The relationship of the different conjugated members to the quenching effect has been studied. Fatty acid mixtures having different proportions of isomers with 2, 3, 4, 5 and 6 conjugated double bonds were obtained by conjugation or by treatment of conjugated mixtures with heat, oxygen,

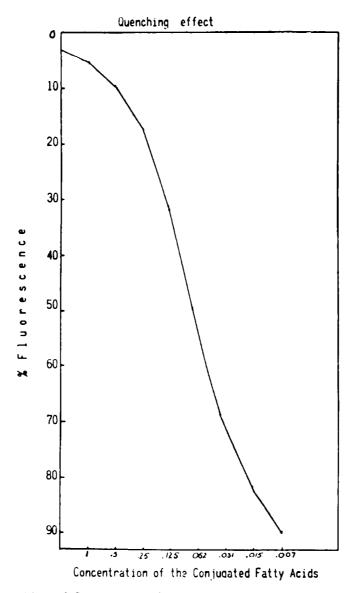


Fig. 282. Quenching of fluorescence of a methylcholanthrene (.0062%) solution in alcohol by different concentrations of conjugated fish oil fatty acids.

chlorine or sulfur. Changes in the proportions of the di-, tri-, tetra-, pentaand hexaenes were followed by means of spectral analyses. The changes in the height of the peaks in these curves corresponding to the different conjugated polyenes were then compared with the changes in the quenching effect of the corresponding fatty acid mixtures. Figure 283 shows the spectral analysis of samples obtained at various intervals during the action of oxygen upon a mixture of conjugated fatty acids. As seen, oxygen induces unequal changes in the height of the peaks in the curves of which correspond to di-, tri-, tetra-, penta- and hexaenes. Fig. 284 shows the quenching activity of the mixtures. It can be seen that a parallelism exists between the relative proportions of the tetraenic component and the quenching activity of

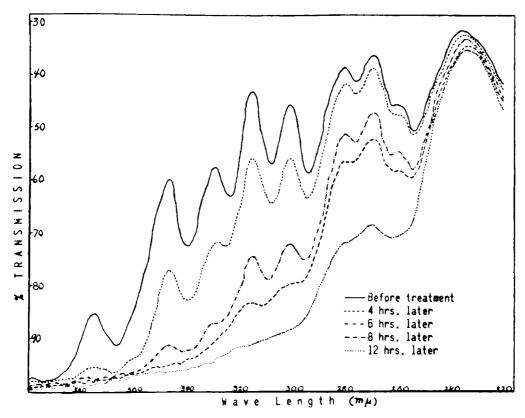


Fig. 283. Changes in the absorption spectra of a mixture of conjugated fish oil fatty acids induced by treatment with oxygen. The treatment has a greater effect on the higher unsaturated members, with the proportion of tri-, tetra-, penta-, and hexaenes decreasing as treatment continues, as seen by the reduction in the height or even disappearance of the peaks. After 12 hours of treatment, the conjugated dienes are the only ones not yet influenced.

Dilution 0.002% in ethyl alcohol.

the mixtures. In this experiment it appears that the quenching effect could also be related to the presence of conjugated pentaenes. Evidence available from other experiments do not, however, sufficiently support this.

We studied in a similar way the effect induced by the treatment—with sulfuric acid—of a mixture of conjugated fatty acids of cod liver oil. Fig. 285 shows part of the occurring changes and Fig. 286, the quenching effect.

Similarly, we studied the changes in the quenching effect during the conjugation with KOH of cod liver oil fatty acids in ethyl alcohol. Fig. 287



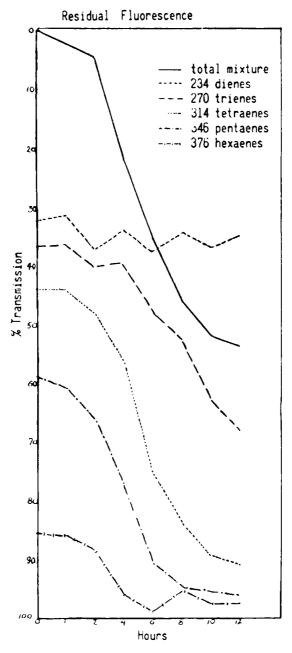


Fig. 284. The relationship between changes of the di-, tri-, tetra-, penta- and hexaenic peaks as found on spectral analysis of samples of conjugated fish oil fatty acids treated for different lengths of time with oxygen and the quenching effect of the same samples. A close parallelism exists between the decrease in the proportion of tetra- and pentaenic peaks and the quenching activity of the mixture.

shows the conjugation effect and 288, the quenching effect of the preparation at different moments, in various dilutions.

The entire problem was simplified by studying a pure conjugated tetraene. We have obtained pure tetraenic parinaric acid from akariton fat

of Parinarium laurinum seeds. In addition, we have prepared almost pure tetraenes utilizing the technique described by Maury, Brode and Brown. Unfortunately, with the last method, the results were less favorable, the proportion of tetraenes beginning to decrease long before the conjugated dienes and trienes have disappeared. Pure tetraenic conjugated acid has shown that the quenching action is related almost entirely to the tetraenic component alone, and in a mixture it is largely parallel to the content in conjugated tetraenic fatty acids. Fig. 289 shows the quenching curve induced by parinaric acid.

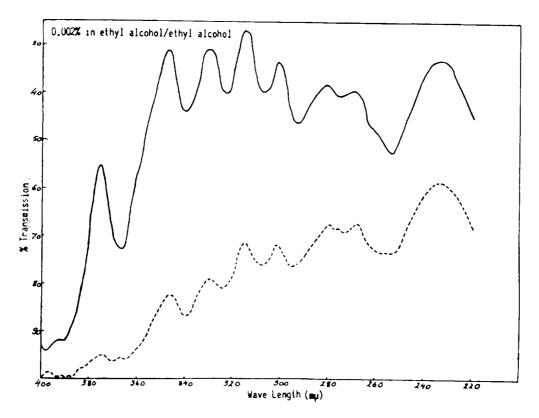


Fig. 285. The changes in the spectral analysis of a mixture of conjugated cod liver oil fatty acids, induced by the treatment with sulfuric acid. The treatment leads to unequal decrease in the amount of different conjugated members. Only two curves are shown: at the beginning of the treatment and at 260 minutes.

Conjugated Fatty Acids and Induced Carcinogenesis

We have investigated the influence exerted by the fatty acids—conjugated or not, and their mixture, upon the induction of tumors by carcinogens. From the various experiments, some were eliminated, either because the dose of methylcholanthrene employed did not produce tumors in a sufficient number in control animals to permit any conclusive comparison, or the death rate from intercurrent causes was abnormally high so that the entire experiments had to be discarded.

