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Author
Calvin, Melvin

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Radiation Laboratory and Department of Chemistry
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ABSTRACT

This paper constitutes a review of the experimental events that led our laboratory to focus its attention on thioctic acid, a discussion of some of the purely chemical and physical properties of thioctic acid that this awakened interest prompted us to investigate, and a brief description of some of our recent biological investigations with thioctic acid.

THIOCTIC ACID: PHYSICS, CHEMISTRY, AND BIOLOGY[*]

Melvin Calvin

Radiation Laboratory and Department of Chemistry[†]
University of California, Berkeley, California

April 2, 1956

In introducing the work we have done on thioctic acid, I think it would be best to divide the discussion into three more or less distinct parts: The first would constitute a description of the experimental events that led our laboratory to focus its attention on thioctic acid; the second would be a discussion of some of the purely chemical and physical properties of thioctic acid which this awakened interest prompted us to investigate; and, lastly, a return to the biological aspects and a description for you of some of our own more recent biological investigations with thioctic acid.

Our interest in this material (thioctic acid) came about in the course of the investigation of the mechanism by which green plants can convert electromagnetic energy into chemical energy in that they are able to reduce carbon dioxide with water to produce reduced carbon in the form of carbohydrates (and other reduced materials) together with molecular oxygen. This investigation of the way in which CO_2 is reduced has reached a certain terminal phase, and we are now able to write down all the reactions through which carbon passes on its way from carbon dioxide to reduced carbon. The way in which this was determined is, of course, a long story and not for today's discussion. I would like to show you the end results of this work, however, so that you will see what our starting point may be. Figure 1 gives a picture of what the path of carbon is, on its way from CO_2 to carbohydrates. (This is not directly pertinent to what follows, but gives an idea of what the significance is of the term "photosynthetic carbon cycle.") Carbon dioxide enters the cycle by reacting with ribulose-1,5-diphosphate, a five-carbon sugar, to give two molecules of 3-phosphoglyceric acid (PGA). The 3-PGA can then be reduced by the well-known enzyme system, triose phosphate dehydrogenase, with reduced pyridine nucleotides as reducing agent and with the assistance of high-energy phosphate (adenosine triphosphate, ATP) to produce 3-phosphoglyceraldehyde. This 3-phosphoglyceraldehyde may then undergo a series of four simultaneous reactions. The first is isomerization to a keto triose which then can condense by the well-known enzyme aldolase to hexose, which by a sequence of transformations can lead to sucrose.
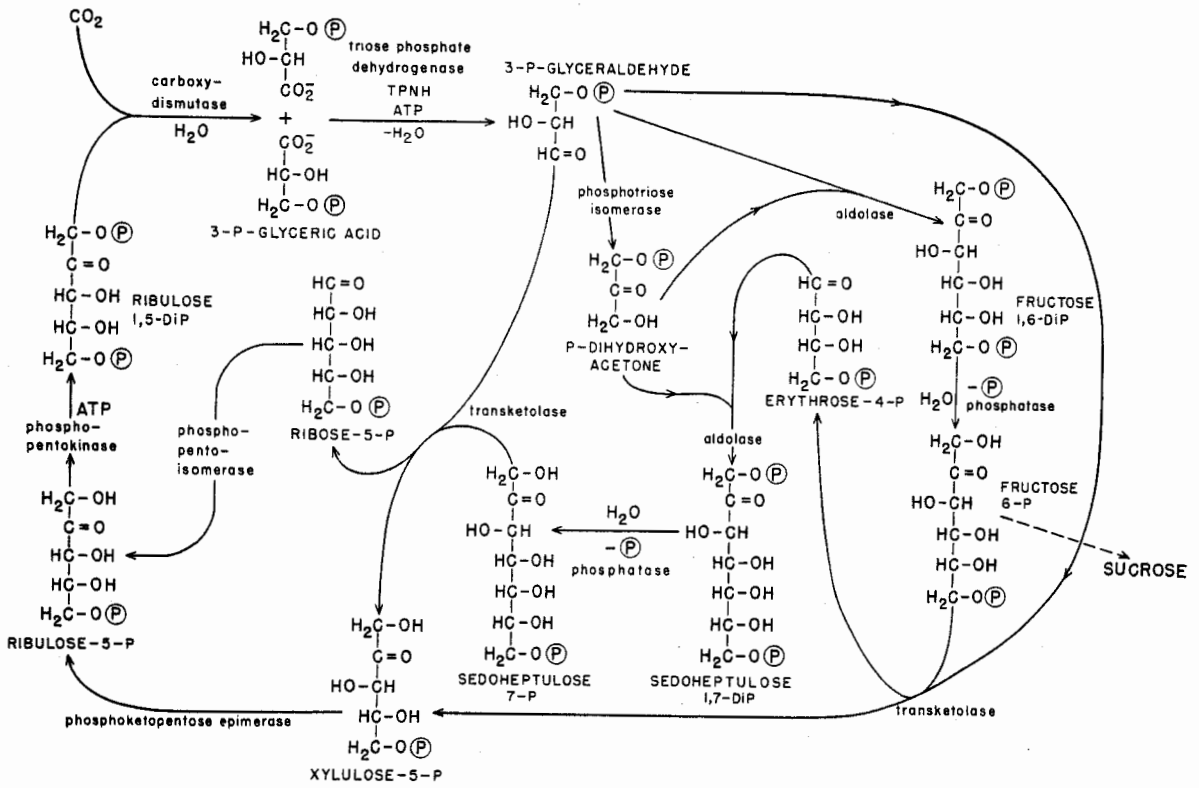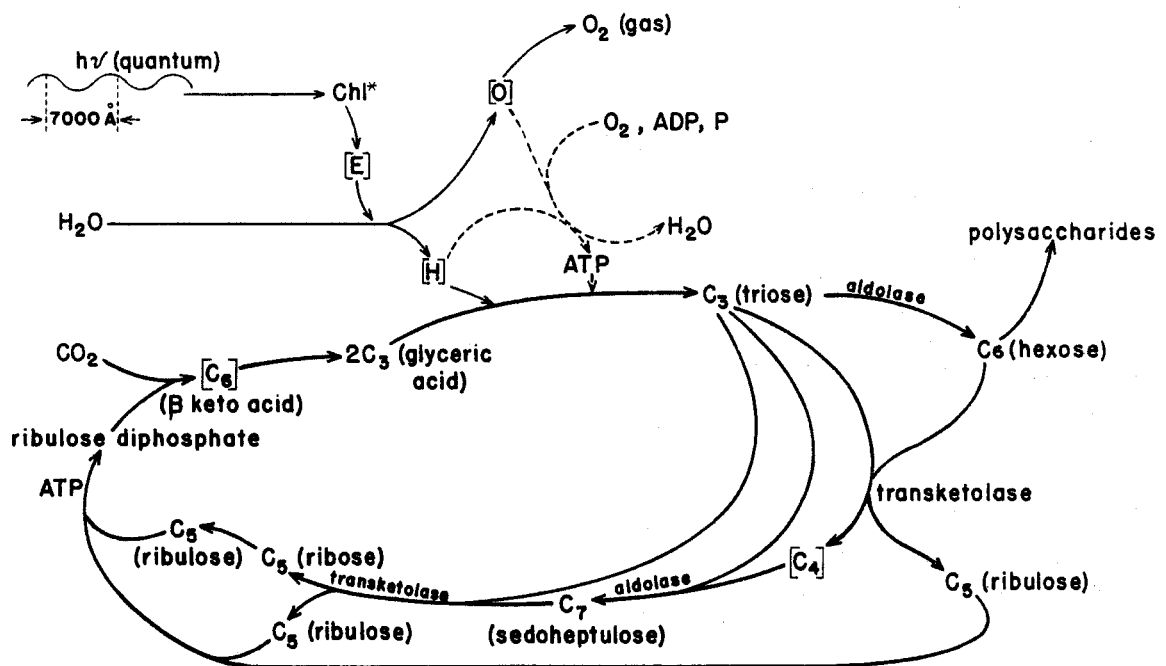
Fig. 1.   The photosynthetic cycle.

The hexose may lose one phosphorus group to form hexose monophosphate which, then, under the influence of the enzyme transketolase, reacts with triose phosphate to form a pentose phosphate and a tetrose phosphate. The tetrose phosphate under the influence, again, of aldolase (the same enzyme that made the fructose diphosphate) can now condense with a keto triose phosphate to ketose diphosphate. It also may lose one phosphoric acid group, in the same way the hexose did, to produce sedoheptulose-7-phosphate, which can subsequently undergo the same transketolase reaction as did the hexose at this point to produce two pentoses: one keto pentose and one aldo pentose. Finally, the keto pentose may be phosphorylated with ATP to produce again pentose diphosphate, thus completing the cycle. What is required here in order to make the cycle go is reduced pyridine nucleotide and ATP; these are the two reagents that are used up and must be supplied by the light.

