foreseeable future. It is apt to hold it static. In its field soap is low in cost and efficient. For example, it is improbable that any substantial part of the commercial laundry market will be lost to synthetics.

The first call on the fats and oils is for edible purposes. A short world supply appears probable for a few years ahead—how far ahead ties in with political problems and hoped-for improvements in standards of living, not nationally but internationally. These are beyond chemistry.

The present trend is to supplement our normal supply of soap with synthetics and to accompany that with the development of more and more applications for all kinds of surface-active agents. Synthetic detergents themselves are being studied with a view to bringing their general cleansing ability up to that of soap in soft water. This apparently can be done by special building. Combinations of anion-active agents with nonionics also show greater detergent ability than either one alone; the combination is not merely additive but is more—it is synergistic. Research activity in this field is still intense.

There seems to be little doubt that the percentage of the total detergent market retained by soap will decrease further. How much further is beyond guess, the rash guesses of a few years ago have already been exceeded. The trend away from natural products of agriculture or animal husbandry has been going on for decades. This competition of soap and synthetic detergents is but one small part of it. But in an expanding economy that does not predict that the soap industry will go into a decline. It merely suggests that in the economist's terms, it is becoming a mature industry.

Biological Synthesis of Fatty Acids[1]

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ALTHOUGH little is yet known of the details of the mechanism of the conversion of carbohydrate to fat, the problem has engaged the attention of investigators since about 1850 and is thus one of the oldest of biochemical studies. Previous to this date it had been assumed that body fat was simply derived from dietary fat. Impressed by the large amounts of milk fat continually secreted by the lactating cow on ordinary pasture, Liebig was led to believe that this fat must have been formed from carbohydrate in the diet. Soon after (1860) Lawes and Gilbert (1) performed their classical experiments at Rothamsted with oxen, sheep, and pigs in which careful balance studies of carbohydrate, protein, and fat left no doubt that body fat can be synthesized from dietary carbohydrate. Later studies by several investigators with many animal species thoroughly confirmed their conclusion. In shorter term experiments with the dog Morgulis and Pratt (2) observed, under appropriate feeding conditions, respiratory quotients much greater than unity, which would be required for the carbohydrate to fat conversion. In plants also there is abundant evidence for fat formation from carbohydrate. Seeds during ripening undergo a change from high-carbohydrate, low-fat content to high-fat levels. Studies with seeds included not only those which were still attached to the plant but also separated seeds in which there could be no doubt about the carbohydrate to fat transformation occurring within the seed. Respiratory quotients of 1.5 or greater have been noted during active oil formation.

The formation of fat in various microorganisms would seem to offer ideal test systems for study since the composition of the medium can be so readily controlled and since evidence for the precursor of fat synthesis might be obtained. Many yeasts and fungi synthesize large quantities of fat from relatively simple media. The many contributions in this field cannot be reviewed here, but classical among these investigations has been that of Haehn and Kintoff (3) with *Endomyces vernalis* in which it was shown that acetaldehyde, lactic acid, pyruvic acid, glycerol, aldol, and ethyl alcohol could serve as fat precursors. A strict comparison of their relative efficiency as precursors was difficult however because of different toxicities of the substances tested. A review of subsequent investigations with different microorganisms seems to show that in addition to glucose itself, pyruvate, alcohol, and acetate were the most active as fat precursors. There is little doubt however that the limitations of toxicity and permeability differences make this type of experimentation dubious for the selection of the probable immediate precursor.

Older Theories of Fatty Acid Synthesis

Hexose Condensation Theory: An early and obvious suggestion for the mechanism of fatty acid synthesis was that hexose units combined directly to form a C_{18} precursor of stearic and oleic acids. Emil Fischer (4) first proposed this theory and also postulated the mixed condensation of hexoses and pentoses to explain the occurrence of such acids as palmitic. The predominant occurrence of C_{12}, C_{18}, and C_{24} in nature has been offered as evidence for condensation of hexose units, but more direct evidence was lacking. Reichel and Schmid (5) and Embde (6) found that fructose was more readily converted to fat than other monosaccharides, and this has been offered in support of hexose condensation.

