ULTRAVIOLET ABSORPTION SPECTRA OF FATTY ACIDS AND THEIR APPLICATION TO CHEMICAL PROBLEMS[1]

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CONTENTS

I. INTRODUCTION

The relation of spectral characteristics to the structure of organic compounds was first pointed out by Graebe and Liebermann in 1868 (37). Witt, in 1876, recognized the existence of special structures responsible for specific absorption of light and named them chromophores. According to the earliest theories, double bonds were essential to chromophores and that view is held today.[2] The simplest and most common of these are ethylenic and carbonyl groups. The position and intensity of absorption bands depend upon the number of such groups, their position with respect to each other (conjugated or unconjugated), and the nature of the substituent groups attached to the unsaturated carbon atoms.

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[2] Infrared absorption is not being considered here.

Absorption spectroscopy advanced so rapidly that by 1918 Watson's (37) book on *Colour in Relation to Chemical Constitution* was adopted as a reference text by organic chemists. Quantitative spectrophotometry is now rapidly displacing ordinary colorimetry as an analytical tool. However, the early work was confined to the visible and near ultraviolet by the limitation of light sources, spectroscopes, and recording devices. Gradually, reliable measurements are being extended toward the Schumann region through those wave lengths at which the unsaturated fatty acids absorb. In 1918 Watson stated that unsaturated aliphatic hydrocarbons do not show selective absorption in the ultraviolet and cited this as an exception to the general rule that unsaturation is the cause of such absorption. Since that time it has been repeatedly demonstrated that such compounds do absorb strongly in the far ultraviolet, and it is in this region of the spectrum that all of the fatty acids must be studied.

In this laboratory both the photographic and the photoelectric instruments have been used, essentially as recommended by Henri (18), Hogness *et al.* (20), and Harrison *et al.* (16). Modifications in methods now used in our laboratory will be described in a future publication.

The solvent used has an effect on the absorption bands and should be mentioned. This is especially noticeable in the case of salts of the fatty acids dissolved in water, as compared with the acids or esters in organic solvents (25).

The absorption values given in this paper are molecular extinction coefficients (ϵ), absorption coefficients ($E^{1\%}_{1\text{ cm.}}$), or specific absorption coefficients (k). All values are calculated from Lambert's and Beer's law,

$$\log_{10} \frac{I_0}{I} = \epsilon cl$$

ϵ is obtained when the concentration is expressed in moles per liter. For $E^{1\%}_{1\text{ cm.}}$ and k, concentrations are expressed as grams per 100 cc. and grams per liter, respectively. All values are calculated to a cell thickness of 1 cm.

Below 2300 Å. the errors increase and many curves are semi-quantitative. With substances having very low absorption, impurities of high absorption may mask the true bands. Substances with strong bands must be so diluted that they are likely to be oxidized or otherwise changed in the solvent. Carr and Walker (9) point out that the absorption curve of 3-heptene is markedly affected by the presence of so-called peroxides which form readily in most olefins.

II. QUANTITATIVE ABSORPTION SPECTRA

The saturated hydrocarbons, alcohols, and ethers are so transparent that, when pure, they are excellent solvents for measurements at 2000 Å.

or above. With the introduction of a carbonyl group, specific absorption bands appear, as shown in figure 1 (19). Curves for other aldehydes, ketones, and acids have been collected by Henri in the *International Critical Tables* (19). Ketones and aldehydes absorb in a region at which the carboxylic acids are transparent. Comparisons of various carboxylic acids are given in figure 2.

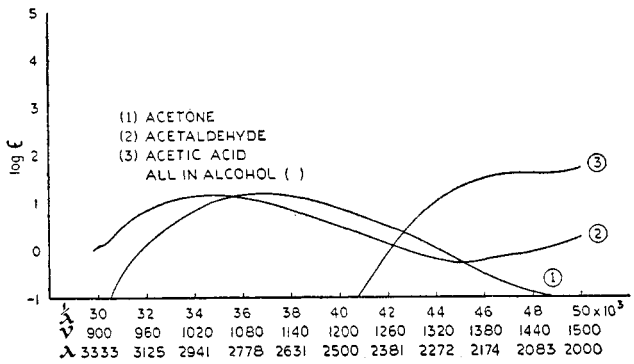

FIG. 1. Absorption coefficients of acetone, acetaldehyde, and acetic acid (after Henri)

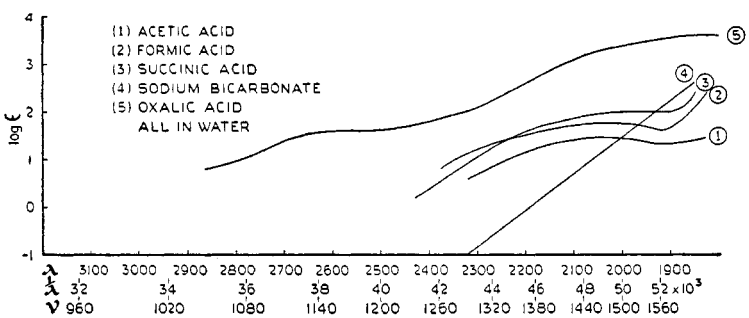

FIG. 2. Ultraviolet absorption spectra of some carboxylic acids (from Ellinger)

Henri (18) established that at the same wave length ϵ(succinic) = 3 × ϵ(acetic) and ϵ(tricarballylic) = 9 × ϵ(acetic). The very high absorption of oxalic acid is attributed to the conjugated carbonyl groups. Without unsaturation the absorption curves of all fatty acids should closely resemble that of acetic acid. This is indeed the case.

The single ethylenic group of olefins introduces absorption bands, as illustrated in figure 3. The position and intensity of these bands are largely controlled by the number of alkyl groups attached to the unsaturated carbon atoms and are only slightly affected by the number of carbon

atoms in these groups. The quantitative curves for 3-heptene and tetramethylethylene are shown in figure 13.