Schematically this cycle is represented in Fig. 2, which shows the relationship between the cycle and the absorption of light which must ultimately supply the necessary pyridine nucleotide and ATP. An investigation of the effect of light upon the behavior of the carbon cycle at this point—in order perhaps to discover something of the chain of events from excited chlorophyll through the energy converter, which may react with water to produce oxidizing and reducing agent—led us to an interest in thioctic acid. Clearly, since there must be a connection between the photochemical process and the carbon cycle, it should be possible, from studies on the effect of change of the light intensity upon the carbon cycle, to gain information about the energy-conversion process. The result of an observation of the changes in the phosphoglyceric acid and other radioactive compounds before and after illumination was the way in which this was done. Results of these experiments are shown in Fig. 3. While the light is on, the PGA that is in the cyclic system very soon becomes saturated with radioactive carbon, because the carbon is passing through the phosphoglyceric acid in a cyclic manner on its way from CO_2 to sugar. On the other hand, while the light is on, the appearance of labeled carbon in citric and glutamic acids, one a member and one a relative of the tricarboxylic acid or Krebs cycle, is very slow; that is, when the light is on, radioactive carbon entering as CO_2 passes through PGA, but not very rapidly into citric and glutamic acid. The moment the light is turned off, not only does the phosphoglyceric acid concentration rise by approximately a factor of two, but the citric acid concentration also rises, eventually by a factor of more than ten, and its rate of appearance by a factor of more than twenty.

It is clear that the effect on the rate of appearance of radioactive citric acid from phosphoglyceric acid cannot be a simple change in dynamics, since it would be a factor of only two, while here the factor is twenty. It follows that there must be some catalytic "valving" action of the light. Figure 4 represents the citric acid cycle. Here it is clear that the carbon from the phosphoglyceric acid enters into the citric acid cycle, and as the reaction proceeds, we arrive at ketoglutaric acid, which is a close relative of glutamic acid. These are two acids that we have used as indicators of the appearance of radiocarbon in all the acids of this cycle.

Figure 5 shows the general relationship between the photosynthetic cycle (Fig. 1) and the Krebs, or tricarboxylic acid, cycle (Fig. 4). While

Fig. 2. The photosynthetic cycle in relation to quantum
absorption and conversion.

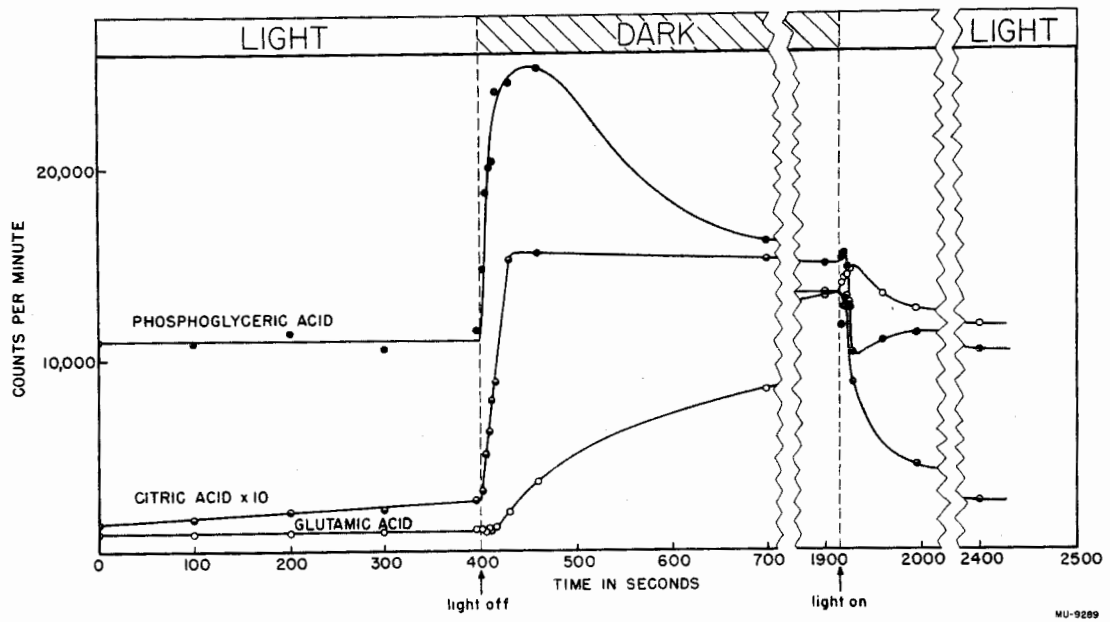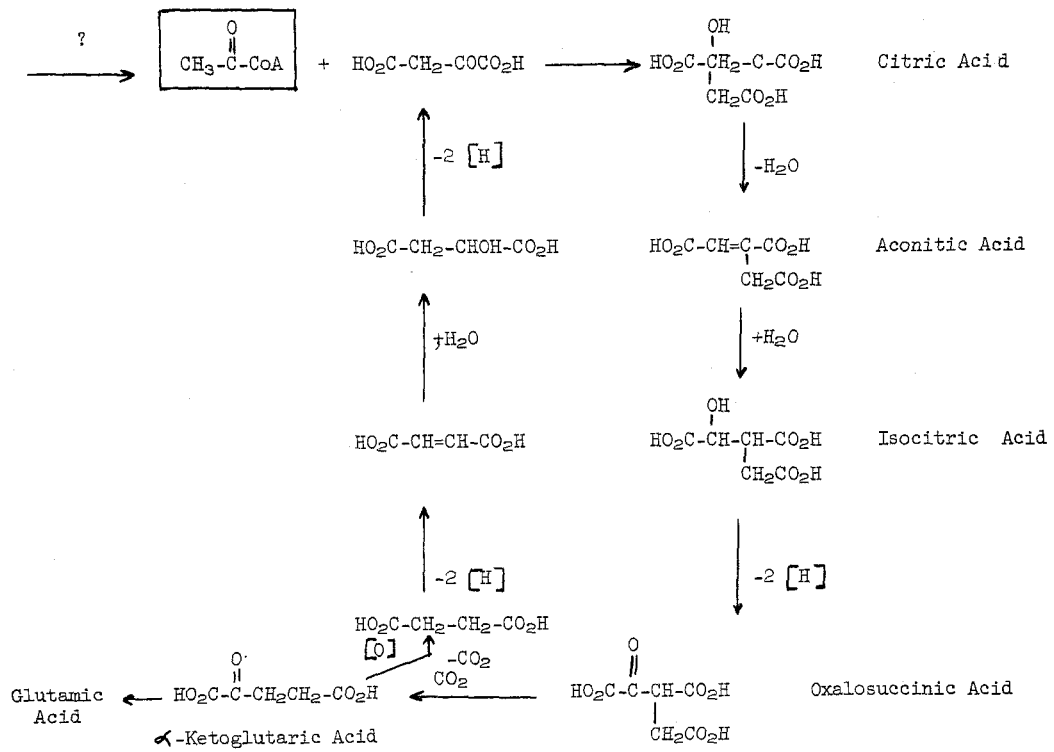Fig. 3. Light-dark changes in concentration of phosphoglyceric
acid, citric acid, and glutamic acid.
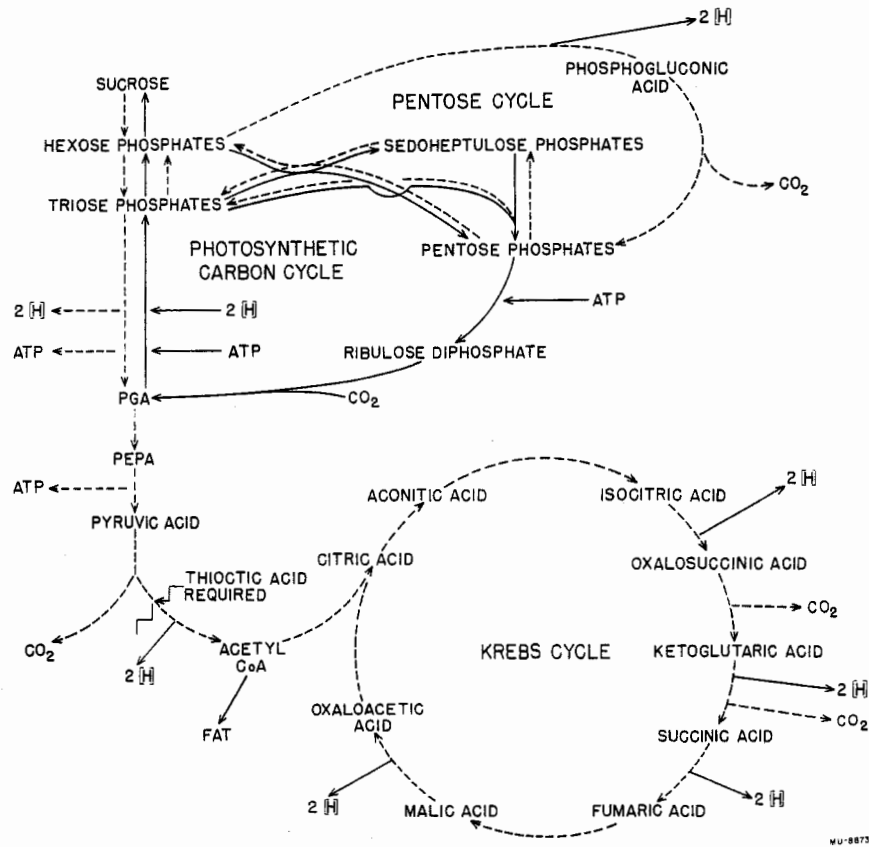
Fig. 4. Tricarboxylic acid cycle.