The experiments that were satisfactorily completed are summarized



in the following three tables. In the first group of experiments (TABLE XXXVIII), 4 groups each composed of 40 adult Swiss mice (20 male and 20 females in each group) were employed. Each animal received in the right flank a single subcutaneous injection 0.2 mg. of methylcholan-

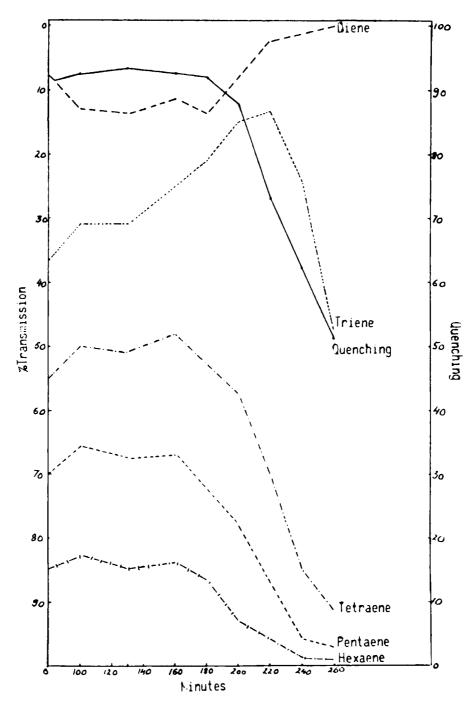


Fig. 286. The changes in the quenching of the treated mixture parallel the changes induced in the amounts of conjugated tetra-, penta- and hexaenes.

threne as a 0.2% solution in tricaprylin. These animals also received subcutaneous injections of a mixture of fatty acids extracted from cod liver oil, or a mixture of cod liver oil fatty acids conjugated by treating them with KOH. The fatty acids were administered as a 5% solution in cotton-

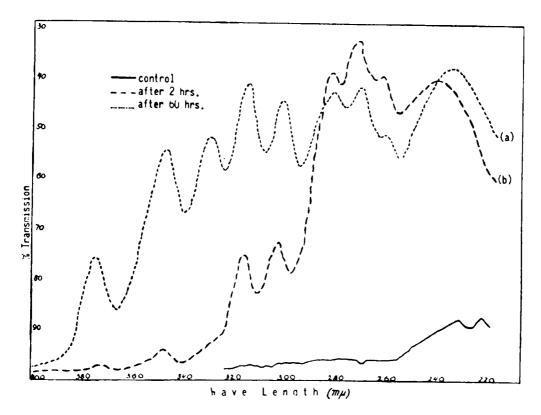


Fig. 287. Spectral analyses of fatty acids of cod liver oil, treated with KOH in ethyl alcohol. They show the appearance of high amounts of tetra-, penta- and hexaenes.

seed oil. Animals treated with fatty acids received 0.3 cc. of this solution in the contralateral side twice a week for three months. The control animals received the same volume of cottonseed oil in the same number of injections. In addition, one group of animals treated with the conjugated fatty acids received four injections during the two weeks preceding the

TABLE XXXVIII

	Died		%		
Treatment	Without Tumors	Tumors	With Tumors		
Cottonseed oil-controls	10	12/30	40		
Fatty acids from cod liver oi	1 3	18/37	48		
Conjugated fatty acids from					
cod liver oil	6	7/34	20		
Conjugated fatty acids from					
cod liver oil (*)	14	3/26	11		

^{*} Received 4 injections of fatty acids before methylcholanthrene was administered.

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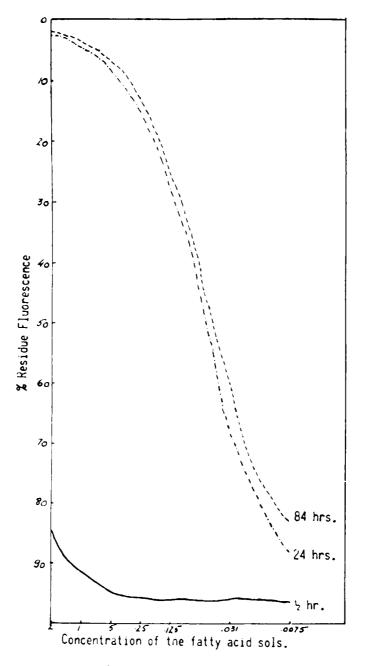


Fig. 288. Changes in the total quenching capacity of samples of cod liver oil during isomerization with KOH in ethyl alcohol. While the quenching effect is reduced—even for high concentration—for the sample having only ½ hour of conjugation, it is high for that obtained after 24 hours. It remains almost the same for the sample after 84 hours of conjugation. The quenching appears related to the presence of conjugated isomers, with 4 or more double bonds.

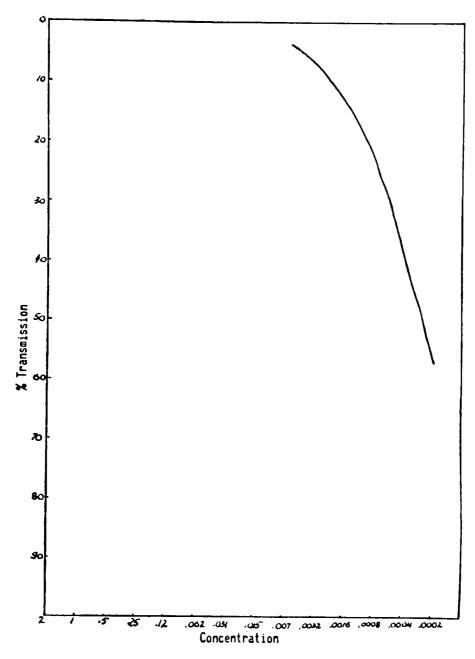


Fig. 289. Quenching effect of parinaric acid upon the fluorescence of methyl-cholanthrene. The relationship between the quenching effect and the presence of conjugated tetraenes is seen in the fact that parinaric acid has a quenching effect of 96.2 for a dilution of 0.006% and still one of 62% for a dilution of 0.0002%.

methylcholanthrene injection. Thirty-three animals died without tumors during the course of the experimental period. The number of animals surviving for five months plus the number in which tumors developed during this period of time are listed for each group.

In a second group of experiments (TABLE XXXIX) six groups of 40 mice each received one subcutaneous injection of 0.25 mgm. of methylcholanthrene of an 0.2% solution in tricaprylin and a second similar injection one week later. Groups were treated twice weekly for three months with 0.3 cc. of 5% solutions of the following fatty acids in cottonseed oil: Fatty acids from cod liver oil, conjugated fatty acids from cod liver oil, eleostearic acid, linoleic acid and conjugated linoleic acid. A control group of animals received 0.3 cc. of cottonseed oil in the contralateral flank twice weekly for three months.

