upon phages, and no change in the receptivity of rabbits to smallpox virus. With either oral or parenteral administration, the immediate effect upon pain was manifest. Oral administration of ½ cc. of a 10% solution of sodium thiosulfate in water, or intramuscular injection of ½ cc. of the 4% solution, was usually followed by a definite effect upon pain in less



than 10 minutes. As this was found to be opposite for the two patterns of pain, sodium thiosulfate was used even for diagnosis of pain pattern. Pain of an alkaline pattern increased while a decrease occurred in pain of an acid pattern. The second day wound crust pH increased manifestly with the use of this substance.

Cellular and nuclear changes following administration of thiosulfate preparations were similar to those produced by mercaptans. The effects upon the lymphatic system and on liver regeneration, however, were minimal. Convulsions induced by thiamine were controlled well by thiosulfate in doses of 120 mgr. per 100 gram of body weight. Injected simultaneously with administration of thiamine, thiosulfate prevented convulsions in a high proportion of animals (17/20).

The effect upon radiation lesions was less manifest. An increase in wound size and prolonged ulceration occurred only with use of relatively large amounts of sodium thiosulfate daily. Doses above 40 mg./100 gm. of body weight were needed to obtain these effects. The healing of a simple wound was retarded only with large doses, around 50 mg./100 gm. of body weight. On the other hand, when very small doses were administered, such as 5 mg./100 gm., the healing effect was enhanced. Effects upon tumors were less manifest in animals. Slight and inconsistent changes were seen in grafted tumors. Very often in the same experimental group, tumors disappeared in some animals while in others the growth rate was only slowed or remained unchanged. The erratic results on tumors in animals produced by thiosulfates were similar to those seen for many of the sulfur preparations, and appeared as characteristic for this group. Repeated injections of thiosulfates in tumors were seen to induce the disappearance of the tumor if growth was slow enough to permit such injections for several weeks. At the systemic level, the most marked effect, other than that on sulfhydryl index, was on surface tension which usually dropped with the administration of a sufficiently large amount.

Most of the research on thiosulfate was done with sodium salts. In a few cases, very high dosage, such as 6-10 grams daily, produced moon-face and slight leg edema, apparently related to sodium retention. This disappeared with cessation of the medication.

Changing the cation of the thiosulfate from sodium to magnesium appeared to increase, sometimes markedly, the results obtained in our experiments. Potassium thiosulfate seemed to be more effective, especially against pain. Its use however, has been limited by disadvantages. When administered parenterally, it causes considerable local pain at the site of injection as most potassium salts do. If administered to patients having

pain or other symptoms of an alkaline pattern or systemic manifestations corresponding to type D, a more marked increase in intensity of symptoms occurs than for the sodium or magnesium salts.

We also investigated sodium tetrathionate. Except for lower dosage requirements, no other advantages were found in its use. Its relative instability is a handicap.

Alpha-Thio-Fatty Acids

In other studies, we tried to introduce sulfur into the fatty acid molecule, this time changing the polar group itself. With the sulfhydryl replacing the hydroxyl of the carboxyl group, a bivalent negative sulfur was introduced, thus realizing a thionic group. (R-COSH)

We prepared several members of this thionic acid series corresponding to various saturated, polyunsaturated and even conjugated fatty acids. We studied in particular the effect of hexylthionic acid, corresponding to caproic acid. The results observed were essentially the same as those seen with the other bivalent sulfur containing lipoids mentioned previously. In addition to influencing pain and systemic changes, hexylthionic acid produced some interesting effects upon experimental tumors, reducing the growth of a few of them. However, there were no important differences from the effects of the other sulfolipoids.

Another entire series of products was prepared by introducing a thiol group at the C_2, or alpha position, of various fatty acids, with the intention of creating a more complex polar group similar to that present in alpha hydroxy or alpha amino compounds. Alpha-thio-fatty acids were thus obtained for the entire homologous series of saturated, and for many of the nonsaturated, fatty acids. Some members of this series of alpha-thio-fatty acids, such as caproic, caprylic and myristic, were studied extensively both in animals and humans. From the biological point of view, however, they showed no manifest differences over the thiolipoids previously discussed.

Thioglycolic Series

All these researches with limited biological results brought us to the study of lipoids in which the thiol represents a polar group but in which a secondary polar center is present in the molecule. Many such synthetic thiolipoids were prepared in our laboratory with the hope that they would prove biologically more effective and would have alkylating activity as well. Two series appeared to be interesting, since they were being active particularly at lower levels of organization. This led us to utilize them also on a larger scale in clinical work. While consistent results were obtained

on pain and systemic changes, the influence upon animal tumors was erratic and no different from that of other preparations with thiol groups or the sulfur compounds mentioned above. There were marked effects in some animals with tumors; in others with the same tumor treated identically, there were no effects at all. In humans the effects on pain, tissular, organic and systemic levels were similar to those of many other sulfolipoids.

Starting with these substances, derivatives were prepared. One group comprised derivatives with a special character. In order to have only one active polar group, one of the two polar groups had to be blocked. For thioglycolic acid which we studied, either the thiol or the carboxyl group could be blocked, leaving the uncombined radical as the active polar group. Since we were interested in substances having the thiol group as active polar radical, the carboxyl group was blocked by replacing its hydrogen with a methyl group. Methylthioglycolate has been thoroughly studied in our laboratory. Its pharmocological activity is similar to that of the other thiol preparations mentioned above.

Other thioglycolate esters with ethyl, propyl or butyl instead of methyl, were prepared and studied but showed no advantage over the methyl ester. We tried to obtain the allyl ester in order to have a more potent secondary center but we were unable to synthesize it.

In the same group of agents we studied another substance, beta mercaptopropanoic acid, having a thiol and a carboxyl as polar groups. Used uncombined, it could be seen that here again, as with thioglycolic acid, it is the carboxyl that acts as active polar group while the thiol acts as a secondary energetic center at the nonpolar group. This acid is very toxic in animals, producing as a peculiar effect, manifest muscular spasms. The compound also produced abnormal muscular rigidity, seen immediately after death. In nontoxic doses, it showed a marked influence upon tumor growth. Many tumors disappeared; in many others, a reduction in size occurred. Impressive results were obtained in spontaneous mammary carcinoma in mice where a fairly high proportion of tumors disappeared (28/40). Repeated injections into these animals gave good results if the growth was slow enough to permit treatment for a period of at least a month. The preparation, however, showed toxic effects in animals with tumors, producing weight loss similar to that produced by the thioglycolic series. To change the polar group and have the thiol act as such, we blocked the carboxyl with a methyl in some experiments and with an allyl group in others. But the influence upon tumors in animals was reduced through these changes.

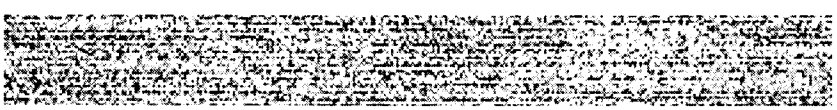
Relationship to Sulfur Metabolism

The study of lipoids with sulfur posed the problem of the relationship between their structure and biological activity. We mention this here because it not only explains the influence exerted by these agents but also because it indicates the manner in which this research has had to be developed. We have seen that, according to the systematization of the elements, sulfur represents a nonmetal anti A element, active especially at the metazoic level. It is its action as an isolated element that appears interesting, in addition to the metabolic changes which it induces in the organism when present in a negative lipoid.