Fig. 5. Some relationships between the photosynthetic cycle and
the Krebs cycle.

the light is on, carbon may enter the photosynthetic cycle but must go through the major pools of carbohydrates, proteins, and fats in the organism and plant before it returns to the tricarboxylic acid cycle. But the moment the light is turned off, a new path for carbon, leading it from the PGA of the photosynthetic cycle to the tricarboxylic acid cycle, is opened without going through the major storage pools of the plant. In an attempt to understand this "valving" mechanism of the light we focused our attention some two or three years ago on thioctic acid.

The reason for this interest is that the only way in which carbon enters the tricarboxylic acid cycle in a noncatalytic fashion—that is, in a mass-transfer fashion—is through the condensation of acetyl Coenzyme A with oxalacetic acid (Figs. 4 and 5). It thus became necessary for us to discover what the valving mechanism would be which would control the passage of PGA into pyruvic acid and acetyl CoA and thus get to the tricarboxylic acid cycle.

The photosynthetic cycle is the source of carbon, and the phosphoglyceric acid, by two successive changes, can be converted into pyruvic acid, which then undergoes oxidative decarboxylation in which thioctic acid acts as a coenzyme to produce acetylthioctic acid and CO_2. That the thioctic acid was the coenzyme for the pyruvic acid oxidase was recognized by Gunsalus and his co-workers, and was at that time given the name "pyruvic acid oxidase factor." The four names shown on Fig. 6 represent something of the history of this substance. Thioctic acid was recognized as a cofactor for pyruvic acid oxidation and as a growth factor for Tetrahymena and given the name Protogen A; it was recognized also as an acid during the course of isolation of fatty acids and given the name of α-lipoic acid; and, finally, when its structure was known and it was synthesized, it was given the name of 6-thioctic acid.

The fact that it operated in this way to make acetylthioctic acid was readily demonstrated in our laboratory by the use of radioactive pyruvic acid and the isolation of the acetylthioctic acid from such a reaction mixture. Immediately we supposed that this was the route for the passage of carbon from the photosynthetic cycle into the Krebs cycle as seen in Fig. 5. (You can now see how this route controls the direct passage of carbon from the photosynthetic cycle into the tricarboxylic acid cycle.) In order for carbon to go through this path, it is necessary for the thioctic acid to be in the disulfide form to carry out the oxidation that is required to make acetyl-thioctic acid. The thioctic acid is reoxidized from the reduced form--the dithiol form--by an enzyme system which will take the two hydrogens, transfer them to the pyridine nucleotide, and return the sulfur into the five-carbodisulfide ring. If the amount of disulfide present in the steady state is reduced, then the rate of passage of carbon through this sequence of reactions must, of course, be reduced. It was proposed that the mechanism of control by the light of this passage of carbon (the dotted line of Fig. 5) lay in the ability of the light to shift the equilibrium from disulfide to dithiol.

About the time we recognized and proposed the mechanism described above, the synthetic thioctic acid became available. We obviously had to understand something of the chemistry of this substance--its peculiarities and its vagaries--so we undertook to study both thioctic acid itself and its
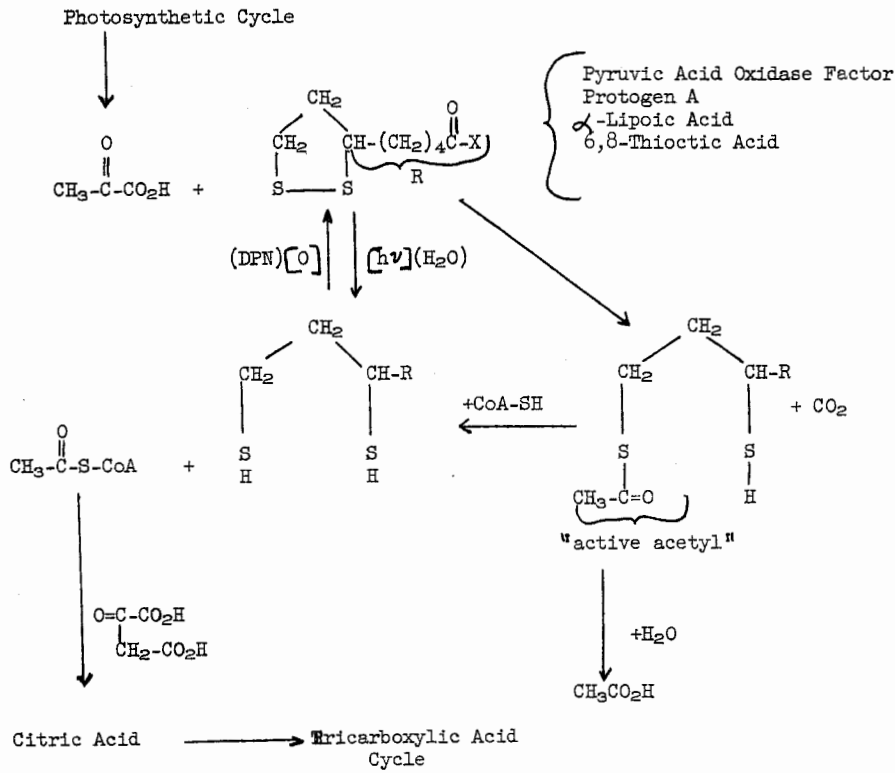
MU-11187

Fig. 6.   Mechanism of photochemical control of the relationships
between the photosynthetic cycle and the tricarboxylic acid cycle.

isomeric relatives, the six- and seven-membered rings, as well as the five-membered ring and some model substances without the side chain.

The first peculiarity that we recognized about the thioctic acid was its color. We had available samples of both the five- and the six-membered rings, and upon inspection it was clear that the five-membered ring had a bright yellow color, whereas the crystal of the six-membered ring appeared colorless. Now, normally, such a difference in two samples would not trouble an organic chemist a great deal, since the appearance of a yellow color in organic crystals is quite common when crystals have remained in the laboratory for some time. In fact, the people at both the Lederle Laboratories and the University of Illinois, who had also observed this characteristic, had made such an interpretation of the difference. But it occurred to us that one could easily determine whether this was so or not by taking a complete absorption spectrum of the material. The results are shown in Fig. 7. There is a definite difference between the absorption spectra of the 6-thioctic acid and of the 5-thioctic acid; the seven-membered ring finally reaches a spectrum resembling that of an open-chain disulfide. It became clear immediately that if these spectra were indeed something characteristic of the sulfur ring itself, we were then observing a specific characteristic of the size of the ring and not of the carboxyl group.

The obvious way to test this was to make the model substances which have no side chains on them, and this we did. Figure 8 shows the spectrum of trimethylene disulfide, of tetramethylene disulfide, and n-propyl disulfide. We actually made the pentamethylene disulfide as well, but its spectrum is not given here because it was never purified sufficiently to be sure of the absolute values of the extinction coefficients. You can see (Fig. 7) that the spectra are indeed characteristic of the substances and not dependent on the side chains. An examination of the structure of these model substances produces the information that the bond angles between the carbon-sulfur bonds in the disulfide must be very nearly in the same plane in the case of the five-membered ring, but may be rotated out of that plane more and more as one goes to bigger rings and finally to the open-chain disulfides.

