obtained from these acids by changing the carboxyl into a primary alcohol through treatment with lithium aluminum hydride. Especially effective influences are obtained using acid lipidic fractions of refractive species in order to reduce the virulence, or insaponifiable fractions of especially sensitive species or of chicken embryos to enhance virulence. Treatment of the host with lipoacid preparations, repeated for several passages of the virus, has reduced virulence to the point where the virus no longer is pathogenic. This treatment seems to provide a method for reducing virulence and obtaining nonpathogenic live virus vaccines.

Chapter 6, Note 33. **Microbes, Phages and Lipids**

We have investigated the influence of various insaponifiable fractions upon the relationship between microbes and bacteriophages. Coli bacilli, of a strain which has shown considerable resistance to phage, were grown in broth to which insaponifiable fractions from different sources—such as human placenta, eggs, butter—were added in colloidal suspensions. While in these media the microbes showed a higher susceptibility to being attacked by phage than the controls grown in simple broth, only very few microbes if any remained resistant. In another experiment, this same strain of coli resistant to phage was grown in successive passages in broth containing insaponifiable fractions. After several such passages, the microbe was grown in simple broth and its sensitivity to phage was tested in this medium. While the phage used appeared unable to attack the microbes of the untreated cultures, lysis was manifest in the treated coli. Thus, insaponifiable fractions increased the sensitivity of the microbes to bacteriophage, not only as an immediate effect, but also as a transmissible character.

Chapter 6, Note 34. **Lipids and Survival Time of Tetrahymena**

Various fatty acids have shown a special influence on survival time of tetrahymena pyriformis. Survival time was determined by keeping a few drops of culture in a capillary closed at one end and by daily examination of the mobility of the tetrahymena. By adding progressive amounts of an agent and withdrawing such a capillary after each addition, we also were able to determine the influence exerted by different concentrations.

In general, addition of very small amounts of fatty acids was found to prolong survival. Similar effects were obtained with hydropersulfide, and even with a solution of saponines which are known to bound sterols in insoluble combinations. The most marked effect was obtained with a fatty acid having a relatively low number of carbons. The addition of heptanoic acid in progressive amounts greatly increased longevity. The longevity increased as the concentration increased up to a point, after which it declined rapidly when greater concentrations were used. Of all the substances studied, heptanoic acid appeared to be most effective in increasing the longevity of tetrahymena pyriformis.

Chapter 6, Note 35. **Lipids, Temperature and Tetrahymena**

We have investigated the influence exerted by different lipids upon the capacity of tetrahymena pyriformis to resist increased temperature. In general, exposure of a culture of tetrahymena pyriformis to increased temperature induces rapid death. Kept in capillaries so a temperature of 37°C could be quickly attained, tetrahymena died in about 15 minutes. By growing tetrahymena for several successive generations in a medium containing insaponifiable fractions, increased sensitivity to temperature was obtained. In some experiments, death occurred within five minutes after exposure to 37°C. On the other hand, growth in media to which fatty acids were added markedly increased resistance, and survival after more than ½ hour of exposure to 37°C was seen.

Chapter 6, Note 36. **Pain Induced by Lipids**

During treatment of cancer patients with lipids, especially in exceptionally large amounts, some who had been pain-free before, experienced pain. Correlation could be established between the appearance of pain and the administration of lipids since pain increased with each new injection. Furthermore, a difference was seen to exist between the pain induced by the administration of fatty acids and insaponifiable fractions. While the former had an alkaline pattern, the second had an acid one. In several cases, pain subsided with discontinuation of the medication; in others, it persisted. The pain always was controlled by the administration of the opposite lipids, fatty acids for the acid type of pain and sterols for the alkaline.

Chapter 6, Note 37. **Lipids and Wound Healing**

The influence exerted by lipids upon wounds was followed by the changes induced in the healing processes. In order to compare the wounds, one square centimeter of the skin was excised down to the aponeurosis on the back of rats and rabbits after mechanical epilation of the skin, and the surface measured daily. We used transparent cellophane on which the exact dimensions of the wounds were drawn. The outlines which corresponded to the surface of the wounds were then passed on paper and cut out. The paper outlines were then weighed to give us a means of comparing the changes in the actual surfaces of these wounds during healing.

While lipid acids in general induce a retardation in the healing of the wound, the administration of lipoids with a positive character were seen to have an opposite effect. It is worthwhile noting that the naturally occurring sterols—cholesterol and insaponifiable fractions—are much more active than the synthetic in inducing rapid wound healing.

We studied the histological and cytological changes in parallel incisions made on the back of rats and rabbits and excised at intervals. In animals treated with sterol preparations, a difference was seen between the healing

of epithelial and connective tissue wounds. While healing of the former was enhanced, healing of the latter was not influenced. On the other hand, the higher alcohols, such as polyunsaturated alcohols or butanol, were more active in increasing healing of connective tissue wounds than of epithelial. It is interesting to note also that in the wounds treated with the sterol preparations, the scar showed an epithelium with many more layers than that of the normal surrounding skin. In rabbits, instead of two or three layers, there were more than ten.

Chapter 6, Note 38. **Liver Regeneration**

The ability of rats to regenerate almost ¾ of their liver in a short time has made them valuable for the study of the factors which intervene in cellular multiplication and differentiation. A study which we made in collaboration with E. F. Taskier has shown the importance of biological age of the individual in these processes. Regeneration is rapidly completed in young animals; much more time is required in the old. Liver regeneration has been seen to be related to the appearance of fats in the form of droplets filling up liver cells. Regeneration follows this first phase. The appearance of the fatty droplets provides a means of judging the velocity of regeneration. The importance of age is shown by the fact that fatty droplets appear early in the liver cells of very young animals, filling up the cells, in the first 24 hours. They appear later—in about two days—in young adults; in three days for middle-aged rats; after the fourth day in old animals.

The influence exerted by administration of different agents upon regeneration could also be judged through the changes induced in the appearance of the fatty droplets. The administration of insaponifiable fractions in general has induced an earlier appearance of the fatty cells. Injection of 2 cc. of a 10% solution of the insaponifiable fraction of human or even cow placenta was seen to induce an early appearance of the fatty droplets and a filling up of the liver cells. Even in old rats such changes occurred on the second day, contrasting markedly with control rats of the same age in which this would happen on the fourth day or later. Under the influence of these insaponifiable fractions, the old animals behave like youngsters, from the point of view of liver regeneration.

The opposite effect was exerted by the administration of 1 cc. of a 10% solution in oil of the lipoacids of human placenta or of a 10% solution of cod liver oil fatty acids. Appearance of fatty droplets was delayed. In young animals, the droplets were not seen until the third or even the fourth day. With a high dose such as 2 cc. twice a day, of the same preparation in animals of 150 grams, no fatty droplets appeared at all. It is interesting to note that in animals treated with such large doses of lipoacids, regeneration still takes place even without the appearance of the fatty droplets. In these cases the liver cells are comparatively very small and have compact nuclei, instead of the reticular aspect of the nuclei in the controls.

It is also to be noted that a parallelism was seen between the appearance of fatty droplets in liver cells and the richness of adrenals in sudano-

phil granules. In cases in which the administration of large amounts of lipoacids was followed by nonappearance of fatty droplets in the liver, the adrenals were found to be entirely depleted of fats.

The influence exerted by the lipids upon liver regeneration confirms the antagonistic role of the two groups of lipids in aging processes. The administration of insaponifiable fractions produces a regenerative response characteristic of young animals, while lipoacids produce the response of aged animals. We have applied these findings to other processes in which age is known to be a major factor—such as in the healing of wounds, and especially of fractures, in older people, where administration of insaponifiable fractions has been seen to change an atonic lesion into a rapidly healing one.

The study of liver regeneration has also indicated qualitative differences between various preparations. It is thus interesting to note that, of all the insaponifiable fractions used, the most active were those from placenta and embryos. The insaponifiable fraction of liver also has shown a special capacity to induce rapid regeneration especially of liver tissue. Higher alcohols have shown much less regenerative effect than the insaponifiable fractions.

Chapter 6, Note 39. **Lipids and Convulsions**

The administration of insaponifiable fractions of placenta or organs sometimes produces no observable manifestations in rats and mice, except an exophthalmia. However, more profound changes occur, since injections of thiamine in doses otherwise harmless are followed by lethal convulsions in these animals. 80 to 100 milligrams of thiamine/100 gr. of body weight produce lethal convulsions in rats or mice who received 1 cc. of 5% insaponifiable fractions of placenta per 100 gr. of body weight in daily injections for a week. In controls, 150 milligrams of thiamine per 100 gr. of body weight were necessary to induce fatal convulsions.

High doses of insaponifiable fraction of organs alone, also produced convulsions. The injection twice a day of a 5% oily solution of insaponifiable fraction of placenta in doses of 2 cc./100 gr. of body weight was seen to induce lethal convulsions after less than a week of treatment.

Chapter 6, Note 40. **Lipoids and Coma**

The administration of heptanol even in larger doses was not seen to induce somnolence or coma. Intravenous injection of a saline solution of 1 milligram of heptanol per cc. induced death in mice in doses above 0.5 cc. With 0.3 cc., the mice remained in deep sleep, sometimes with respiratory arrest. Most of the animals, however, recovered, starting to breathe in less than half a minute and awakening in about ten minutes. A dose as high as 10 cc. of the same solution, containing 10 milligrams, injected intravenously in rabbits, produced no more than a very short period of inactivity, without inducing sleep. Intramuscular doses as high as 500 mil-

ligrams of heptanol in oil in humans did not produce somnolence. However, after several days of concomitant administration of heptanol and cortisone, even in reduced amounts such as 50 milligrams of heptanol and of cortisone daily, deep somnolence was seen to appear in some patients, and coma in two cases. In one, a man of 85, we were unable to overcome the coma. In the other, administration of cod liver oil fatty acids, sodium thiosulfate, and especially ½ cc. of DOCA (desoxycorticosterol acetate), brought the patient back to normal state.

Chapter 6, Note 41. **Cardiac Rhythm**

The antagonistic influence exerted by the two groups of lipoids was seen to have an especially interesting effect upon the cardiac cells. The importance for the pharmacological study of the lipoids, as well as for the cardiac physiology and pathology of the changes induced, has urged us to study them in more detail.

The principal physiological property of the cardiac cell is its automatism, that is, its capacity to produce the proper energetic influx which when discharged, will induce the contraction of the myofibrils. Through the cytoplasmatic bond formations characteristic of the myocardial cells, the discharged influx passes also into the nearby cells where it acts as an external incitation which, in turn, induces the discharge of the influxes produced by these cells. It is through this progressive discharge of contiguous cardiac cells that the contraction progresses in a centrifugal manner through the heart.

Each cell needs a definite time to "mature" its own influx, a negative period following each discharge. During this refractory period, the cell does not respond to any influx, either from nearby cells or from any external excitation. On the other hand, due to the same progressive maturation of its proper energetic influx, if within a certain time this influx incitation produced in the cell has not been discharged by an influx coming from a nearby cell, the cell itself discharges it. This automatism is common to all cardiac cells. It differs however, from one cell to another in the time necessary for the influx to mature, that is, in the time necessary to bring the cell out of its negative refractory period or to discharge its own influx, if not discharged by an external incitation to the cell. A cell has a high automatism if it has a short refractory period, if it rapidly produces its influx, and if it discharges it early. A cell has a low automatism if its negative period is long and if it requires a long time to discharge its own influx if not discharged by an influx coming from the nearby cells. The rhythm of the contractions of the entire heart will be given by the discharge time of the cells with the highest automatism.

If groups of cells have an abnormally low automatism and their negative period is so long that these cells will still be in the refractive negative period when the influx from nearby cells arrives to them, they will not be discharged by this flux. If the group of cells represents a part of the heart

through which the influx has to pass in order to attain the entire heart, it will block its propagation.

The normal cardiac physiology results from the inequality of the automatism of the different cardiac cells. Those with the highest automatism will represent the pacemaker for the entire heart contraction. Under normal conditions the cells of the sino-auricular node show this highest automatism. Other cells with an automatism lower than that of the pacemaker, but still sufficiently high to be out of their refractive period, will respond when the influx started by the sino-auricular node arrives to them. The automatism of the other centers present in the heart—Aschoff-Tawara's node, Hiss's band, its branches, Purkinge's cells—progressively lower than that of the sino-auricular node, will supply an influx if that of the sino-auricular fails to reach them in due time.

Under abnormal conditions, this automatism is influenced. It can be either increased or decreased. In general, if the automatism of cells other than those of the sino-auricular node, is increased above that corresponding to the rhythm of this node, their influx will be prematurely discharged. If the cells around it are out of their refractive period, this influx will propagate and induce a contraction. They appear as abnormal pacemaker centers due to their premature discharge and also to their ectopic position. The resulting contractions will be manifested as extrasystoles, if the abnormal discharge appears as an isolated event, or as paroxysmal tachycardia if the abnormality persists. In auricular fibrillation, this abnormality takes place in a larger group of cells. Oppositely, a lowered automatism affecting an entire group of cells will result in a blockage of the passage of the normal influx due to this lengthened negative period.

The factor which appears to govern the differences seen in automatism of the various centers in the heart, is the degree of differentiation of the respective cells. As a general rule, a less differentiated cell has a higher automatism, while a more differentiated cell has a lower automatism.

We have seen that up to a certain point, the properties related to the degree of the differentiation of these cells can be connected with youth characters. The changes seen in heart cellular physiology, and especially those which appear under abnormal conditions, can be conceived as taking place through changes in the degree of the differentiation of the cells. We have seen above, in the study of the influence exerted by lipids, that while the unsaponifiable fraction induces a "prolonged youth" with a degree of the dedifferentiation of the cells, the acid lipid fractions induce a process similar to a more rapid aging, respectively a more advanced differentiation. This effect was seen also to be general for the respective positive and negative lipoids. While for other cells such a change may be uneventful, for the cardiac cell it will be marked by a change in automatism.

From this specific point of view, we have studied the influence exerted by different agents upon the heart, seeking in the changes induced, modification corresponding to an increased or decreased automatism. Clinical observations have shown such correlation. Extrasystoles were seen to appear

in subjects who had previous extrasystoles, when lipoids with positive polar groups were administered in high doses. They disappeared when the medication was stopped and reappeared when medication was resumed. In cases with previous auricular fibrillation we have seen it reappear with high doses of positive lipoids, disappear with cessation of the medication and reappear when medication was resumed for even a short time. This was fully controlled by the administration of lipoids with negative polar groups.

In hundreds of electrocardiograms taken of experimental animals, such a correlation between the administration of lipoids and induced arrhythmias was investigated in collaboration with I. Eroglu. We studied thus various substances, lipoids with positive or negative characters administered intraperitoneally or intraveneously in rabbits. An extremely high amount of the agent was necessary to influence the cardiac rhythm in normal animals. It was usually near a lethal dose and in general, proportionately many hundred times that used therapeutically in humans. In repeated injections however, changes could be induced with relatively smaller doses. In sufficient doses, the positive lipoids were seen to induce extrasystoles. Figures 292 and 293 show such changes obtained with huge doses of butanol and glycerol administered intravenously. (Page 714)

In animals, the negative lipoids induce a dromotropic negative effect, leading to auricular contractions not passing to the ventricles. Huge doses were seen to induce a bigeminated pulse.