It seems that the organism generally metabolizes bivalent negative sulfur by changing it ultimately to the hexavalent form. Ferguson and du Vigneaud have studied the metabolism of methionine and cysteine which are the principal sources of sulfur in the organism. (125, 126) While the evidence we have on this subject is too limited to provide more than a working hypothesis, it would indicate that other compounds with lipidic character—the thiol-containing lipids—have more important biological activity.

The metabolism of these compounds varies among individuals, especially those with abnormal conditions. The study of the excretion of sulfhydryls through the urine, expressed as the sulfhydryl index, has served us as a guide in studying the metabolism of sulfur up to a point. The high excretion of the thiol group seems to be related not only to low oxidation but to an abnormal form, as mentioned above, since with the exaggerated excretion, the thiol level in the blood is reduced. This form is to be considered as an excremental one. This seems to be confirmed by the fact that the administration of bivalent sulfur, even in a large amount, is not followed consistently by its elimination in the form of thiols in the urine. In some subjects, an impressively high proportion of the thiols administered appears in the urine, and this is true even for relatively small doses of thiols or of bivalent sulfur as in thiosulfates. In other subjects, on the contrary, even when larger amounts are administered, the increase in elimination is minimal or does not occur at all. The abnormality in sulfur metabolism, which appears to be a limited capacity to oxidize it to the hexavalent positive form, also means an exaggerated intervention of the thiol group as such in the economy of the organism. This occurs along with symptoms and signs, previously noted, corresponding to an exaggerated oxidative intervention of fatty acids, in which processes the thiols probably take part.

We tried to study the capacity of the organism to fully oxidize thiolic



sulphur by following the response to the administration of a known amount of sulfur in bivalent negative form. The change of the sulfhydryl index of urine would serve as a tolerance test for thiol metabolism.

After injections of 80 mg. of sodium thiosulfate, the differences in the capacity of various organisms to metabolize it could be seen and related to pathological conditions. This concept of thiol metabolism can be the basis for understanding an abnormal form of thiolic sulfur which may be involved in the pathogenesis of abnormal conditions. Substances containing a thiol group, such as methionine, cysteine and particularly glutathione, are present in sizable amounts in the organism, but it is not this form of thiol that intervenes in the abnormal metabolism. A large amount of the normal form of thiol is present in the blood of subjects with a low urinary sulfhydryl excretion. When another form, the abnormal one, intervenes, it is excreted in the urine. The organism eliminates this "abnormal" compound with sulfhydryls. It seems quite probable that this abnormal thiol compound is in a lipidic form since the sulfhydryl-containing compound is readily extracted by ether from the urine. Its affinity for the lipidic system would explain the influence exerted upon fatty acids and the oxidative processes occurring in the lipidic system. The thiolipoids intervene catalytically in the oxidation of the fatty acids, as seen in experiments in vitro.

Thus, the thiol group in lipoids containing bivalent sulfur rather than metabolized sulfate would increase catabolic metabolism. Although the thiol in this abnormal form is largely eliminated by the urine, apparently as a defense mechanism against its pathological activity, some of it is probably retained in the cellular lipids where it continues its activity. Circulation of sulfur in thiolic lipidic form, with consequent impairment of its change from the bivalent negative sulfur into the hexavalent positive sulfur, would thus appear as the fundamental source of the participation of sulfur in the abnormal pattern. The influence exerted by administration of thiosulfates upon the sulfhydryl index can serve as an indication for these specific changes.

Sulfur is an anti-A element and it is active as such in all the forms in which it exists in the organism, although the intensity of its action varies at different hierarchic levels. The activity of thiol as an anti-A factor can be related to the influence exerted by carcinogens or other agents upon the biological activity of this radical, especially when it is taking part in the formation of enzymes. This relationship explains the results obtained by repeated injections of organ or tumor cells treated in vitro with agents having a thiol as polar group. The heterogenization induced leads to the appearance of severe changes resulting from the allergic reaction.

It is interesting to note that sulfur has an anti-A tendency even in the hexavalent positive form in which it appears as sulfate. The sulfate ion has a capacity in the organism to inactivate lipoids of a positive character. The sulfate becomes bound to such substances, thereby tending also to facilitate their excretion in urine. Many of these substances are eliminated in combination with the sulfuric radical in the forms called "sulfo-conjugated."

In view of this, the effect of sulfate ions appears to parallel that of the fatty acids. Both oppose substances having a positive polar character; that is, both are biologically antagonistic to anti-fatty acids. In the sense that they oppose antifatty acids, both the bivalent negative and hexavalent positive sulfur have anti-A activity, the first directly and the last indirectly.

The characteristic influence exerted by sulfur in its different forms is based on its action as an agent inducing changes toward increased homotropy, by acting at levels above the cellular. This is a typical example of the relationship between an element's activity and its place in the periodic chart. Sulfur is a member of the series with homotropic action; it belongs to the period which corresponds to the metazoic compartment and thus acts above the cellular level.

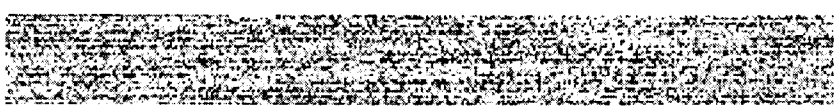
Selenium Lipoids

The influence exerted by bivalent sulfur upon oxidation processes in which fatty acids participate, served as a guide for further research. Seeking agents that would act at a still lower level of the organization, even below the cells, we considered other substances which also affect oxidation processes. Theoretically, at least, it appeared possible to induce changes at a compartment below the metazoic, at which sulfhydryl-containing compounds act.

We have discussed previously the systematization of the biological activity of elements, their fundamental anti-A and anti-D influence, their distribution among the various levels of the organism, all related to their atomic structure and their place in the periodic chart.

All of this led us to investigate selenium which is the nonmetal element next to sulfur in the sixth series of Mendeleeff's periodic table—the series with an anti-A character in which oxygen is the first member. According to its period, selenium belongs to the cellular compartment.

The first problem was the nature of the compound in which it would be active. We were particularly interested in using bivalent negative selenium because of the activity of bivalent negative sulfur. However, we did investigate the selenic and selenious acids. These acids or their sodium salts



have limited effect upon viruses and microbes. An interesting effect is seen in Tetrahymena pyriformis, where a manifest cellular vacuolization is induced. The influence upon tumors, pain, organic and systemic levels is less manifest and toxic effects are great. Therefore, we prepared lipoid compounds with a predominant nonpolar group and with a negative bivalent selenium. We utilized, on a larger scale, hexyl and heptyl diselenides synthesized in our laboratories by M. Bier.

Hexyl and Heptyl Diselenides

Studies have shown that hexyl and heptyl diselenides are lower in acute and chronic toxicity than selenic and selenious acids and their sodium salts. In wounds and tumors, these selenium preparations induce a relatively limited fixation of chlorides, the increase above controls being only about 16%. In only a very few cases could any direct effect upon pain be observed within a few hours. However, the long range effects after several days of administration, seemed to be superior to those of various fatty acid preparations. This was true both for the decrease in intensity of pain with an acid pattern and the increase of pain with an alkaline pattern. The effects persisted for many days. In animal tumor experiments, there were relatively slight changes in growth or survival time.