In considering biochemical reactions analogous to the type of reaction leading to carbon-to-carbon linking of hexose molecules, the reverse aldolase reaction comes to mind (see Figure 1). There is no experimental evidence for this reaction involving hexoses rather than trioses, nor for the existence of the highly hydroxylated intermediates which would result. The latter cannot constitute evidence against the hypothesis since intermediates are notoriously missing for the support of any theory of fatty acid synthesis or

[1] Presented at 22nd fall meeting, American Oil Chemists' Society, New York City, Nov. 15-17, 1948.

oxidation; nevertheless in view of the lack of direct evidence for the hexose condensation theory of fatty acid synthesis, other theories are more generally favored.

Aldol Condensation: Since the two-carbon substances, ethyl alcohol and acetate, proved to be efficient fat formers in microorganisms and further since the naturally occurring fatty acids always contain an

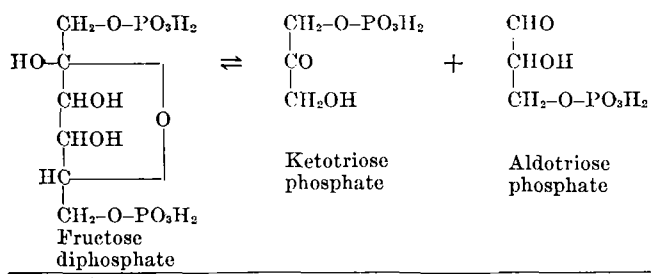

FIG. 1. Aldolase reaction.

even number of carbon atoms, a two-carbon precursor for fatty acid synthesis was early suggested. Actually Nencki (7) suggested acetaldehyde as the precursor before the above facts were established. He prepared the substance aldol by the condensation of acetaldehyde in alkaline solution. Internal oxidation and reduction in this compound could result in butyric acid. Other workers extended this hypothesis. Thus Raper (8) succeeded in condensing two aldol molecules with the formation of a hydroxylated caprylic aldehyde, and Smedley (9) and Kuhn and co-workers (10) have shown that condensation of crotonaldehyde, which is readily prepared by dehydration of aldol, results in unsaturated straight chain aldehydes (see Figure 2). Beta-reduction of either type of compound could produce the saturated carbon chain of fatty acids, the reverse process of beta-oxidation which is so widely accepted as a mechanism of fatty acid oxidation.

Fatty aldehydes are recognized as components of the "aldehyde phospholipids" (11), but the biochemical significance of these compounds is not yet clear. Ehrlich and Waelsch (12) have concluded from isotope studies that the fatty aldehydes are not principally concerned with fatty acid metabolism. A serious objection to the acceptance of an otherwise attractive theory of fatty acid synthesis is that acetaldehyde condensation remains on a chemical level, and this reaction has not been demonstrated to occur in biological systems. Acetaldehyde itself is very toxic to living organisms and has never been recorded as an efficient fat-former. Thus the aldol con-

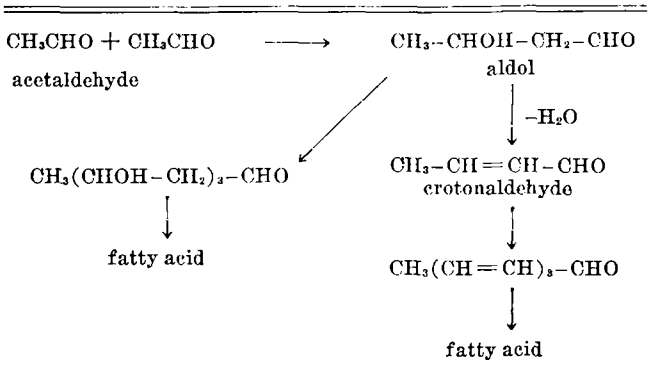

FIG. 2. Aldol condensation mechanisms.

densation theory of fatty acid synthesis is not yet sufficiently substantiated to be completely acceptable.

Pyruvic Acid Condensation: Smedley and Lubrzynska (13) suggested that pyruvic acid might condense with itself to form higher keto acids, and experimental support on the chemical level was offered. Although this theory was not widely accepted, it comes into possible consideration again in view of the biological activity of the postulated type of intermediate. Thus the condensation of pyruvate molecules results in α,γ-oxidized acids, and α,γ-diketo acids are now known to be hydrolyzed by a liver enzyme (14) to pyruvic acid and a lower fatty acid. In view of the biological activity of these compounds it is not impossible that the reverse of this reaction is significant in the biological synthesis of fatty acids.

