Report of Uniform Methods Committee Meeting, Washington, D.C.

The Uniform Methods Committee met April 3, 1968, in Washington, D.C., with the following members present: D. L. Henry, K. E. Holt, L. D. Metcalfe, R. J. Houle, E. Handschumaker, R. A. Marmor, and E. M. Sallee.

1) *Oxygen Bomb Method for Fat Stability.* The members discussed this method which had been proposed as a replacement for the AOM method now in standards. Mr. Marmor reported that the bomb method is not likely to be used very much. It is quite different from the procedure used by cereal chemists. Consensus of the chemists' thoughts was that since the method is nonspecific and a procedure is already in use, a change should not be approved unless there is an evident demand for it. It was agreed that the method should be published in the Journal but not added to the Methods book at this time.

2) *Oxirane Oxygen.* Mr. Holt reported that the HBr procedure now in the AOCS Methods is precise but that the reagent is not stable and results tend to be low on some samples. The method does not work successfully on some types of material. The Jay method has been collaboratively studied in the past and appears to be more accurate than the current method. The subcommittee is still studying the matter but is expected to recommend a change.

Communications. As complexity of committee activities increases, there has been a problem in maintaining communication between the technical committees, the Uniform Methods Committee and the officers of the Society. Annual reports supplied by each technical subcommittee to its parent committee and then to the Chairman of the Uniform Methods Committee should improve the liaison.

4) *Physical and Chemical Characteristics of Oils, Fats and Gases.* Section I of the Methods book contains the accepted range of fat characteristics for many natural oils. The data were critically reviewed in 1962. Usefulness of the information in its present form is questioned. Mr. Holt moved and Mr. Handschumaker seconded the motion that the table be removed from the Methods book. The motion was approved when all present, except Mr. Sallee, voted "aye." Mr. Holt then moved and Mr. Handschumaker seconded that a new technical committee be organized to collect current information on composition of oils and that such information be published in the Journal.

5) *Method Cc 8e–63.* This method describes a modified bleach test for soybean oil. The reference to the lye table in Ca 9a-52 is somewhat ambiguous. The committee agreed to an editorial change which would transfer the appropriate lye dosage table to Method Cc 8e-63.

6) *Aflatoxin.* It was reported that the Aflatoxin Committee has a revised method ready for submission. A mail vote will be taken so that the procedure can be included in 1968 revisions.

7) *To Be Studied.* The following areas of interest were suggested by committee members as needing attention at present.

The Antioxidant Committee Chairman, E. R. Sherwin, has requested release from his assignment. The information accumulated by the committee should be organized for publication.

The application of current techniques to the measurement of solid fats should be considered. Nuclear magnetic resonance and differential thermal analysis may offer advantages over Method Cd 10–57. R. T. O'Connor will be asked to survey the need for technical committee work.

Since the retirement of E. W. Blank, the Glycerine Analysis Subcommittee has been without a chairman. Correspondence from the ISO indicates an interest in glycerine methods in Europe. I. A. Wolff should appoint a new subcommittee chairman if there is need for work in this country.

8) *Oxygen Bomb Method for Fat Stability.* The Uniform Methods Committee discussed the oxygen bomb method which had been proposed as a replacement for the AOM method now in standards. It was reported that the bomb method is not likely to be used very much. It is quite different from the procedure used by cereal chemists. Consensus of the chemists' thoughts was that since the method is nonspecific and a procedure is already in use, a change should not be approved unless there is an evident demand for it. It was agreed that the method should be published in the Journal but not added to the Methods book at this time.

Fat Stability
Oxygen Bomb Method

Principle. Six grams of sample is dispersed on filter pulp in a glass liner which is placed in a bomb under 50 psi oxygen. The bomb is placed in a boiling water bath. The interval between placing the bomb in the bath and the end of the first hour in which a pressure drop of 2 psi occurs is the oxygen bomb hours.

Scope. Applicable to all normal fats and oils of animal and vegetable origin intended for human consumption.

A. Apparatus

1. Oxidation stability of gasoline apparatus made to ASTM D 525 specifications.
   a. Bomb.
   b. Bomb tubes and covers, Pyrex.
   c. Wrench, for closing bomb.
   d. Table socket, or equivalent.
   e. Pressure recorder, 4 pens, or equivalent; 12 in. chart, 0 to 200 lb divisions; 24 hr chart.
   f. Flexible metal hose.
   g. A safety valve set at 150 psi is recommended to protect the recorder. This would be inserted in the line between the bomb and recorder. A suitable safety valve is available from the Imperial Brass Mfg. Co., 6300 W. Howard Dr., Chicago, Ill. 60648. Cat. No. 268P.
2. Oxygen stability bomb bath for ASTM D 525.
3. Gaskets for bombs, Teflon, 1–31/32 inches I.D., 2–7/32 inches O.D. and ⅛ inch thick. May be obtained from Garlock Packing Co. 830 Lee St., Elk Grove, Ill.
4. Wrenches, end open type.
   a. ½ inch on one end and 9/16 inch on the other end.
   b. ¾ inch on one end and 13/16 inch on the other end.
5. Oxygen reducing valve, two-stage, with regulator and gauge for control and indicating the reduced pressure over the range of 0 to 200 lb.
6. Air hose, capable of operating with 150 psi.
7. Quick change coupler for air hose. Air hose with quick change coupler is used to connect bomb to reducing valve on oxygen cylinder. Male plug of coupler is attached to the bomb.
8. Analytical Filter pulp, Ash-Free S & S No. 289.