The study of the intervention of lipoids has led to a new therapeutic approach. Extrasystoles, paroxysmal tachycardia and auricular fibrillation were seen to respond well to the administration of lipoacids and lipoids with negative polar groups, while partial blocks were influenced by lipoids with positive polar groups.

Chapter 6, Note 42. **Some General Considerations of the Role of Lipids in Blood Physiology** *

Lipids and Red Cells "In Vitro"

Among the first experiments concerning the influence exerted by sterols and polyunsaturated fatty acids in vitro, were those concerned with the effects upon red cells. We have noted that when citrated blood is kept for two hours at 37°C in a test tube, the walls of which have been coated with crystals of cholesterol or with nonsaponifiable fractions obtained from various natural sources, the red cells become more swollen and turgescent, and less crenated than those not treated. Seen under the dark field microscope, the cell crown appeared uniformly more refringent. It was also noted that the treated red cells failed to form rouleaux or conglutinates similar to those seen as sludge in vivo. At the same time, the cells appeared richer in their sterol content. None of these changes were observed when the red cells were separated from their plasma and washed with saline and kept in a saline solution when treated with sterols, in the manner mentioned above.

* Delivered at Gordon Research Conferences, Kimball Academy, Meriden, New Hampshire. 1955

Opposite effects were observed when fatty acids were added to blood. As the direct contact with the red cells produces hemolysis, the following technique was used. Fatty acid preparations especially as mixtures obtained from blood or cod liver oil, were added to heparinized or citrated plasma, thoroughly agitated and the excess separated by centrifugation. The plasma so treated was then added in various proportions to citrated or heparinized blood from the same subject. This portion of this blood was centrifuged and the treated plasma added to the supernatant plasma from which the same amount was withdrawn. The added plasma was mixed with the supernatant plasma and then this was mixed with the red cells. In this way hemolysis was prevented. Small amounts of treated plasma caused the red cells to shrink in size and frequently become crenated. In addition, a strong tendency to conglutinate which exceeded that noted in corresponding control specimens, was observed. When the quantity of fatty acid-treated plasma exceeded a certain amount, hemolysis was induced. The addition of these two groups of lipids to red blood cells, have appeared to exert frank antagonistic effects.

Sedimentation Rate

The two groups of lipids were also found to influence oppositely the red cell sedimentation rate in citrated blood. When citrated blood samples having high sedimentation velocities, were treated with cholesterol or an insaponifiable fraction in the manner described above, the speed of sedimentation was markedly reduced. TABLE XXVIII shows results obtained

TABLE XXVIII

RED CELL SEDIMENTATION RATE (mm. hr.) SAMPLES TREATED WITH UNSAPONIFIABLE FRACTION OF BLOOD LIPIDS

Control	Treated
110	12
96	19
81	18
48	15
18	10
12	6
9	7
8	8
6	5

mentation rate tended to increase to abnormal values. This varied with the amount of treated plasma added. (TABLE XXIX)
in different blood samples in which the sedimentation rate during one hour, was measured by the Westergren method. In general, it can be seen that the higher the sedimentation rate of the untreated sample, the greater was the effect of adding sterols.

On the other hand, when polyunsaturated fatty acids were added in the manner already described, to citrated blood from healthly subjects, the sedi-

TABLE XXIX

RED CELLS SEDIMENTATION RATE—mm./1st hour

Fatty Acid Used	Control	Quantity of Treated Plasma Added to 5 cc. Citrated Blood		
		1/4 cc.	1/2 cc.	1 cc.
Stearic	9	8	9	9
Palmitic	9	10	9	10
Linoleic	9	15	18	22
Linolenic	9	15	21	25
Cod Liver Oil	9	20	36	Hemolysis

Red Cell Volume

The same opposite effects of sterols and fatty acids were further observed upon the volume of red cells, as determined by the hematocrit, or also when the sedimentation in tubes was observed over a 24 hour period. (TABLE XXX) Sterol-treated blood showed a significant increase in red

TABLE XXX

CHANGES IN VOLUME OF RED CELLS IN CITRATED BLOOD TREATED IN VITRO
(Sedimentation After 24 Hours)

Substance Used	Control	Treated
Unsaponifiable fraction of blood	53	66
Stearic Acid	53	54
Saponifiable fraction of blood	53	50

cell volume, while on the other hand, with the addition of polyunsaturated fatty acids, the red cell volume decreased. This agrees with a retention of water by the cells in general when richer in sterols, which Schaeffer has described as the lipocytic index.

On further analysis, these effects of fatty acids upon red cells mentioned above could be related to the polyunsaturation of these acids, since by treating the blood under the same conditions with saturated members, such as palmitic or stearic, these changes were not obtained.

The treatment of red cells with conjugated fatty acids, especially trienes, induces a marked vacuolization. This is seen to occur through an accumulation of part of the content of the red cell in droplets, strongly stained with eosin. *(Fig. 264a)* Similar changes are seen to occur in vivo. In lesions characterized by a predominance of fatty acids or induced by the administration of conjugated fatty acids, such vacuolated red cells are often seen. We used their presence, together with other characters, for the pathological diagnosis of the type D present in a lesion. *(Fig. 264b)*

Red Cells, Plasma and Lipids

As most of these changes did not occur with the red cells in suspension in different isotonic saline solutions, we have attempted to explore the re-

lationship of plasma to red cells and lipids. This was done in the following manner. The cholesterol content of red cells was seen to be progressively lowered by repeated washings with isotonic saline. When the amount of cholesterol is reduced below a certain level, hemolysis ensues. Standardizing these washings by replacing the plasma with an equal amount of saline, hemolysis is usually obtained in some bloods after 1 or 2 washings while in most after more than 10 washings. This occurs when the cholesterol con-

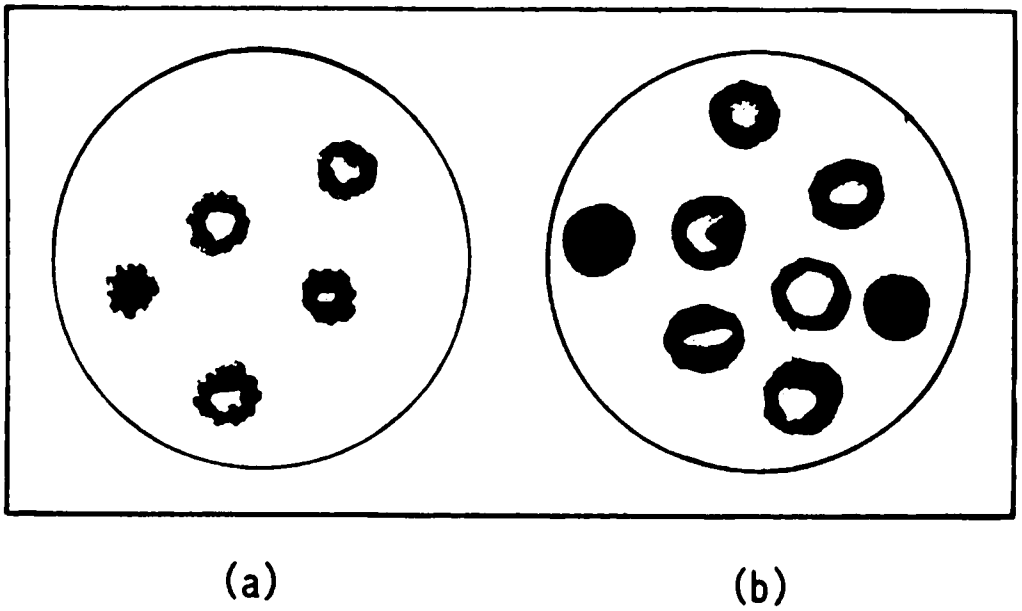

FIG. 264. The treatment of red cells in vitro with conjugated fatty acids (trienes) induces the appearance of vacuoles. (a) In spontaneous lesions characterized by an off-balance type D with a predominance of fatty acids or in lesions induced by the administration of conjugated trienic fatty acids, vacuolated red cells are seen. (b)

tent of the red cells falls below 58 mgm. %. When the separated plasma is again added to these repeatedly washed and consequently cholesterol-impoverished blood cells, their cholesterol rises. (TABLE XXXI) By further additions of new portions of the same plasma, the cholesterol content of the red cells can be progressively elevated to the original values. Apparently cholesterol passes readily from the plasma to the red cells. This

TABLE XXXI

INFLUENCE OF CITRATED PLASMA UPON RED CELL CHOLESTEROL

Before Treatment	186 mgr.%
After 10 Washings	62 "
After First Treatment with Plasma	111 "
After Second Treatment with Plasma	142 "

was confirmed by measuring the cholesterol content of the plasma before and after it was mixed with cholesterol-impoverished cells. After treating a portion of citrated plasma several times with other portions of cholesterol-impoverished red cells, the amount of cholesterol in the plasma was markedly reduced. The addition of the unwashed red cells to this cholesterol-impoverished plasma raised back its cholesterol content. The content could be increased still further almost back to the previous values by repeating this procedure. (TABLE XXXII) It was clear that a balance in

TABLE XXXII

INFLUENCE OF RED CELLS UPON PLASMA CHOLESTEROL

Before Treatment	226	mgr.%
After Fifth Treatment with Washed Red Cells	96	"
After First Addition of Unwashed Red Cells	163	"
After Second Addition of Unwashed Red Cells	180	"

the cholesterol content seems to be realized between plasma and red cells through possible passages in both directions. This fact would imply that the red cells may serve as a buffer reserve for rapid changes occurring in the plasma sterols.

The role of red cells in the transportation and distribution of fatty acids through the blood not only appears more evident, but also indicates a selective intervention. When plasma was treated as mentioned above with fatty acids that were easily identifiable, and then mixed with the red cells, an unequal distribution between plasma and red cells was seen. The different influence of the different fatty acids became obvious. Saturated fatty acids could not be found in the red cells when these acids were used, while unsaturated fatty acids seemed to be selectively retained. Fatty acids such as oleic, linolenic, eleostearic, or norbixine were found convenient to be used for this purpose. They were easily identified, the first through its chemical character, the second after conjugation, and spectral analysis, the third through its characteristic absorption in ultraviolet light, and the last through its color in chromatographic column. After mixing with the red cells, they were found unequally and selectively fixed to the red cells: least for oleic, fixation was found to increase with the degree of desaturation. The same fixation was seen to occur in vivo. When animals were treated with saturated fatty acids, these substances did not appear in the red cells. When treated with oleic, linolenic, eleostearic acid or norbixine, the content of these acids in the red cells was found to be for the last acids as much as five times higher than in plasma. A selective fixation of those fatty acids on the red cells could thus be recognized. This appeared still more striking when compared with that of cholesterol. In animals treated with cholesterol, the relative proportions between the proportion in plasma and cells

was not seen to be altered by a total increase in cholesterol. It appears that the red cells have the capacity of selectively fixing from the plasma certain fatty acids, particularly the more unsaturated ones.

Lipids and Blood Oxygen Transport

One of the most interesting observations and one of the simplest in vitro experiments that indicates these opposite effects of sterols and fatty acids is their influence upon the oxidation processes in which red cells intervene. When a sample of ordinary venous blood is treated with a preparation of cholesterol or nonsaponifiable fractions, using the above mentioned technique and after its separation from the cholesterol it is agitated with air or oxygen which is passed through these samples, the color becomes a bright vermilion red, and this persists for a long time. When the same venous blood is treated with a preparation of polyunsaturated fatty acids as mentioned above, the color becomes very dark, almost black purple. When air or oxygen is passed through these samples, the blood becomes lighter in color for only a short time, the darker color reappearing within a few minutes. One is immediately impressed by the similarity of the cholesterol-treated blood to arterial blood, while the fatty acid-treated blood is similar to venous blood, and especially to the color of venous blood in cases of shock.

We tried to tie in these findings with the observation of Binet concerning the changes in blood fatty acids when passing through the lungs. He has been able to show that the amount of the polyunsaturated members appears to be reduced by the passage of blood through the lungs. We could show that the red cells leaving the pulmonary vascular bed are somewhat richer in the unbound cholesterol than they have been in the blood which entered the lungs. The lipid content is altered in an opposite way as the blood travels through the general circulation. That is, the polyunsaturated fatty acid content is increased in the red cells while the quantity of free cholesterol seems to be diminished. The sterol-richer red cells appear capable of retaining for a longer period of time, the amount of oxygen which hemoglobin has fixed, while a rapid reduction of oxyhemoglobin is seen in the red cells when the polyunsaturated fatty acids intervene. This led us to consider an intervention of these two groups of lipids in relation to the oxygen transportation by the red cells. Bearing in mind the fact that while cholesterol reduces cell permeability and polyunsaturated fatty acids increase it, an alternating intervention of these lipids seems to play a role in a better distribution of oxygen. The oxygen which is fixed by hemoglobin when the red cells have passed the lungs, is largely retained as such by the intervention of the sterols until they reach the point in the tissues where liberation of oxygen is necessary, this being favored now by the intervention of the fatty acids.

Lipids and State of Shock

The abnormally dark color of the blood resulting from its treatment in vitro with polyunsaturated fatty acids has suggested the intervention of such

substances in those clinical conditions in which similar color changes are noted in the blood as in shock. We will present our studies in shock below. For the moment we will only note that in the state of shock experimentally induced by trauma, burns or irradiation, or found in terminally ill adrenalectomized animals, these animals have not only a high fatty acid content, but that the kind of fatty acids encountered are not the same as in normal animals. We have discussed these abnormal fatty acids above. The existing differences have been shown by measuring the quantity of oxalic acid that is produced when these fatty acids are submitted to a careful standardized oxidative fission. The oxidative fission of the fatty acids not only from their entire body but even from their blood has shown that for normal animals, no oxalic acid could be found, leading to the assumption that no conjugated fatty acids are present. On the other hand, oxalic acid appeared when fatty acids obtained from animals in shock or from their blood were broken down with the analytical method utilized.

Of particular significance for the pathogenic role of these fatty acids is the fact which we will discuss again below, that death appears to ensue when the conjugation of fatty acids reaches a certain value, which is approximately the same whether the animal has been traumatized, burned, irradiated or adrenalectomized, and independent of the fact that death occurs in a short time or several days. It corresponds to 14-17 mgm. of oxalic acid per gram of fatty acids. It is also interesting to note that these abnormal fatty acids were found to be more abundant in the red cells than in the plasma.