No manifest influence was seen at the organic level. With relatively large amounts of these agents, an involution of the lymphatic system was obtained. Thymus, lymph glands and spleen were markedly reduced in size in animals dying after acute toxicity tests, and adrenals were small and appeared to be depleted of their sudanophilic content. In rats, a frank lymphopenia followed administration of larges doses of these preparations, and eosinophilopenia also was uniformly seen. Changes in urine analyses also were obtained with high doses.

It is noteworthy that administration of diselenide to a subject with a type A pattern induces the appearance of oxidizing substances in the urine, as one of the first changes.

Effects at the cellular level are seen even with microgram dosages. Vacuolization occurs in the cellular cytoplasm. It is interesting to note that, despite the cellular vacuolization, pericellular edema occurs. The fact that these selenium compounds are active in small doses may be an indication that they act entirely at the cellular and not the metazoic level. This effect at the cellular level is confirmed by the fact that almost constantly the administration of selenium if in sufficient amount is followed by a manifest increase in serum potassium values, and a decrease in the amount in red

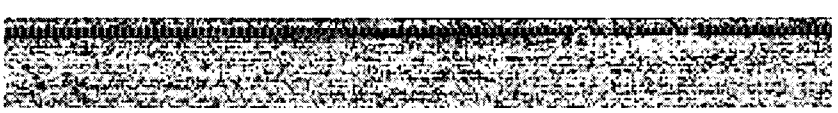
cells. This change in serum potassium is apparent before any other change, and is generally obtained with relatively very low doses of selenium.

The effects upon cells of another lipoidic selenium preparation, with selenium this time as the polar group, warrant mention. The preparation, synthesized in our laboratory, is hexylselenoic acid in which the hydroxyl of the carboxyl group has been replaced by a SeH radical. A manifest effect is produced by this agent in animals with ascites tumors. Intraperitoneal injection leads almost constantly to the disappearance of such tumors, even if the compound is used after ascites is already present. We used this product to bind selenium in vitro to cancerous cells as will be seen below.

Tetrahydronaphthalene Perselenide

The fact that in the first phases of the defense mechanism the organism uses fatty acids acting largely through their products of oxidation, has directed us—as we have seen above—to search in the therapeutic approach for agents having as pharmacodynamic activity an intervention of peroxides. In a further step, parallel to lipidic peroxides, we investigated similar products in which, instead of peroxides, persulfides were present, sulfur being the element immediately above oxygen in the VIth series of elements. We studied thus the persulfides, among which the tetrahydronaphthalene persulfide has been an interesting compound. Its activity was explained, according to the biological systematization of the elements in which oxygen corresponds to the organism level, while sulfur represents a metazoic element. Following the same line, we searched similar compounds for selenium —an element still higher in the VIth series, which corresponds to the cellular level. We thus prepared and studied perselenides by bounding selenium to tetraline in the same way as was done for oxygen and sulfur.

The effects of the perselenides on microbes or animals were similar to those of the other selenium preparation discussed above. Tetraline perselenide showed low toxicity in animals, ¼ cc. of the 10% solution of the product obtained having 25 mg. selenium %, was not toxic in intraperitoneal injections in mice. Administered orally in humans, in doses from $\frac{1}{50}$-2 cc. of the solution containing 25 mg. of selenium per 1 cc., repeated even several times a day, did not show toxic effects. The influence exerted on pain and systemic changes took some time to appear as with the other selenium preparations. The influence exerted upon the growth of experimental tumors in animals was more manifest than for the other selenium preparation. Similar results were obtained with the perselenides of naphthalene and other aromatic hydrocarbons.



An investigation of the influence exerted by the immediately heavier member of this VIth series, tellurium, is in progress.

The foregoing data on lipoids with negative character indicate that their activity generally is related to changes in processes in which ultimately an intervention of oxygen takes place. This brings us to the first member of the sixth series, oxygen, a nonmetal with D inducing biological activity.

We studied the effects of ionic oxygen, using compounds which liberate oxygen readily. These included hydrogen peroxide as well as peracids and their salts, such as perchloric, perboric, persulfuric and periodic.

The changes induced by these substances upon microbes, viruses and cells are similar to those obtained with polyunsaturated fatty acids and all are catalogued as radiomimetic. This fact tends to confirm the importance of oxidation changes in the pharmacodynamy of fatty acids. The effects upon pain and at organic and systemic levels also were similar to those of polyunsaturated fatty acids. It is interesting to note in the same frame of activity the appearance of oxidizing substances in the urine following the oral administration of these agents in higher doses.

We investigated the effects of turpentine oil which is known to induce the appearance of peroxide in vitro. Highly oxidized through treatment with oxygen or especially bound to sulfur, turpentine oil has shown interesting pharmacological activity. An old therapeutic device was parenteral administration of turpentine oil to stimulate the defense mechanism in cases of septicemia. However, we saw no such stimulating effect in the fight against cancerous cells. The influence exerted upon the cellular level of the organization was quite reduced. The action of atomic oxygen appears to be different from that of the molecular, as we will see later.

ALKYLATING AGENTS

We investigated, as compounds with negative character, certain alkylating agents, choosing from the large number available those which also showed lipidic properties. We were especially interested in two members, sulfur mustard and epichlorohydrine. Sulfur mustard contains, along with one active polar chloroethyl group, a second represented by the bivalent sulfur polar group. It has the effect at different levels of organization of producing an offbalance with predominance of the acid lipids. We will discuss briefly here some of the experiments in which this influence upon the body lipids has been observed.

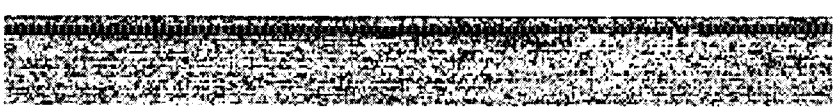
Sulfur Mustard

In studying sulfur mustard, we were first interested in its effects upon body lipids and, through them, upon the lipidic system of organisms. In inducing sulfur mustard's characteristic skin lesions, an interesting relationship was observed. Pure sulfur mustard was applied to mechanically epilated skin of rats. If a sufficient amount—2 to 3 drops—was used and spread on one square cm., the animal died. However, the time of death varied. If the lesion showed a massive necrosis, followed by deep ulceration, similar to a burn of the third degree, the animal died in about three weeks. However, if the lesion was only erythematous, similar to a burn of the first degree, the animal died in only 3 to 4 days. It seemed as if the lesion itself intervened secondarily in the pathogenesis of changes leading to death. Sick but still living cells appear to have an activity which is highly detrimental. The abnormal cells apparently produce substances which are responsible for rapid death of the animal. In widely necrotic tissues, these abnormal but still living cells are limited in number; in an erythematous lesion, they form the lesion itself. This correlation of toxicity with local lesion was confirmed by the fact that excision of the lesion itself, if performed in time, prevented death in some animals. The administration of ferrous sulfate to rats having sulfur mustard applied to their skin was seen to induce the erythematous form of the lesions, with death in 3-4 days.

The similarity between the influence exerted by mustard burns and caloric burns with a sufficiently extensive first degree burn producing more rapid death than a third degree burn, was of interest.

Analysis of the body of an animal killed by a mustard burn reveals abnormal amounts of unsaturated fatty acids and reduced amounts of sterols. In some cases, where death occurred after more than three weeks, body sterols were found to be almost completely lacking. In these cases, almost no insaponifiable fraction could be found. The lesion itself, especially an erythematous and edematous one, was very rich in unsaturated fatty acids. Histological study of these skin lesions revealed changes similar to those obtained through the intradermic injection of concentrated solutions of body acid lipidic fractions. The study of these lesions further revealed that the lesions themselves were separated from the organism by a barrier of adipous cells, the result of an exaggeration in number of the cells of the subdermic fatty layer.