TABLE XXXIX

	Died	%		
Treatment	Without Tumors	Tumors	With Tumors	
Cottonseed oil—controls	4	31/36	86	
Fatty acids from cod liver of	il 3	33/37	89	
Conjugated fatty acids from c	od:			
liver oil	6	15/34	44	
Eleostearic acid	10	24/30	80	
Linoleic acid	0	29/40	72	
Conjugated linoleic acid	6	25/34	73	

In the third group of experiments (TABLE XL), four groups of 30 mice each were employed. Mixtures of the methylcholanthrene and of the fatty acids used were prepared by adding 0.5 cc. of the 5% fatty acids in cotton-seed oil solutions to 0.25 mg. of methylcholanthrene of 0.25% solution in tricaprylin. The injections were made subcutaneously immediately after mixing. Each animal received three injections at intervals of one week and

TABLE XL

Treatment	% Residual Fluorescence		Tumors	% With Tumors
Methylcholanthrene + cottonseed oil	95	6	33/44	86
Methylcholanthrene + fatty acids from fish oil	85	4	20/46	43
Methylcholanthrene + conjugated fatty acids from fish liver oil	19	4	6/46	13
Methylcholanthrene + eleostearic and conjugated linoleic acid	92	11	24/39	61

the observations on tumor incidence were followed for 5 months. The fatty acids employed were fatty acids from fish oil, conjugated fatty acids from fish oil, a mixture of equal parts of eleostearic and conjugated linoleic acids, and cottonseed oil as a control. The quenching effect is shown as the percent of residual fluorescence of methylcholanthrene when mixed with the fatty acid mixtures.

These results indicate a certain relationship between the quenching action of the conjugated fatty acids upon hydrocarbon carcinogens and the ability of fatty acids to reduce the carcinogenicity of these hydrocarbons. It is not sufficient to have a conjugated fatty acid present, in order to have the effect upon carcinogenesis. Eleostearic acid did not significantly reduce the incidence of tumors and conjugated linoleic acid was no more active than its nonconjugated isomer.

Conjugated fish oil fatty acids (which contain di-, tri-, tetra-, pentaand hexanes) when mixed with methylcholanthrene reduced the tumor incidence to 13%, while a mixture of eleostearic and conjugated linoleic acid (di-, and triene conjugated acids) which have a limited quenching action gave an incidence of 61%. Although the incidence of tumors was much lower in the group receiving conjugated fish oil fatty acids, the nonconjugated fatty acids from the same source has a limited influence upon the cancer inducing property of the hydrocarbon. When fatty acids were not mixed with the carcinogen, but were injected separately, the nonconjugated acids appeared without effect.

Statistical analysis of the data from these three experiments show the following: the results are significant for the group treated with conjugated fatty acids from cod liver oil before and after methylcholanthrene was administered as compared with control group treated with cottonseed oil in Experiment I ($\chi^2 = 6.65$ on basis of tumor/no tumor). In Experiment II,

TABLE XLI

QUENCHING OF METHYLCHOLANTHRENE 0.062% IN ETHYL ALCOHOL
BY SUBSTANCES OTHER THAN FATTY ACIDS

Substance	% Dilution Used	Fluorescence
Glycerol	5.0	106.8
n-Butanol	4.5	96.4
Butyl mercaptan	1.0	102.5
Hexyl mercaptan	2.0	92.0
Dodecyl mercaptan	2.0	82.0
Hexadecyl mercaptan	2.0	70.0
Na thiosulfate	.05 cc. from	97.0
	50% solution	
Ethyl sulfate	1.0	95.0
Nitrogen mustard	0.1	79.9
Allyl K xanthate	1.0	3.8
Nitromethane	1.0	7.4
Ethylene trithiocarbamate	1.0	.2
Cholesterol	1.0	93.0



the results are very significant for the group treated with conjugated fatty acids of cod liver oil as compared with the control group ($\chi^2 = 13.09$). In Experiment III, the results are very significant for all three groups in which fatty acids were added to the methylcholanthrene ($\chi^2 = 13.3$, 41.56 and 8.32 respectively).

When comparison is made on the basis of tumors/no tumors between groups receiving nonconjugated and conjugated isomers of the same fatty acid mixtures, the results were significant in all three experiments ($\chi^2 = 8$ in Experiment II, 22 in Experiment III).

In the light of the relationship between quenching activity and the reduction of the carcinogenic activity, we are investigating different other agents. Table XLI shows the values of this effect.

Chapter 12, Note 5. Lipids and Tumor Chlorides

We submitted groups of mice grafted with DBA mammary adenocarcinoma, to treatment with various lipoacids or positive lipoids preparations. After ten days of treatment the tumors were removed and analyzed for their content of chlorides, using the Volhard technic, in which the titration of silver nitrate was made electrometrically. As lipoacids, we used for experiment cod liver oil fatty acids, lipoacids from human placenta and butyl-mercaptan; for lipids with a positive character, we used cholesterol, insaponifiable fractions of human placenta and butanol. In all cases treated with lipoacids, the amount of chlorides was higher than in untreated controls. With cod liver oil fatty acids, values as high as 135% above those of controls were found. With the lipoacids of human placenta, the average value was 114% above that of controls; with mercaptans, 78% above. The influence exerted by the opposite lipids was much less manifest. With cholesterol and insaponifiable fractions, chloride values were 20% below those of controls; with butanol, 33% below.

Chapter 12, Note 6. a-OH Fatty Acid and Experimental Tumors

We studied the influence exerted by the series of alpha OH fatty acids, saturated and unsaturated, upon the evolution of different tumors in mice and rats, to find that only one member has a manifest effect which is limited to a single tumor. Subcutaneous grafts of 6C₃HED lymphosarcoma in C₃H mice grew with abnormal rapidity. 48 hours after the transplant, the tumor could be felt. A very soft, highly edematous and, for this reason, diffuse tumor developed rapidly so that death occurred usually around the tenth day. This tumor was especially resistant to most of the chemotherapeutic agents tested. Daily administration of a 5% solution of alpha OH caprylic acid in a dose of 0.2-0.5 cc., started even the fifth day after the graft when the tumor was already well-developed, was followed by its rapid involution and disappearance in a high proportion of cases (55/60). In the few cases in which the tumor persisted, its evolution was very much changed. The animals remained alive for more than a month. If, after three

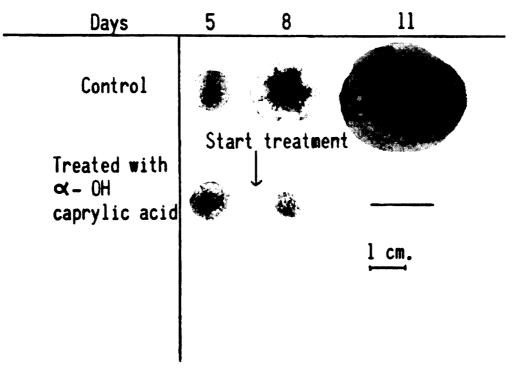


Fig. 290. The administration of alpha OH caprylic acid in mice bearing 6C₃HED lymphosarcoma tumor, induces the disappearance of the tumor in a high proportion of cases.

or four days, when the tumor was already highly reduced, the administration of the preparation was discontinued, the tumor began to grow again, but much more slowly than usual.