Newer Developments in Biological Fat Synthesis

Until quite recent times fats were regarded primarily as metabolically rather inert storage materials, made when an excess of calories were consumed, and borrowed upon in starvation. Possibly this was a mental deterrent in attacking problems of fatty acid synthesis, but more likely was the fact that substances of biological origin which might logically be considered as intermediates in the synthesis or degradation of the higher fatty acids were generally lacking. This situation was in marked contrast for example to the intense activity which occurred in intermediary carbohydrate and protein metabolism, afforded in large part by the discovery of intermediates of obvious importance.

There is no doubt that the introduction of isotopes in biochemistry brought a new era to studies of biological fat synthesis. From some of the earliest studies of Schoenheimer (15) it became evident that fat, even the depot fat, is in an active (dynamic) state of flux. By enriching the body fluids with heavy water, a rapid incorporation of deuterium into the fatty acids of neutral fat was noted. Thus it was estimated that more than half of the fatty acids in the liver of an animal on a low-fat diet was synthesized in one day, and in about one week in the depot fat.

These early studies also gave suggestions as to the mechanism of fatty acid synthesis. When the incorporation of deuterium in the saturated fatty acids had reached a maximum value, the deuterium concentration in the fatty acids was one-half that in the body water, which can only mean that the fatty acids were formed by the coupling of small chemical units. Even more significant for this conclusion were the studies with deuterio-palmitic acid. After feeding this acid, the amounts of deuterium found in the stearic acid isolated from the depot fat left no doubt that the C_{18} acid had been synthesized directly from the C_{16} acid. Thus there are biological mechanisms available for synthesizing higher fatty acids by the coupling of small, two-carbon units.

Acetic Acid as a Source of Higher Fatty Acids: The earlier isotope experiments and the probability of acetic acid as a product of fatty acid oxidation led to the testing of acetate as the probable C_2 precursor of fatty acids. In the first experiments of Bloch and Rittenberg (16) the administration of deuterio-acetic acid (CD_3COOH) containing only a moderate excess of deuterium did not result in a significant incorporation of deuterium in the fatty acids

These experiments might have discouraged further testing of acetate, but Rittenberg and Bloch (17, 18) continued work with acetate containing C^{13} labeled acetate, and with acetate labeled both with C^{13} in the carboxyl group and a great (77%) excess of deuterium in the methyl group. In analyzing for the distribution of C^{13} in the fatty acid, there was twice as great an excess in the carboxyl group as in the whole molecule. Thus it is indicated that the labeled carbon of the acetate carboxyl group occurs in alternate atoms of the fatty acid molecule.

In considering the mechanism to explain this picture, the Claisen type condensation resulting in acetoacetic acid is most favored, especially since the acetate to acetoacetate conversion has been clearly demonstrated (see below).

The Rittenberg and Bloch experiments with acetate containing both C^{13} labeled carboxyl and deuterium labeled methyl are of interest. In the fatty acid synthesized there was a considerably lesser proportion of deuterium to C^{13} than in the acetic acid. Since the deuterium of the acetic acid is not exchangeable with hydrogen of the medium, this loss of deuterium indicates either a) that the acetic acid is first converted to a compound with exchangeable hydrogen, or b) that an intermediate produced possesses exchangeable hydrogen in a position corresponding to the methyl group of the acetic acid precursor. It is known that the α-hydrogen atoms of β-keto acids are enolizable and therefore exchangeable.

Acetoacetate Formation from Carbohydrate Precursors: Condensation of two acetic acid molecules by a Claisen type condensation results in acetoacetic acid:

$$CH_3CO-\overline{|OH|}\ \overline{|H|}-CH_2-COOH \longrightarrow CH_3COCH_2COOH$$

Evidence for this reaction is not lacking. Lehninger (19) demonstrated the enzymatic conversion of acetoacetate to acetate, the reverse of the above reaction. Soodak and Lipmann (20) have recently shown that a pigeon liver extract, in conjunction with the new pantothenic acid containing coenzyme (coenzyme A), brings about the synthesis of acetoacetate from acetate. In kidney slices Medes, Weinhouse, and Floyd (21) have demonstrated by isotope dilution technique a rapid and quantitatively important conversion of acetate to acetoacetate. In liver, which is considered the most likely site of fatty acid synthesis, *in vitro*, experiments favor pyruvate rather than acetate as the most active precursor of acetoacetate. Thus the "insoluble residue" of liver, used for studies of fatty acid oxidation by Lehninger (22), produces a rapid and quantitative conversion of pyruvate to acetoacetate. This introduces the possibility that an active two-carbon intermediate of pyruvate metabolism rather than acetate itself is the active condensing agent in acetoacetate synthesis.