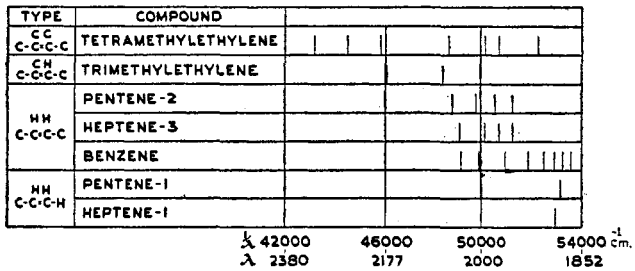

FIG. 3. Absorption bands of olefins in the Schumann ultraviolet (redrawn from Carr and Walker)

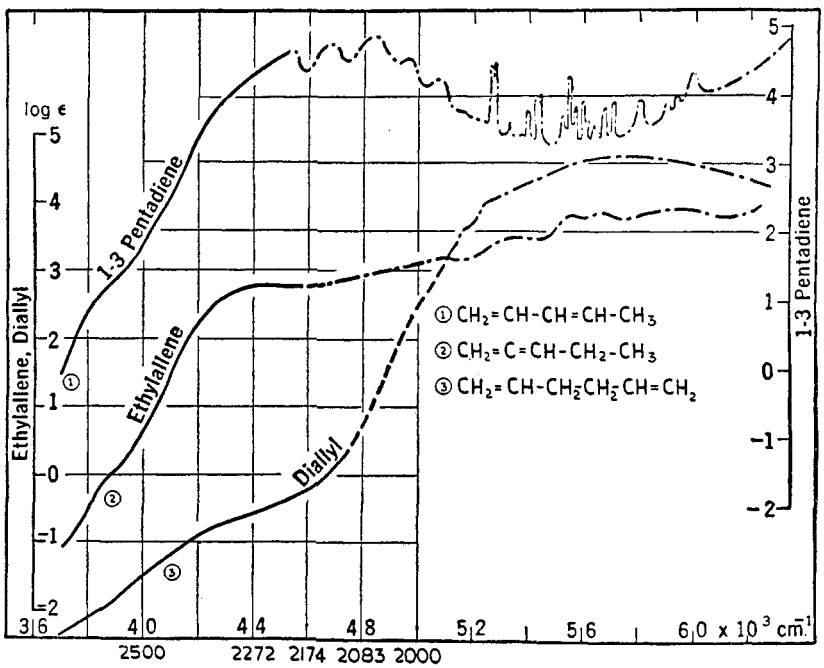

FIG. 4. Absorption curves of hydrocarbon dienes (adapted from Carr and Stücklen). The dotted portions of the curves were run in the vapor phase and give estimated intensities not as quantitative as the measurements on liquids and solutions (solid portions).

The effect of the position of two ethylenic linkages in hydrocarbons is shown in figure 4 (8). When the double bonds are isolated (as in diallyl), there is no mutual interaction and the curve resembles that of 1-pentene

(10). When the double bonds are adjacent (as in ethylallene), the absorption is greatly intensified in the longer wave lengths but is still far less than that of a conjugated system (1,3-pentadiene).

The mixed fatty acids of natural oils exhibit absorption bands due to both the carboxyl and the ethylenic groups. Free fatty acids, glycerides, or other esters may be used more or less interchangeably in these studies. However, if natural oils are used, the absorption by compounds other than the glycerides may predominate. This is the case in many of the curves

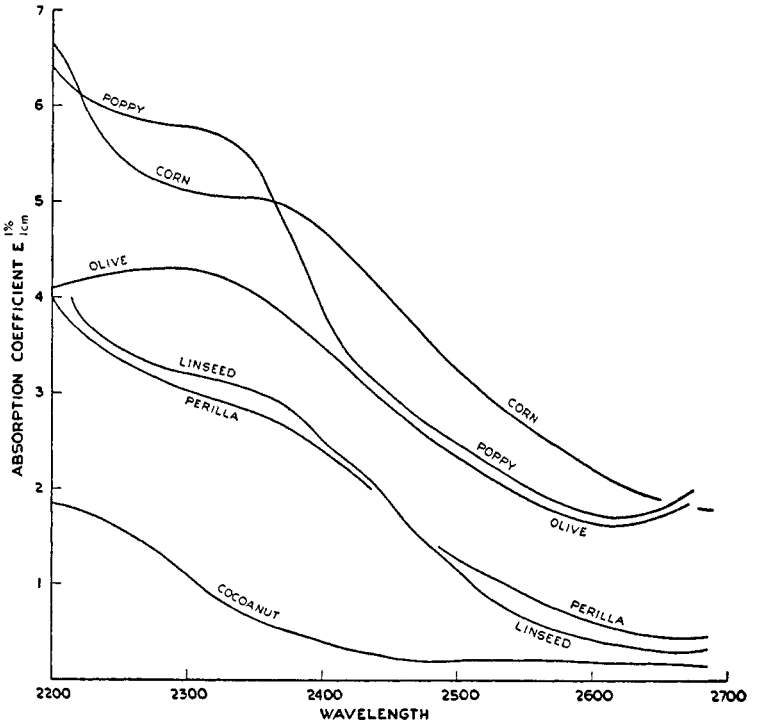

FIG. 5. Absorption spectra of some oils (University of Minnesota)

in the literature, especially at wave lengths longer than 2300 Å. It is clear from figures 5 (unpublished data from our laboratory) and 6 (5) that, although the highly unsaturated oils absorb more strongly than the saturated oils at wave lengths longer than 2250 Å., there is no direct relationship between the iodine number and the degree of absorption. In natural oils and impure preparations the bands at the longer wave lengths are apparently due to substances other than pure unsaturated fatty acids. Hulst (21) removed the impurities by absorption on bleaching earth (figure 7). He distinguishes between "chemically pure" and "optically pure"

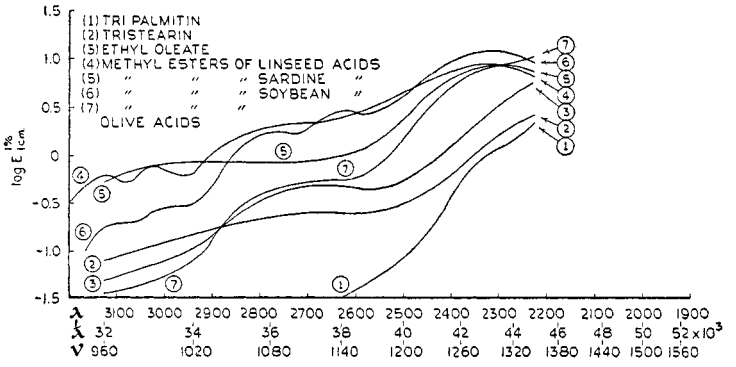

FIG. 6. Absorption spectra of natural fatty acid esters (drawn from data of Bradley and Richardson).