Figure 9a is a photograph of such models. The open-chain disulfide is to the left and shows that a carbon-sulfur bond is indeed a 90° bond, whereas when you introduce the ring, a four-carbon ring, the biggest angle that one can get between the carbon-sulfur bonds is 60°, and, finally, when one ties them together with three carbon atoms the largest angle that one can get between these two bonds is 40°. In fact, the 40° angle constitutes a very strained position in terms of carbon-hydrogen interaction. This fact seemed to us to constitute one of the essential characteristics of this five-membered ring. Figure 10 shows the spectrum of the disulfide monoxides of the five-membered ring and of the six-membered ring, and here you see that the five-membered ring has a nice maximum at about 2500 Å and the six-membered ring has no maximum but a broad absorption. In this case, you will see, the spectra are inverted: The bigger the ring is, the higher the absorption and the further to the visible; the smaller the ring is, the lower is the absorption and the further towards the ultraviolet. The difference between the spectra in alcohol and in water is interpretable in terms of the polarities of the solvent and in the fact that the strain does not exist in the disulfide monoxide.
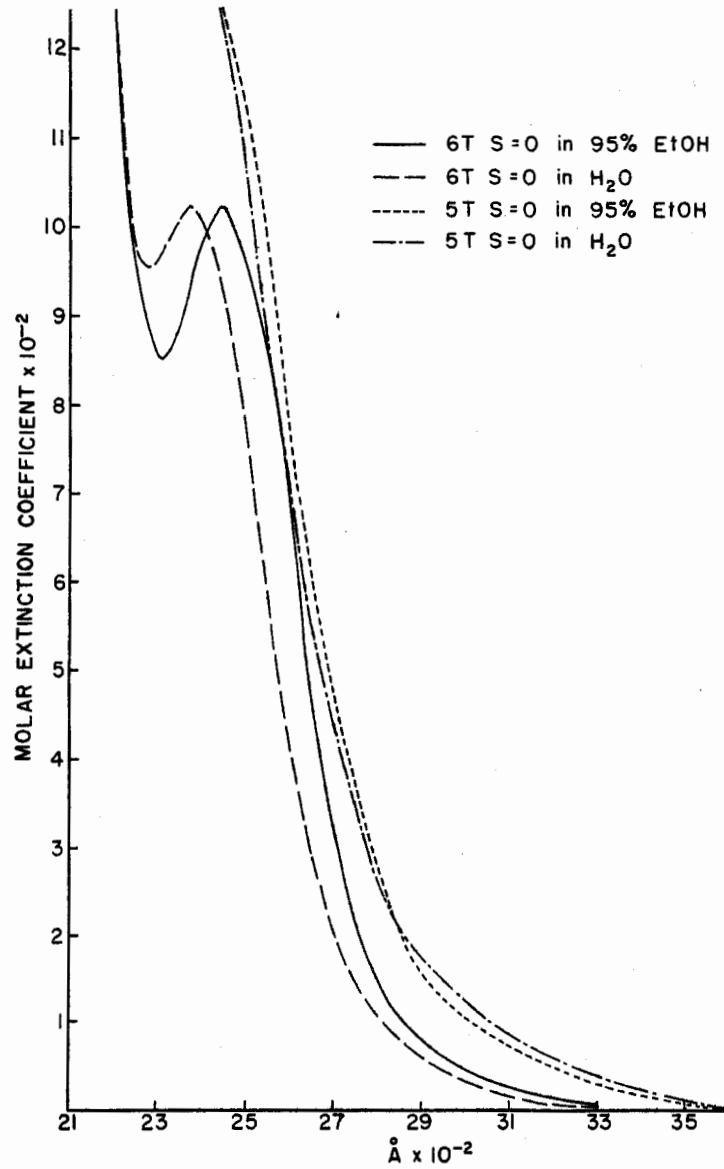
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Fig. 7. Absorption spectra of thioctic acid sulfoxides.
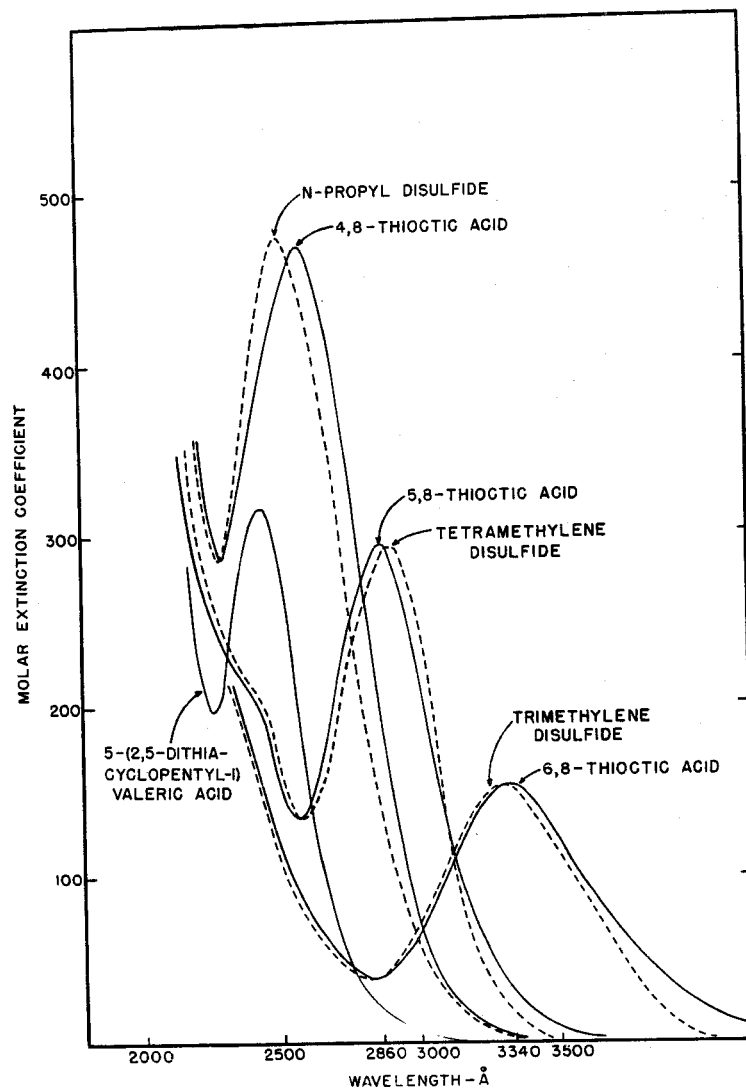
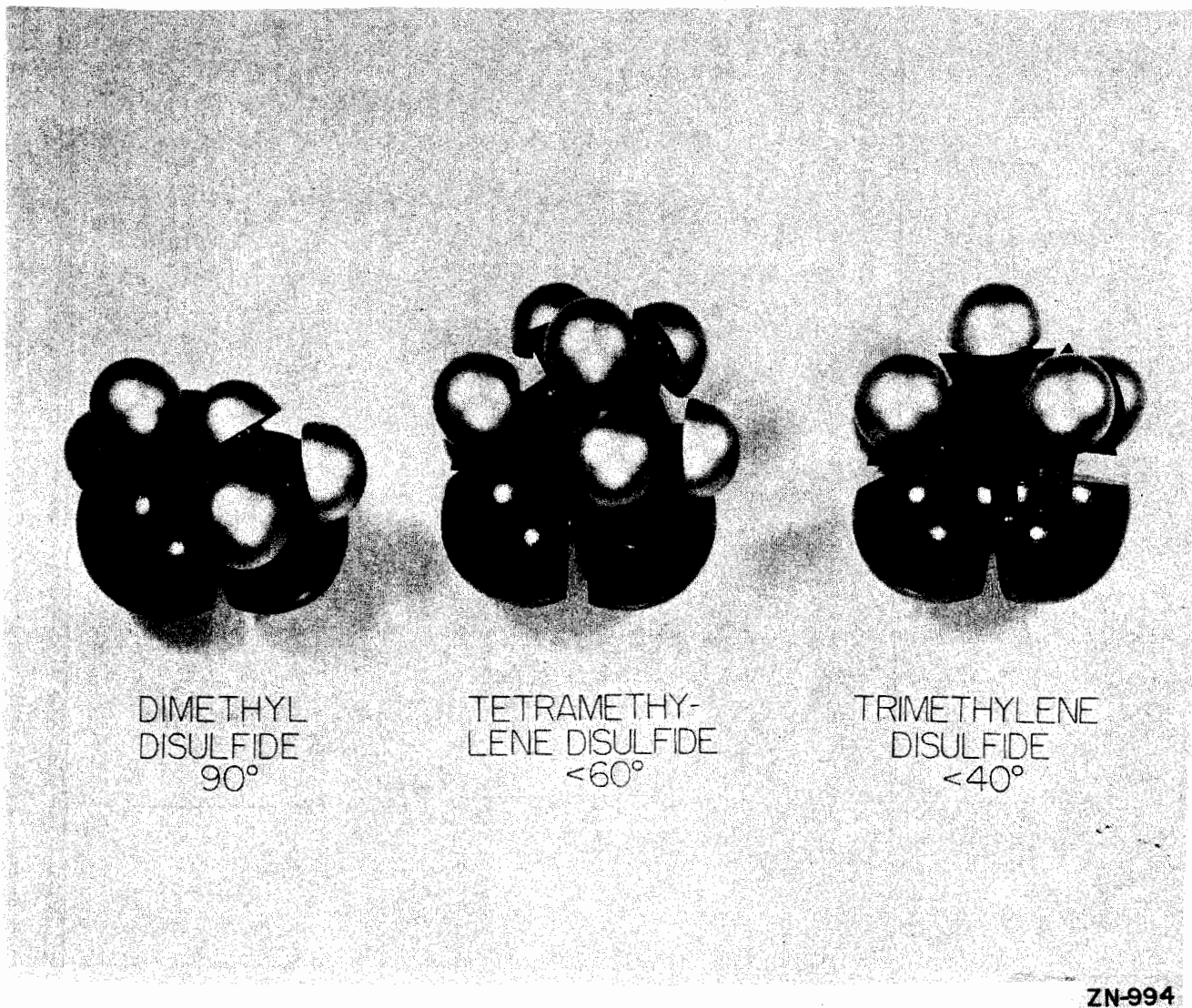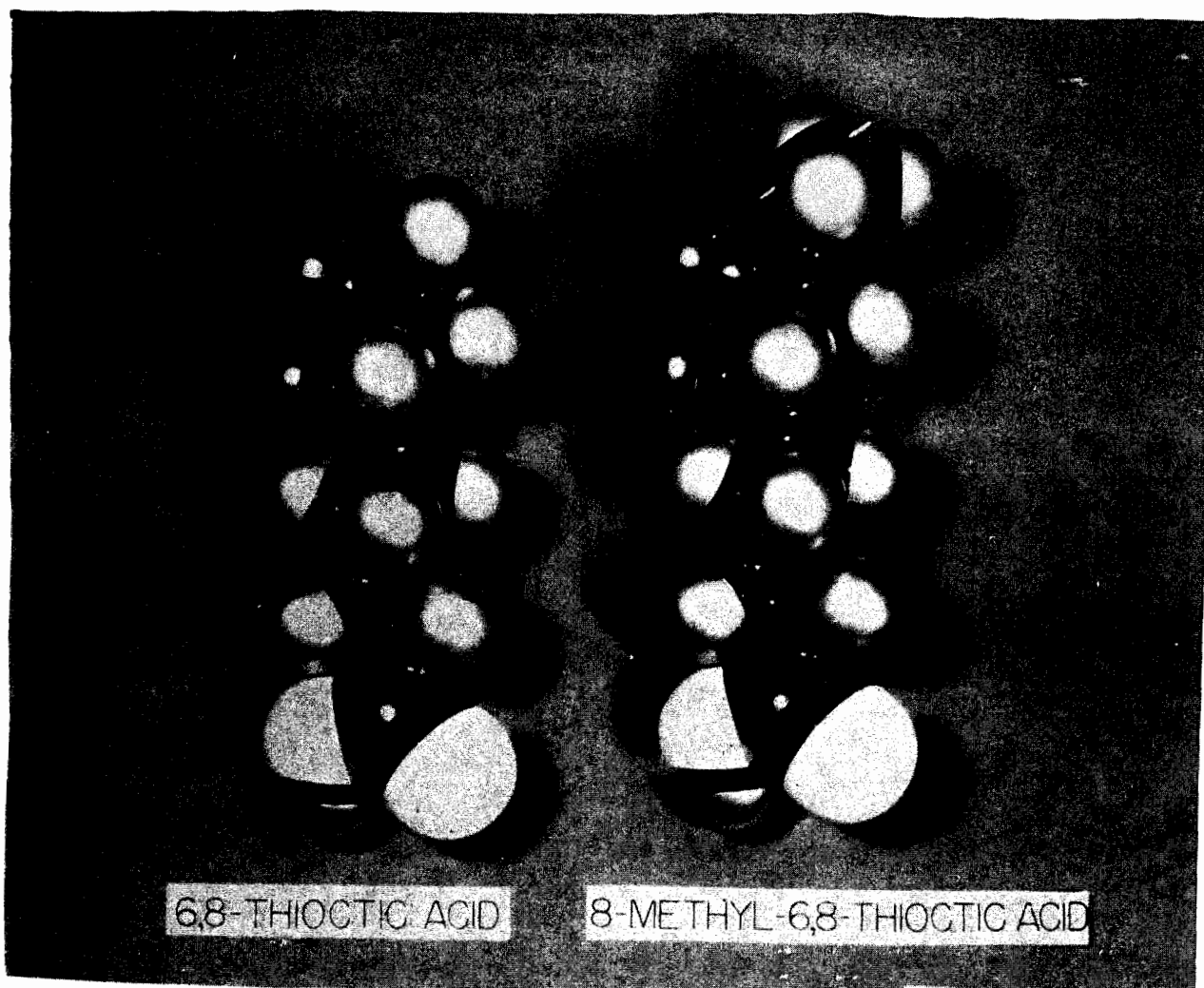Fig. 8. Absorption spectra of thioctic acid and model substances.