Effect upon Leucocytes

The biological antagonism between sterols and fatty acids has appeared in the influence upon other blood constituents. We have observed that the administration of sterols tends to elevate the total white blood cell count and especially the number of neutrophilic granulocytes. Polyunsaturated fatty acids, on the other hand, produce rapid leucopenia, and again it is the neutrophile elements that are first affected. A hyperleucocytosis often was seen following the neutropenia induced by polyunsaturated fatty acids if small amounts are administered. This effect could be considered as being reactional to the first leucopenia, since it is retarded or even prevented if large doses of these fatty acids are injected. (TABLE XXXIII) It is also interesting to note that a deviation to the right, in Arneth's formula, was seen after the treatment with fatty acids; and to the left after treatment with sterols. Thus, this concords well with the antagonistic effects upon the aging process seen for these lipids and which is discussed below.

Lipids and Blood Serum Cholesterol

Further study of the relationship between blood and lipids has permitted the recognition of several peculiarities concerning the blood serum which when related to abnormal conditions, acquires a special significance. Policard has observed that when crystals of cholesterol are added to blood sera, two opposite changes can ensue. In one, a precipitate appears while

TABLE XXXIII

EFFECT OF LIPIDS ADMINISTERED IN VIVO UPON THE TOTAL NUMBER OF LEUCOCYTES

Unsaponifiable Fraction of Blood—10% Solution—5 cc. I.P.		
Before Administration	14,600	12,000
2 Hours Later	18,400	19,000
7½ Hours Later	26,000	22,600
Saponifiable Fraction of Blood—10% Solution—5 cc. I.P.		
Before Administration	13,200	16,200
2 Hours Later	11,000	6,800
7½ Hours Later	6,000	5,100
Stearic Acid—10% Solution—5 cc. I.P.		
Before Administration	16,100	14,200
2 Hours Later	12,800	15,100
7½ Hours Later	15,000	12,000

the serum cholesterol content decreases. On the contrary in the other, a part of the added cholesterol passes in solution into the serum, thereby causing an increase in cholesterol content. When animals were treated with large amounts of sterols for a long time, their sera showed this tendency to precipitation when in contact with cholesterol in vitro, while the sera of animals treated with large amounts of fatty acids showed the capacity of dissolving more cholesterol. We believe that this capacity of sera to precipitate in the presence of cholesterol may be correlated with the clinical conditions present in arteriosclerosis when acute episodes occur.

These studies of the role of lipids in blood physiology suggest that the general antagonism between sterols and polyunsaturated fatty acids also intervenes in other important processes of blood physiology. It has thus raised the question of the role these lipids may play, through their opposite effects, in different metabolic balances of the body governed by blood changes.

Chapter 7, Note 1. **Analyses Used for the Study of Hemoshock**

Besides body temperature and blood pressure, the following blood analyses were made: complete blood count; coagulation time; clot retractability; values of albumin and globulin, total and free cholesterol, free fatty acids, degradated proteins, antitryptic power of the serum, esterase amylase, potassium, sodium, calcium and glucose. Most of these analyses were made in venous blood samples obtained every five minutes, during the fifteen minutes preceding and the half hour following the noxious intervention.

Chapter 7, Note 2. **Morphine and Shock**

The possibility of inducing hemoshock simply through an intravenous injection of colloidal metals has provided a useful method to study the conditions under which hemoshock can be induced or suppressed. We have investigated a series of agents to determine their influence upon shock. Adrenalin, quinine, ephedrine and atropine have not changed the course of clinical and hematic manifestations. On the other hand, morphine, as well as other opium derivatives, completely prevented the development of these manifestations. Subcutaneous injection of 2 centigrams of morphine

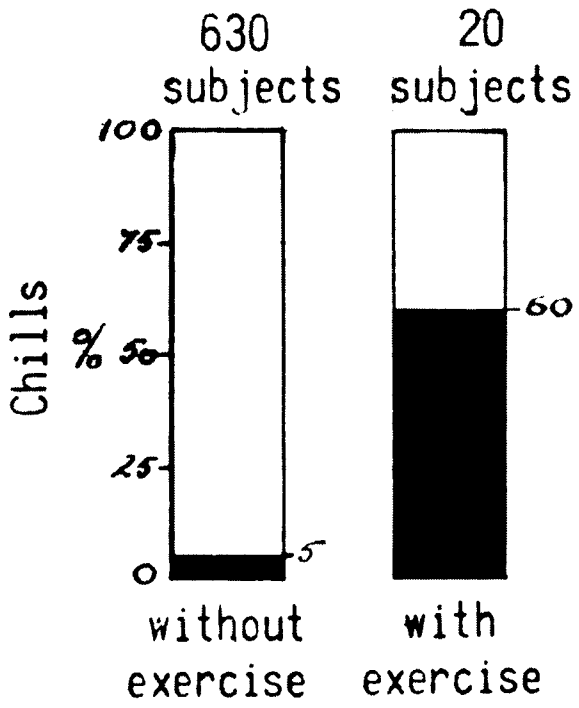

FIG. 265. The proportion of chills appearing after direct transfusion (with the Jubé syringe, injecting 500 cc in less than 10 minutes) increases manifestly after exercise (walking in the room).

sulfate fifteen minutes before the intravenous injection of the metal always suppressed the manifestations as did an intravenous injection of 5 milligrams of morphine sulfate. Analyses have shown no leucopenia, to occur. Similar effects were obtained, with none of the changes characteristic of hemoshock, for instance in transfusions where 500 cc. were injected in less than ten minutes and where morphine prevented the frequently observed chills. The influence of morphine upon the leucocytes has been confirmed in the following experiment.

In rabbits, a pleural exudate was obtained by an intrapleural injection of broth, 16 hours later, pleural punctures furnished fluid rich in leucocytes. As we have noted above, the addition of a colloidal suspension of silver

proteinate (collargol) was followed by the appearance of rapidly growing vacuoles which led to bursting of leucocytes. *(Fig. 76)*

The addition of even minimal amounts of morphine or other opium derivatives entirely prevented these changes in leucocytes. No lysis nor vacuoles were seen.

Chapter 7, Note 3. **Physical Exercise and Shock**

The study of hemoshock has shown the influence exerced by physical exercise upon shock. A leucopenia was observed in normal subjects after an intensely sustained physical effort, such as after running for five minutes, a fact which led us to try to see what influence exercise would have on the shock induced by the intravenous injection of colloidal metal. The shock was seen to be much stronger than usual. The chill which followed 25 minutes later was also proportionately severe. We have since correlated the appearance of hemoshock with exercise in patients having direct transfusion. If the patient exercised immediately after the transfusion, a chill consistently followed about a half hour later. *(Fig. 265)*

Chapter 7, Note 4. **Lymphocytes and Effects in Vitro**

The capacity of lymphocytes to hydrolyze even higher esters can be demonstrated by having lymphocytes separated and their activity tested. Fluid obtained from tuberculous pleural effusion rich in lymphocytes was centrifuged and the fluid decanted. The centrifugate was then put on a plate of beeswax, covered with a cup and left for several hours at 37°C. A clearly visible depression appeared where the lymphocyte preparation had been added.

Chapter 7, Note 5. **Lipids and Immunity**

Three groups of five rabbits each, of the same sex and weight, were injected intravenously on two consecutive days with the same amount of a suspension of killed Eb. Thyphi. One group was kept as control, receiving daily injections of 1 cc. of cottonseed oil. Of the other groups, one was injected subcutaneously daily with 1 cc. of a 5% solution in cottonseed oil of the acid lipids mixture obtained from human placenta. The third group received daily 1 cc. of a 5% solution in cottonseed oil of the insaponifiable fraction of the same origin. Every second day, venous blood was obtained and the agglutinating power of the serum determined. Figure 265A shows the average values for each group.

Chapter 7, Note 6. **Microbes Treated with Lipids**

The injection of microbes killed by heat and treated in vitro with various lipids and lipoids had an interesting effect on the appearance of antibodies. Eb. typhi, cultivated on agar and suspended in saline so as to give

nephelometrical values corresponding to 30 mil. per cc., were killed by heating for 1 hour at 62°C. Different portions of this suspension were treated by mixing them with preparations of the acid lipidic or insaponifiable fractions of various origins, such as human placenta, cow, carp or rabbit organs; entire bodies of guinea pigs or rats; entire bodies of squid; seeds of Bixa orellana; microbes such as Esch. coli, B. subtilis, and tubercle bacilli. Fatty acids such as oleic, linoleic, eleostearic or mixtures of acids obtained from

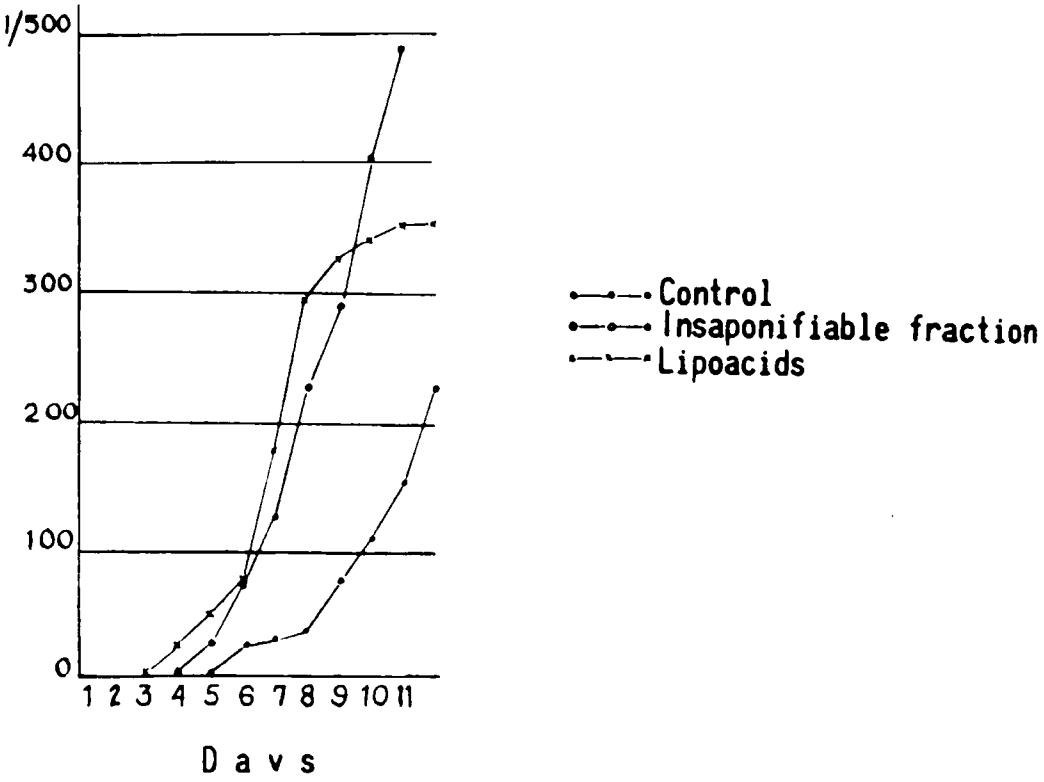

FIG. 265A. *Lipids and agglutinines.* Influence exerted by the administration of unsaponifiable lipid fraction and the acid lipid fraction of human placenta upon the appearance of *agglutinines* against Eb. typhi vaccines injected intravenously. Each curve corresponds to the average value of 5 rabbits. The agglutinines appear earlier and their amount increases more rapidly in the animals treated with lipids than in the controls.

cod liver oil or butanol or heptanol also were used. 5 cc. of a 2% alcoholic solution of the lipoids were introduced in 110 cc. distilled water and the solvent eliminated by boiling the mixture to bring the amount to 100 cc. 1-5 cc. of these milky preparations were added to 5 cc. of the microbe suspension and incubated at 37°C for 24 hours. The treated microbes were then separated by centrifugation, the fluid decanted and the microbes resuspended in the same amount of saline. They were used in doses from 1/10-1 cc. for subcutaneous injections in rabbits, repeated every third day for two weeks. As controls, animals were injected with the same amounts of suspension of untreated microbes.

For the first two weeks small amounts of blood were obtained every third day from the ear veins of the treated animals. Blood was obtained from the heart every second week for 8 weeks. The agglutinins were determined for all the samples. In the two-week samples, the presence of immune antibodies was determined by the capacity of different amounts of the sera to prevent a lethal infection in mice given intraperitoneal injections of standard amounts of living microbes. The agglutinins were seen to appear earlier than in controls—in a manner similar to that seen when lipids were injected in the animal as related in Chapter 7 Note 5. It was especially in the immune protective antibodies where the difference was manifest. It was not only an earlier appearance of these antibodies, but the protection against a lethal injection was obtained with much smaller amounts of serum.

Chapter 7, Note 7. **Skin Allergy**

An aqueous extract of squid body was prepared by blending it and extracting with saline in a proportion of 1/20. After filtration, the mixture was centrifuged and the supernatant fluid put in ampules with methiolate added as a preservative. Some of the ampules were tyndallized by heating them at 56°C one hour daily for four consecutive days.

1/10 cc. of these preparations was injected intradermically in various subjects who also received control injections of saline. Immediate and 24 hour reactions were noted. An induration present the second day was considered as a positive cellular response, while the immediate appearance of a hive was considered as a reaction taking place in the metazoic compartment.

Twelve days after a first injection in exactly the same place, a second injection was given with the same material. The immediate and the 24 hour reactions were judged. If the reaction was negative, a third injection was made, ten days after the second, in the same place. It could be seen that in normal individuals, the second sometimes, and the third injection always induced second day induration which persisted for several days. In cancer patients, including those in terminal condition, the injection of this antigen induced virtually the same reaction as in normal individuals.

Chapter 9, Note 1. **Hemoglobinuria a frigore (244)**

Certain information about shock which has emerged from the study of a rare condition is worth noting here. When a patient with paroxysmal hemoglobinuria—also known as both hemoglobinuria a frigore and cold hemoglobinuria, immerses his hand in icy water, he experiences a chill a half-hour later which is followed by the appearance of hemoglobin in the urine. Classically, this phenomenon was considered to result from the intervention of a hemoshock. We investigated such "attacks" of hemoglobinuria in three cases, inducing and studying the phenomenon several times in each subject. Usually, observations were carried out during a three hour

period after the immersion of the patient's hands for ten minutes in icy water and included the following procedures:

1) Measurements of blood pressure and temperature every 5 minutes;
2) Determinations at ten minute intervals, of coagulation time, clot retraction, white cell count and differential; serum hemoglobin content, serum proteins, antitryptic power and esterase—all measurements being made on venous blood.
3) White cell count and differential measured on capillary blood obtained every 10 minutes by finger puncture at 5 minute intervals after withdrawals of venous samples.
4) Tests for the presence and amount of hemoglobin in the urine at 15-minute intervals.

Coagulation time was established in the centrifuge tube and was related to the moment when blood ceased to flow if the vertical position of the tube was changed. Clot retraction was determined by centrifuging the coagulated blood after 2 hours at room temperature and measuring the amount of serum obtained from 15 cc. of blood. The serum and urine hemoglobin content was determined photometrically. For total protein content, we used both the refractometric index of the serum and gravimetric measurements after adequate precipitation. For antitryptic power we determined the inhibitory effect of the serum upon the digestion of a solution of casein by trypsin. The quantity of esterase present was determined by the changes upon ethyl-butyrate.

The data obtained were plotted as curves with time as common abscissa. Parallel variations were observed in all three patients during repeatedly induced attacks.