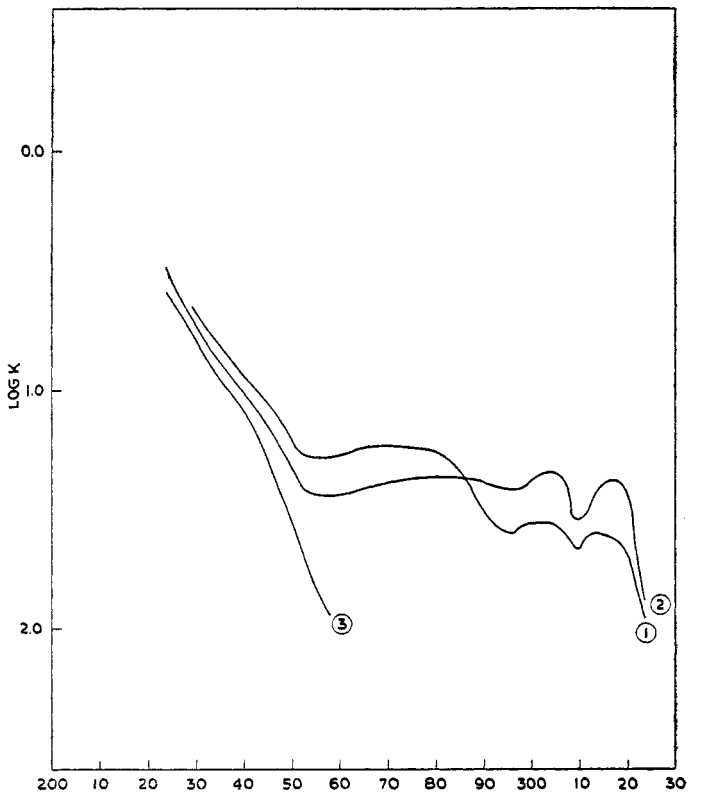

FIG. 7. Absorption spectra of (*1*) distilled ethyl linolenate, (*2*) ethyl linolenate after treatment with 10 per cent of bleaching earth, (*3*) ethyl linolenate after treatment with 100 per cent of bleaching earth (redrawn from Hulst).

ethyl oleate. Extensive spectrographic data for natural fats and their mixed fatty acids are given by Gillam *et al.* (15). Additional curves for plant and animal fats will be found in the reviews by Ellinger (14).

Much of the data for fats and oils is of limited value because the measurements do not extend far enough into the ultraviolet. Many curves stop at 2500 Å. The same is true for the purified fatty acids, and this accounts for the limited material available for comparison.

The saturated fatty acids show small differences in position and height of the absorption bands. Bielecki and Henri (3) compared formic, acetic, propionic, butyric, and valeric acids and found a regular shift toward the red with added —CH_2— groups and a rise in absorption maximum. Henri (18, page 93) states that for the same absorption there is a shift of $\Delta\gamma = 30 \times 10^{12}$. However, Ley and Arends (25) have made an extensive study of some acids and show that this general rule can not be applied to the higher fatty acids (figure 8). The similarity of acetic and palmitic acids in alcohol is shown by the following data:

Log ϵ	0.35	0.60	0.90	1.10	1.30
λ acetic acid	2411	2390	2350	2315	2269
λ palmitic acid	2428	2400	2360	2325	2295

They, too, find a large shift toward the red for butyric acid in water, but from figure 8 it is clear that in alcohol the higher fatty acids do not continue the shift postulated by Henri. Great similarity in the absorption spectra of all the fatty acids in alcohol was also found by Ramart-Lucas *et al.* (34) for acetic, butyric, hexanoic, octanoic, myristic, and palmitic acids between 2200 and 2555 Å. They say that CH_3— and —CH_2— groups can not be considered chromophores in this region of the spectrum.

The unsaturated fatty acids absorb more strongly than the saturated, since the effects of the ethylenic linkages are added to the carbonyl absorption. The magnitude of these effects depends upon the number and position of double bonds, as shown by Carr and Stücklen for hydrocarbon dienes (figure 4). Figure 9 presents some absorption curves of well-known fatty acids. All the curves are redrawn from Hulst, except curve 9 which is taken from Kaufmann *et al.* (24). Not shown here is the curve for ricinoleic acid, which Hulst found to be so much like that of oleic acid that he concluded that the hydroxyl group had no characteristic influence. The same may be said about licanic (couepic) acid, which is 4-ketoeleostearic acid. Steger and van Loon (36) compared it with β-eleostearic acid and found the curves to be almost identical. From figure 9 it is clear that the unsaturated acids fall into three groups: those with isolated double bonds (unconjugated) and those with the C=C—C=O or C=C—C=C

groupings (conjugated). A discussion of the conjugation of ethenoid and carbonyl groups is given by Morten *et al.* (32) in a spectrographic study of keto–enol isomerism. These authors formulate the hypothesis that "the