Acetoacetate to Fatty Acids with the Lower Fatty Acids as Intermediates: Given acetoacetate as a first stage in fatty acid synthesis, how might higher fatty acids be formed? Acetoacetate might first be reduced to butyric acid before the latter condenses again with acetate. The resulting β-keto hexanoic acid would then be reduced to hexanoic, etc., as pictured in Figure 3. According to this scheme the lower fatty acids are intermediates in the formation of the higher ones.

The work of Barker and associates (23, 24) with *Clostridium Kluyveri* might be interpreted as support of the above theory. Employing this organism Barker, Kamen, and Bornstein (23) were able to isolate both butyric and hexanoic acids as products of the metabolism of acetic acid and ethyl alcohol. When the acetate was carboxyl-labeled (C^{14}), the butyric acid isolated was found to contain C^{14} almost equally distributed between the carboxyl carbon and the β-carbon, as expected from a Claisen type of acetic acid condensation. In the same type of experiment the hexanoic acid isolated contained about one-third of its C^{14} in the carboxyl carbon. Also when carboxyl-labeled butyric acid was incubated with ethyl alcohol, hexanoic acid was formed, and the C^{14} did not occur in the carboxyl group. This synthesis apparently involved condensation of the carboxyl group of butyric acid with the methyl group of acetate (from oxidation of the alcohol), again by the Claisen type process.

The role of alcohol in these experiments appears to be not only as a source of acetic acid, but its oxidation appeared necessary for the condensation reactions. In a later paper by Bornstein and Barker (24) evidence was presented to show that the reactions in this bacterium are oxidation-reduction processes in which ethyl alcohol is oxidized to acetic acid and the fatty acids are formed by successive condensation and reduction reactions. Since hydrogen gas was also identified as a product of the reaction, there is some doubt whether this bacterial reaction can be extended with probability to higher organisms.

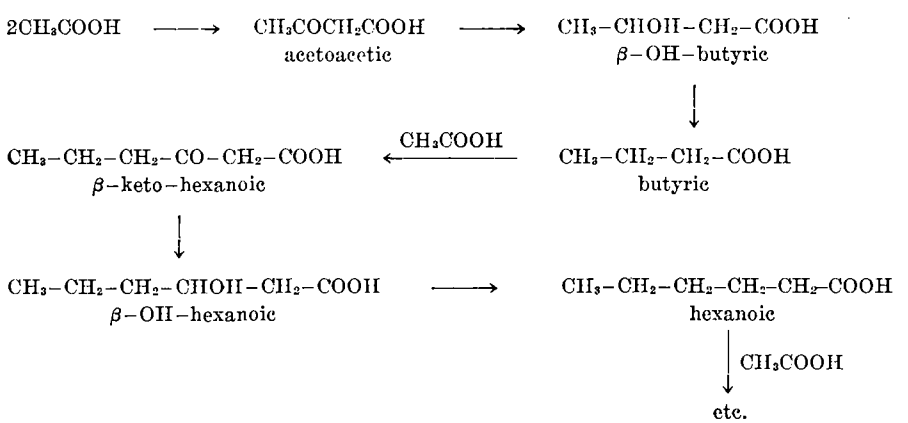

FIG. 3. Synthesis of higher fatty acids through lower homologues.

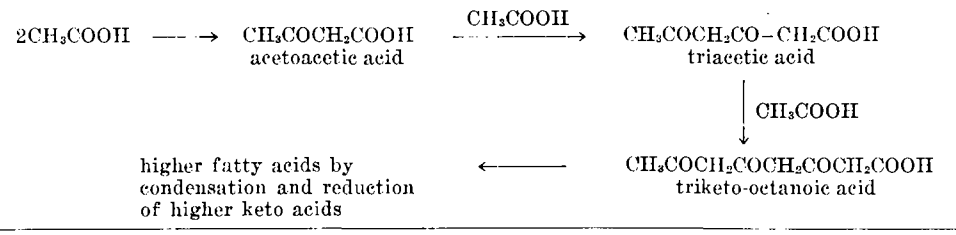

FIG. 4. Synthesis of fatty acids by successive condensation of acetic acid.