For almost all analyses, except for the presence of hemoglobin in urine, the variations indicated a diphasic phenomenon. *(Fig. 266)* The first phase was characterized clinically by hypotension and slight hypothermia. The characteristic analytical changes were leucopenia, prolonged coagulation time, reduction in clot retraction, lower refractometric serum value, lower antitryptic serum power and increased serum esterase. During this first phase of the diphasic phenomenon, hemoglobin also appeared in the serum and, when abundant in the serum, also was found in the urine. The first phase was followed by a second 5 to 10 minutes later. The clinical manifestations were a sensation of chill, varying from very slight to severe, followed by temperature elevation and slight hypertension. Analytical changes in the opposite direction from those noted during the first phase could be seen. Hyperleucocytosis, reduced coagulation time, higher retraction of the clots, elevated refractometric value and antitryptic power, and reduced esterase content were characteristic of the second phase. Hemoglobin present in the serum in the previous phase disappeared at this time.

The most interesting finding in paroxysmal hemoglobinuria was that two or three such distinct diphasic episodes followed each immersion. In all cases, the first diphasic complex appeared in about 10 minutes after

immersion. It was relatively mild and lasted in general about 10 minutes, after which all values returned to pre-attack.

About a half-hour later, however, a second diphasic complex, much more intense in its manifestations, was noted. Hypothermia and hypotension were more marked. Leucopenia was more intense, the number of leucocytes falling to as low as 200 per cubic mm. The quantity of hemoglobin in the serum was very high, with hemoglobin spilling over into the urine in large amounts. Serum antitryptic power decreased to much lower levels than during the first diphasic complex. In some cases the coagulation time increased to 15 minutes and the clot almost failed to retract. These changes

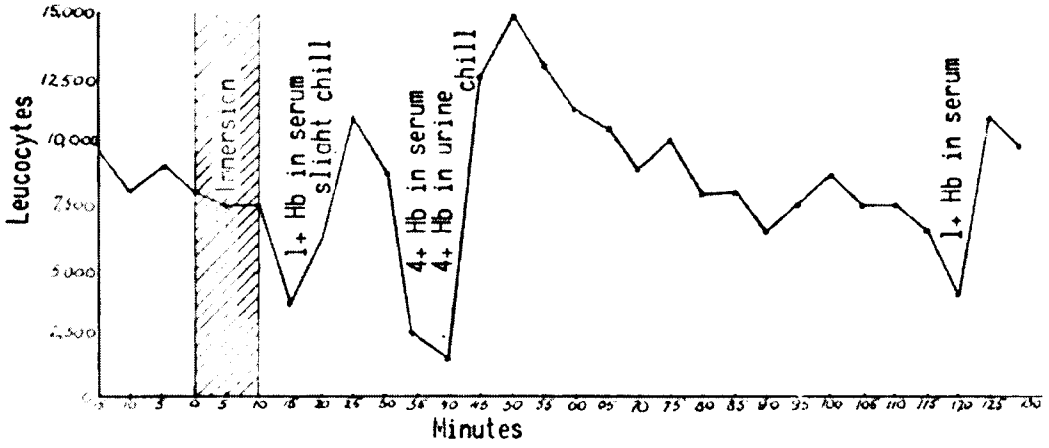

FIG. 266. The clinical data and analytical changes of blood and urine following the immersion in icy water of the hand of a subject with hemoglobinuria a frigore reveal three distinct diphasic phenomena corresponding respectively to three separate hemo shocks. Their intensity appears correlated to the degree of the occurring leucopenia.

were followed by the second phase of the complex, with severe chill and manifest changes in the analyses in the opposite direction. It is the second phase of the diphasic complex that is recognized clinically as the "attack" of hemoglobinuria. The chill was often very intense, followed by a temperature above 39°C, and hypertension. Leucocytes increased to as much as 20,000 per cmm., blood coagulation time was abnormally shortened and clot retraction increased. Hemoglobin disappeared rapidly from the serum. The albumin content as well as the antitryptic power of the serum increased, while the esterase content fell. In about 30 minutes, however, all these changes were dampened and the blood slowly regained its normal characteristics. This period, with almost all manifestations slowly returning to normal often was followed by a third diphasic complex, not clinically evident but revealed by hematological findings. In most cases, it appeared about two hours after immersion. While it was much milder than the first two, its diphasic character was quite clear. Occasionally the patient reported a slight sensation of cold. The amount of hemoglobin in the serum was less

than during the first complex, and hemoglobinuria was never seen. Figure 266 shows these findings in a typical succession of these complexes.

An analysis of these cases indicated two striking characteristics. One was the time sequence of the three diphasic changes which was uniform for all attacks in all subjects. The second was that although they differed in intensity, all the attacks were qualitatively alike.

In further studies we tried to understand the meaning of these changes. Similar changes, but without hemoglobin in serum and urine, and with only slight chill as a clinical manifestation, could be observed when the hands of normal individuals were placed in icy water for 10 minutes. Two similar diphasic phenomena were seen to appear. While their intensity was greatly reduced, the time of appearance of the two diphasic phenomena observed

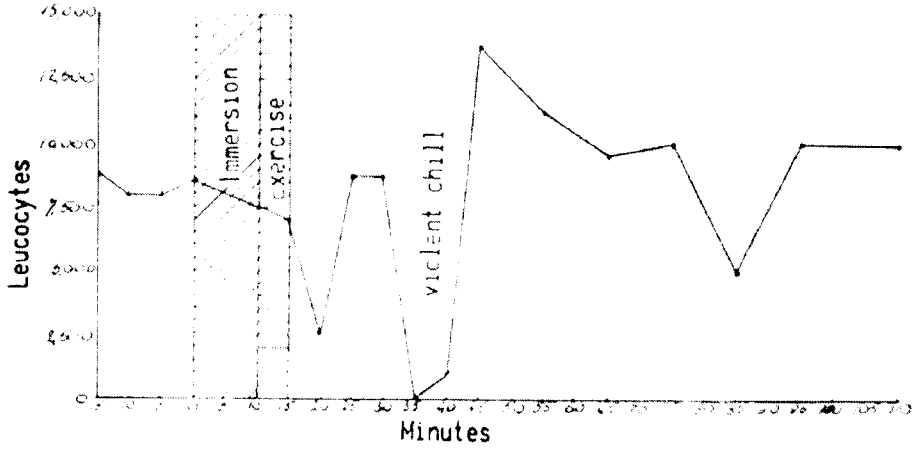

FIG. 267. In the patient with hemoglobinuria a frigore, exercise after immersion of the hand in icy water induces a violent chill with abundant hemoglobinuria.

was basically the same as in patients with cold hemoglobinuria. This would indicate that the leucolysis in hemoglobinuria a frigore corresponds to a physiological response which induces hemolysis because the red cells have been sensitized by cold. This sensitization is recognized in the Donath-Landsteiner reaction. It appears probable that leucolysis liberates the complement necessary to induce the hemolysis of the sensitized red cells.

We have tried to correlate these changes with other processes encountered in normal and abnormal physiology. In differential studies of blood smears obtained during an induced attack of hemoglobinuria, nuclear shadows were observed in the smears at the time of the leucopenia of the first phase. This was exceptionally manifest for the second diphasic complex, when a marked leucopenia occurs. We have seen above that this leucopenia has been correlated to the lysis of the leucocytes.

In patients with cold hemoglobinuria, we were able to show that, if with any physical exercise after the hands were out of the icy water, such as even walking in the room, the severity of the attack induced was much greater than when they were allowed to rest quietly. Not only was the

severity of the chill and the degree of hemoglobinuria corresponding to the second diphasic phenomenon greatly increased, but all other changes were similarly intensified. The number of leucocytes decreased to less than 200 per cubic mm. Blood coagulation time went to values as high as over ½ hour with almost no retraction of the clots. The antitryptic power of the serum, obtained only after centrifugation, reached the lowest values observed. Figs. 266 and 267 show the changes for the same subject with and without physical exercise. These findings could explain observations indicating the importance of rest, after blood transfusions. With the direct transfusion method, where 500 cc. of blood was administered in less than 10 minutes, chill was seldom seen in patients resting quietly, while it was constantly seen to appear in subjects taking any exercise immediately following the transfusion. (Fig. 265)

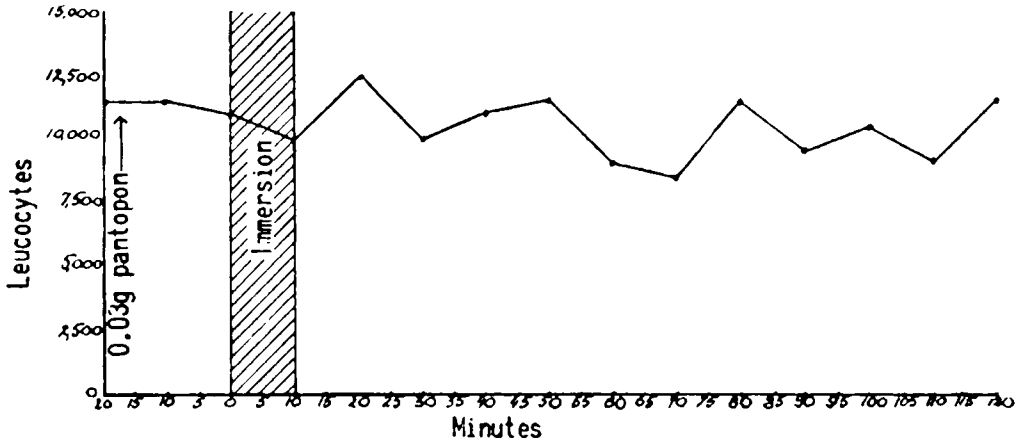

FIG. 268. The administration of 0.03 gm. Pantopon prevents the appearance of manifestations after the immersion of the hand in icy water, in the subject with hemoglobinuria a frigore.

This relationship of leucolysis to the pathogenesis of the diphasic complex phenomenon in hemoglobinuria a frigore was confirmed when the attack could be prevented by a pretreatment which influenced the leucocytes. The study of the influence exercised by various agents in vitro upon the leucocytes of a pleural effusion preparation from rabbits, when collargol solutions were added, has shown that only morphine and other opium alkaloids were able to prevent lysis. Adrenaline and quinine—among others—appeared inactive. Administered to patients with paroxysmal hemoglobinuria, these last substances did not prevent attacks. However, morphine in minimal doses, such as 5 mgr. (1/12 g.) or less, by intravenous injection, entirely prevented the development of any clinical manifestations as well as analytic changes. Minimal or no leucopenia was seen, and there were no changes in coagulation time, retraction of the clot, antitryptic power, refraction index of the serum, esterase, etc. *(Fig. 268)*

In addition to confirming the hypothesis that leucolysis intervenes in the

pathogenesis of the diphasic complex, the influence of morphine has furnished a means of preventing the attacks.

The presence of two or three successive diphasic phenomena in the case of paroxystic hemoglobinuria attack has revealed another important relationship. As mentioned above, hemolysis has been seen to occur only in the first phase of the diphasic phenomenon. Hemoglobin disappears from the serum between the complexes. It is classically accepted that in cold hemoglobinuria two factors must intervene to produce hemolysis. One is sensitization of the red cells which results from the influence of cold; the other is the presence of the complement. It appears that while sensitization persists for a while after exposure to cold, hemolysis will occur when the second condition is also fulfilled. Complement is released in the first phase of the diphasic phenomena since it is during the first phase that hemolysis occurs through the changes induced by the lysis of the leucocytes. The correlation between these two processes is clear from the fact that, under the influence of morphine, leucolysis does not take place and consequently hemolysis is prevented.

Chapter 9, Note 2. **Lipids and Rouleaux and Sludge Formation**

Human blood obtained through venous puncture was mixed in the syringe with 1/10 of its volume of 1.5% sodium citrate, passed into several centrifuge tubes and separated into plasma and cells. The plasma from the various centrifuge tubes was placed in separate test tubes. One of these samples of plasma was treated with a mixture of conjugated fatty acids of cod liver oil, another with a lipoacid preparation of human placenta, a third with a preparation of the insaponifiable fraction of human placenta. As control, the plasma was treated with liquid paraffin. The mixtures, frequently shaken, were kept in a warm bath at 37°C for one hour, after which they were centrifuged and the oily material separated. The plasma treated with the lipoacids was then added in proportion of 10% to untreated plasmas which then were reunited with their red cells. The plasma treated with the insaponifiable fraction was mixed directly with its respective red cells. Plasma and red cells were shaken for five minutes and left at room temperature for another ten minutes. One small drop of this blood obtained with a platinum loop or a capillary pipette was mixed with two drops of saline on a slide covered by glass and examined under the microscope. While the controls showed few short rouleaux, the blood treated with insaponifiable fraction showed isolated cells. In the blood treated with placenta lipoacids, almost all of the red cells formed rouleaux; in blood treated with conjugated fatty acid, almost all cells formed sludges.

Chapter 9, Note 3. **Dark Color of the Blood in Shock**

In order to determine why dark-colored blood is seen in shock and the role of fatty acids, the following experiments were performed.

Venous blood of patients in severe state of shock was drawn and mixed

with 1/10 of its volume of a citrate solution. The hematocrit value was determined and saline added in order to bring it to the value of the normal blood. Through these blood samples, oxygen was passed for five minutes at the rate of 50 cc. per minute. At the same time, blood from a normal person was obtained and similarly treated. The changes in color of the two samples after cessation of oxygenation were compared. While the normal blood needed almost ten minutes to return to the previous color, the blood from the patient in shock was back to the deep dark color in less than three minutes.

Blood samples from subjects in shock were treated in vitro with unsaponifiable fractions and subsequently with oxygen for five minutes at the rate of 50 cc. per minute. While the color became red immediately, the time required for it to return to the previous dark color was entirely different from that of controls. As against a few minutes for controls, more than twelve minutes were required for the blood treated with insaponifiable fraction. From these experiments it appears that the dark color of blood in shock results from changes in the red cells and not because of impaired circulation, and that these changes can be related to the intervention of fatty acids. This was confirmed by the fact that the dark blood of shock patients, if treated in vitro with insaponifiable fractions of placenta, for instance, loses its characteristic color.

Chapter 9, Note 4. **Induction of Acute Shock**

Acute shock can be induced by intraperitoneal injections of mixtures of conjugated fatty acids. The preparation largely used was a 10% solution in oil of fatty acid of cod liver oil conjugated through treatment with KOH in ethylene glycol or in ethyl alcohol. For a rat of 200 grams, 8 cc. of this preparation injected at once was able to induce an acute shock.

Chapter 9, Note 5. **Induction of State of Shock**

State of shock was induced by the repeated administration of a mixture of conjugated fatty acids obtained from cod liver oil. To insure a progressive systemic absorption, the preparation was injected subcutaneously. The injection in rats of 1 cc. per 100 gram of body weight of the 10% solution of these fatty acids in oil, repeated every hour was seen to induce after 3-5 injections a state of shock. The addition of 4% of sodium thiosulfate in a dose of 5 cc. per 100 gram of body weight in rats, was seen to favorize the appearance of this state of shock.