DIMETHYL
DISULFIDE
90°

TETRAMETHY-
LENE DISULFIDE
<60°

TRIMETHYLENE
DISULFIDE
<40°

ZN-994

Fig. 9a.   Models of various disulfides.
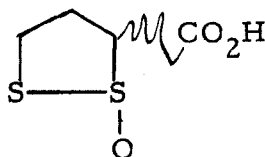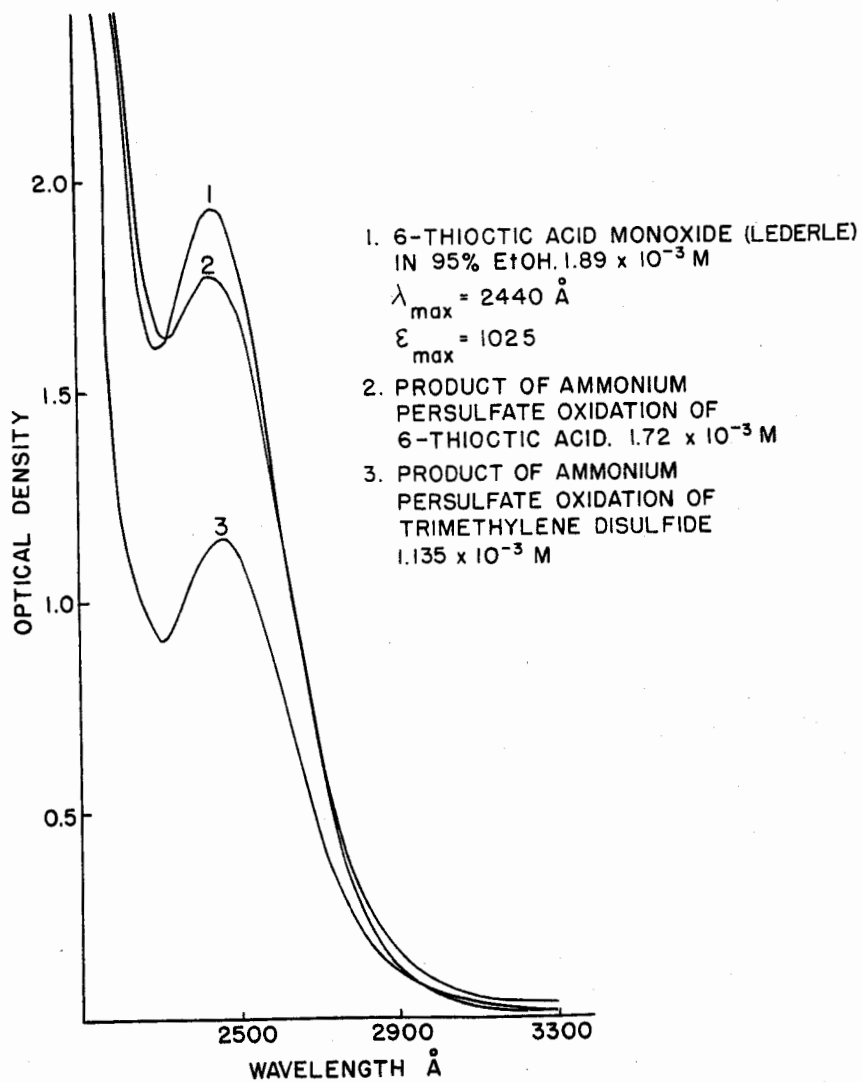
Fig. 9b. Models of thioctic acids.