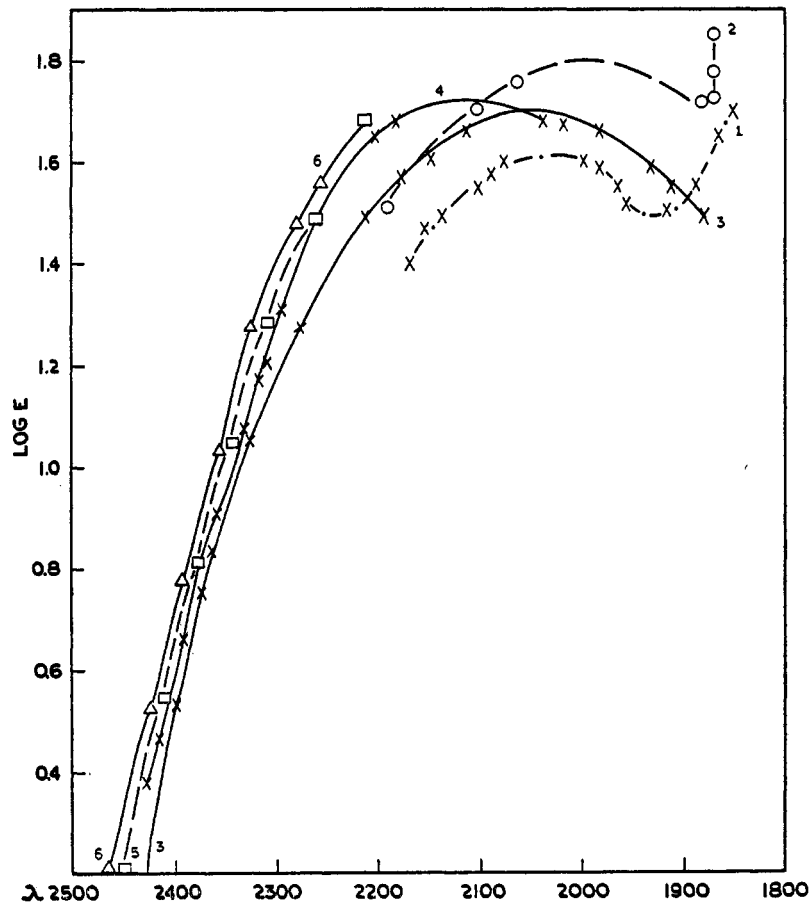

FIG. 8. Absorption spectra of some saturated fatty acids in alcohol, water, and hexane. Curve 1, acetic acid in water (Ley and Arends); curve 2, acetic acid in hexane (Ley and Arends); curve 3, acetic acid in alcohol (Ley and Arends and Henri); curve 4, palmitic acid in alcohol (Ley and Arends); curve 5, butyric acid in alcohol (Henri); curve 6, valeric acid in alcohol (Henri).

different energy levels characteristic of the carbonyl group are the same, or very nearly the same, as those characterizing the ethenoid linkage." It is clear, however, that in a diene like 9,11-linoleic acid the first strong absorption band is shifted much more toward the red. This shift is such

that when measurements do not go below 2200 Å. the maximum absorption of only the conjugated polyenes is observed. The unconjugated acids absorb only slightly more than saturated acids, and the curves for α, β-forms are still rising toward a maximum below 2100 Å. (see figure 12). Figure 10 shows the change in position and intensity of absorption bands with increasing numbers of conjugated double bonds (35). Such smooth curves do not represent the true spectra of conjugated trienes and tetraenes, as shown in figure 9. Kaufmann *et al.* (24) found the number and the intensity of bands in decatetraene to correspond almost exactly with those in parinaric acid, with only slight shifts in position (figure 10a).

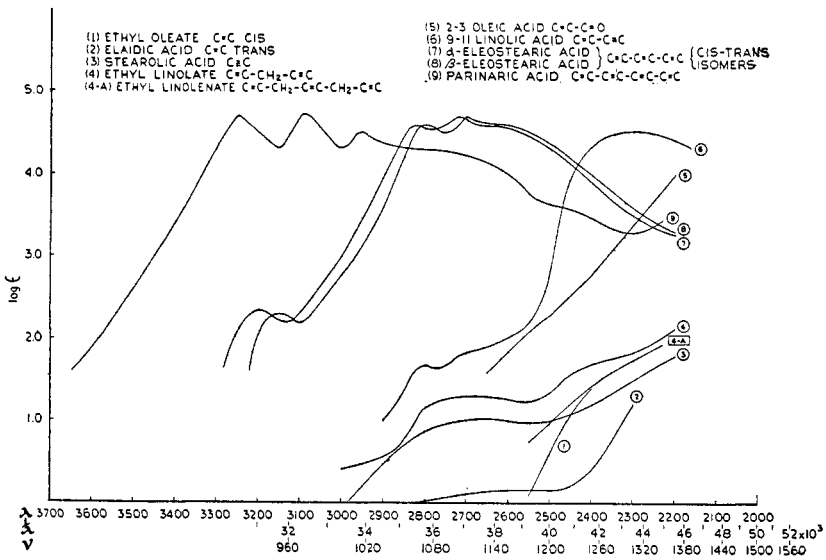

FIG. 9. Absorption spectra of some unsaturated fatty acids (redrawn from Hulst and Kaufmann *et al.*)

Because of possible practical applications, more detailed studies have been made of the natural unsaturated acids, α, β-unsaturated acids, and conjugated dienoic acids. These results are summarized in figures 11, 12, 13, and 14. There is close agreement between our 10,12-linoleic acid and linoleyl alcohol (22) and Hulst's 9,11-linoleic acid. The shift of λ_{max} may be due to *cis–trans* isomerism (see below). For comparison a curve for dimethylbutadiene is included (19). Except for Hulst's curve, the α, β-unsaturated acids (figure 12) show fairly close agreement, but these values indicate that much more quantitative work is needed.

By the use of the Littrow photographic apparatus the absorption curves for common unsaturated acids have been extended to 2100 Å. in this

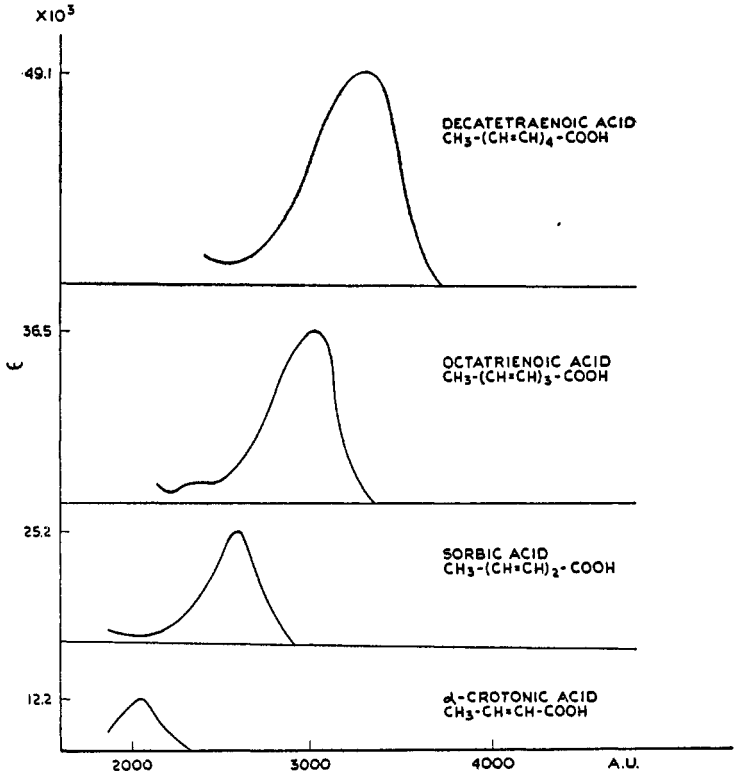

FIG. 10. Absorption spectra of some conjugated acids (redrawn from Smakula)