We studied these important changes from several points of view. We could show that an exaggeration of the adipous layer underneath the skin occurs when lipoids with negative character, such as polyunsaturated fatty



acids, thiolipoids, etc. act upon the skin. Thus, this subcutaneous adipous formation appears to be a defense weapon, designed to keep such lipoids from passing into the organism. The defense appears to be unequal for males and females, as shown in the following study.

In collaboration with the late Prof. R. Leroux of the Faculty of Medicine in Paris, we studied histological changes in the ears of rats after local application to the skin of a small amount of pure sulfur mustard. Normally there are no adipous cells in the pavillion itself except at its base. Twenty minutes after the application of sulfur mustard on the skin of the ear, 2 or 3 layers of adipous cells were seen in the connective tissue between skin and cartilage. (Chapter 6, Note 22)

Curiously enough, the rapid appearance of adipous cells 20 minutes after application occurred in females and not in males. The spaying of females or castration of males did not change this response even after a lapse of months. The administration of male sex hormones to female rats—spayed or not—or of female sex hormones to males—castrated or not—also produced no change. It was only by the administration to males, over a period of days, of a sufficiently large amount of the insaponifiable fraction obtained from the bodies of rats that this rapid response was induced. The administration to females of the acid lipid fraction obtained from rat bodies was seen to prevent the rapid adipous response. This difference in response between males and females, can be related to the differences in the amounts of members of the two groups of lipids ordinarily found in males and females, as mentioned above.

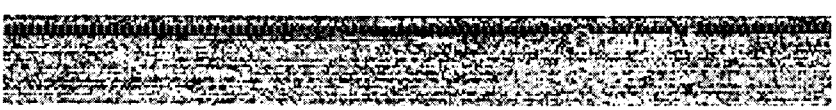
We studied sulfur mustard from the point of view of pharmacological activity. Doses of 100 mcgr./100 gr. of body weight (of a 0.1% solution of sulfur mustard in oil) were nontoxic in rats and mice. Except for an intensive local reaction at the injection site, no important immediate changes were obtained in humans when 1 to 3 cc. of the 0.2% solution was injected intramuscularly. The influence upon pain—a decrease in the intensity of acid pattern and an increase for the alkaline pattern—was only temporary. The influence on tumor evolution was not sufficient to warrant clinical use of this agent, especially in view of the persistent and intensive systemic changes toward an offbalance of the type D which appeared after a few days. Through its anti-A action, which is the most intensive of all agents tested, sulfur mustard remains one of the most interesting substances for experimental studies, especially for the effects exerted upon the anti-fatty acids.

Epichlorohydrine

The importance of the relationship between the energetic centers present in alkylating agents and their ability to produce type D offbalance, led us to study a group of these substances which, at once, have both short molecules and two polar groups in close proximity. The desire to have such an agent with lipoidic property as well led us to study epichlorohydrine which corresponds to propane and has an epoxy group binding C_2 and C_3, while C_1 binds a chlorine. Soluble in neutral solvents, epichlorohydrine becomes soluble in water only after hydrolysis. Its biological activity differs from other chlorohydrines such as chloropropanediol or trichloropropane, both of which can be considered to be closely related to the substance produced by hydrolysis of epichlorohydrine. The acute toxic dose of epichlorohydrine was found to be 6 mgr./30 gr. for mice and 25 mgr./100 gr. for rats by intraperitoneal administration; 22 and 35 mg./100 for mice and rats by subcutaneous injection. In tests for chronic toxicity, it was apparent that doses of 5 and 1.5 mgr. injected daily were well tolerated respectively by rats and mice. With higher doses, the animals became rapidly emaciated before dying. Used orally in drinking water, a solution of 1/3000 was well tolerated by rats and mice even for months. With the use of solutions of 1/2000, only a few animals did not lose weight, while a solution of 1/1000 invariably induced weight loss.

There were no effects observed upon microbes or bacteriophage.

It appears that epichlorohydrine, acting below the morphological levels, induces changes similar to those seen for other alkylating agents. However, it is not upon the desoxyribo-nucleic acids present that an important action is seen but in the lipidic system at these lower levels. Epichlorohydrine seems to act also at other levels. The influence upon pain—an increase for alkaline pattern, a decrease for acid—was more noticeable than for sulfur compounds. Delayed effects, however, were more obvious than immediate ones. The influence upon wound healing was similar to that of polyunsaturated fatty acids. Cancerous cells, such as those from mouse ascites, were destroyed in vitro by a 0.5 solution of epichlorohydrine. The effect in vivo upon sarcoma 180 or Ehrlich ascites tumors was most interesting. Administered by subcutaneous or intramuscular injection, epichlorohydrine had no effect on the tumor even in doses as high as 2.5 mgr. daily. However, when administered in drinking water in a 1/1000 solution, it prevented the development of ascites in 19/20 animals. But the toxicity was too high. A 1/2000 solution, used as drinking water, controlled the condition in more than 50% of the animals, while a 1/3000 solution showed favorable re-



sults in only a few animals. Under the same conditions, there was no apparent effect upon solid tumors in mice, even those induced by subcutaneous injection of ascites tumor cells.

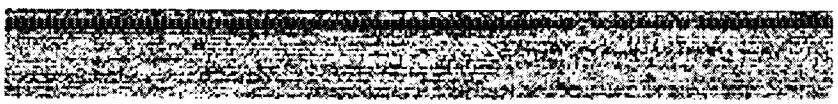
In humans, all effects upon the tumors were interesting and will be discussed below. The influence upon systemic patterns was relatively reduced except for a marked effect upon the elimination of calcium in urine, obtained even with small doses which produced no other changes. Repeated injections of organ cells treated in vitro with epichlorohydrine were able to induce severe degenerative changes in the respective organs. Experiments with tumor cells treated and administered in the same manner are still in progress.

THE ELEMENTS

We have discussed previously the method used to classify the elements in accordance with their predominant biological intervention. The place of the elements in the periodic chart, which establishes the relationship between their structure served as a further basis for this systematization. The capacity to induce changes towards an offbalance of the same type was found to be a common property for elements in the same series in the periodic table. The series could be separated into two groups, one Ht (from heterotropic) inducing an A and the other Hm (from homotropic) inducing a D offbalance. Elements systematized as different periods in the chart have been found to have predominant activity in various compartments or groups of levels which form the hierarchic organization of complex organisms. We have related each element to a compartment (or sometimes even to a level), which didactically is called the compartment (or the level) of the element. We have tried to utilize this systematization in the study of the pharmacological activity of elements.

From the beginning, several basic facts about biological activity became apparent. Often the elements, used as such, do not induce the changes which characterize their physiological activity. Basically elements act in normal physiology through specific compounds suited to the compartment or level to which they belong. In general, knowledge of the level of an element permits us to identify also the proper compound and its activity. The intervention of an element at levels of the organization other than its own can be understood only in terms of the relationship between the element with its characteristic compound the proper level and the compounds present at other levels.

Two factors are fundamental in determining the activity of an element: a) its availability and b) the possibility to enter into its proper combination.