It would be of interest to draw the structural formula of the monoxide:

One immediately recognizes that there is here a very polar bond and that in all probability the absorption of light in the monoxide involves a decrease in polarity. The higher the polarity of the solvent, the lower will be the energy of the ground state, and therefore in the more polar solvent the energy of the ground state will be lowered and the spectra shifted towards the ultraviolet, which is quite the opposite of the way in which the strain and the solvent affect the spectrum of the disulfide itself. Now this monoxide has appeared in all of the work on thioctic acid; it appeared in the beginning under the name of β-lipoic acid, and its biological function is still unknown.

Now the next sequence of studies involved the fact that the disulfide itself is very labile--very sensitive to light--and we performed an investigation of the photochemical reaction of this disulfide. This was rather easy to do because, if one illuminates the disulfide with light which it absorbs, the spectrum of the disulfide disappears gradually with the appearance of an isobestic point, indicating quite clearly that the photoreaction is a simple one. Figure 11 shows how the trimethylene disulfide, that is, the model substance, is bleached by light of 3650 $\overset{o}{A}$ wavelength;with light absorbed at shorter wavelengths it is bleached more rapidly. The same experiment performed with thioctic acid itself (see Fig. 12) shows the initial thioctic acid and the disappearance of the disulfide band as one illuminates the substance. Again, the spectral changes are very clear and clean, giving an isobestic point. It is perhaps interesting to mention that we have measured the quantum yield for the disappearance of the disulfide in alcohol solution, and we have found it to be very nearly unity throughout the absorption spectrum on both sides of the maximum, as well as at the maximum, indicating that very nearly every molecule that absorbs a quantum anywhere in this band cannot return to the ground state as disulfide but reacts with the solvent. This initial reaction with the solvent is to the production of a thiol on one sulfur atom and a sulfenic acid, or ester, on the other sulfur atom. But this compound--this thiol-sulfenic acid--is a very unstable material and undergoes a succession of "follow reactions" that are very complex. Without going into this we can simply point out that the photochemistry so far discussed involves directly the absorption of light by the thioctic acid.

We now also attempted to see if it was possible to transfer energy from a sensitizer to thioctic acid and produce this same sort of change. As sensitizer, for obvious reasons, we chose a rather stable porphyrin molecule which we could make synthetically, a zinc tetraphenylporphyrin salt. We sought to demonstrate a direct energy transfer between the porphyrin and the thioctic acid in true solution; however, in the presence of molecular oxygen, the disulfide is very sensitive to photooxidation catalyzed by the zinc porphyrin and can produce oxidation of the disulfide monoxide as shown in Fig. 13, as shown by isolation and other ways. Figure 13 shows that the photosensitized oxidation of the disulfide gives the monoxide —one atom of oxygen per molecule

1. 6-THIOCTIC ACID MONOXIDE (LEDERLE)
   IN 95% EtOH. 1.89 x 10^{-3} M

   λ_{max} = 2440 Å

   $\mathcal{E}_{max}$ = 1025

2. PRODUCT OF AMMONIUM
   PERSULFATE OXIDATION OF
   6-THIOCTIC ACID. 1.72 x 10^{-3} M

3. PRODUCT OF AMMONIUM
   PERSULFATE OXIDATION OF
   TRIMETHYLENE DISULFIDE
   1.135 x 10^{-3} M

MU-6989

Fig. 10.   Absorption spectra of disulfide monoxides in alcohol.
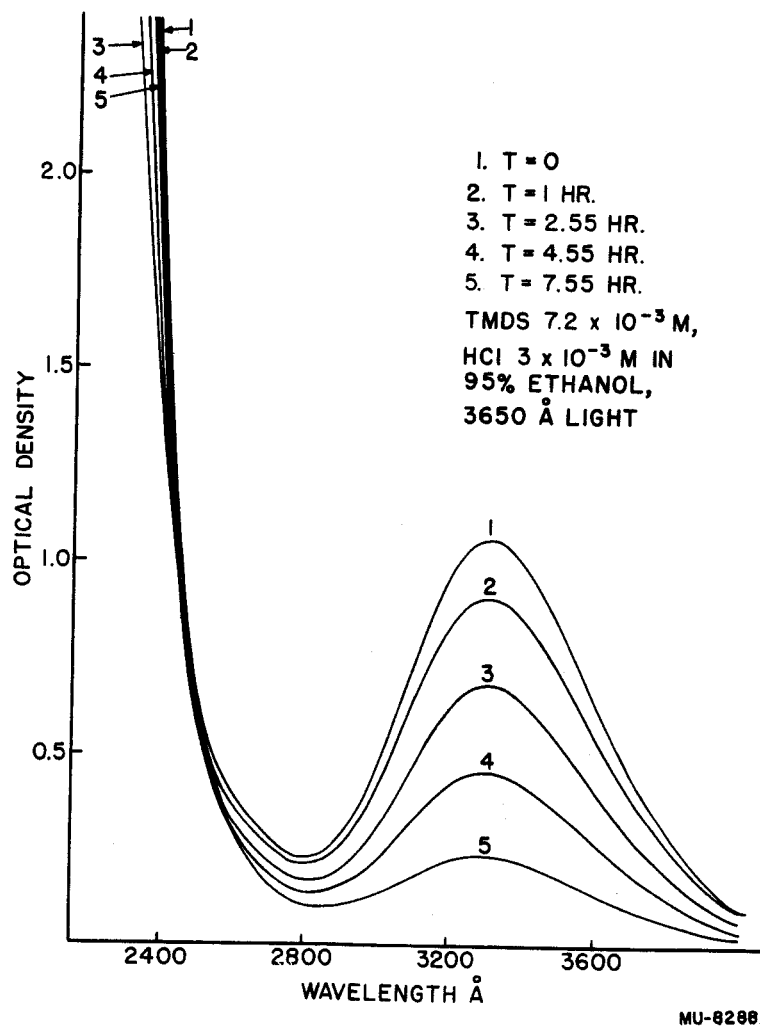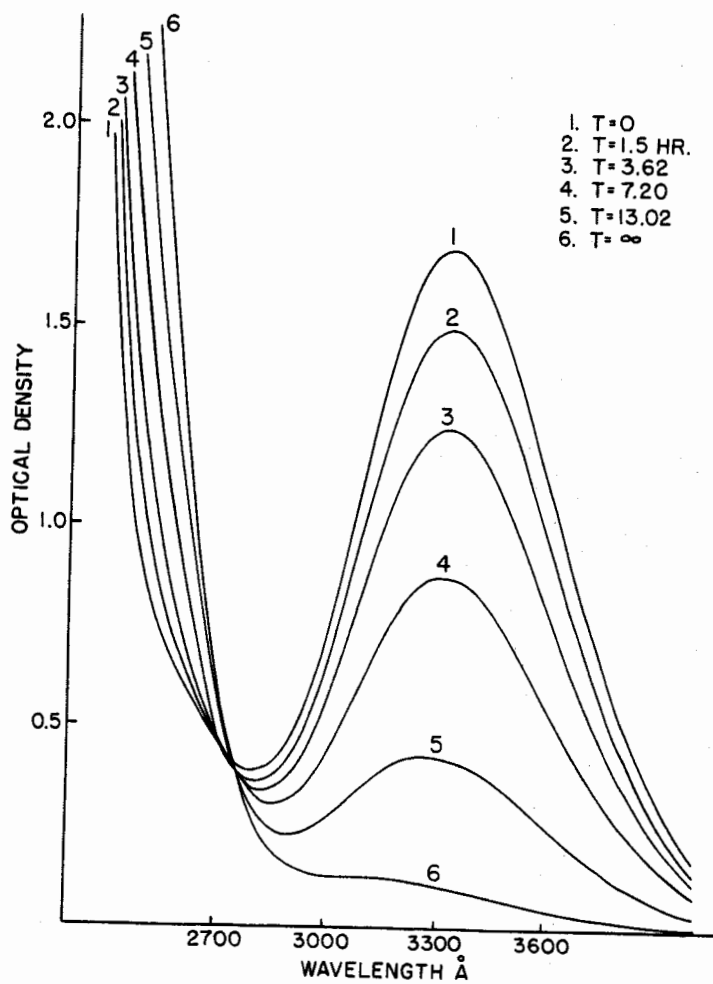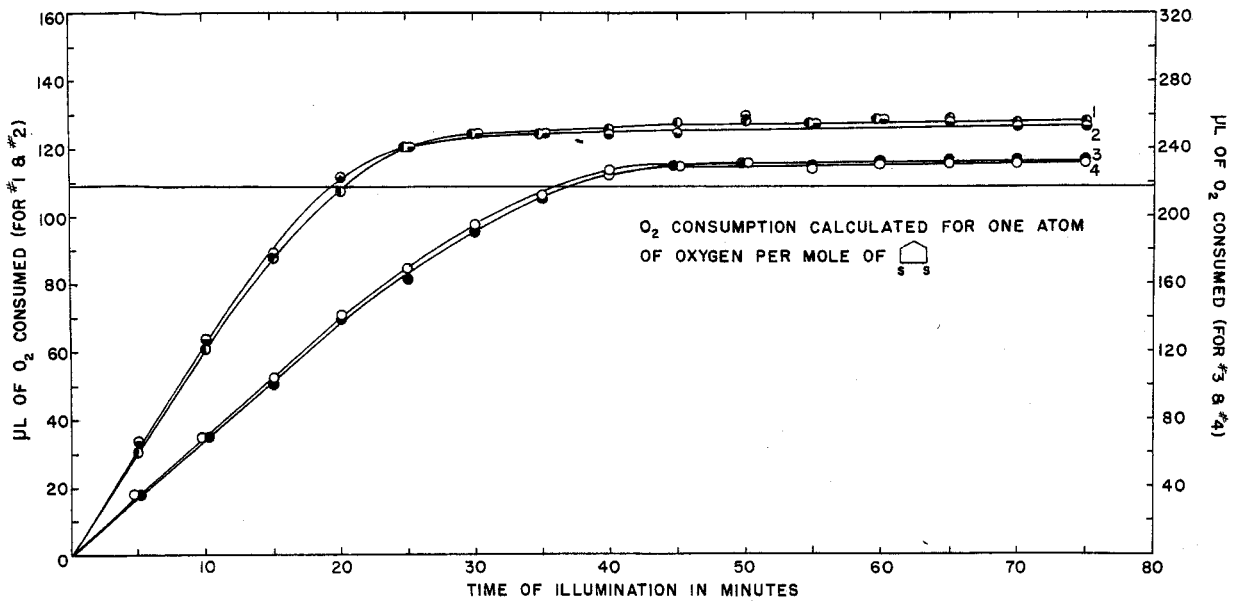
Fig. 11. Representative family of spectrophotometer curves in photolysis of trimethylene disulfide.