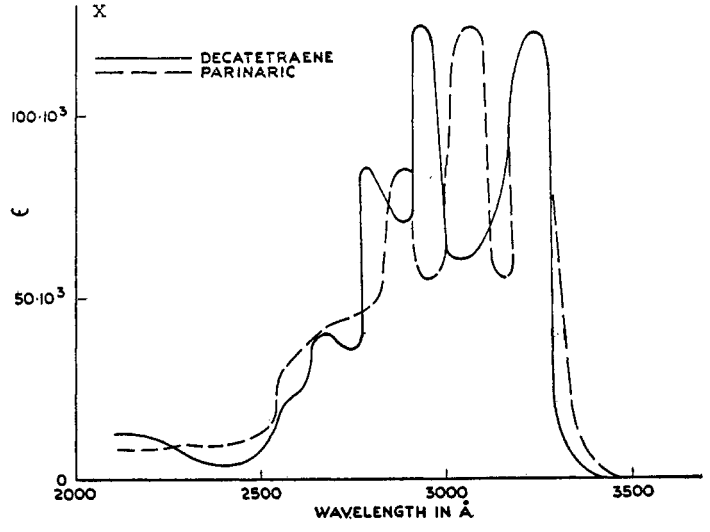

FIG. 10a. Absorption spectra of decatetraene and parinaric acid (from Kaufmann *et al.*)

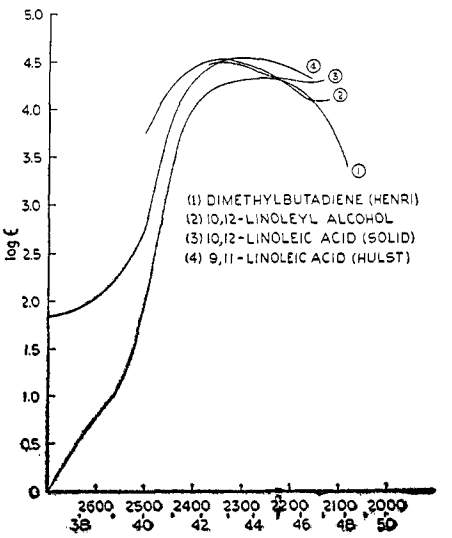

FIG. 11. Absorption spectra of some dienoic compounds. The work on the 10, 12 compounds was done in this laboratory.

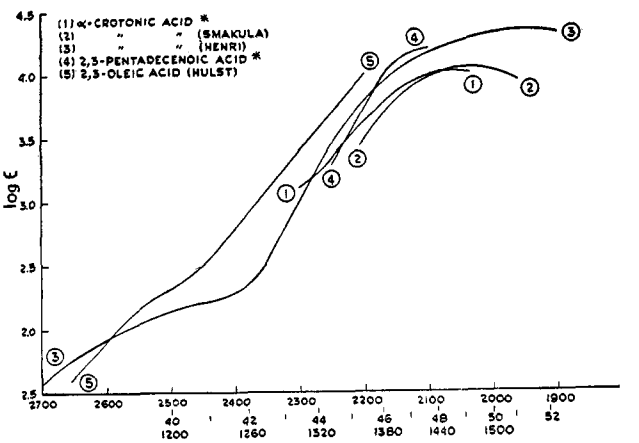

FIG. 12. Absorption spectra of some α,β-unsaturated acids. The starred curves are from this laboratory. The authors are indebted to Dr. Walter Lauer for the sample of 2,3-pentadecenoic acid.

laboratory. Acids of the highest purity available have been used.[3] The data are summarized in figure 13. For comparison, the curves of 3-heptene

[3] We are indebted to Dr. J. P. Kass for these preparations and to Dr. Richard Barnes and Mr. Irving Rusoff for most of the spectrographic work.

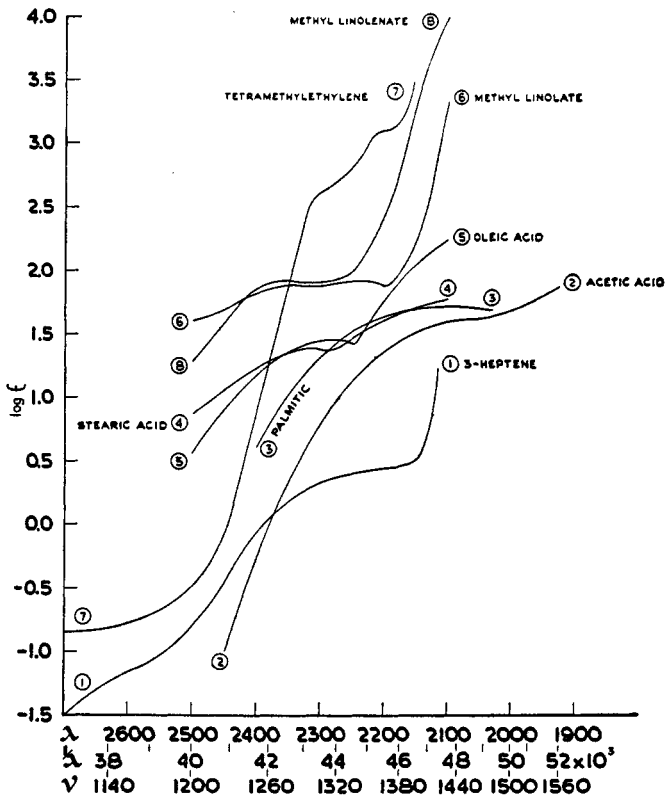

FIG. 13. Absorption spectra of some fatty acids and ethylenic hydrocarbons. Curves 1 and 7 are from Carr and Walker; curve 2 from Henri; curve 3 from Ley and Arends; curves 4, 5, 6, and 8 from this laboratory.