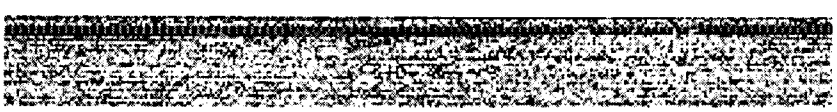
The nature of any abnormality in activity of an element can be recognized only by relating it to these two factors. The amount of the element available and the capacity of entities of the level to which the element belongs to manufacture the proper compound, ultimately governs the amount of the element at its own and at the other levels.

Under normal conditions, the entities of the proper level utilize only the amount of the element needed to maintain the normal constants. The rest of the element usually is eliminated. An excess of the element thus does not induce a permanent excess at the proper or higher levels under normal conditions. Such an excess normally is only temporary. A persistent excess of the element at the proper level and at the higher level indicates an abnormal general availability. A persistent excess at the higher level only corresponds to a qualitative deficiency at the proper level. The organism maintains an excess of the element at the higher level in an effort to compensate for the qualitative deficiency at the proper level. Thus, an excess of an element at the higher level indicates a qualitative deficiency at the proper level, only if the value of this level is found low.

When there are low values at higher levels, we also need to know the amount present at the proper level in order to interpret the abnormality. Low values of the element at higher levels with a low amount of the proper level indicates a quantitative general deficiency, while a high value at the proper level permits us to recognize a qualitative excessive utilization of the element at the proper level. For example, in cancer, copper is low in tumor cells and liver, but abnormally high in blood. When a high amount of copper appears in blood, the diagnosis of a qualitative deficiency in utilization in cells or an abnormal general amount can be made by investigating the amount in cells. A low amount of copper as found in tumors and liver cells in subjects with cancer make the qualitative nature of this deficiency evident. The trouble lies not in too little copper available in the body but in the lack of the capacity of the cancerous cells to manufacture the compound through which copper becomes active, in this case, catalase.

A normal amount of an element at its proper level reflects normal utilization. The pathological amount can result from a quantitative or qualitative abnormality. Any anomaly in an element not only means an inadequate amount of it at its own proper level but in other levels as well. A quantitative deficiency results in an insufficient amount at its proper level. A qualitative deficiency—that is, incapacity of the entities to manufacture the proper compound—also leads to a reduced amount of the element at its own level.

Thus, in both cases, quantitative and qualitative deficiency, the amount



of the element present at its own level is low. However, in quantitative deficiency there is also a low amount of the element in the hierarchically superior level, while an increased amount at this superior level occurs when there is a qualitative deficiency at the proper level.

The same opposite variations between the amounts at the two levels is seen in the case of an excessive utilization of the element at its proper level. The amount of the element at the superior level is also high, if a quantitative excess is present but this amount at the superior level is reduced as a means of controlling the excessive utilization at the proper level if a qualitative anomaly occurs. This makes it possible to recognize the quantitative or qualitative nature of the abnormality in utilization of an element at its proper level by determining its amount both at this level and at the next superior level. Too much of the element present at the proper level indicates either excessive amount present or excessive utilization, while too little at the proper level can be due either to a qualitative or to a quantitative deficiency. A low amount at the higher level indicates either a quantitative general deficiency or a qualitative excessive utilization at the proper level. An excess amount at the higher level indicates either a quantitative general excess or a qualitative deficiency at the proper level.

This relationship, which is also critical for the understanding of the pharmacology of the elements, can be summarized in the following table:

Amount of Element at the Proper Level	Amount of Element at the Higher Level	Interpretation: Occurrence at the Proper Level
high	high	quantitative excess
low	high	qualitative insufficiency
high	low	qualitative excessive utilization
low	low	quantitative deficiency

From a practical point of view, we must have information on the amount of the element both at the proper level and at a higher level. We found that for the elements proper to the cellular level, such information can be obtained by comparing the amounts in plasma and red cells (or total blood). It is not the ratio between these values—as often supposed—which is important, but rather the values themselves. For changes at the systemic level, the comparison can be made between blood and urine, the latter corresponding to the level above the systemic.

The importance of this concept can be seen in the following examples. Potassium is a cellular level element. In cancer, in offbalance type A, potassium is present in abnormally high quantity in proliferating cells. It

is also found in high amounts in blood red cells. In these cases, potassium is found in low values in the hierarchically higher level in the blood plasma or serum. The abnormality does not reside in a simple hypokaliemia, but in excessive utilization of potassium at the cellular level, a low amount of potassium in red cells also would indicate a potassium deficiency. A high amount of potassium in serum and red cells can be interpreted, as mentioned above, as corresponding to a quantitative excess. The reduction in the quantity of potassium in red cells, together with an excess in the serum, indicates a qualitative deficiency at the proper level.

We have the true picture of the situation if we consider that "qualitative" excess or deficiency is determined by the ability to form the proper compounds. While it is the element as such which has to be administered in order to correct a quantitative deficiency, other factors must be changed to overcome qualitatively deficient utilization, excessive utilization, and quantitative excess. *(Fig. 127)*

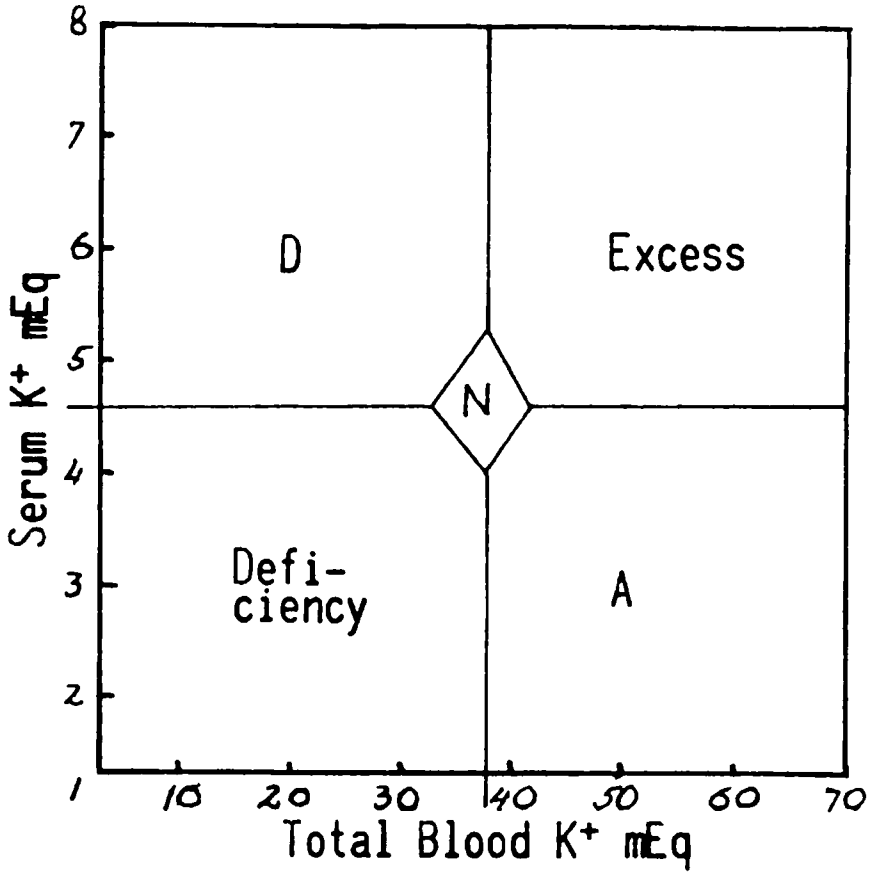


FIG. 127. The relationship between the amount of potassium in serum and in total blood permits to indicate the existing condition as being in normal limits, in quantitative deficiency or excess, or in an offbalance type A or D.