Fig. 12. Photolysis of thioctic acid in 95% ethanol by 3650 Å light.

MU-8289

Fig. 13. Photooxidation of S —— S catalyzed by zinc
tetraphenylporphin.

of sulfur—and you can see that the reaction proceeds quite nicely up to the point of oxidation to the disulfide monoxide, and then it ceases. We were able to isolate from such a reaction mixture the monoxide, so there is little question that the porphyrin can catalyze the photooxidation of the disulfide very cleanly to the monoxide. Other dyes would cause a similar reaction, and this is certainly at least one of the reasons why the monoxide always shows up in the experiments in which the disulfide, or in which the thioctic acid, is isolated.

The next reaction is one in which we studied the direct nonphotooxidation of disulfide with persulfate (see Fig. 14). Here, our method was again spectroscopic and the procedure was very simple: we observed a spectrum of the disulfide, then added the persulfate and watched the disappearance of the spectrum of the disulfide and the appearance of the spectrum of the monoxide. We measured the rate of disappearance of the disulfide, and the kinetics of this have been determined. We have examined such oxidation reactions for the disulfides with the results shown in Table I. (This is the reaction of the disulfide plus the persulfate in acidified water to produce the monoxide.)

The kinetics is very simple; the reaction is bimolecular in disulfide and persulfate, and Table I reproduces the bimolecular rate constants for a series of disulfides. The five-membered ring (regular thioctic acid) has a very high rate constant and is very easily oxidized by persulfate. When one enlarges the ring to the six-membered ring, the rate drops by a factor of approximately thirty. When one enlarges the ring still further to a seven-membered ring, it is barely attacked at all by the persulfate. Finally, if there is no ring at all--an open-chain disulfide--again the rate is approximately zero. This shows that the strain introduced by closing the ring down to a five-membered ring leads to very easy oxidation by persulfate in a quantitative way. In addition to that, we have in Table I a fifth compound, 8-methylthioctic acid, a compound containing one more carbon atom on the far side of the ring from the carboxyl chain. Here the strain seems to have increased by roughly a factor of two, at least as far as it is measured in terms of the rate of oxidation. This 8-methylthioctic acid has some anti-thioctic properties, but they are very weak.

Figure 9b is a photograph of the models of 8-methylthioctic acid and thioctic acid itself, showing the position of the methyl group here as compared with thioctic acid, and a reason for the somewhat greater steric effect arising from the methyl group upon the oxidation of the sulfur. Not all of the evidence has been given to demonstrate the existence of the strain in the disulfide bond when one closes it into a five-membered ring.

In order to complete this phase of the discussion I think it is worth while to give you what evidence we have regarding the quantitative measurements of the strain energy in this ring. One way of determining this strain energy would be the determination of equilibrium constants in which one side of the reaction contained the five-membered ring and the other side did not, but did contain the same number of disulfide links; it is very easy to set up what looks like this system, and we were able to do it by allowing the trimethylene disulfide to react with mercaptoethanol and a few other simple mercaptans.
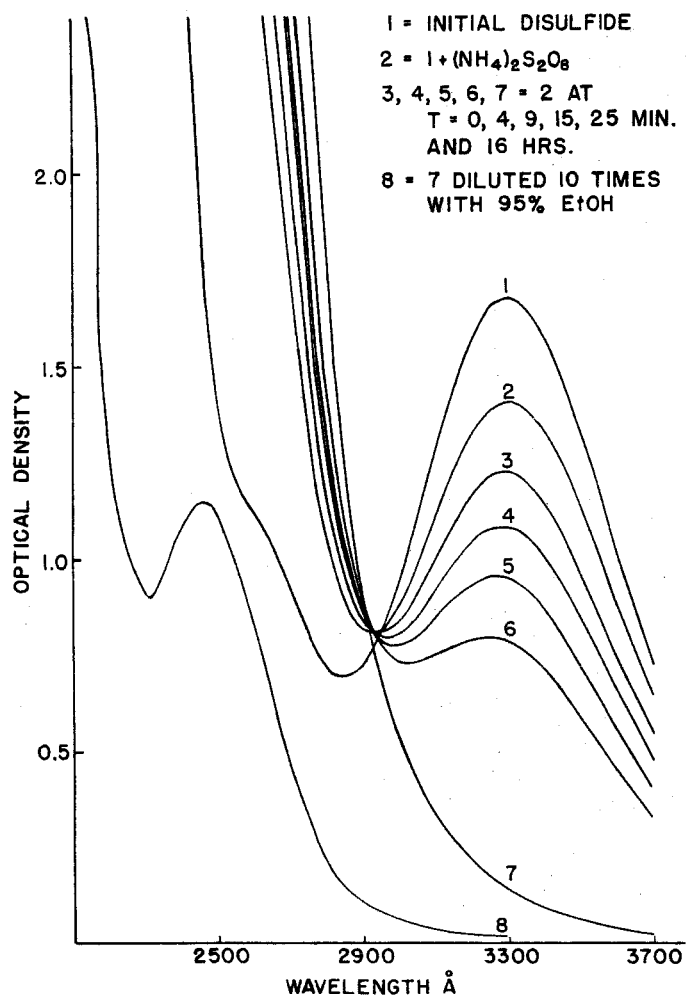
Fig. 14. Oxidation of trimethylene disulfide by ammonium persulfate.

Table I

Kinetics of disulfide reaction with persulfate,

$$\overset{\boxed{}}{S—S} + S_2O_8^= + H_2O \xrightarrow[\substack{25°C \text{ in } H_2O \\ (0.1\,\underline{N}\ H^+)}]{k} \overset{\boxed{}}{S—S}=O + 2\,HSO_4^-\ .$$

$$-\frac{d(S\underline{\quad}S)}{dt} = k\left[S—S\right]\left[S_2O_8^=\right]$$

Compound	$k(m^{-1} \cdot 1 \cdot min^{-1})$
6,8-Thioctic acid (5 ring)	141.0
5,8-Thioctic acid (6 ring)	4.2
1,2-Dithiaheptane-4-carboxylic acid (7 ring)	$< 10^{-6}$
8-Methyl-6,8-thioctic acid (5 ring)	220.0
di-n-Propyl disulfide	~ 0

In Fig. 15 you can see that the reaction with mercaptoethanol will lead to the opening of the ring and formation of a new sulfur-sulfur bond. We have the same number and kinds of sulfur bonds on both sides; the difference between the two sides should be the strain energy of the five-membered ring. It was quite easy to measure what looked like an equilibrium here, because the spectrum of the ring has a nice absorption, as shown earlier, at 3300 $\overset{o}{A}$, but there is nothing on the right-hand side that absorbs in this region. So, all that was required was to mix the two components in various ratios and determine by the spectrum the amount of ring disulfide present. One could shift the equilibrium backward by simply diluting the reaction mixture, and you see on the right-hand side the product is one molecule, on the left-hand side two molecules, so that the equilibrium should shift backward by dilution—and indeed it does. Measurements of this equilibrium constant as a function of temperature produce a ΔH for this reaction of about 4 to 5 kcal, which is much smaller than we had originally anticipated. Quite recently, Suner in Sweden has made direct measurements of combustion heats of a series of compounds related to this, and has come to a similar conclusion: that the ΔH—strain energy—in this molecule, measured from the combustion heats, appears to be 3 to 5 kcal. Frankly, I do not understand why it is so small if these measurements are correct. I think it should be larger. Perhaps something has escaped us, but this is the way the experimental facts stand at the moment.