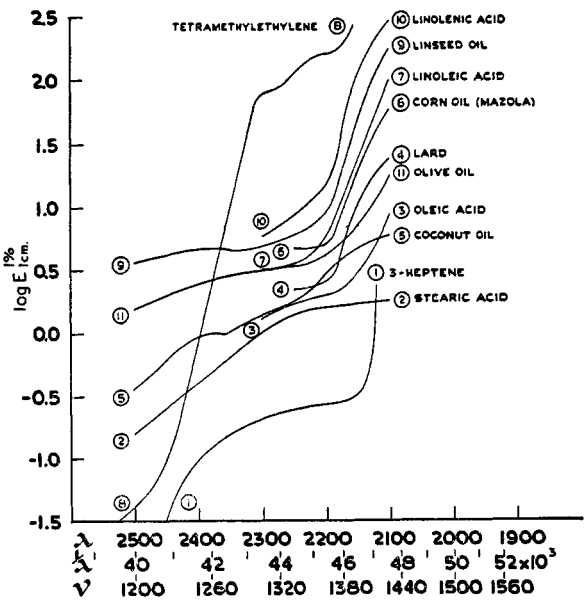

FIG. 14. Absorption spectra of fatty acids and oils, calculated as $E\,^{1\%}_{1\,cm.}$.

and tetramethylethylene from Carr and Walker (9) are inserted. Our stearic acid compares well with the palmitic acid of Ley and Arends (25).

All of the unsaturated acids studied superimpose on the carboxylic absorption a band due to the ethylenic groups. In oleic acid the new band starts at slightly longer wave lengths than that of 3-heptene. In the linoleic and linolenic acids this shift continues, showing the influence of additional unconjugated double bonds. These curves are replotted in figure 14 as log $E^{1\%}_{1\,cm.}$ for comparison with the absorption of some common oils. It is clear that, except for coconut oil which seems to have had an absorptive impurity, the values fall within the range expected from their known composition. It seems that most of the absorption by oils thus far observed in the far ultraviolet (below 2200 Å.) is due to the known fatty acids. Unfortunately, there is no material in the literature to compare directly with these measurements. The curves of Hulst, of Bradley and Richardson, and of Brode and Tyron (6) do not extend to 2200 Å.

III. APPLICATIONS

Numerous applications of spectroscopy to lipid problems have been made and the number is increasing rapidly. Citations here will be limited to a few examples from each category of use with fatty acids, excluding the many applications to the fat-soluble vitamins, hormones, sterols, pigments, etc., in which fields spectroscopy has been an invaluable tool of identification.

A. Analysis

In 1927 Manecke and Volbert first studied the absorption spectra of eleostearic acids, but not until 1934 did Dingwall and Thomson (12) show that the spectra of the acids were so different that the percentage composition of a mixture of the two could be determined spectroscopically.

For some years this laboratory has used spectroscopy for quantitative measurement of different conjugated systems (23, 27), as well as for the proof of the presence of such structures in mixtures. Spectroscopic measurements are unaffected by hydroxyl groups and others which interfere with the maleic anhydride diene number. Since they further distinguish between conjugated dienes, trienes, and tetraenes, spectroscopy is to be preferred to chemical methods of dienometry. Figure 15 compares the chemical and spectroscopic measurement of dienes in some commercial preparations and in two dienoic acids.

We have found that Moore's reaction (see below) can be made reproducible and hence an empirical quantitative measure of linoleic and linolenic acids in oils. In this reaction bonds are shifted to the conjugated position, giving an absorptive diene and a triene from linoleic and linolenic acids,

respectively. Unpublished results from this laboratory show the following content of linoleic acid in commercial oils: cottonseed, 57.5; corn, 65.1; almond, 21.5; peanut, 32.9; sunflower, 56.8; castor, 8.1; and olive, 17.2. The method will soon be checked carefully against standard chemical analyses. It is especially useful in showing the presence of a small amount of linolenic acid, which produces a new band at 2700 Å.

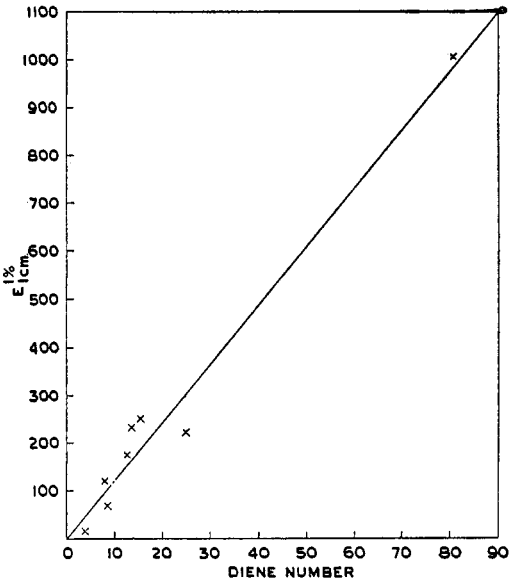

FIG. 15. $E^{1\%}_{1\,cm.}$ at 2325 Å. plotted against diene number. The upper points are from some preparations of fairly pure 10,12-linoleic acids. The lower points are from commercial conjugated preparations.

B. Structure

In addition to the evidence as to conjugation, spectroscopy has been widely used to distinguish the *cis*- and *trans*-forms of ethylenic compounds. This change brings about a shift in the position of the absorption bands, but there is no agreement as to which form absorbs the longer wave lengths. Carr and Stücklen (7) show that in the *cis*-forms of 2-butene and 2-pentene the first absorption band is shifted about 50 Å. toward the red and is very intense. This agrees with the finding of Hulst that the first band for oleic acid (*cis*) appears at longer wave lengths than for elaidic acid (*trans*). From this it would be assumed that α-eleostearic acid is the *cis*-form. Hartley (17) recently used a colorimetric measurement of the degree of conversion of *trans*-azobenzene to *cis*-azobenzene, made possible by the change in visible absorption.

Morton *et al.* (32) have shown the usefulness of the spectrographic method in determining the keto–enol isomerization of oxy acids.