Although these relationships represent the most important aspect of the pharmacodynamy of the elements, still others must be considered. An excess or deficiency of an element at a superior level, even if it serves as a biological defense means to combat an abnormality at a lower level, represents a problem by itself for the superior level. The fact that the element does not belong to this level gives a noxious character to its influence. The influence exerted upon sensitive organs often induces important abnormal manifestations. Hyperkalemia, even originated by an abnormally low utilization of potassium at the cellular level, can lead to serious troubles in the function of the nervous system or of the heart.

Another important aspect of the reactivity of an element is its influence upon levels below its proper level. An element acting at a lower level usually has a biologically opposite effect to what it has at the proper level and therefore is a noxious influence. At the lower level, the A or D type of activity of the element is reversed.

Sodium, for instance, which is an agent of the A type of the metazoic compartment, produces an offbalance of type D at the cellular level, which is hierarchically inferior to its own. Similarly, Mg, which is a D agent at its own metazoic level, has an A inducing activity at the cellular level.

Analysis of the pharmacology of elements in terms of their A or D inducing activity, the level at which they belong and the compounds through which they act, is still only in its early stages although it represents a program of great promise. In the presentation which follows, we will try to interpret the data concerning the elements in terms of A and D inducing activity. We start with Hm elements having a D inducing activity. They parallel in their action the lipoids, with a negative polar group. TABLE XVII lists Hm elements or the D series and relates them through their periods to the organizational compartments.

TABLE XVII

HM ELEMENTS

Compartments	*Metals*								*Non-Metals*	
Organic	Be								C	O
Metazoic	Mg								Si	S
Cellular	Ca	Sc	V	Mn	Co	Cu	Ge			Se
Nuclear	Sr	Y	Nb	Tc	Rh	Ag	Sn			Te
Submorphol.	Ba		Ta	Re	Ir	Au	Pb			
Primary Biol.		Ce	Nd	Sm	Gd	Dy	Er	Yb		
Submolecular	Ra	Th	U	Pu	Cm	Cf				

We have already discussed the pharmacological activity of sulphur and selenium through the compounds in which they enter and will not discuss

them again here. Before analyzing the other elements, it is of interest to emphasize again a principal character of their activity. As most of the elements act through specific compounds, the factors which determine the entry of elements into specific biological combinations appear to be of capital importance. Availability of the element alone is only one factor in its pharmacodynamy. With this in mind, we have investigated some of the elements of this Hm group.

We know little about any influence of berryllium as a metal upon the organism as an entity. Its toxic effects are due to abnormal amounts active at lower levels. In the same period of the chart of elements, we have two nonmetals, C and O, for which the organism represents the proper level. The general pharmacological nature of oxygen is indicated by the role of oxidation in metabolic changes. Oxidation represents the first step toward catabolic, homotropic changes. The respiratory phase in the metabolism of carbohydrates, the oxidative fission of fatty acids, and the oxidative desamination of amino acids represent examples of the fundamental homotropic intervention of oxygen.

Acting at the systemic level, immediately inferior to its own level, oxygen has a different action. According to the rule mentioned above, oxygen, a D agent at its proper level, will have an A activity for the blood, which we will study below together with the other A agents. The homotropic relationship of oxygen to fatty acids was discussed above with the study of the biological role of these substances.

The relationship between CO_2 and fatty acids also is interesting. Large amounts of free fatty acids in the blood were seen to allow better fixation of CO_2 to hemoglobin, just as large amounts of sterols do for oxygen. We have investigated this correlation between CO_2 and fatty acids by keeping a fatty acid, such as linoleic acid, in an atmosphere of CO_2 connected to a manometer. A manifest negative pressure results. The venous blood, rich in CO_2, which also shows a predominance of fatty acids, loses CO_2 and fatty acids during passage through the lungs.

Magnesium

Among offbalance D inducing elements of the II A series of the periodic chart, we first studied magnesium, which belongs to the metazoic compartment and thus, is related to the sea as original environment. Much of the activity of this element can be interpreted as D inducing activity. Mg in many respects is antagonistic to Na, the cation of the same compartment, a member of a series with an opposite A inducing character. High values of

Mg found in blood were related to adrenal insufficiency, (127) while low Mg levels usually occur when blood cholesterol is high. (128)

Excess of Mg was seen to induce adrenal deficiency. We explained this action through the antagonism between Mg and Na. We could thus counteract the salutary effect of NaCl in adrenalectomized rats through administration of magnesium sulfate parenterally. A similar effect was obtained even with oral use of magnesium thiosulfate. *(Note 9)*

The relationship of magnesium to the defense mechanism is of special interest. Mg seems to intervene in the lytic effect of sera upon ascites cancer cells (extensively studied in our laboratory by R. Willheim, P. Fluss and M. Auber) (129) which, as we have seen, represents a characteristic feature of D inducing activity. Similarly, magnesium prevents thrombosis, acting as an antithrombocytic agent. Not only does it prevent the appearance of fibrin, partly preventing the destruction of thrombocytes (130); but it also favors the lysis of already existing thrombi. (131) It appears, therefore, to be a valuable agent in the treatment of thrombosis. (132) Its concomitant action against cholesterol has led to its use in the prevention and treatment of coronary thrombosis.

Magnesium appears to be part of another defense mechanism, the nonspecific one, represented by the properdin system. Properdin is active only in the presence of magnesium; neither Ca nor Na can replace it. (133) Higher amounts of magnesium increase properdin activity.

Magnesium sometimes is seen to parallel the action of Cu, another D inducing element. In animals fed milk too long, leading to a type A offbalance, the amount of magnesium falls along with the amount of Cu. The quantity of magnesium in the blood is low in humans with convulsion. (134) Mg appears to be especially effective in the prevention and treatment of "grass tetany" in animals, which often follows feeding on grass with high potassium content. (135)

Magnesium has a similar antagonism toward K and this can explain its activity in cancer. Lower than normal values of magnesium are found in cancer, as opposed to high amounts of potassium which is an A inducing element, and the low Mg seems to favor cancer growth. Moisture increases the amount of K and lowers the amount of Mg in plants, a fact related to cancer frequency in various geographic regions. (136) The difference between the preventive and curative actions of magnesium is of special interest. Administered after a carcinogen has been applied, magnesium reduces the percentage of cancers induced. (137, 138) It has minimal influence, however, once the tumor has appeared or upon trans-

planted or spontaneous tumors. We will discuss this occurrence below together with the effect of other elements.

It is necessary to bear in mind, when we have to choose nonspecific combinations in which to administer it, that the D inducing activity of magnesium is particularly manifested at the metazoic level. Magnesium sulfate appears to be suitable for parenteral use, while the thiosulfate appears to be suitable for oral administration. In these forms, Mg has been found to induce marked local alkalosis in the second day wound crust pH and to produce salutary effects upon pain of the acid pattern. A marked influence upon thiamine-induced convulsions in rats and mice has led us to use magnesium thiosulfate as a tool not only in tetany but in the treatment of convulsions. It appears to be effective in preventing epileptic seizures and valuable even in cases of status epilepticus. We have utilized the same preparation successfully in cancer when pain and preterminal conditions corresponding to an A offbalance were present. Less important effects are seen for magnesium sulfate and magnesium thiosulfate at lower levels.