The lower fatty acids, butyric and hexanoic acids, are in fact contraindicated as sources of the higher fatty acids. Upon feeding deuterio-butyric acid (CH_3–CHD–CHD–COOH) and deuterio-hexanoic acid (CH_3–CHD–CHD–CHD–CHD–COOH) Rittenberg, Schoenheimer, and Evans (25) were unable to find significant amounts of deuterium in the deposited fat. Thus these lower fatty acids could not have served directly for condensation with acetate toward higher fatty acid production. The authors point out that if these acids were first converted to keto acids, the deuterium might be largely lost by enolization and exchange. Actually all the evidence indicated a rapid oxidation of the lower fatty acids rather than their serving as a source for synthesis of higher fatty acids. There is no sound evidence to conclude whether the naturally occurring lower fatty acids, for example butyric acid in cow's milk fat or caproic acid in goat's milk, arise from condensation of smaller molecules or from degradation of higher units.

Polyketo Acids as Intermediates in Fatty Acid Synthesis: If the lower fatty acids are themselves not intermediates in the synthesis of higher fatty acids, it seems possible that acetoacetate might undergo further condensation with acetate without prior reduction to butyric acid. Polyketo acids would be intermediates, a mechanism which has been previously suggested (26) and is illustrated in Figure 4.

According to this theory β,δ-diketo hexanoic acid is the direct product of the condensation of acetoacetate and acetate and incidentally would also be the product of "multiple alternate oxidation" of hexanoic acid. This compound (triacetic acid) has been synthesized in our laboratory (27) and an analytical method developed (28) for its determination in biological materials. It was then found that triacetic acid was readily metabolized by liver (29) and an enzyme was isolated from this tissue (30) which hydrolyzes triacetic acid to acetoacetate and acetate. This is the reverse of the reaction postulated for the synthesis of fatty acids. The exact significance of triacetic acid has not yet been determined, whether it is involved in synthesis, oxidation, or both, but at least one essential requirement has been met, namely that the compound is biologically active.

Requirements for Fatty Acid Synthesis: Of the common food materials, fat possesses per gram of material the greatest energy content. Synthesis of fatty acid must therefore require considerable energy, a biological process which undoubtedly requires the complex organization of the cell. With our present knowledge of coupled reactions and the role of energy-rich phosphate compounds, the time nevertheless seems ripe for a demonstration of fatty acid synthesis in relatively simple biological systems.

Some of the replacements for the organization of the cell are recognized. With the energy requirements of fatty acid synthesis, it seems certain that adenosine triphosphate, or a coupled reaction in which energy-rich phosphate is produced, is involved in the synthesis. The hormone insulin may also be concerned. As early as 1940 Drury (31) suggested that insulin is directly involved in the utilization of sugar for fat synthesis. Further, in experiments of the type introduced, Schoenheimer, Stetten, and Boxer (32) showed that in alloxan diabetes the incorporation of the deuterium of body water in the depot fatty acids was decreased to only 5% of normal. This of course does not prove a direct action of insulin in the fatty acid synthetic process since a lack of carbohydrate intermediates for the synthesis might also yield the same experimental result.

Recent tissue slice experiments however do indicate a rather direct function of insulin in fatty acid synthesis. Thus Bloch and Kramer (33) studied the incorporation of carboxyl labeled acetate in the fatty acids of rat liver slices in the presence of other carbohydrate intermediates. Pyruvate was particularly effective in increasing the incorporation of acetic acid, and the addition of insulin caused a further doubling of this incorporation. In these experiments the role of insulin seems more specifically concerned with fatty acid synthesis. It will be noted in these studies that acetate itself was not readily incorporated. Here again therefore it is indicated that one of the two carbon units undergoing condensation may have to arise in special form from pyruvate or at least be closely allied to the energy derived from pyruvate breakdown. In this connection it is of interest that, after feeding rats pyruvate with carbonyl labeled carbon (CH_3–$C^{14}O$–COOH), radioactivity was found in the saturated fatty acids of the liver and carcass (34). In fact the bulk of the C^{14} recovered was found in the fatty acids.