C. Chemical reactions and changes during processing

In 1931 Gillam *et al.* (15) showed the presence in butter of spectroscopically active acids with an absorption maximum at 2300 Å. Booth *et al.*

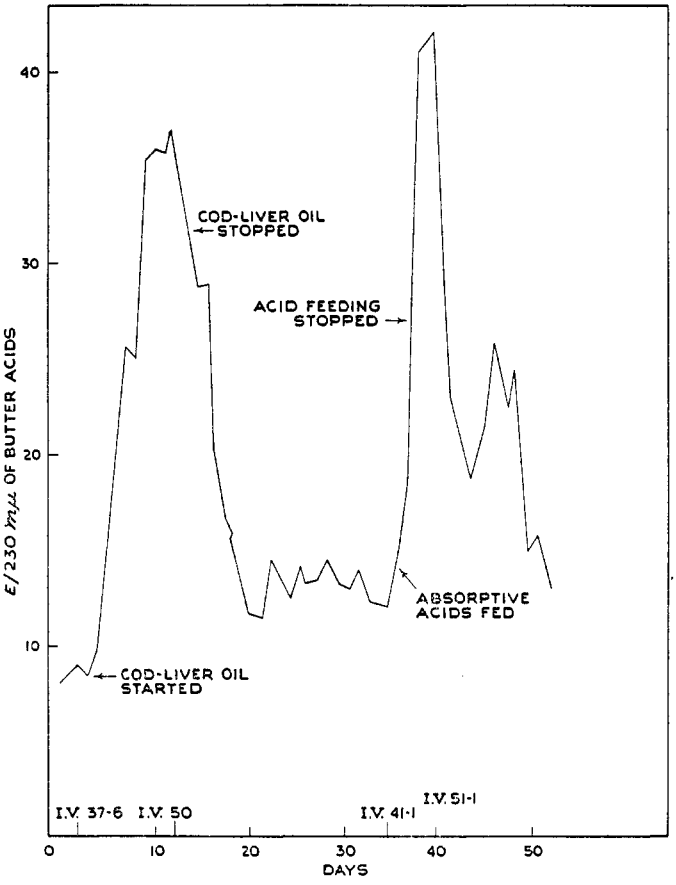

FIG. 16. Effect of feeding cod-liver oil and spectroscopically active acids on butter acids (from Dann *et al.*).

(4) demonstrated the seasonal variation of these acids, with absorption values ($E\,{}^{1\%}_{1cm.}$) running from about 25 in the summer to about 10 in the winter. The same authors (11) also demonstrated the effect of feeding cod-liver oil on butter fatty acids, shown in figure 16.

During the course of these investigations the above workers found that

prolonged saponification increased the absorption of the fatty acids. This led to an extensive investigation by Moore (29), who showed that the effect increased with unsaturation. Some of his data are given in table 1. He further showed that from linseed oil new acids were produced which resembled eleostearic acid. Edisbury *et al.* (13) applied the saponification technique to fatty acids of cod-liver oil and concluded that cyclization

TABLE 1

Effect of unsaturation on absorption

OIL	IODINE VALUE	$E^{1\%}_{1\text{ cm.}}$ AT 2325 Å. AFTER 24 HR. SAPONIFICATION
Coconut	8–9.5	2
Olive	79–88	12
Peanut	83–100	51
Cottonseed	108–110	120
Soybean	122	120
Linseed	171–201	170

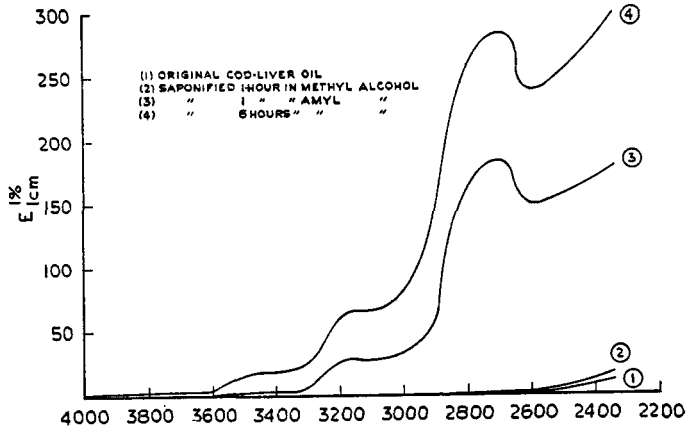

FIG. 17. Absorption spectra of cod-liver oil acids after prolonged saponification (drawn from Edisbury *et al.*).

of the natural C_{20} and C_{22} acids is the most plausible explanation of the changes in absorption spectra. The reaction takes place quickly at higher temperatures, as shown in figure 17.

Vacuum distillation of the methyl esters of highly unsaturated fatty acids brings about changes in the absorption spectra. Gillam *et al.* (15) found a decrease in absorption during distillation, presumably due to decomposition of the active groups. On the other hand, Norris *et al.* (33) have shown that there is some increase in absorption of the highly