Chapter 9, Note 6. **Influence of Fatty Acids Upon Traumatic Shock**

Rats of 250 grams were introduced in the Collip-Noble Drum with their forepaws taped with adhesive and submitted to 500 falls at a rate of 40 per minute. 50% died of acute shock in less than two hours. If 2 cc. of a preparation of 10% cod liver oil fatty acids in oil per 100 grams of body

weight was injected intraperitoneally or even subcutaneously ½ hour before the animals were placed in the drum, more than 50% of the animals died during the trauma itself and the fatality rate in some experiments approached 100%. Bleeding from the nose and mouth was dark in color and smaller in quantity than in untreated animals. If the same amount of the lipoacid preparation was injected immediately after the animal came out of the drum, it also increased mortality within the first two hours. For some animals, death occurred in a few minutes after the injection.

Chapter 9, Note 7. **Influence of Unsaponifiable Fractions Upon Traumatic Shock**

The influence exerted by the unsaponifiable fractions upon traumatic shock appeared evident in rats submitted to 5-700 falls in the Collip-Noble drum. 10% solutions in sesame oil of the unsaponifiable fraction of human or cow placenta, of eggs or of butter, were used in these experiments. 1 to 5 cc. of these solutions were injected intraperitoneally at different intervals before or after trauma. The injection of 2 cc., ½ hour before trauma, was seen to entirely prevent lethal shock (0/20) in a group of experiments where the mortality of controls was 18/20. The same results were obtained with 2-3 cc. of the preparations injected immediately after the animals were taken out of the drum. Doses as high as 5 cc. injected one hour after the animal was removed from the drum, protected only a few animals (11/20) and generally only those without symptoms of shock. Once the symptoms of shock were present, the effect of the unsaponifiable fractions was greatly reduced. (From 2/20 to 5/20 in different experiments.)

Chapter 10, Note 1. **Oxalic Index**

The need to have quantitative information about the amount of double bonds present in the organism or in its lipoacids has led to a method of analysis based on the fact that molecular breakdown or fission will furnish characteristic components. With fission occurring at the level of the double bonds, the fraction corresponding to a conjugated double bond will appear as oxalic acid. The problem was to obtain this fission with a carboxyl corresponding to each carbon and without having artificially induced displacements of the double bond which is a frequent result of treatment.

We employed the following technique. Fatty acids from an organism or any other preparation were neutralized with the exact amount of sodium carbonate necessary. This amount was established through the neutralization index of the substances to be treated. After sufficient dilution, an excess of sodium carbonate was added with the aim of obtaining an alkaline medium. After bringing the solution to 4°C potassium permanganate was added until further discoloration of the permanganate stopped, after which 20% more of the amount already used was added. The mixture was kept refrigerated at 4°C for 16 hours, after which the excess of permanganate was reduced by sodium bisulfide. The liquid obtained was filtered and the

precipitate washed. The liquid was extracted first with ether to eliminate the higher fatty acids, after which it was submitted to distillation in order to eliminate the volatile fatty acids. In the remaining part, the oxalic acid was precipitated with calcium chloride. From the precipitate, the part corresponding to calcium malonate was separated from calcium oxalate, by using the difference in solubility at the boiling temperature. The oxalic acid was then titrated in the usual manner. The amount of oxalic acid divided by the quantity of lipoacids used represents the oxalic index of the preparation. Carlos Huesca Mejia and Daisy Franco have widely studied the changes of this oxalic acid index in our laboratories.

Pure nonconjugated fatty acids treated in this manner yield no oxalic acid. When linoleic acid is conjugated (*e.g.* by treating with KOH in ethylene glycol) oxalic acid is found in the fission products in amounts that gradually change as the treatment continues. (TABLE XXXIV)

TABLE XXXIV

THE QUANTITY OF OXALIC ACID PRESENT AFTER OXIDATIVE FISSION AND IODINE NUMBER OF SAMPLES OF LINOLEIC ACID CONJUGATED FOR DIFFERENT PERIODS OF TIME. (LINOLEIC ACID MIXED WITH EQUAL QUANTITIES OF KOH: DISSOLVED 5% IN ETHYLENE GLYCOL; CONTINUOUS TREATMENT IN REFLUX.)

Time	Oxalic Acid mg/gm of Fatty Acid	Iodine Number
Before treatment	0	180
After 30 minutes	117	119.8
" 1 hour	205	115.1
" 2 hours	114.2	94
" 4 hours	119.4	96
" 8 hours	99.9	91
" 12 hours	92	86
" 24 hours	85	81.7
" 36 hours	80	76
" 48 hours	77.3	76.5
" 144 hours	40.8	57.3

The quantitative relationship between known proportions of conjugated fatty acids and the oxalic acid obtained through their oxidative fission has been studied.

Autolytic changes have been found to influence the nature of the fatty acids extracted from tissues. Studies have shown that formalin fixation does not significantly change the fatty acids present. (TABLE XXXV)

It has been noted that relatively stronger methods of conjugation, utilizing KOH at higher concentration and higher temperature, are necessary to conjugate the fatty acids of normal tissues, than are needed for the fatty acids extracted from pathological tissues (burn, shock, adrenalectomy, tumor necrosis) which can readily be conjugated by much milder procedures.

TABLE XXXV

EFFECT OF FORMALIN UPON THE QUANTITY OF OXALIC ACID PRODUCED BY OXIDATIVE FISSION OF A MIXTURE OF CONJUGATED FATTY ACIDS. (5 cc. ALIQUOTS OF A MIXTURE OF CONJUGATED FATTY ACIDS WERE MIXED IN A STOPPERED CYLINDER WITH 10 cc. OF A 20% FORMOL SOLUTION. THE CYLINDER WAS SHAKEN AND SAMPLES WERE REMOVED AT FREQUENT INTERVALS FOR DETERMINATION OF OXALIC ACID.)

Time	Mgm Oxalic Acid/gm Fatty Acids
Before mixing	121.7
After 1 hour	121.8
" 24 hours	120.5
" 48 hours	120.9
" 72 hours	118.9
" 144 hours	121.9

Chapter 10, Note 2. **Irradiation and Oxalic Index**

Forty male albino rats separated in groups of 10 were irradiated with nonfiltered X-rays from a 200,000 v. machine, receiving in one session a dose of 1500 r. which is considered a lethal dose. Similar experiments were repeated several times, some with the radiation dose obtained from radioactive cobalt. The four groups of animals were first mixed together and then separated in four big cages. The animals and controls were kept on Purina Chow and water as libitum. Two of the controls, two of the treated animals, and any others approaching death were sacrificed daily.

Each dead animal was saponified separately. The total amount of acid lipids extracted was analyzed for conjugated fatty acids, using the method indicated previously in which the amount of oxalic acid which appears as the result of oxidative fission is measured. The values were expressed in oxalic index, which corresponds to the amount of oxalic acid in milligrams per gram of fatty acids. Figure 85 shows the results of two such experiments.

Values found for normals were zero or less than 0.5, but a constant increase of the oxalic index was noted after irradiation. While irregular values were still seen during the first three days, all were above 3 on the fourth day and continued to increase constantly afterward. Death occurred when the oxalic index reached a critical point which was found to correspond to 14-17 mgr. of oxalic acid per gram of the fatty acids of the entire body.

Chapter 10, Note 3. **Oxalic Index in Sublethal Irradiation**

We studied the changes in oxalic acid in animals treated with an amount of radiation below the lethal doses. The oxalic acid rose after treatment with values much lower than those seen when lethal doses were used. Three groups of ten animals each were radiated with 600 r., and every few days

two of the animals were sacrificed along with two of the controls. Figure 91 shows the values obtained for these animals. It could be seen that although indices of 6 and 7 were found, these never reached the critical point of 14, and that the indices went down in an irregular fashion. While some animals had values of around 4 and 5 after the tenth day, others had high values at the same time.

The administration of polyunsaturated conjugated fatty acids (1-2 cc. daily of a 5% oily solution of conjugated cod liver oil fatty acid) induced death in a high proportion (16/20) of animals irradiated with otherwise nonlethal doses such as 800 r. In these animals the oxalic index was high, often showing values above 17.

Chapter 10, Note 4. **Radiation-induced Offbalances**

Through the routine analytical technique used for the study of the offbalances, we studied the changes occurring in a group of 56 subjects with different conditions, submitted to radiotherapy alone. We tried to correlate the clinical noxious effects of radiation to these offbalances.

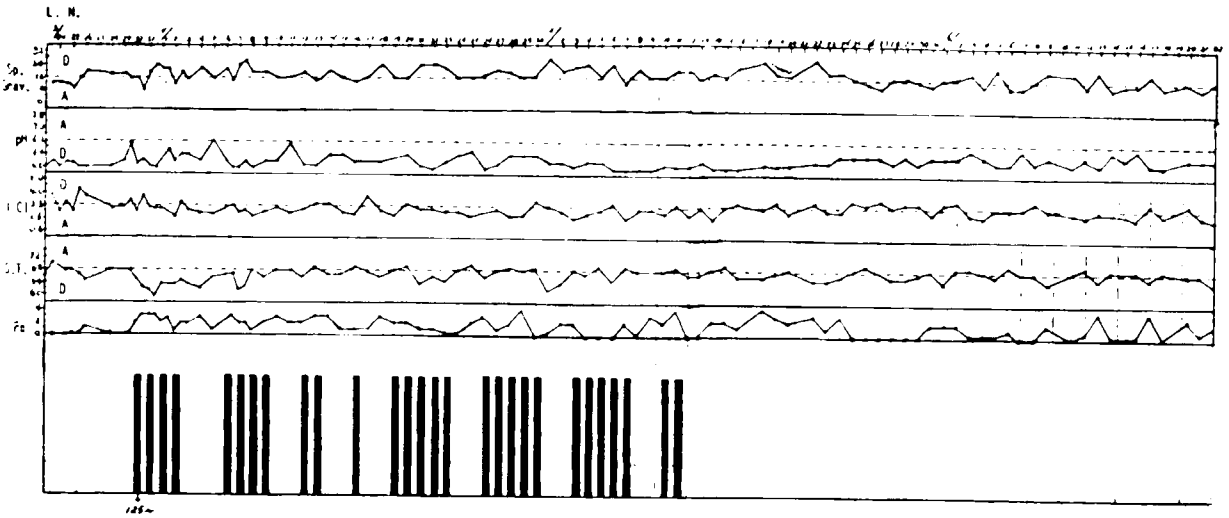

FIG. 270. L.N., 59 years old. Cancer of the breast. Mastectomy two months earlier. No recurrence. Treated with radiation, 125r per seance. Entirely uneventful except moderate erythema. Minimal or no changes in the analyses.

In this part of the study, we limited ourselves to the consideration of the immediate changes. Figs. 270 and 271 show the analyses in two cases in which no clinical, local or systemic changes were observed during and after the radiation. It can also be seen that no changes occurred in the analyses; they remained within normal limits during the entire period of observation. It is especially noted that the peroxides were present, almost constantly in high amounts in the urine.

Fig. 272 shows a case who died during the radiotherapeutic treatment, probably directly influenced by it. All the analyses show that a change to-

ward offbalance type D had occurred. Among these, we want to single out the urinary surface tension and the urinary chloride index, both evidencing very manifest changes toward the patterns corresponding to type D. The peroxides were on the contrary almost completely missing from the urine.

Another case *(Fig. 273)* had severe clinical reaction to radiation which persisted until the death of the patient. Again, we judged these effects of radiation mainly through the changes occurring in the two analyses, urinary surface tension and chloride index, which show the same shift toward a strong offbalance type D. The peroxide reaction became negative at the end.

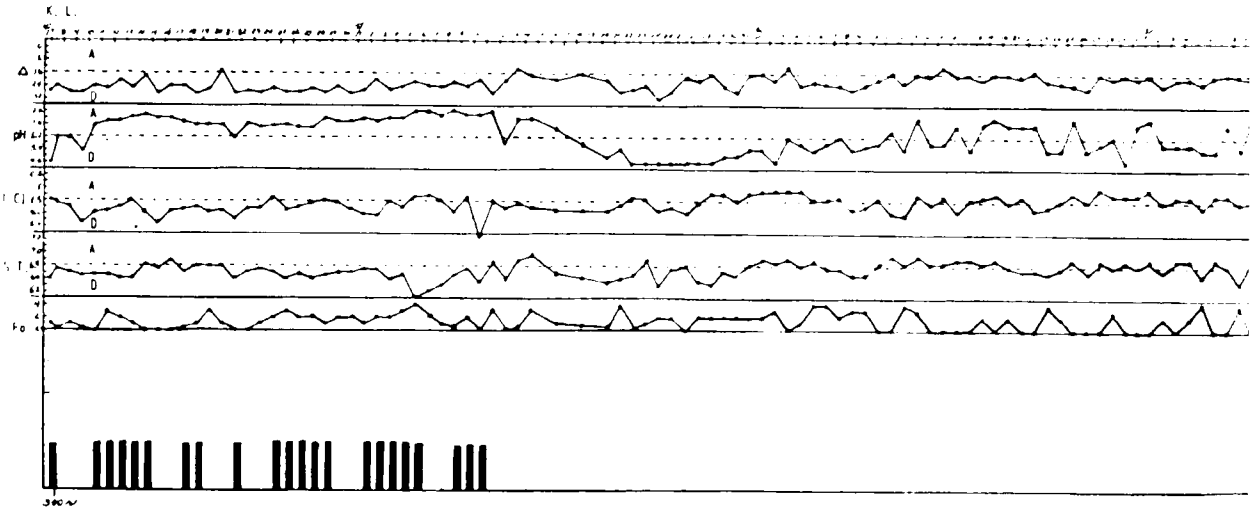

FIG. 271. K.L. 52 years old. Cancer of the hypopharynx. Treated with 300r for each session. The high specific gravity shows a tendency toward the type A, a month after the completion of the treatment.

While specific gravity and ST show a slight offbalance D during the treatment and the pH a manifest change toward the type D, following the radiation all the analyses show a change toward the type A, one month after completion of the radiation. Peroxides persisted in the urine. Clinically the entire evolution was eventless.

When the different tests were discussed, we mentioned that each one of the analyses used furnished information concerning changes which take place at a specific level of the organization. This would explain why the noxious effects are seen to be serious when the changes take place concomitantly in different analyses, that is, at different levels, indicating thus a more complete offbalance. When this concomitance does not exist, when the abnormal patterns concern only one analysis, the clinical manifestations are seen to be less serious. This was seen true in the case shown in Fig. 274. The importance of this concomitance in the changes present is seen in Fig. 275. In this case, although manifest changes corresponding to type D are seen for some analysis, they do not coincide. The peroxide reaction in the urine is constantly positive. This seems to permit the patient to withstand the noxious effect of radiation. The evolution of the changes induced by radium application is shown in Fig. 276.