The vitamin pantothenic acid, in the form of coenzyme A (coacetylase), may also be involved in fatty acid synthesis. This coenzyme has a role in both pyruvate and acetate metabolism (35, 36) and in acetylation reactions involving acetate and sulfanilamide or choline (37). Soodak and Lipmann (20) have recently shown that coenzyme A is necessary in the formation of acetoacetate from acetate and, if this reaction is a prototype of biological fatty acid synthesis, the importance of pantothenic acid is obvious.

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Report of the Committee on Analysis of Commercial Fats and Oils

Fall Meeting, November 1948

Determination of Free Fatty Acids for Refining Test

THE present method for the determination of the free fatty acids in crude vegetable oil, Ca 9a-41, page 4, D (for refining test) specifies the use of a 7.05-gram sample inspection of free acid content. Since many crude oils run too low for this method to be entirely satisfactory, the Committee recommends that Method Ca 5a-40 be used instead and that Method Ca 9a-41 be rewritten accordingly.

SUBCOMMITTEE ON F. A. C. COLOR METHOD:

(E. W. Blank, Chairman)

Copies of a questionnaire relating to the F. A. C. Color Method were submitted to approximately 50 users of the F. A. C. Color Standards. Much of the criticism of the F. A. C. Color Standards centers around the 11A, 11B, and 11C standards. A suggestion has been made that these standards be redesignated. Your chairman has been unable to obtain any unanimity of opinion on this proposal. It may be pointed out that irrespective of how the standards are designated, the problem of interpretation remains a personal one. The problem was thoroughly discussed in a meeting of the subcommittee at New York and plans made for proceeding with the problem.

SUBCOMMITTEE ON DETERMINATION OF PEROXIDE VALUES:

(A. R. Baldwin, Chairman)

Three separate studies of the Wheeler method for determining peroxide values have been made by the collaborating groups. The extent of agreement within and among several laboratories was evaluated in the first test. The data shown below indicate that agreement on duplicate samples within each laboratory was very good and that all results agreed surprisingly well.

The second series was designed to note the effects of varying the reaction time from one to five minutes and of varying the interval between water addition and titration. Differences between values obtained at one- and five-minute reaction times were not as large as were expected. However, in general, the peroxides appeared to be somewhat higher after five minutes of reaction than after one minute. There was greater variation among laboratories on corn oil than on lard, but the duplicability within individual laboratories again was very good with an average difference between duplicates of about 0.5 Me/Kg.

Peroxide Values of Oxidized Fats
(Unaltered Wheeler Method)

Collaborator	1	2	3	4	5
Peroxides (Me/Kg)					
Lard	25.8	27.0	26.5	25.2	24.5
	26.1	26.0	24.5	25.0	24.7
	26.4	25.7	25.5	24.9	24.5
	26.5	25.0	25.3	25.4	.....
			25.6		
Ave.	26.2	25.9	25.5	25.1	24.6
Corn oil	21.8	23.3	23.4	20.7	19.7
	22.2	23.0	23.1	20.9	20.0
	22.0	22.9	23.4	20.9	20.2
	22.1	22.0	23.4	21.5	.....
			23.2		
Ave.	22.0	22.8	23.3	21.0	20.0

The lapsed time between water addition and titration up to five minutes had no effect on the peroxide values of lard, but wide variation was found between titration immediately and after five minutes for corn oil. Titration immediately after water addition would seem to be indicated from these results.

The third series of samples was distributed for the purpose of studying the effects of sample size (1.0 to 10.0 g.) on peroxide values. Several reports in the literature have indicated that with increased sample size the apparent amount of peroxides is reduced significantly. For both lard and corn oil the peroxide values, when 10 grams of fat were used, ranged 10 to 20 per cent lower than when only one gram of fat was analyzed. In fact, sample size thus far in the investigation of the Wheeler peroxide method appears to be the most significant variable.

SUBCOMMITTEE ON UNSAPONIFIABLE MATTER:

(C. P. Long, Chairman)

During the year, cooperative work was done on two samples of tallow. These were analyzed by the A. O. C. S. Method Ca 6a-40 and the modified S. P. A. ethyl ether method official for the A. O. A. C.