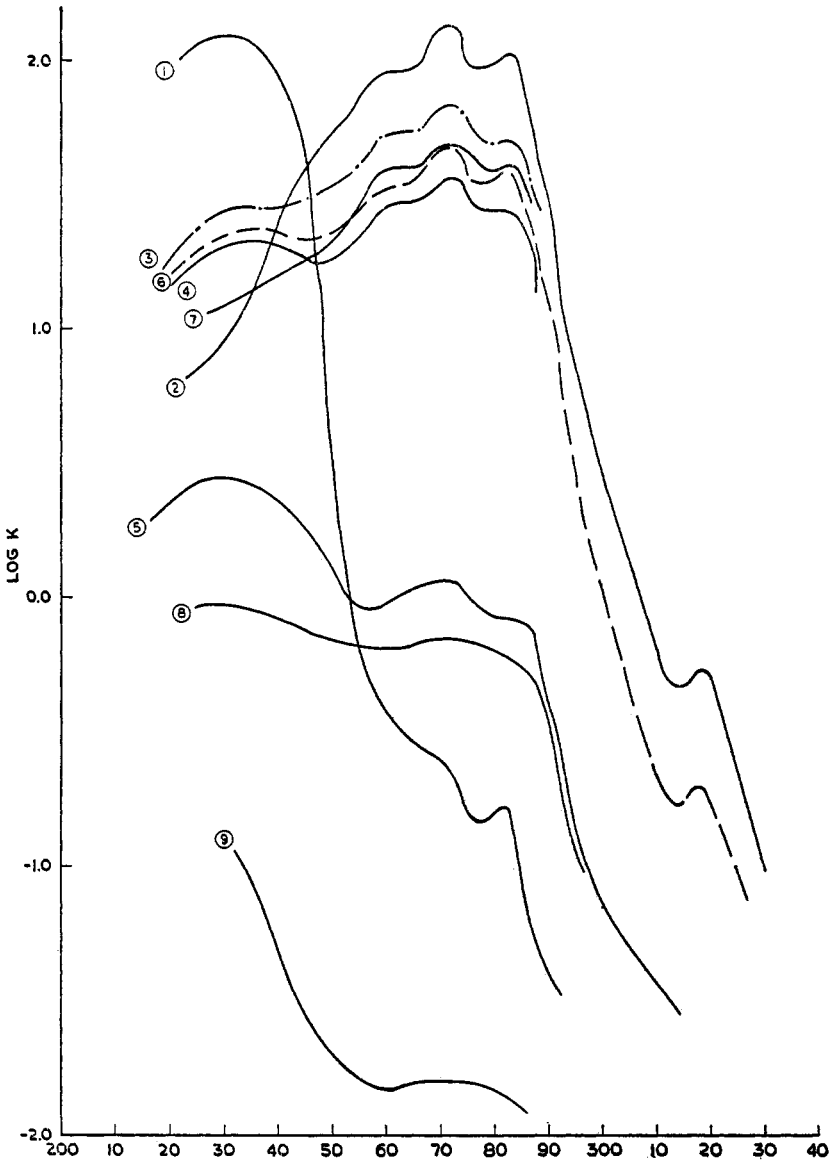

FIG. 18. Changes in absorption spectra of tung oil during hydrogenation (Hulst). Curve 1, 9,11-linolic acid; curve 2, China wood oil; curve 3, hydrogenation product A_1 (Normann); curve 4, hydrogenation product B_2 (Normann); curve 5, hydrogenation product C (Normann); curve 6, hydrogenation product b_5 (Wilbuschewitch); curve 7, hydrogenation product IX (high pressure); curve 8, hydrogenation product —(high pressure); curve 9, glycerols of palm oil.

unsaturated fatty acids of linseed and cod-liver oils due to the heating required for distillation. Bradley and Richardson (5) have also used the spectrographic method of following changes in drying oils during heating.

In 1935 Hulst (21) used absorption spectra for the detection of impurities in fatty acids and for following the changes in tung oil during hydrogena-

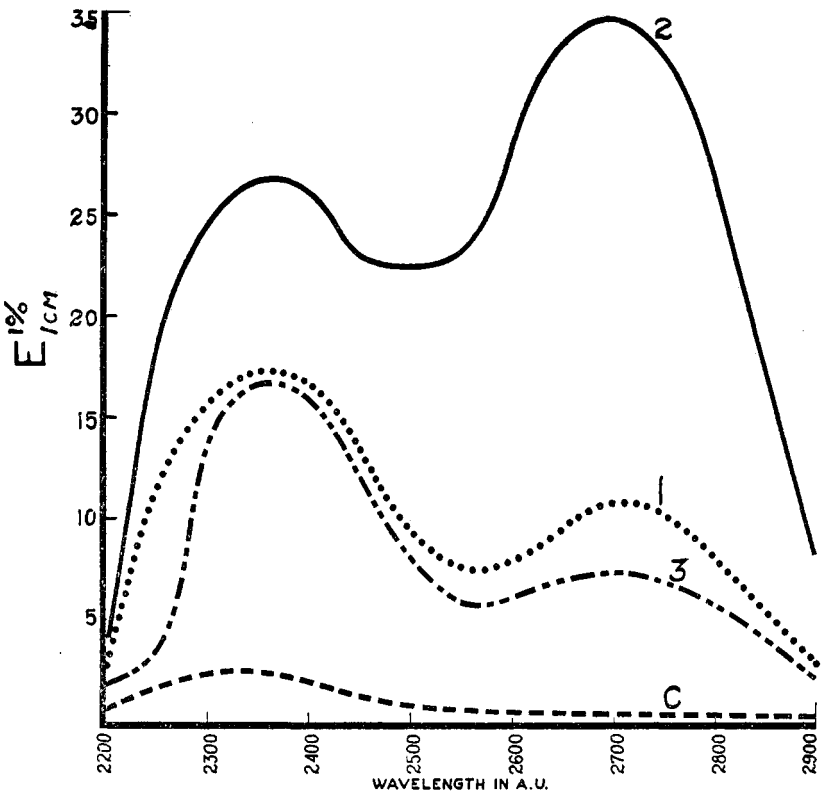

FIG. 19. Absorption curves of lipids extracted from adipose tissue of three experimental rats and a control. Curves 1, 2, and 3 represent rats killed 24, 48, and 70 hr., respectively, after consumption of the first tung oil. Curve C is the control on stock diet, but with no tung oil.

tion. Partial hydrogenation eliminates the absorption band at 2700 Å., with the production of a new band at 2300 Å. (figure 18). Moore (31) has recently repeated the work for comparison with changes which take place when tung oil is fed to rats.

D. Biological tracers

The work of Moore and coworkers (11) showed that spectroscopically active fatty acids appear in butter when fed to cows. Following this

example Miller and Burr (28) fed tung oil to rats, which were killed and analyzed at daily intervals thereafter. The rate at which this material was deposited in the different tissues is indicated by the absorption spectra shown in figure 19. It seems that one of the first metabolic activities is the destruction of one double bond of the eleostearic acids (possibly hydrogenation), resulting in a dienoic acid with absorption maximum at 2300 Å. Moore (30) has recently observed the same phenomenon in the fat of hens' body tissues and eggs. Such changed fat does not appear in the milk of the cow after the feeding of tung oil.

Because of poor edibility and the above spectral changes, tung oil is not an ideal tracer. However, corn oil fatty acids which have been changed by our modification of Moore's reaction do serve well for such work. In this laboratory several papers (1, 2, 26) have been published which show the rates of transfer and phosphorylation of such fatty acids in normal and abnormal rats.

IV. CONCLUSION

With the improvement of apparatus for rapid quantitative measurements of absorption spectra in the far ultraviolet, many problems of lipid chemistry and physiology can be attacked in a more satisfactory manner than is possible without this tool.

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