The possibility to evaluate through urine analyses the noxious effects of radiations, has appeared especially important for the prevention and even treatment of the serious inconveniences during radiation therapy. By observing the changes in the analyses, particularly of the urinary surface tension and chloride index, valuable information can be obtained, permitting one to guide the application of these therapeutic agents. By being easily and continuously informed about the occurring changes, we need no longer consider the amounts of radiation to be administered as standard values for each patient. Through analytically guided radiotherapy one can replace the common pattern presently used for all patients by

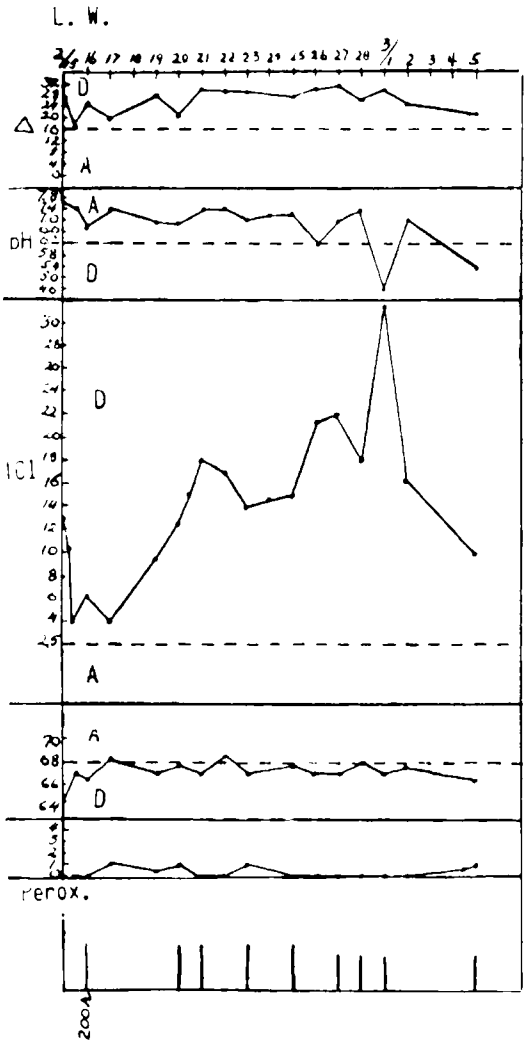

FIG. 272. L.W., 58 years old. Cancer of the lung. After receiving only 1000r, very weak. Much worse after, with very rapid downhill course. After the treatment on 3/5, the patient entered into coma and died 3/7. The extremely high values for Cl I and high specific gravity characterize the analytical changes. It is interesting to note the negative reaction for peroxides for almost the entire time.

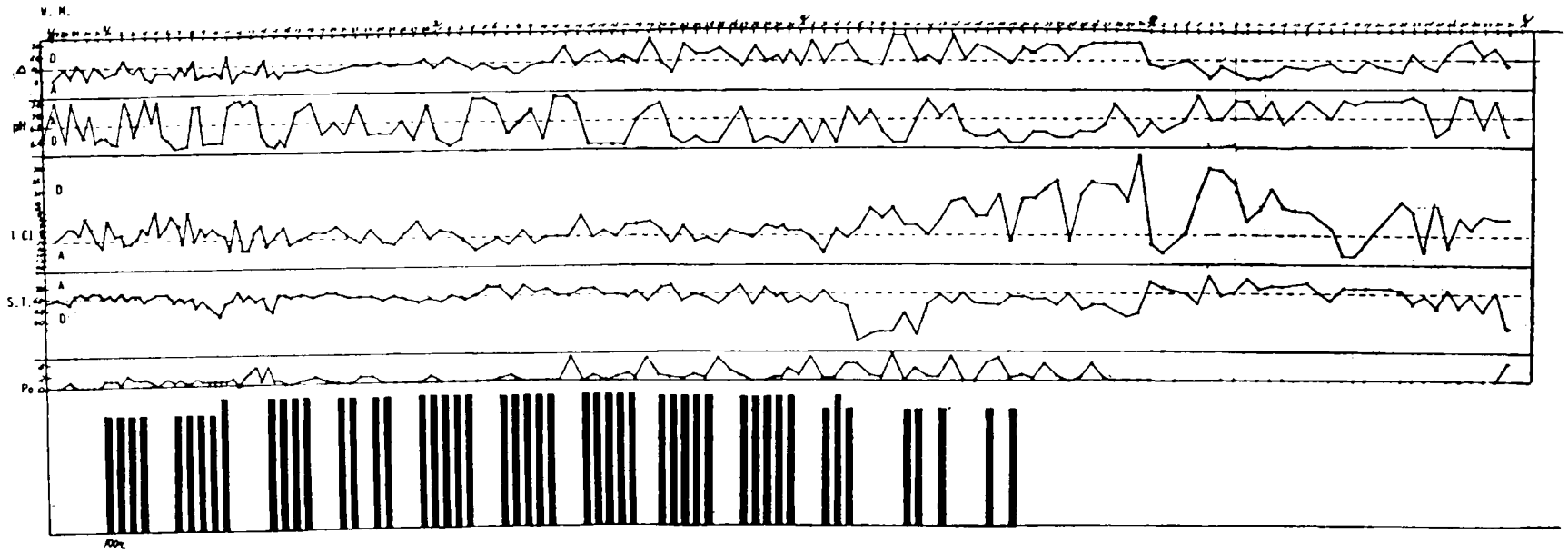

FIG. 273. W.M., 50 years old. Cancer of the ovary with abdominal metastases. Repeated doses of 100 and 120r. Almost uneventful for more than 1½ months of treatment after which the specific gravity analyses pass into the D pattern. After two months, the Cl index and ST show manifest concomitant changes toward the D pattern, a fact which coincided with a worsening of the general condition. In spite of changes in these analyses toward more normal values, the condition *worsened* with a lethal issue. The peroxide negative reaction—of bad prognostic in radiotherapy—is to be noted.

individualized treatments adapted to the need and the response of each subject.

Continuously followed analyses permit their utilization as a guide for more general application of radiotherapy. When the surface tension and chloride index remain within normal values, treatment can be continued with administration of doses above those originally intended, without any danger of serious noxious effects. A change toward low surface tension

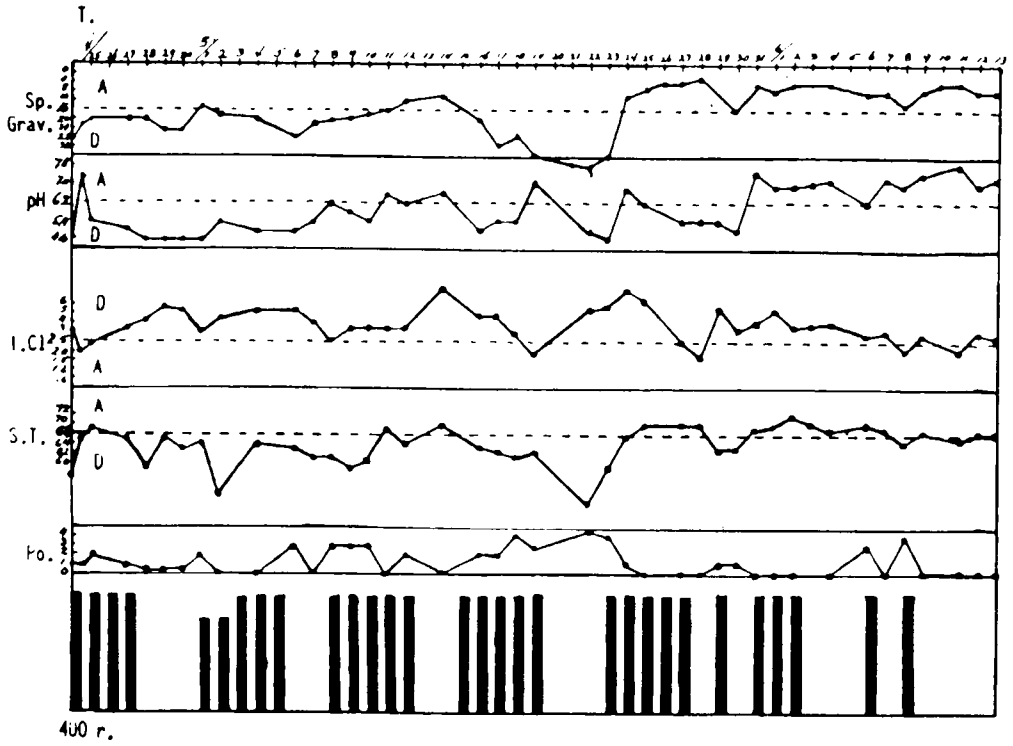

FIG. 274. Cancer of the cervix intensively irradiated. Although changes occur in the analyses, the passages in offbalance D do not coincide in the different tests. With the continuation of the treatment, the patient passes in offbalance A. No clinical, noxious manifestations were seen.

below 65 dynes/cm. or high chloride index above 5, represents a warning which should not be ignored. The treatment should be discontinued, the dosage reduced or the sessions more widely spaced, even if the desired radiation dose has not yet been attained. Concomitant changes of the analyses should constitute a serious warning even when the general clinical condition does not indicate any abnormality. The bad prognosis of persistent strong "D" offbalance during radiation is related to the progression of the anomaly as described in the experimental studies. For this reason, a persistent strong offbalance D seen for the urinary surface tension and chloride index indicates the need for the administration of lipoids with positive character, even if the clinical manifestations are not too serious.

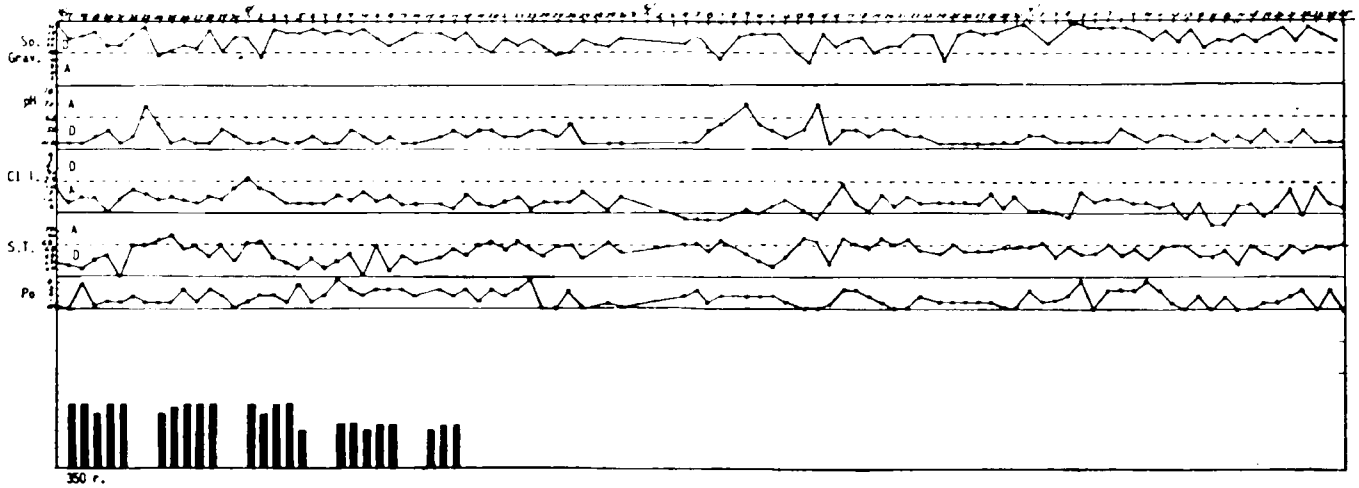

FIG. 275. Cancer of the larynx. No changes in the analyses. Entirely without clinical noxious reactions. (The Cl Index values are inverted.)

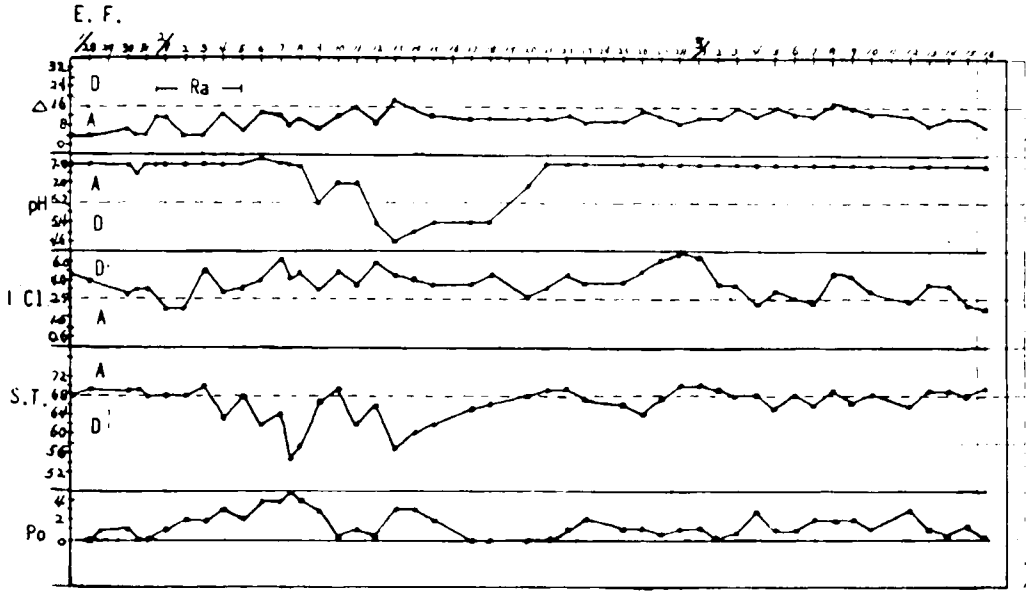

FIG. 276. F.E., 64 years old. Cancer of the fundus of the uterus, treated with 45 mgr. radium for 122 hours in situ, with a total of 5490 mgr. hours. Felt subjectively well after radiation without any complaint. The analyses show a manifest change for almost one week from the type A to type D. For the S.T. it starts 2 days after the insertion of radium with the values dropping from 70 to 55. For the pH, the change started a few days later with the values passing from 7.8 to 4.6. The changes of Cl I show values as high as 7 without however, having the changes coincide with the pH and S.T. 16 days after radium was taken out, the analyses went to previous values, except the peroxides, which remain present.