Chapter 12, Note 7. Hydropersulfides

The existence of different bonds between unsaturated fatty acids and oxygen led us to study different bonds similarly occurring between the same fatty acids and sulfur, the second member of the oxygen series. The treatment of polyunsaturated fatty acids or their triglycerides with sulfur has shown that two different formations can be obtained. By heating the mixtures above 110° but below 125° C, precipitated sulfur is incorporated without a manifest change in color or other properties. The iodine number is not changed. When conjugated fatty acids or their triglycerides are treated, no changes are seen in the spectral analysis. By heating above 130° C, the color of the preparation changed progressively reaching deep red-brown if the treatment is sufficiently prolonged. Concomitantly, the iodine number decreases progressively and eventually reaches zero. The spectral analysis of the conjugated fatty acids shows the peaks going progressively down until no more conjugated formations are present, indicating that these changes affect the double bond.

The analogy between the fixation effects of oxygen and sulfur has sug-



gested that the first bond corresponds to a hydropersulfide similar to a hydroperoxide. The second bond would represent a fixation of the sulfur at the level of the double bond itself, similar to a peroxide. Studies of similar bonds of sulfur were made in tetralin where hydropersulfides were obtained. The study of the properties of all these preparations seems to confirm the hypothesis that the compounds obtained are hydropersulfides.

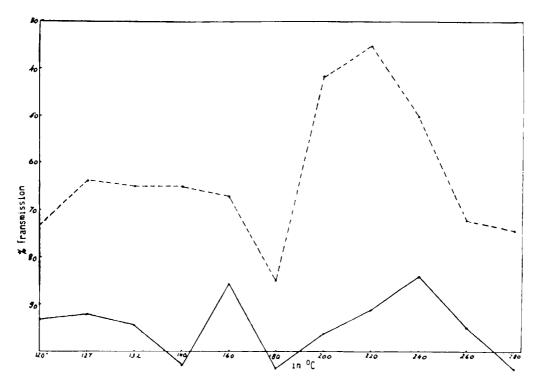


Fig. 291. Quenching of methylcholanthrene fluorescence in samples obtained when 0.5% sulfur in cottonseed oil was heated from 120°C to 280°C. The samples were dissolved in ether-alcohol mixture and mixed in equal part with .0125% methylcholanthrene. The existence of concomitant changes in the oil with sulfur and in the oil alone, indicates that the variations correspond primarily to changes which take place in the oil itself when heated.

We studied the changes in the quenching effect upon the fluorescence of methylcholanthrene which occur when sulfur added to a mixture of triglycerides is heated. This was compared with the effect of heating upon cottonseed oil alone. Fig. 291 shows the results of this analysis.

Chapter 12, Note 9. Magnesium and Adrenalectomy

The biological antagonism between homotropic magnesium and heterotropic sodium, both acting at the metazoic level, has led us to the study of the specific influence exerted by magnesium upon the recovery processes in adrenalectomized rats. It is known that, while an adrenalectomy is not always fatal in old rats—death occurs uniformly in younger animals weigh-

ing less than 150 grams. The administration of 1% sodium chloride as drinking water is known to protect the adrenalectomized animal and, if administered for a sufficient length of time, to prevent death. The administration of magnesium sulfate by repeated injections of .5 cc. of a 10% solution per 100 grams of body weight or even orally as .5—1% in drinking water has an antagonistic effect to that of sodium chloride. A 75% mortality rate in older animals receiving magnesium sulfate as compared to a 20% rate in the untreated was seen. Similarly, in young animals receiving magnesium sulfate in addition to salty drinking water, the mortality rate in some experiments was over 80%.

Chapter 13, Note 1. Glycerol and Chills

In one of a group of severely burned subjects, who were at that time under our care and had been experiencing several chills a day, an injection of glycerol solution had been given by coincidence just at the moment when a chill was starting. While such a chill always previously had lasted for more than ten minutes in this patient, it stopped almost immediately after the glycerol injection.

An experiment was set up to confirm or negate this correlation. As soon as any patient of this group felt the sensation or premonition of a chill, he was given either an intramuscular injection of 3-5 cc. of a 20% solution of glycerol in saline or 3 cc. of saline alone as placebo. In almost every case, the chill was cut short by the glycerol while the placebo had no effect. Less striking but still interesting effects were obtained when 20 to 30 drops of glycerol were given orally in 50 cc. of water against an oral solution of 1% sugar in water as a placebo.

Not one of the other substances used at this time, such as adrenalin, quinine, pilocarpine or pantopon, orally or parenterally influenced a chill once it had begun. Later, butanol also was found to have an effect similar to that of glycerol although less manifest.

Since the first experiment with glycerol, we have tried it in many patients subject to repeated chills and have frequently obtained the same results. We have tried to explain glycerol's effect upon chills by considering the role of chills in the defense mechanism. Chill would mark the beginning of the second phase of the diphasic defense phenomenon. (See Chapter 5.) It brings various constituents, especially those which have to replace constituents altered in the first hydrolytic phase. Among them are agents especially able to influence the free fatty acids liberated in the first phase. Apparently, the fact that it takes some time for the anti-fatty acid agents to pass into the circulation, most of them coming from the RES cells, makes the chill last so long. The immediate presence in the blood of a sufficient amount of glycerol, which is a relatively efficient anti-fatty acid agent, climinates the need for liberation of body anti-fatty acid agents. Thus, with glycerol, the chill would no longer be required to induce liberation of such agents and would stop.



Chapter 13, Note 2. Influence of Glycerol Upon the Cardiac Rhythm

Figure 292 shows the electrocardiogram of a rabbit receiving a solution of 20% glycerol intravenously. Frequent extrasystoles appeared. It was interesting to note that, at the same time, the animal became somnolent.