Calcium

The biological activity of calcium, another member of the II A series and a D inducing element belonging to the cellular level, also is of interest.

In its absorption by grass from the soil, Ca parallels Mg, another D inducing element, but opposes K, an A inducing element. Grass tetany is thus induced by high K and low Ca and Mg values. Ca, which is another D inducing element like copper is antagonistic to zinc, an A inducing element. It has been observed that cancer is less frequent in the so-called calcerous clay regions where the soil is formed by limestone. (139) Together with other minerals, an optimum of calcium in soil may help to prevent cancer. While SiO_2 favors cancer, Ca appears to prevent it. Calcium also is an antagonist to zinc which, in high doses, seems to favor the development of cancer. (140) The relationship between Ca and K has permitted us to be more precise about the role of Ca in cancer pathogenesis. As opposed to K, which increases by as much as 60% in tumors, the content of calcium decreases by 44%. (141-147)

Confronted with K and Ca changes, it appeared interesting to see to which element we could directly attribute the increase in malignancy. In the regeneration of liver cells, where rapid growth without malignancy takes place, potassium is increased while the amount of calcium is unaltered. Similarly in other rapidly growing but normal cells, calcium is not diminished while K is increased. Potassium thus appears to be related to the process of cellular growth and multiplication which represents also an

added factor in transforming noninvasive into invasive cancer. However, potassium is not directly related to the cancerous character of the cells. On the other hand, reduced amounts of calcium appear to be peculiar to the cancerous process. (147) The reduced calcium in cancer is not due to a lack of the element in the organism since calcium is not only available but even apparently present in excess at the systemic level. As we have shown, a high urinary calcium index, indicating exaggerated excretion, is present in the type A offbalance. The anomaly resides in the low capacity of the cancerous cells to fix and properly utilize calcium. As calcium acts at the surface of the cell and its deficiency reduces cellular adhesiveness, (148, 149) lack of cellular calcium can be seen to increase the invasiveness of cancer cells and the tendency to metastases. Deficiency of calcium in cells appears related to the character of youth while excess seems to result in rapid aging.

The anomaly induced by the qualitative deficiency in calcium thus appears to be at the cellular surface. Related to it also are manifestations at the tissular level.

Administration of any calcium salt induces a manifest increase in local alkalosis *(Fig. 128)* of the second day wound crust pH. It appeared interesting that in bone lesions, especially in bone cancer metastases, the offbalance type A is characterized by an osteolytic process, the D type by an osteoplastic one. The local acidosis present in lesions with an A type of offbalance explains the mobilization of calcium in these osteolytic processes. Ca is deposited in important amounts in metastases with a type D offbalance, a fact which can be related to the local alkalosis resulting from the abnormal metabolism. This alkalosis represents a condition favoring the precipitation of calcium. Indirectly, the deposit of calcium in bone metastases appears to correspond to the D pattern of tissular abnormality. Calcium has a D inducing activity even in this case.

With calcium excreted in excess through the urine, the problem of calcium pharmacology in the A type of cancer is related to the form in which it acts at the cellular level, which appears qualitatively impaired. As the quantitative decrease must be considered to be a consequence of qualitative insufficiency and not a general quantitative deficiency, the problem is not to provide calcium but to find a way to insure better utilization at the cellular level. It is for this reason that administration of most calcium salts does not influence the evolution of experimental or clinical cancer, but has a preventive effect upon the induction of tumors through carcinogens. Administered after the injection of the carcinogen, calcium

appears to reduce the percentage of positive results. Administered after the tumors have appeared, the influence is minimal or nil.

As we have mentioned above, an excessive calcium excretion is, in itself, sufficient to indicate the existence of a deficiency in calcium utilization at the cellular level without a calcium deficiency in the organism. The therapeutic indication is for agents able to influence the fixation of calcium at the cellular level. Fatty acids which change the cellular metabolism so

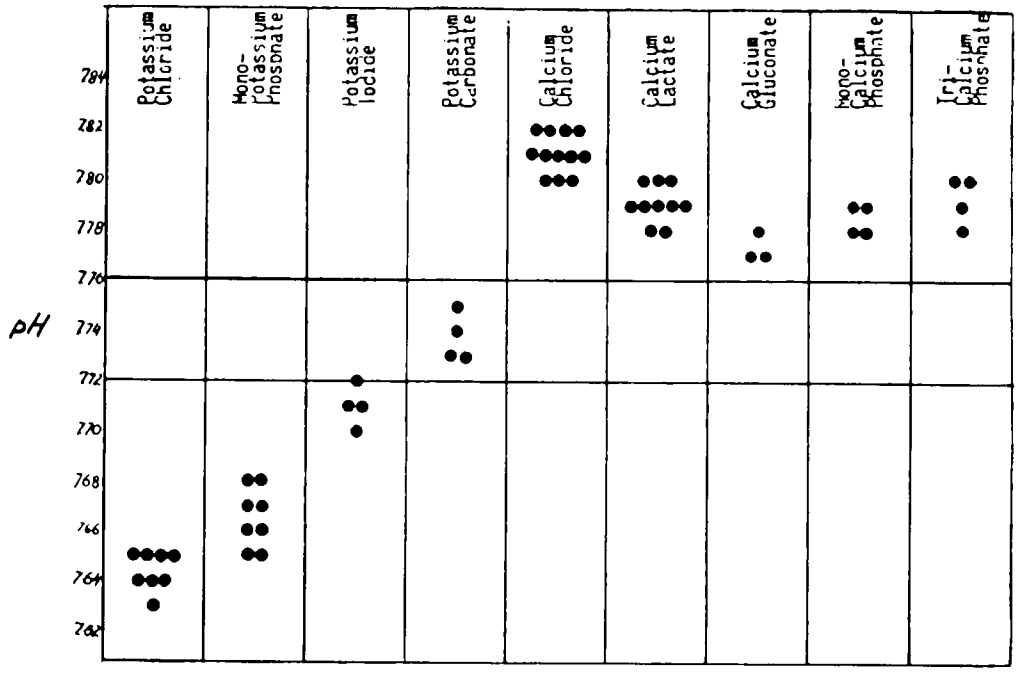


FIG. 128. The influence exerted by two elements, potassium and calcium, upon the second day wound crust pH shows a frank tendency toward acidification for potassium and alkalinization for calcium. For different salts, the differences result from the unequal influence exerted by the anion which works additively to that of the cation.

as to induce local alkalosis, have appeared to be the most active agents. Testosterone, and calciferol have appeared helpful but not nearly as active as fatty acids.