Chapter 11, Note 1. Carcinogenic Activity of Urethane

The interesting research of Berenblum has brought an important contribution not only for the largely debated role of urethane as carcinogen, but also for the problem of carcinogenesis in general. The fact that croton oil, applied to the skin, induces the appearance of malignant tumors in animals previously fed with urethane, concords largely with the concept of plural changes taking place in carcinogenesis. The analysis of the influence exerted by carbamic acid upon amino-acids would place the intervention of this agent at the first members of the biological realm. It can thus be seen that the bond between the amino-acid group and the carboxyl and amine groups of carbamic acid occur in a way similar to that which occurs between two amino-acids with the big difference that in the first case it would result in the appearance of the CNCN formation. *(Fig. 277)* As mentioned above, this CNCN formation represents the group which charcterizes the first biological entity. The place of this CNCN group, not at the end of the molecule opposed to the carboxyl as in the alkaline amino-acids, but as corresponding to the bond which results in polymers, represents the anomaly, which according to the work hypothesis we advance, would correspond to the first cancerous entity. The fact that the carcino-

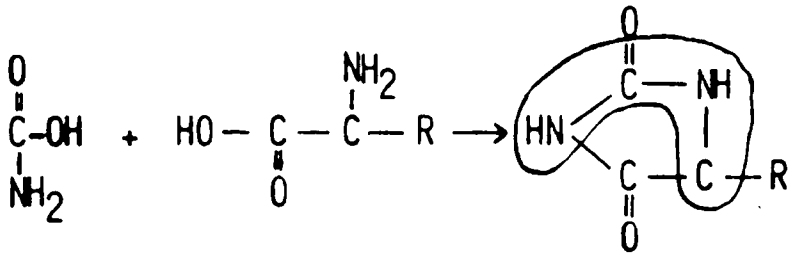

FIG. 277. The bond between the carbamic acid and an amino-acid group leads to the formation of the NCNC group.

genic activity of urethane takes place at the lowest levels of organization, explains the necessity that a certain time separates its intervention from that of croton oil, which would act only at the higher levels, probably inducing the passage from noninvasive to invasive phase. This time is necessary for the first cancerous changes to build up the series of cancerous hierarchic entities since the cocarcinogen, croton oil, would act only in those more evoluted cancerous entities. In experiments in course, the passage of the noninvasive urethane-induced carcinogenesis entities into invasive cancer, is successfully induced by preparations of unsaponifiable fractions.

Chapter 11, Note 2. **Constitution of Viruses**

It is superfluous to emphasize the interest with which viruses are being studied from all points of view. Their role in carcinogenesis has placed them in the limelight of cancer research, and any contribution concerning their constitution or activity is of great interest. A much debated initial problem concerns the nature of viruses and their place among the other entities. (293)

Two fundamental groups of constituents—DNA and proteins—(301, 289) have been recognized to take part in the formation of the viruses. These two groups could be separated and reunited, reproducing the original virus with all its characters. Futhermore, new viruses could be created when fractions resulting from different viruses were bound together. (289) However, the fact that a part of the virus, the DNA fraction, was seen to be furnished by the constituents of the host cell, and the protein directly by it (312), has raised the question of the nature of the virus itself. Some workers have gone so far as to see the viruses as parts of the constituents of the cells. By considering the viruses in the concept of the hierarchic organization presented above, a new aspect emerges.

According to this organizational concept, a virus represents an entity that has reached a certain step in the hierarchic evolution, and remained there throughout its individualization. Like all entities, a virus can be conceived to be formed by a principal part bound to a secondary part, the ensuing entity limited by a boundary formation. The principal part would be formed by a group of immediately inferior entities in the hierarchic

scale. In the case of viruses, such inferior entities would be formed by what we could call "proviruses," which correspond to characteristic DNA formations. The principal part of the virus would be formed by the grouping together of provirus entities, proper to each virus. The secondary part is conceived to result from the immediate environment of these entities, taken directly from the host's own protoplasmatic or nuclear formation in which the principal parts are present as free entities. This secondary part is represented by the protein fraction furnished as such by the invaded nucleus or cell protoplasmatic formations. This protein fraction conserving its characteristics can be recognized and identified.

Having nuclear formations, nuclei and protoplasmatic formations as their environment, the principal part of the virus, the proviruses, multiply as proper hierarchic entities. These proviruses will leave the host usually when the cell bursts, bound this time to the proper secondary parts directly furnished by the host entity. Under these conditions, the principal part multiplied in the protoplasmatic formations or in the nucleus and the secondary parts, furnished as such by the host, would form an immediately higher entity, the virus. In the multiplication of the virus (299), the pattern followed is the same as that of other hierarchic entities. This has to occur in the proper environment which, for the viruses, is the immediately higher hierarchic entity, the nuclear level. This is represented in microbes by the individual itself, and in cells by the nucleus or by the protoplasmatic formations, which we consider to belong to the nuclear level, due to their ribonucleo-proteins. It is in these nuclei or protoplasmatic formations that the viruses multiply. This explains the development of viruses in cells in compact groups, which would correspond to parasitated protoplasmatic formations and not in a diffuse form in the cytoplasma. The virus loses its secondary part upon entering the entity where it will multiply, but will take it back when it leaves its host, becoming again the entity, virus. The parasitated entity has contributed two parts to the multiplication of the virus—one, indirectly, by furnishing material which will be utilized by the provirus and transformed into its specific DNA, and two, the secondary part, directly formed by its own proteins. Like all the secondary parts, that of the virus directly furnished by the host will surround the group of proviruses forming the principal part.

Chapter 12, Note 1. **Lipids and Cytolytic Activity of Sera** (245, 246)

The fact that cancer cells are found circulating in the blood without inducing metastases throughout the body, as might be expected, has made various workers investigate the means by which the organism rids itself of these cells and which might represent part of a general process through which the organism could fight cancer. Research to confirm the capacity of blood sera for influencing the lysis of cancer cells has been carried on in our laboratories by Robert Willheim and co-workers. Sera of both normal individuals and cancer patients were found to induce lysis of cancer cells in vitro. Ehrlich, Krebs and Sa 180 ascites in mice were used, as well

as cells obtained from solid tumors with encephaloid character. Tumors obtained from biopsies or autopsies of human beings also were used. Lytic activity was determined through the dilution of sera which later was mixed with a suspension of a known number of cells. After incubation for one hour

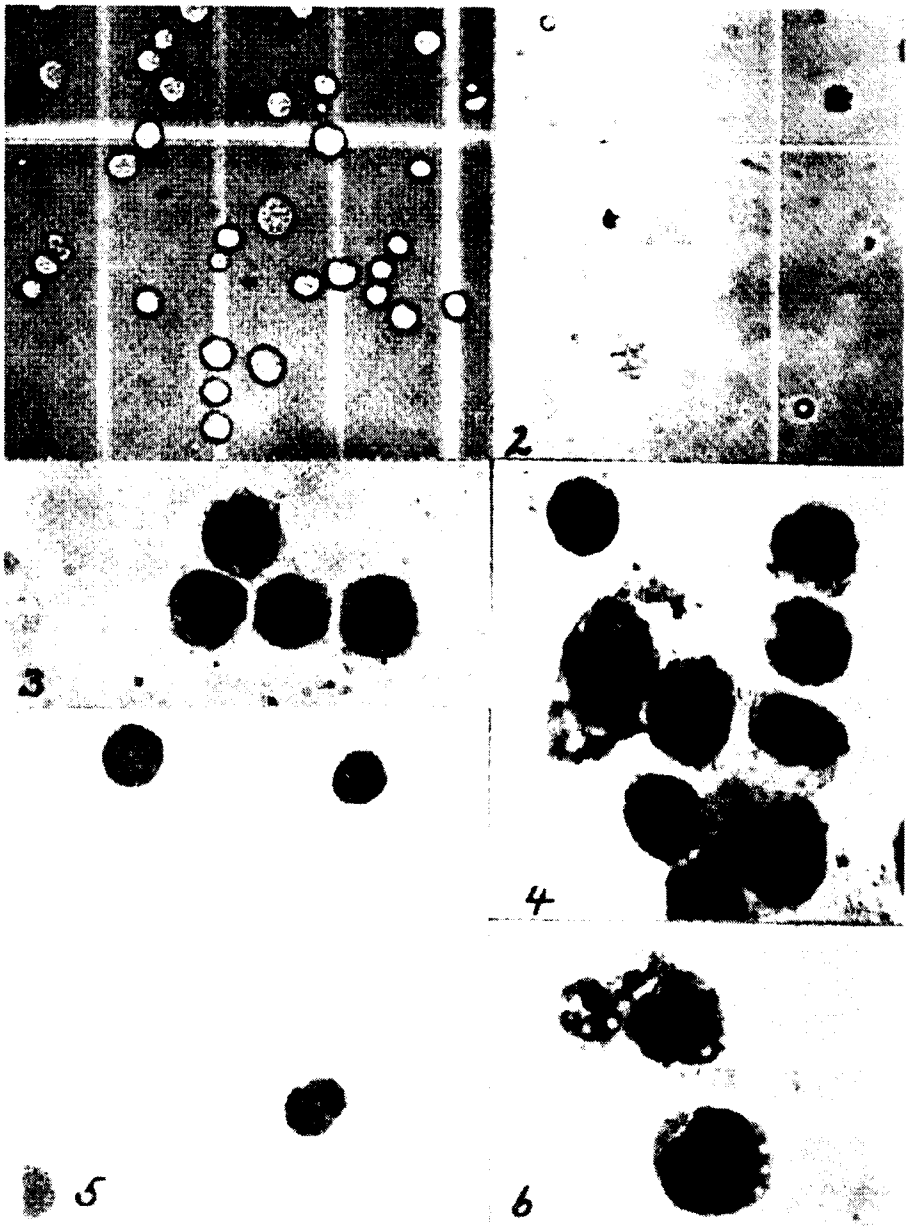

FIG. 278. Lytic action of human serum upon Sa 180 ascites cells.
(1) Normal SA 180 Cells X 300, not stained.
(2) Lytic effect of Serum on SA 180 X 300, not stained.
(3) Normal SA 180 Cells X 1000, Giemsa stain.
(4) Lytic effect of Serum on SA 180 Cells X 1000, Giemsa stain.
(5) Normal Krebs Cells X 1000, Giemsa stain.
(6) Lytic effect of Serum on Krebs Cells X 1000, Giemsa stain.

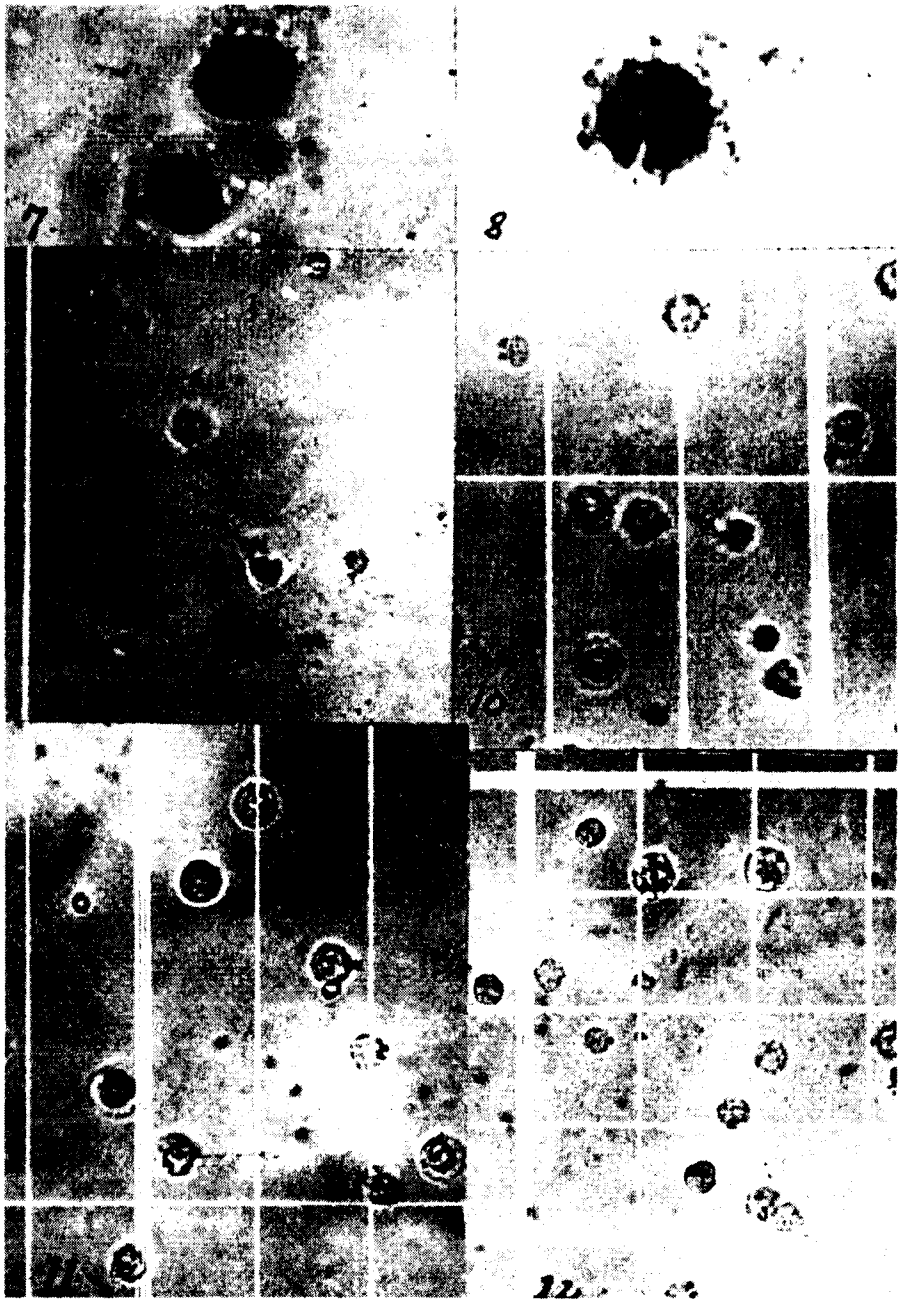

FIG. 279. Continuation—

(7) Normal SA 180 Cells X 1800, Giemsa stain.

(8) Carcinolytic effect of Serum on SA cells X 1800, Giemsa stain.

(9) Normal Lysis of SA 180 Cells as control in Polysaccharides Inhibition-Studies on Carcinolysis X 300, not stained.

(10) Effect on Carcinolysis by the Polysaccharide Dextran X 300, not stained.

(11) Effect of Levan on Carcinolysis. Note the clearly distinct outline of unaffected cells X 300, not stained.

(12) Effect of Mucin on Carcinolysis X 300, not stained (evidence of inhibited Lysis.

at 37°C the product was examined and the cells counted in an adequate chamber. *(Fig. 278)* Relatively big variations were seen in different individuals, the results being negative for 1:1 dilution in some cases and positive in 1:32 and even higher for other individuals. No correlation could be established between this lytic power and the clinical condition of an individual.

With R. Willheim and M. Auber (247) we showed that the addition of a suspension of unsaturated fatty acids is able to induce such a lytic property in sera without this capacity, while the addition of insaponifiable fractions or of cholesterol inhibited lytic activity. With R. Willheim and M. Auber we (248) have investigated the problem of the correlation between the lytic activity and the structure of different fatty acids and their sodium soaps. The higher members of the fatty acid series have shown intense lytic activity. It could be shown that the lipoidic character of the fatty acids increases their lytic capacity. The value of the lipoidic character of the fatty acids used has appeared when members with the same number of carbons but having an hydroxyl or carboxyl in their molecule, were tested. With disappearance of lipoidic character, lytic property disappeared. The research covered not only cancer cells but also liver, red blood cells and lymphocytes. It could be seen that a correlation exists between the rapid growth character of these cells and the capacity of the fatty acids to attack them. It is interesting to note that cancer cells treated in vitro with fatty acids having lytic activity no longer produced cancerous growths when transplanted to animals. (249)

Chapter 12, Note 2. **Fatty Acids Transportation in the Blood**

It appeared especially interesting to study the distribution and transport of polyunsaturated fatty acids in the body in view of the fact that parenteral administration of the acid lipidic fraction of various organs to subjects with acute pain had an effect within only a few minutes. The rapid action was independent of the nature of the induced change, that is, decrease of the intensity of pain of acid pattern, and increase of pain of an alkaline pattern. This effect in opposite directions, occurring after the same short interval and seen in hundreds of cases, eliminated the possibility of a psychological factor, as suspected at the beginning.