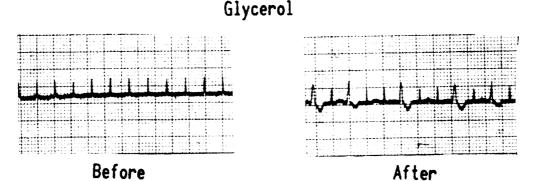


Fig. 292. Electrocardiogram of a rabbit receiving intraveinous injections of a solution of glycerol 20%, characterized by the appearance of extrasystoles. (a) before treatment. (b) after 30 cc.

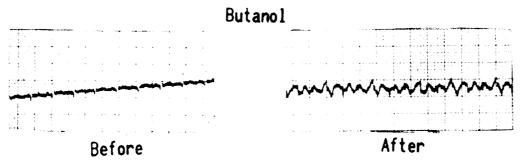


Fig. 293. Extrasystoles appear after the intraperitoneal injection of butanol in very high doses. (a) before treatment. (b) after 1.6 gram/1000 gr of animal.

Chapter 13, Note 3. Glycerol Induced Convulsions

Repeated injections of glycerol in rats were seen to induce convulsions. Using rats weighing 200-250 grams, 5 to 10 cc. of the 20% solution of glycerol in saline were injected intraperitoneally. The injections were repeated once or twice a day. After several days of treatment, usually from 3 to 5 days, one of the injections was followed within a few minutes by a severe convulsion, lethal for most of the animals. In the surviving animals, the next injection was always followed by a lethal seizure.

Chapter 13, Note 4. Suspensions of Lipoids

In order to obtain colloidal suspensions, various lipoids were dissolved in alcohol and a certain amount of the alcohol solution mixed with water, saline or isotonic solutions. From the resulting milky suspensions, the al-



cohol was eliminated by boiling under reduced pressure. To insure almost complete elimination of the solvent, an excess of water was added and the excess was then eliminated through boiling. The use of acetone or ether as solvent gave much less favorable results.

Relatively stable suspensions were obtained by mixing some lipid preparations such as mixtures of unsaturated fatty acids with a 0.5% solution of cellulose gum. Such stable suspensions could not be obtained with preparations of positive lipids.

Chapter 13, Note 5. Cholesterol Induced Convulsions

When relatively larger doses, such as 5 cc. of 2% cholesterol daily, were administered repeatedly to rats of around 250 gr. of weight, convulsions appeared after 4 to 8 days. They were induced earlier in females than males. The first convulsion always was lethal. Convulsions also occurred in humans after repeated injections of cholesterol in doses as high as 20 cc. of the 2% solution in oil. Even small doses, such as 2 or 3 cc. of the same solution, induced convulsions in patients with brain metastases or in those who had had previous convulsions.

Chapter 13, Note 6. Treatments In Successive Generations

The relatively short survival time of animals bearing transplanted tumors has been a handicap for the study of the influence exerted by many agents. Effects requiring some time before they can be induced are thus missed. Changes which occur in tumors—such as the tendency to ulcerate after treatment with fatty acids—have been found to be transmitted in successive generations of the tumors. This has led us to carry on treatment beyond the survival time of one individual host in order to study the influence of various agents. In one group of experiments, this was done through treatment of the successive hosts of serial transplants. In another group of experiments, the treatment was applied to the transplants themselves in successive hosts.

Mice with grafted tumors were treated with the chosen agents. When the tumor in a treated host, or in a control, had grown to 1½ centimeter diameter, it was removed. Part of it was used for further transplants, part for microscopic studies. The rest of the animals were kept until death and the survival time was noted. Transplants of the tumor from treated animals as well as from controls were grafted in new animals and the treatment continued for the new hosts. This procedure was repeated for successive generations. In other experiments, the successive transplants were dipped, prior to grafting, in an oily solution or in a suspension in saline of the agent being tested. The procedure was repeated continuously for both treated animals and controls, and growth and survival time were noted. The following experiments are characteristic.

Using the insaponifiable fractions of human placenta in an oily solution



of 5%, or in a saline suspension corresponding to two milligrams of the material per cubic centimeter, the following results were observed in the case of Ehrlich mammary carcinoma in mice. No changes in survival time, evolution of the tumor, gross or microscopic character were seen in the first and in some experiments even in the second generation. Usually with the third generation, the survival time was reduced, the tumor growing much more rapidly and killing the animal in around 20 days. The malignant character of the tumor was seen to increase in the subsequent transplants and in the fifth generation in some experiments, killed the animal in

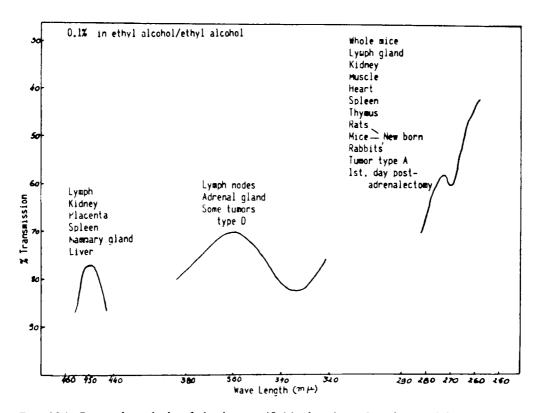


Fig. 294. Spectral analysis of the insaponifiable fraction of various origins. It shows a characteristic peak at 450 m μ , another at 360 and another at 272. The organs in which they are especially present are indicated.

less than a week. The morphological change observed in these successive transplants were also characteristic. The tumor was seen to change from solid to encephaloid. The adenocarcinomatous character was thus altered and the degree of undifferentiation was increased by passing through the third, fourth and sixth generation. At the sixth generation in some experiments—and the fifth or eighth in others—microscopic examination showed that sarcomatoid portions were present in the tumor. The malignancy appeared to be at its maximum in these tumors. Transplants of tumors with sarcomatoid microscopic character, if treated in the same manner, gave negative grafts. Thus, it appears that the treatment with the insaponifiable

fractions has progressively increased the malignancy until the moment when sarcomatous character appeared after which negative transplants were observed.

The treatment of a tumor with lipoacid preparations of human placenta has produced opposite changes manifest even in the first transplants. These

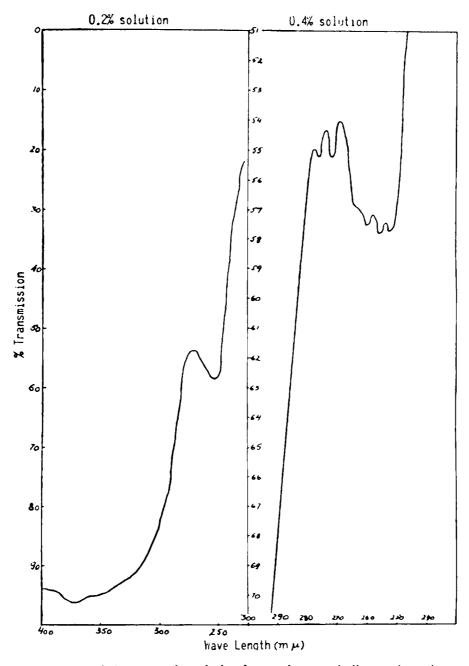


Fig. 295. Details of the spectral analysis of rat colostrum indicates the existence of a formation with three peaks in the region 290-250 m μ with some similarity to the conjugated trienes.

increased in the second generation. After the second and very rarely after the third grafts, negative transplants were obtained. We used this method of treating tumors through successive generations routinely. The results obtained for different agents are discussed in the text of this publication.