While high urinary excretion of Ca indicates a type A offbalance, low or no urinary excretion can result either from an excessive cellular utilization of calcium or from a type D offbalance at the cellular level. Other analyses can be used to indicate the probable occurrence. With calcium excretion low and other analyses indicating type D offbalance, all the chances are that the low excretion is part of the D offbalance. If, on the contrary, only the Ca excretion is low and the other values correspond to

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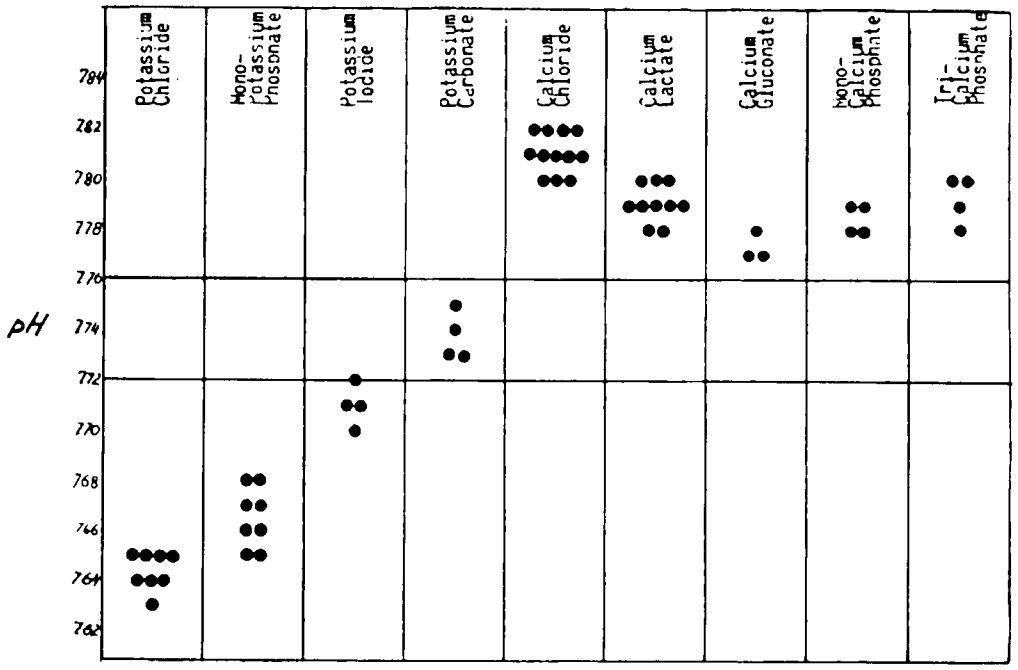

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A type of offbalance, the probability is a quantitative lack of calcium in the organism. This can be corrected by the administration of calcium parenterally or orally in any absorbable form. Low excretion as well as some symptoms can be overcome in a short time through the administration of sufficient calcium. In the opposite type of case, with metabolic calcium retention, administration of calcium will induce an increase in the intensity of symptoms.

Excess of calcium in the urine thus corresponds to lower values at the cellular level and need for fixation of calcium. This indicates again that the problem is not the amount of calcium present but the deficiency in its utilization.

Copper

Copper, from the IB series and another anti-A element of the cellular level, appears indispensable for the synthesis of heme of catalase. A deficiency of copper results in reduced activity of this enzyme. Similarly, the synthesis of hemoglobin is possible only in the presence of copper. Cytochrome oxidase contains Fe and Cu. Cu deficiency reduces liver cytochrome oxidase. (150, 151, 152) Cu is present in blood serum bound to a protein to form ceruloplasmin which also acts as an oxidase. (153) Cu, while favoring the synthesis of these substances, intervenes ultimately in the processes which lead to the active catabolic intervention of oxygen. In this way, Cu acts as a D inducing agent. Cu intervenes actively in the metabolism of sulfur. (154, 155) The transformation of sulfhydryl to disulphide is slow and incomplete when there is a copper deficiency. The same deficiency reduces the formation of phospholipids as seen in rat liver. (156) Indirectly, Cu favors anti-A activities.

The influence of Cu upon Ca metabolism also is indirect. We have seen that a local deposit of calcium in bones corresponds to a local D pattern. Deficiency in copper, a D inducing agent, permits the appearance of local A conditions, which in the case of bone, will result in a lack of calcium, the opposite of what is seen in local D offbalance. This correlation explains why, in spite of sufficient P and Ca, the lack of copper induces an osteomalacia with bone fracture and symptoms of rickets, as seen in animals with an indirect Cu deficiency caused by an excess of molybdenum, (157, 158, 159) an A inducing agent. The administration of copper helps to repair these fractures. (157) Parallel reductions in Cu and Mg occur in milk-fed calves. (160) A richness of zinc, like molybdenum, can provoke a deficiency in Cu and Ca. With copper deficiency and low catalase, resistance to infections is lowered. In brucellosis, a deficiency of Cu and Ca

coincides with reduced concentrations of Mn and Co in the blood and pituitary glands. (161, 162, 163) Cu, Ca, Mn and Co are all metals of the D inducing group.

In cancer, a qualitative deficiency of copper is found. A frank reduction of the catalase content is seen in cancerous cells as well as in the liver of cancerous subjects. (164, 165) On the other hand, Cu content is considerably increased in the blood of these subjects, with values even three times greater than normal, often encountered. (166, 167, 168) These values return to normal if the cancerous tumor disappears. (169)

In cancer, copper deficiency at the level of the cells is manifest, shown not only by reduced catalase but also by upsets in the cytochrome oxidase system, the heme system, the SH metabolites, and the phospholipids. The deficiency, however, is only local and consequently qualitative since an excess of Cu is found at the immediately superior level, the blood.

A local abnormality residing in inadequate capacity to utilize copper thus appears related to cancer. In normal animals, copper in excess can be utilized and is able to prevent the appearance of tumors. This explains why Cu, which protects rats against carcinogenetic azodyes, (170, 171, 172) does not influence the tumors once they have been induced, *i.e.*, once the qualitative insufficiency is present. The recognition of this difference between the form in which copper is utilized by the normal animal and the deficiency in the cancerous entities, has been the basis for a series of studies concerning this key problem in the pathogenesis of cancer. The therapeutic use of copper—and of other elements which we will discuss below—is not a quantitative but a qualitative problem.

Manganese and Cobalt

Two other elements of the cellular compartment have appeared interesting. Manganese by intervening as a catalyst in processes resulting in an activation of oxygen, indirectly manifests D inducing character. Its presence in smaller amounts in tumors or cancerous organs than in controls, has been considered. Just as with copper, no effects are seen in treating tumors with manganese compounds although a certain degree of preventive action is obtained in tumors induced by carcinogens. Similarly, with cobalt we have obtained a certain degree of prevention against tumor induction by carcinogens but no effect upon the evolution of tumors once they have appeared. No effects have been seen in transplanted tumors.

Heavier Elements

We have studied the activity of elements corresponding to the lower levels—such as strontium and tin for the nuclear; barium, gold and lead for the submorphologic; and cesium for the primary biological compartments. The lower the level of the element, the greater appears to be the preventive effect against induction of tumors by carcinogens. But the minimal, or complete absence of effect upon already existing tumor cells remains unchanged.

As mentioned above, we connected this paradoxical activity to the fact that, while normal cells are able to manufacture compounds through which the appearance of a cancerous entity can be prevented, these compounds are no longer formed if an entity is already cancerous.

These have been the considerations forming the basis for an entire series of studies of the role of various elements in the pathogenesis of cancer.

Elements in the Pathogenesis of Cancer

Investigations have been made of the amount and form of the element present in the normal animal as compared to the cancerous animal. The quantitative and especially qualitative differences have been seen to indicate the site of the abnormality largely responsible for the lack of influence exerted by the element. The results explain why the administration of the element alone is unable to influence the evolution of an already existing cancerous process. More interesting, they show what compound of the element could have an influence. This research, which is in progress, opens the door for possible therapeutic applications. It is through such compounds, present in the normal and lacking in the cancer-stricken animal, that attempts are being made to influence the evolution of cancer. An important step has been the finding that suitable compounds can be obtained by the treatment of fresh organs in vitro with some of the elements. Their study may make possible synthetic preparation of suitable compounds. The few results already obtained in experiments with animals confirm that D-inducing activity represents a factor which the promising elements share.