The fact that the change occurs at the level of the painful lesion itself raised the question of rapid transport between the injection site and the lesion. In order to investigate it, we used two fatty acid preparations containing easily identifiable substances, norbixine and polyconjugated fatty acids. Norbixine could be identified by its characteristic color while the polyconjugated fatty acids were identified by their specific curves in spectral analysis in ultraviolet light.

Adult New Zealand rabbits were injected intraperitoneally with 8 cc. of 0.3% solution of bixine in sesame oil. The injected animals were bled at different intervals by heart puncture. The red cells were separated from the plasma by immediate rapid centrifugation. Each fraction, plasma and red

cells, was hydrolyzed separately with 5% KOH. The acid lipidic fractions obtained as a solution in benzene was passed through a chromatographic column with alumina. Bixine was easily recognized because of its red color. After elution with chloroform the amount present was determined photometrically. Similar experiments were made by using a 10% solution of eleostearic acid in oil. The acid lipidic fractions obtained separately from the red cells and the plasma were submitted to spectral analysis and the presence and amount determined by the characteristic peaks. Both norbixine and eleostearic acid were seen to appear in the red cells in less than two minutes, the amount increasing rapidly.

A marked difference was found between the amount of these fatty acids in the red cells and in the plasma of the same blood, for all the samples. The red cells contained 5 to 6 times as much of the injected lipids as the plasma. This unequal distribution, also seen for other highly unsaturated fatty acids, indicates an important physiological role for the red cells which has not been recognized before. Red cells appear to be preferred vehicles for transporting polyunsaturated fatty acids through the blood.

Chapter 12, Note 3. **Conjugation Method**

Spectral analysis of a mixture of fatty acids such as obtained from cod liver oil has shown that prolonged conjugation sometimes is detrimental for some members. Prolongation of conjugation was found necessary, however, since the members with a lower number of conjugated double bonds needed more time to appear. We investigated the factors which would intervene in these changes. High temperature was seen to affect the polyconjugated formations. With ethylene glycol or glycerol as solvent, conjugation took place rapidly, but the peaks of tetraenes and especially pentaenes and hexaenes were seen to go down rapidly. This was not seen to occur if the temperature of conjugation was lower. In this last case, the conjugation was seen to take much more time. This study led us to use ethyl alcohol as a solvent. Maximum conjugation however, required a longer time, usually around 100 hours. With this method we could obtain from the same preparations much higher amounts not only of conjugated pentane and hexane but also diene and triene. *(Fig. 280)* We also utilized the same method for analytical purposes with the same good results.

Chapter 12, Note 4. **Quenching Action and Anti-Carcinogenic Effect of Conjugated Fatty Acids**

We have investigated the influence exerted by different fatty acids, conjugated and nonconjugated, upon various carcinogens. In a first group of experiments we studied this influence in vitro, and chose the quenching of the fluorescence of the carcinogen as criterion of activity.

This part of the research was made in collaboration with C. Huesca-Mejia and P. Teitelbaum.

The quenching effect has been studied as follows:

The fluorescence of carcinogenic hydrocarbons is measured by means of the fluorescent attachment to the Beckman spectrophotometer using a wave length around 365 mμ. The sensitivity of the apparatus is adjusted to show a value of 100 for the fluorescent light utilizing the concentration of the hydrocarbon having the maximum fluorescence. The carcinogenic hydrocarbons are dissolved in alcohol, iso-octans or cyclohexane, the last two being purified and thus rendered optically inactive by passage through a silica column.

The fatty acids are dissolved in varying dilutions in the same solvents and added to the solution of hydrocarbon carcinogen which has been previously chosen to give a fluorescent value of 100. The fluorescence of the mixture is immediately determined. The quenching effect of the fatty acid is shown by the percent of the residual fluorescence of the carcinogen when mixed with different solutions of a mixture of conjugated fish oil fatty acids. TABLE XXXVI shows the quenching effect of mixtures of fatty acids conjugated by treatment with KOH upon different carcinogenic and related hydrocarbons.

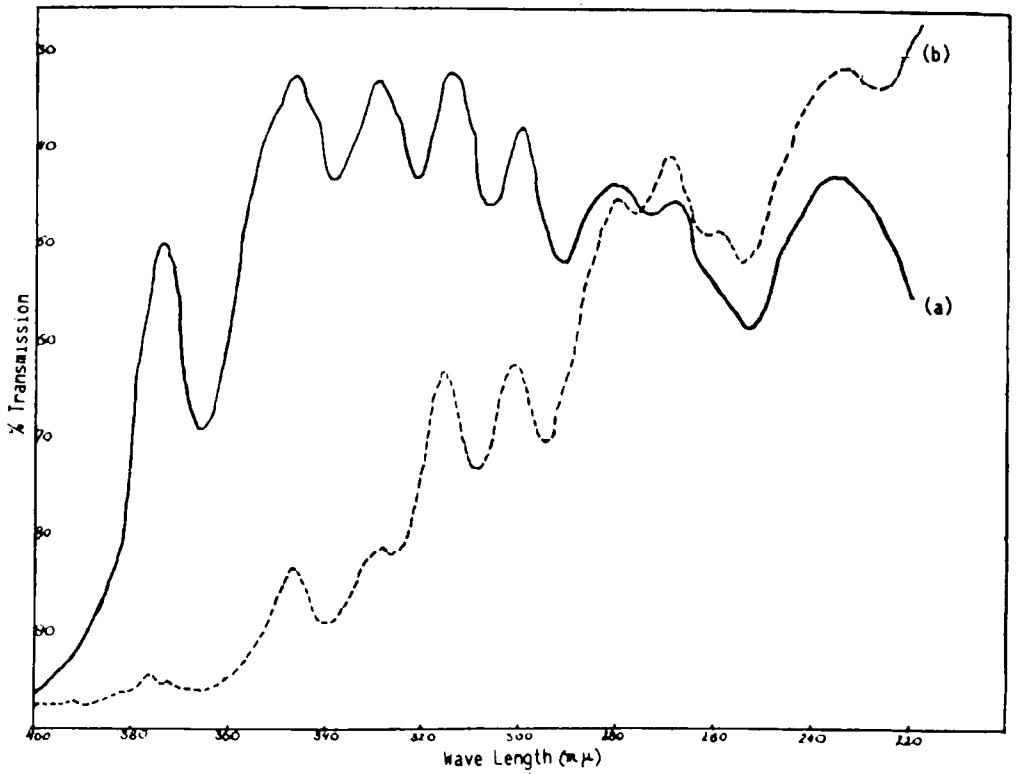

FIG. 280. Conjugation method. The use of ethylic alcohol as solvent for conjugation changes the amount of polyconjugated members obtained for cod liver oil fatty acids (a) as compared with those obtained when the conjugation is made at higher temperatures using ethyleneglycol, (b) glycerol or other solvents.
Dilution 0.002% in ethyl alcohol.

Table XXXVI

Quenching Effect of Conjugated Fatty Acid Mixtures Upon the Fluorescence of Carcinogenic and Related Hydrocarbons

Hydrocarbon	% Concentration	Quenching Agent	% Residual Fluorescence
		Mixture of conjugated fatty acids from cod liver oil	
Methylcholanthrene	0.0062	(0.1% solution)	25.0
3, 4 Benzpyrene	0.0062	"	36.0
1, 2 Benzanthracene	0.0031	"	24.0
1, 2, 5, 6 Dibenzanthracene	Sat. Sol. (alcohol)	"	15.2
9, 10 Dimethyl -1, 2 Benzanthracene	0.0062	"	23.0
		Mixture of conjugated fatty acids from fish oil	
Benzanthracene 12 ol -7 Methylacetate	0.005	(0.2% solution)	5.7
7 Chloro-10 Methyl -1, 2 Benzanthracene	0.005	"	7.9
1 Cholanthrene -3 Methyl	0.005	"	10.0
Benzo (d) Pyrene -5 Methyl	0.005	"	11.2
3, 10 Dimethyl -1, 2 Benzanthracene	0.001	"	6.5
7 Cyano-10 Methyl -1, 2 Benzanthracene	0.0057	"	4.0
5 Chloro-10 Methyl -1, 2 Benzanthracene	0.0025	"	5.8
6 Chloro-10 Methyl -1, 2 Benzanthracene .*	0.01	"	5.2
4 Methoxy-3, 4 Benzpyrene	0.005	"	13.0

* Fluorescence set at 45.

TABLE XXXVII

QUENCHING EFFECT OF VARIOUS NONCONJUGATED AND CONJUGATED FATTY ACID MIXTURES UPON THE FLUORESCENCE OF CARCINOGENIC AND RELATED HYDROCARBONS

Hydrocarbon	% Concentration	Fatty Acids from	Conjugated Fatty Acids from	B-Eleostearic Acid	Linoleic Acid	Conj. Linoleic Acid	Oleic Acid
		Cod Liver Oil					
		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Methylcholanthrene	0.0062	92.5	25.0	95.8	95.0	89.8	—
3, 4 Benzpyrene	0.0062	82.8	36.0	100.0	—		—
1, 2 Benzanthracene	0.0031	93.4	24.0	94.8	100.5	97.0	100.8
1, 2, 5, 6 Dibenzanthracene	Sat. Sol. (alcohol)	89.0	15.2	100.0	97.0	98.0	100.0
9, 10 Dimethyl -1, 2 Benzanthracene	0.0062	90.8	23.0	97.8	93.5	100.0	96.0
		Fish Oil					
		0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Benzanthracene 12 ol -7 Methylacetate	0.005	66.0	5.7	89.0	87.0	80.0	92.1
7 Chloro-10 Methyl -1, 2 Benzanthracene	0.005	67.0	7.9	84.0	85.0	87.0	87.0
1 Cholanthrene -3 Methyl	0.005	110.0	10.0	110.0	110.0	110.0	110.0
Benzo (d) Pyrene -5 Methyl	0.005	88.0	11.2	88.0	70.0	90.0	91.0
3, 10 Dimethyl -1, 2 Benzanthracene	0.001	72.0	6.5	63.0	78.0	74.0	73.0
7 Cyano-10 Methyl -1, 2 Benzanthracene	0.0057	83.5	4.0	91.0	90.2	88.5	91.0
5 Chloro-10 Methyl -1, 2 Benzanthracene	0.0025	89.0	5.8	92.0	95.0	92.0	93.5
6 Chloro-10 Methyl -1, 2 Benzanthracene *	0.01	47.0	5.2	43.0	45.0	42.0	45.0
4 Methoxy -3, 4 Benzpyrene	0.005	88.0	13.0	86.0	90.0	89.0	91.0

* Fluorescence set at 45.

Conjugated Fatty Acids and Quenching

Nonconjugated fatty acids such as linoleic acid, linolenic acid, arachidonic acid, mixed fatty acids from body liver oil and cod liver oil have a limited quenching action. Conjugated dienes such as isomers of linoleic acid or conjugated trienes such as eleostearic acid obtained through conjugation of linolenic acid or extracted from China wood oil also have a limited quenching effect upon hydrocarbon carcinogens and related compounds. (TABLE XXXVII) The same is true for mixtures of conjugated dienes and trienes.

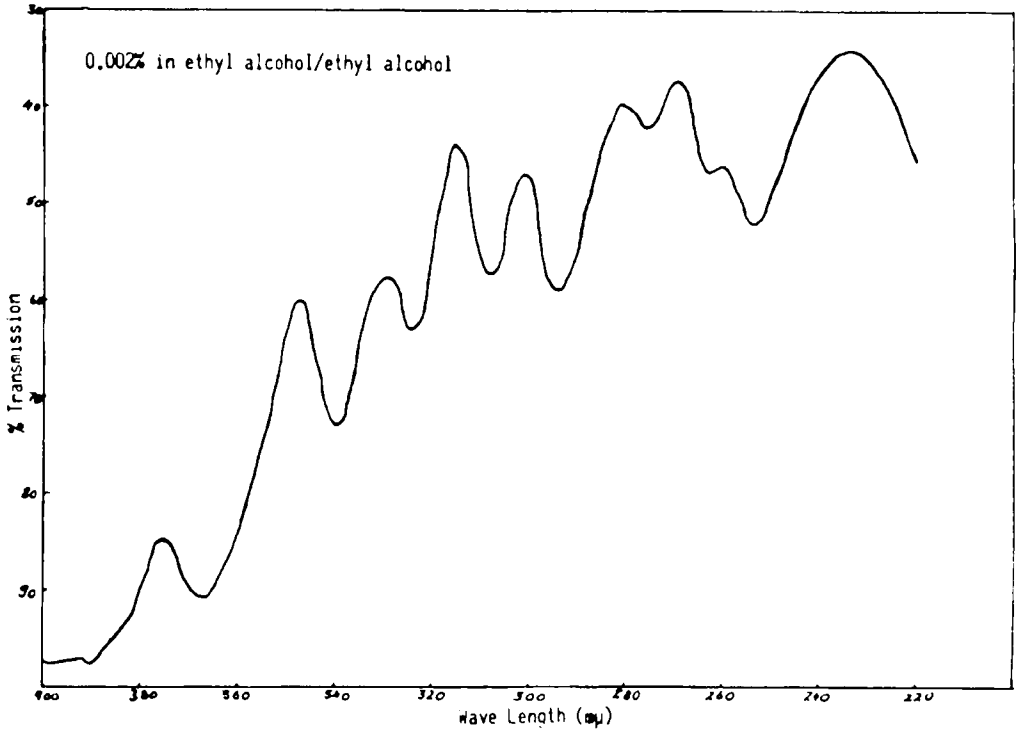

FIG. 281. Spectral analysis of conjugated cod liver oil fatty acids shows the presence of conjugated di-, tri-, tetra-, penta- and hexaenes.

Fatty acid mixtures having conjugated di-, tri-, tetra-, penta- and hexaenes as shown by spectral analysis *(Fig. 281)* have been found to exhibit a high degree of quenching activity *(Fig. 282)* (TABLE XXXVII) when mixed with hydrocarbon carcinogens.

The quenching action of fatty acids upon the fluorescence of hydrocarbon carcinogens appears to be nonadditive. When the incident ray is passed first through an 0.2% solution of conjugated fish oil fatty acids in alcohol and then through an 0.012% solution of methylcholanthrene in alcohol in separate vessels, the residual fluorescence is 81%. When the same two solutions are mixed together in one cell, the residual fluorescence is 11.2%.