Chapter 13, Note 7. Conjugated Trienic Alcohols

Spectral analysis has permitted us to recognize the presence, in certain mixtures of the insaponifiable fraction, of several peaks, some especially interesting. Characteristic peaks were seen at 450, 360 and 272 m μ , as shown in Figure 294. In the first analysis, one was identified as corresponding to a peak of 2720 Angstroms. In more complete further spectral analyses, it could be seen to correspond to a conjugated triene with its characteristic three peaks. The fact that it corresponds to a substance with positive polar group explains why, compared with conjugated acids, the curve shows a marked displacement of the peaks toward higher wave lengths. (Fig. 295) This can be related to the different influence exerted by the electrically opposite polar groups. This compound was first found in the colostrum obtained from the stomach of newborn rats on the first day. In smaller amounts, it has been seen in other samples of milk or butter, and in pork kidneys. It has been found less frequently in growing tumors and is even rarer in growing animals.

The same spectral analysis has permitted us to recognize other peaks and relate them to the different sources from which the unsaponifiable fraction was obtained. Fig. 294 shows these peaks and indicates their correlation with the origin of the material.

Chapter 13, Note 8. Toxicity of Butanol in Humans

A group of advanced schizophrenic patients (221) were given 500 cc. of a 6% solution of n-butanol in saline intravenously, the entire amount being injected in 30 minutes. The only manifestation which could be considered to parallel the toxic effect in animals was a very short period of somnolence which, in only one or two cases, could be considered as sleep. Usually, even with doses of 500 cc. of a 6% solution administered intravenously in less than 25 minutes, it was not possible to obtain even this transitory somnolence. No toxic effect was noted when the same dose was again administered 24 to 48 hours later, and repeated several times. Except for an inflammation of the vein which appears only if hundreds of cc. of a solution above 6% is injected, no other noticeable effects are observed.

The intravenous administration, in postoperative cases, even of 15 gm. of butanol diluted in about 2-3 liters of saline per day, repeated for four and even five consecutive days, has been entirely free of any toxic effect.

Intramuscular administration was observed to be well tolerated even for higher concentrations of butanol. We obtained concentrated aqueous solutions by dissolving butanol in a 35% solution of sodium benzoate in water. Preparations containing more than 30% butanol seemed to induce

necrosis when administered intramuscularly in animals and to induce pain at the site of injection in humans. A 30% solution of butanol, however, was well tolerated. Daily administration of subnarcotic doses for long periods to mice caused no toxic effects. On the other hand, repeated injections with narcotizing doses were toxic and even led to death of the animals after several days.

Chapter 13, Note 9. Butanol and Leucocytes

The administration of butanol in solutions of 6.5% in saline intraperitoneally in rats was seen to induce a hyperleucocytosis. 5 cc. injected at once was seen to double the previous amount of leucocytes. This increase

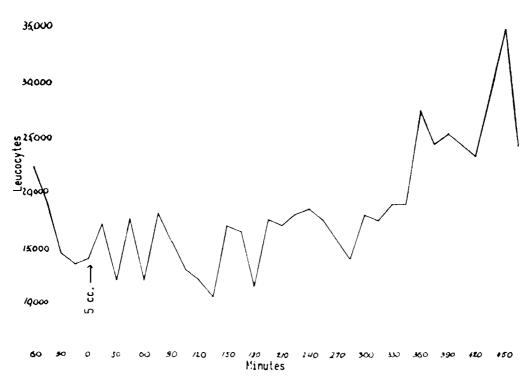


Fig. 296. The administration of 5 cc. of a solution of 6.5% n-Butanol intra-peritoneally to rats induces an increase in the leucocyte number.

started two hours after the beginning of the injections and continued progressively, to reach the value of 34,000, seven hours after the beginning of the injections. The hyperleucocytosis was seen to persist for more than 24 hours. The number of leucocytes was increased in the animal shown in Fig. 296.

A still more manifest effect was obtained with injections of 1 cc. of the same solution, repeated every hour during the day. It is interesting to note that this effect was manifested almost 6 hours after the injection with butanol. Fig. 297 shows an example of these experiments in which the number of leucocytes arrived at 42,500.



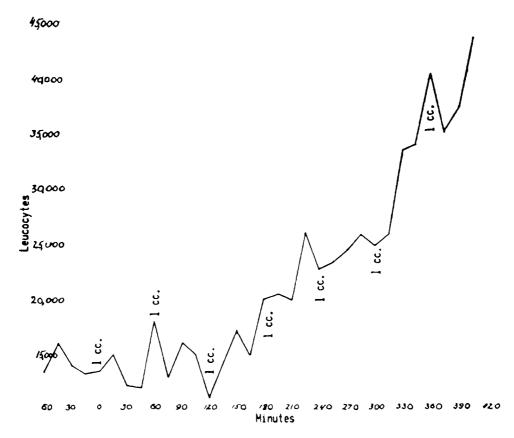


Fig. 297. The administration of 1 cc. of n-Butanol, repeated every hour, induces a sizable increase of leucocytes in rats.

Chapter 13, Note 10. Butanol-Sodium Lactate in Burns

In collaboration with R. Ravich and P. Teitelbaum we studied the effect of various agents upon the survival time of mice to which a severe caloric burn was inflicted. Under ether anesthesia, adult white female mice were scalded until the xyphoid, in water maintained at 90°C. When the duration of this treatment was 4 or more seconds, the animals died in a few minutes in superacute shock. With 3 seconds the animals survived the immediate effect of the burning.

They started to die several hours later, in 6 hours 40% of these animals had died, and at the 18th hour, 90% were dead.

The influence exerted by various agents was studied by injecting the respective solutions 2-3 times a day according to the experiment. The effect was judged according to the survival time. As the animals did not eat or drink and especially due to the local burns did not urinate or defecate, we considered the effects obtained during the first 18 hours, after which the animals were sacrified and used for the study of the chemical changes. Fig. 138 shows the results of such an experiment with sodium chloride, isotonic solution—sodium lactate 6 M solution, butanol 6.5% in saline—and butanol 6.5% in the sodium lactate solution. While sodium lactate

alone seemed even to increase the mortality, and butanol in saline alone influence little this mortality, the effect of the butanol—sodium lactate solution was manifest. The mortality was reduced from beginning to end of the experiment. At the 18th hour it was of 30% instead of 90% for the controls. And of 95% for the animals treated with sodium lactate alone.