Now another piece of quantitative information about the disulfides concerns the oxidation potentials. You can see that we can describe a simple relation between disulfides and dithiols of this sort: We may write the oxidation-reduction potentials for the half reaction,
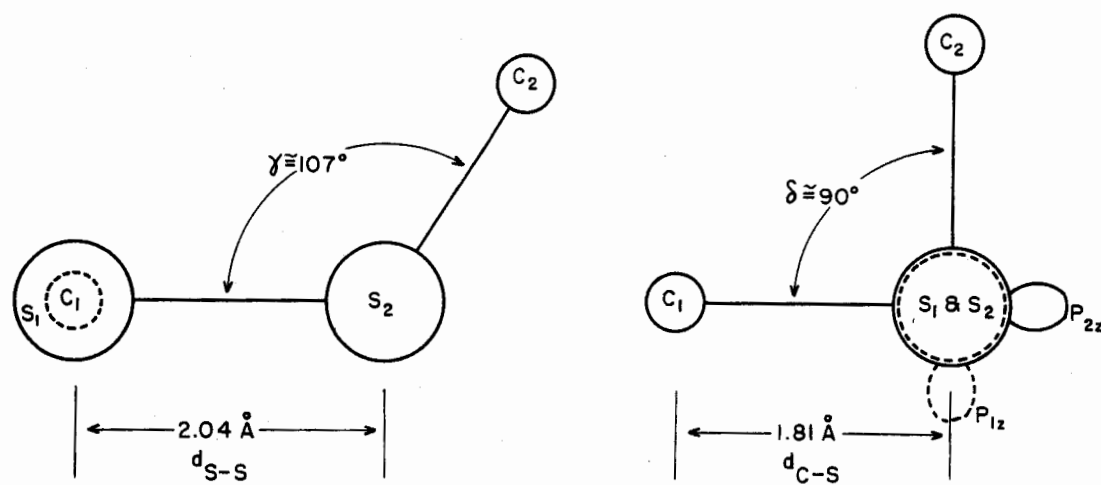
MU-6877

Fig. 16. Geometry of S –S –S –C system.

thioctic acid to a plant should improve the efficiency with which it can use light.

This we have been able to do, and Fig. 17 shows the type of experiment we were able to achieve with a green alga. Here the control is the Scenedesmus without any added thioctic acid; the open circle is the result when one adds thioctic acid to the Scenedesmus culture and measures the rate of evolution of molecular oxygen. You can see that here in ten minutes, for example, almost half again as much oxygen has been evolved from the same algae in the same light when we have added thioctic acid present as when we do not. Another way of expressing this would be to say that the efficiency of the quantum conversion has been improved by 50%, which is quite a large improvement.

Now an experiment such as this demonstrates quite clearly that the thioctic acid is indeed in the sequence of events from the absorption of light to the production of molecular oxygen and has something to do with it. But whether any intermediate hydrogen carrier lies between the light-absorption act and thioctic acid remains to be determined. In order to get this effect, one must allow the algae to be in contact with thioctic acid for at least ten minutes. This suggests that the thioctic acid itself, as free acid, is not effective in this reaction, but that it must be metabolized in some aerobic process to produce the active form of thioctic.

You have seen that it is possible to increase the quantum yield by allowing the alga, the green plant, to metabolize thioctic aerobically. If air is kept away, then this phenomenon does not occur. I repeat this is not the free acid but some complex of it. And therefore we undertook, for this and other reasons, to discover what form the thioctic acid may have, i. e., in what form it exists, in the green plant. In order to do this, we synthetized sulfur-labeled thioctic acid. (I shall not go through the synthetic procedure because there was not anything particularly new in it in the way of synthetic reactions. The only thing that is necessary to say is that we went through the 6,8-dibromide and radioactive sulfur-labeled benzylmercaptan to give the dibenzylmercaptooctanoic acid, which was then reduced with sodium in liquid ammonia to produce the dithiol-labeled thioctic acid. This in turn was oxidized with catalytic amounts of iron and molecular oxygen to the disulfide.) We made some relatively high-specific-activity thioctic acid--I think it was of the order of 50 $\mu c/mg$. The specific activity of the last preparation I was telling you about was 58 $\mu c/mg$, which is high specific activity for materials of this kind. (Incidentally, what I am telling you now has happened since I left Berkeley and I have it from my collaborator, Dr. Hans Grisebach, in various forms--letters, notes, etc.)

The algae were inoculated with this sulfur-labeled thioctic acid and the fractionation of the organisms was made, dividing them into plastid and nonplastid materials. It was found (Table II) that between approximately 5% and 10% of the incorporated thioctic acid is in the plastids, about 20% in the supernatant material, and the remainder in the insoluble compounds. From all three, upon acid hydrolysis (see Table III), one can recover only labeled thioctic acid or labeled thioctic acid monoxide.

Table II

Radioactivity distribution in Chlorella		
	Incubation time	
	3 hours	4 hours
Plastids	7.3%	6.3%
Supernatant	16.8	24.6
Insoluble	76.0	70.0

Table III

Radioactivity distribution of 6-thioctic acid lipid hydrolyzed with commerical Lipase		
	Lipid + lipase	Control (Lipid + 0.1 $\underline{N}$ CH_3COOH without lipase)
Origin	16%	4%
6-Thioctic acid sulfoxide	55	
6-Thioctic acid	11	
Front (lipid)	19	96

This immediately tells us that even after four hours of metabolism the sulfur is not removed from the thioctic acid and incorporated into other compounds such as cysteine, cystine, etc. In the supernatant and insoluble material, the thioctic acid is found in some very easily hydrolyzable form; very mild treatment with dilute acid liberates the free thioctic acid or its oxide. In the plastids, however, the thioctic acid is in quite a different form--it is in the form of a fat or lipid and it is not very easily hydrolyzed. (The complete characterization of this lipid has yet to be made.) But from the lipid one can obtain, again upon complete hydrolysis, only the disulfide or the monoxide, and the hydrolysis rates are given in Table IV. An initial hydrolysis occurs fairly rapidly, and later more slowly; furthermore, in this intermediate phase of the hydrolysis there appears a sulfur-labeled thioctic acid—containing compound which has a chromatography characteristic lying between that of the lipid and that of the free acid. Experiments with lipases in an attempt to characterize the nature of the lipid—thioctic acid complex have been inconclusive because the specificity of the lipases that we had was not sufficiently great. But the characteristics of the hydrolysis curves, taken together with other properties, seem to demonstrate quite conclusively that the thioctic acid in the plastids is all in the form of a glyceride, or at least with its carboxyl esterified. In all probability, it is a glyceride, but this remains yet to be established. It is very difficult to

Table IV

Hydrolysis of 6-thioctic acid lipid with 0.1 $\underline{N}$ HCl at 110°C

Time	Percent hydrolysis
10 min	3.5
30 min	19.5
1 hr	43.5
3 hr	68.5

separate it from the phytol-containing plant pigments, and it requires a double chromatography to achieve the separation, but we can say now that it is not attached to chlorophyll as it would seem.

I think that when we shall know the precise character of the lipid-thioctic acid complex, which is the photochemically important one, we may be able to do experiments with plant fragments instead of with entire plants, adding this particular complex of thioctic acid. (This, of course, will apply to other organisms and functions as well.)

There is much more to say about the photochemical relationships of thioctic acid, but time will not allow it here. We must reserve some time for discussion, and I therefore close these remarks by saying that while we do not yet know the precise position occupied by thioctic acid in the chain of photochemical transformations, we do know that it is in that chain of energy transformation. We have a number of experiments in progress now which may help us eventually to place it precisely in the chain, and we have in the last year suggested that nothing lies between the excited chlorophyll and thioctic acid--that the electron is handed directly from the excited chlorophyll to the thioctic acid. This may not be true, and remains for the future, to be determined.