Chapter 13, Note 11. Effect of Heptanol

Adult rats and mice were injected subcutaneously in the back with 20 cc. and 3 cc. respectively of nitrogen which had been sterilized by being passed through sterile cotton plugs. Into the pouches so formed, a suspension of living coli bacilli—2 cc. for rats and ¼ cc. for mice—was injected. This suspension was obtained from a 24-hour culture on agar and was diluted to provide 10 million microbes per cc. One group of animals was treated by intraperitoneal injection of 1 cc. (for rats) and ¼ cc. (for mice) of sterile sesame oil. The other group received injections of similar doses of 2% heptanol in oil. In some experiments, only one injection with heptanol was given, while in others this was repeated daily or every second day. In the controls no special reaction was seen. In the heptanol-injected animals an exudate appeared in the infected pouch and led to rapid necrosis of the skin. A characteristic of the exudate was the presence of a small number of leucocytes.

Chapter 14, Note 1. Observations of Dr. E. Stoopen

From a series of observations published by E. Stoopen (184), we chose the following:

"Right trigeminal neuralgia for the past 10 years, with short, sharp pains. Neither food intake, nor time of day have ever influenced the pain. The patient was submitted to various treatments such as ultraviolet rays, quinine, neosalvarsan, cobra poison and vitamin B. First alcohol nerve block calmed the pain for 15 months. Second alcohol injection brought no relief. Third block calmed the pain for one year. Fourth alcohol injection calmed pain for three months. The last injection caused, however, trophic ulcerations of the throat and corneal ulcerations with ultimate loss of the sight.

In June 1942, a treatment with glycerin and the insaponifiable lipid fraction was begun. The pain which proved to be of an alkaline type, ceased in 3 days.

In July 1943, the patient had a lumbago attack, a condition from which she had often suffered and which had been both long-lasting and resistant to classical medication. Treatment with the insaponifiable lipid fraction made the pain subside within a few days.

In an attempt to modify the ulcerations in the throat, though the pain had not reappeared, the patient was continuously treated with the insaponifiable fraction and cholesterol, and with large doses of vitamin A. However, there was no effect on the ulcerations.



In February 1944, the pain reappeared. Study of the pain curve revealed it to be of the acid type since the paroxysmal pain corresponds to a very low pH. The patient was given ammonium acetate and lipoesters. Four days later the pain had considerably decreased, and fifteen days later, completely subsided."

"Mrs. W. For eleven years this patient has suffered of a left trigeminal neuralgia. Each year the crisis lasted four to five weeks, during which time the pain always appeared between four and seven in the morning, lasted for one to two hours and then disappeared. The pain was so severe, being almost unbearable. For the rest of the day, the patient only felt a slight sensitivity. Barbiturates taken even in large amounts had no influence on the pain. Removal of the Gasser ganglia was suggested as the sole possible cure by numerous doctors consulted in Mexico and the United States.

The patient came under our care on November 12, 1943. Her most recent crisis had started on October 31. Study of her pain showed it to be of an alkaline character, since pain is quite intense when the urinary pH is high. We recommended glycerine. The patient protested, feeling that a few drops of glycerine would not be able to help her, pointing out that intensive treatment, had given no results. However, on November 15th, when pain started at 5:00 a.m., the patient took the glycerine drops and to her amazement, the pain disappeared within two minutes. She asserted that no medicine had ever been able to stop the pain once it had started. At 7:30 a.m. the pain returned but again decreased after the patient had taken several glycerine drops.

On November 16th, the patient experienced pain at 1:30, 5:30 and 7:30 A.M. During the first two periods of pain, the pain was instantaneously calmed with glycerine and phosphoric acid; the third period of pain was decreased in intensity but lasted for 40 minutes.

On November 17th: pain appeared at 9:00 A.M. but disappeared three minutes after medication of glycerine and phosphoric acid.

On November 18th: pain which started at 8:00 A.M. could not be calmed with medication. Coramine, Cholesterol and insaponifiable fractions were then prescribed.

On November 19th: pain was experienced at 4:00 and 8:00 P.M. but subsided within three minutes after medication of glycerine, phosphoric acid and coramine was taken.

On November 20th: from this day through November 25th, the medication was unable to influence the pain, and consequently the patient became disheartened. A study of her pain pattern at this point indicated that it had changed to the opposite type—from alkaline to a definite acid pattern.

On November 26th: bicarbonate was given at the onset of pain resulting in a considerable decrease in its severity. No further pain was felt on the following days and the crisis was considered ended. In this instance, the crisis had lasted for 27 days (the length usually varied from 27 to 35

days). However, the treatment achieved what had been impossible for all previously tried treatments—the cessation of pain once it had started.

These observations led to the following pertinent conclusions: 1) they showed the existence of typical acid or alkaline pain; 2) the possibility of changing pain from one type to the opposite one, either during the course of the disease or due to medication; and 3) the possibility of eliminating pain with appropriate treatment."

Chapter 14, Note 2. Dr. Welt's Publication on Butanol-Conclusions

"n-Butanol was administered to a large number of patients with pain due to the trauma of various common otorhinolaryngological and ophthalmologic surgical procedures. Pain was relieved in approximately 90 per cent of the patients so treated.

These clinical results were considered in the light of studies by Revici and his co-workers regarding the physiopathology of wounds. The results indicate that the proposed concept of pain has significant practical clinical applications."

Chapter 14, Note 3. Dr. A. Ravich's Conclusions (189)

In his article concerning the post-operative care in prostatectomies, A. Ravich arrives at the following conclusions exemplified in Figure 298.

SUMMARY

"A new concept of the local physicochemical changes occurring within pathological foci as introduced by Revici, has been briefly described. According to this view, pain is the result of local pH changes brought about by the accumulation of acid or alkaline substances within disturbed tissues. Changes in the lipid balance are associated with and may account for these alterations.

"The possibility of correcting or neutralizing such lipid changes has been explored clinically in several series of urological cases. The favorable effects upon pain as well as upon bleeding, wound healing and other important postoperative problems and complications indicate the need for further study along these lines."

Chapter 14, Note 4. Treatment of Post-traumatic Conditions

Of special theoretical and practical interest has been the treatment of traumatic conditions, especially those following surgical procedures. The recognition of the role of fatty acids acting at different levels of the organization and inducing several different manifestations, has led to the concomitant use of various agents proper to the levels. From the various agents studied, heptanol was thus chosen as acting at the cellular level, glycerol, polyunsaturated alcohols and alkaline amino acids and butanol



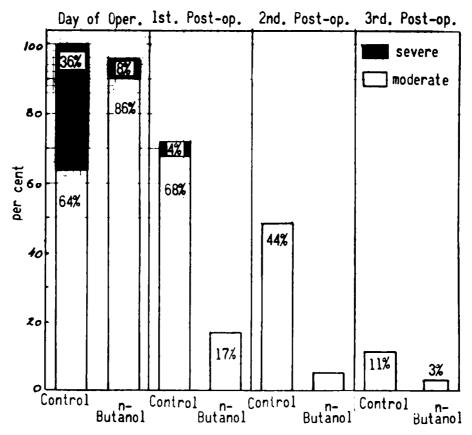


Fig. 298. The administration of n-Butanol after prostatectomy markedly reduces pain. (J. Urol. 62: 629, 1949.)

at the tissular level, glycerophosphoric and organic acids at the systemic level.

The various preparations, obtained by combining or mixing these agents, were administered by intravenous infusions together with glucose and saline or glucose and sodium lactate, in the more severe cases, and intramuscularly or orally in the milder cases. The results obtained with these preparations in hundreds of subjects have been highly satisfactory.

Chapter 14, Note 5. Dr. B. Welt's Conclusions on Hearing

In his studies on hearing, Welt arrives at the following conclusions, communicated at the Brooklyn Eye and Ear Society.

- "1) The ideas, methods and substances devised by Revici have been applied to the problem of impaired hearing, and have shown significant results in improving that function.
- 2) The study has confirmed the dualistic concept about pathological foci, namely that a pathological focus may exist in two states of metabolic imbalance, leading either to a local alkaline or acid change.
 - 3) The substances utilized in this study have been effective in influenc-

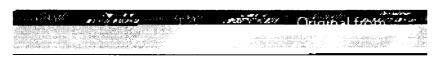


ing the symptom of impaired hearing, and it is reasonable to believe that the pathological structure has also been influenced to some degree.

- 4) Revici's ideas of the fatty acid-sterol imbalance have been confirmed by showing that other instances having similar biologic activities, act in the same manner. Their clinical application confirms this.
- 5) This study shows that both air conduction and bone conduction may be benefited. No method has been found to improve bone conduction up to the present time.
- 6) From the biochemical and chemotherapeutic point of view, this study indicates that the vestibular labyrinth and the cochlea should be viewed as one organ. Phylogenetic, histological, and this clinical study all tend to support this idea.
- 7) The study indicates that people up to 60 years of age may obtain normal audiometric hearing if treated early enough. Children and the younger age groups were those that had the highest incidence of good results.
- 8) Finally, this study has indicated a dual therapeutic attack on a hitherto insoluble problem. It can help us in this otologic problem at any age in life, the formative years, when hearing is vital to the education of the children.
- 9) It should not be construed or inferred, from this communication, that a cure for hearing is implied. The only conclusion to be drawn is that the author has beneficially influenced the impaired hearing function, or induced a remission for varying periods of time at an improved or normal functional level."

Chapter 14, Note 6. Butanol in Plastic Surgery

Following our indications, S. Sher has utilized butanol in post-operative cases. One of the most disagreeable complications seen in plastic surgery of the nose is seventh day bleeding, which while usually not severe, has been known to endanger the lives of several patients. Use of antibiotics has reduced remarkably both incidence and severity of the hemorrhage. However, the prevention of seventh day bleeding has remained a problem for the plastic surgeon. S. Sher has applied our treatment with butanol in almost 2,000 cases. Immediately following surgery, 10 cc. of a 6.5% solution of butanol is injected intramuscularly, the injection being repeated every six hours for the first day. After 24 to 48 hours, the butanol is administered orally in doses of one tablespoonful every four to six hours, and this is continued until after the eighth day following the operation. With this treatment, no severe bleeding has been seen. In several cases when the patient failed to follow instructions and did not continue taking butanol, hemorrhage resulted. In two cases, bleeding was relatively severe, the hemorrhage was brought under control by the intravenous injection of 10 to 20 cc. of the butanol solution. Administration of butanol afterward prevented subsequent bleeding. The value of butanol as a preventive of seventh day hemorrhage thus has been confirmed. (189)



Chapter 15, Note 1. Radio and Chemotherapeutical Essays

Though carried out only at the beginning of these studies, the application of this method, conducted by Leonard B. Goldman, M.D. in 1950 and 1951 appears interesting. As part of this investigation, lipids were used in association with radiotherapy. In the same research, a group of patients were also treated with lipids alone. A report on this series was presented by L. Goldman as part of a symposium on the therapy of advanced cancer patients, before the Radiological Section of the American Medical Association at its annual convention in Atlantic City on June 14, 1951 (327). His results with patients treated with X-ray and lipids and with lipid therapy alone are summarized in Table XVIA and XVIB.

TABLE XVIA

RESULTS OF LIPID THERAPY COMBINED WITH IRRADIATION
(In excess of those expected with irradiation alone)

Type of Malignancy	Total Num- ber	R Num- ber	elief of S Slight	ymptom Mod- erate	s Mark- ed	Tem- porary Ar- rest	Re- gres- sion
Breast	8	7	2	1	4	5	2
Lymphoblastoma	7	4	2	1	1	1	0
Lung	6	5	2	0	3	1	1
Head and Neck	11	4	2	0	2	1	1
Gastro-intestinal	6	4	0	1	3	2	1
Gynecological	3	3	1	1	1	1	0
Genito-urinary	3	0	0	0	0	0	0
Sarcoma	2	1	0	0	1	0	0
Miscellaneous	4	2	1	0	1	1	1
Total	50	30	10	4	16	12	6
		(60%)				(24%)	(12%)

TABLE XVIB
RESULTS OF LIPID THERAPY ALONE

	Total	R	Relief of S	ymptom	s	Tem- porary	Re-
Type of	Num-	Num-		Mod-	Mark-	Ar-	gres-
Malignancy	ber	ber	Slight	erate	ed	rest	sion
Breast	14	7	1	0	6	2	2
Lymphoblastoma	8	5	0	0	5	3	2
Lung	3	3	0	0	3	0	0
Head and Neck	3	2	1	0	1	0	0
Gastro-intestinal	6	3	1	1	1	0	0
Gynecological	3	1	0	0	1	1	1
Sarcoma	1	0	0	0	0	0	0
Miscellaneous	2	2	0	0	2	1	1
Total	40	23	3	1	19	7	6
		(58%)				(18%)	(15%)

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