B. Reagents

1. Oxygen, cylinder gas.

C. Procedure

1. Weight 6 ± 0.1 g of liquid sample heated to 10C above melting point on to 2 g of filter pulp (A, 8) in the glass liner (see Note D, 5). Distribute evenly over top of the filter pulp. Weighing is carried out on a torsion balance sensitive to 0.01 g. The torsion balance is brought to exact balance with 2 g of filter pulp in the glass liner. Six grams is then added to the balance and the liquid sample added evenly over the filter pulp until the scale is again in balance.
2. Insert the glass liner into the stainless steel bomb and place the cover on the liner.
3. Attach the cap containing the pressure line and valve to the bomb. Attach to oxygen cylinder and raise the pressure in the bomb to 50 ± 5 lb (see Note D,

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corporation into the alkane fraction is independent of light. Carboxyl-labeled butyrate, valerate, caproate and caprylate are all incorporated into the extent of 0.01–0.05% of the added activity into long-chain hydrocarbons. The amount incorporated into branched hydrocarbons was 40–90% of that incorporated into normal hydrocarbons. Furthermore, about 80% of the radioactivity of the branched-chain hydrocarbons isolated from the experiment with (8-^{14}C) caprylate is found in the methyl carbon. These results, together with previous findings that the terminal branched portions of the branched-chain hydrocarbons are derived from the amino acids valine, leucine and isoleucine, suggest that the hydrocarbons very likely are produced from the precursors resulting from a condensation of two long-chain fatty acids, at least one being a normal fatty acid. This mechanism is consistent with the observed relative distributions of long-chain fatty acids and of hydrocarbons in tobacco.

SERUM TOCOPHEROL LEVELS OF NORMAL PRESCHOOL CHILDREN AND CHILDREN WITH PROTEIN-CALORIE MALNUTRITION IN SOUTH INDIA. A. Begum (Dept. Nutr. Res., The Christian Med. Coll. and Hospital, Vellore, S. India). *Proc. Soc. Expt. Biol. Med.* **127**, 91–5 (1968). Serum tocopherol levels of 90 normal children, aged 2–5 years, and of 42 children with kwashiorkor were estimated. Of the 99 children, 57 from an orphanage had an average serum tocopherol value of 649 μg/100 ml whereas 42 children from a day-care creche had an average level of 470 μg/100 ml. The patients with kwashiorkor had an average value of 410 μg/100 ml when admitted to the hospital. The estimated dietary intake of α-tocopherol of the normal preschool children was 4 mg per child per day.

THE REQUIREMENT OF FREE FATTY ACIDS FOR THE FATTY LIVER OF CCl_4 INTOXICATION. I. Weinstein, L. Willhite, H. Klausner and M. Heinberg (Dept. Pharmacol., Vanderbilt Univ. School of Med., Nashville, Tenn. 37203). *Proc. Soc. Expt. Biol. Med.* **127**, 850–54 (1968). Livers, isolated surgically from normal animals and from rats intoxicated with CCl_4, were perfused *in vitro* with a medium into which palmitic acid was infused continuously. Livers from normal rats were also treated with CCl_4 *in vitro* by direct addition of the chlorinated hydrocarbon to the medium. Under the conditions of these experiments, poisoning with CCl_4 resulted in inhibition of net release of triglyceride by the liver into the perfusate and simultaneous accumulation of triglyceride in the liver. These observations support the hypothesis that the fatty liver of CCl_4 intoxication results primarily from interference with the biochemical mechanisms involved in formation and release of the triglyceride in the very low density lipoprotein of the serum.

DIETARY PREVENTION OF CORONARY HEART DISEASE: LONG-TERM EXPERIMENT. I. OBSERVATIONS ON MALE SUBJECTS. O. Turpeinen, M. Miettinen, J. Karvonen, P. Roine, M. Pekkarinen, E. Lehtosuo and P. Alivirta (College of Veterinary Med., Helsinki, Finland). *Amer. J. Clin. Nutr.* **21**, 255–76 (1968). The feasibility of primary prevention of coronary heart disease by dietary means was studied in two mental hospitals. In one of them the diet was changed so that most of the milk fat was replaced by soybean oil, whereas the other hospitals were kept as the control without any intentional dietary change. A fall in the serum cholesterol level occurred after the dietary change. The incidence of coronary heart disease assessed on the basis of both electrocardiographic changes and coronary mortality was significantly lower in the experimental hospital.

FURTHER OBSERVATIONS ON THE CLEAVAGE OF BOVINE INSULIN BY RAT ADIPOSE TISSUE. D. Rudman, L. Garcia, A. Del Rio and S. Akgun. (Columbia Univ. Res. Serv., Goldwater Mem. Hosp., New York, N.Y. 10017). *Biochemistry* **7**, 1864–74 (1968). A previous investigation showed that the water-insoluble, lipid-rich fraction of the aqueous homogenate of rat adipose tissue contains a system of peptidases which cleave insulin into numerous fragments. In this study, bovine insulin was incubated for 2 hr at pH 7.5 with this fraction of rat adipose tissue homogenate and the mixture of insulin cleavage products was then fractionated by gradient elution chromatography on DEAE-cellulose columns followed by high-voltage electrophoresis and paper chromatography. In the first experiment 13 peptide fragments of insulin, and in the second experiment 17 peptide fragments, were isolated and their quantitative amino acid compositions were determined. These data, as well as those previously reported on the quanti-

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1 and 2) and allow to escape into the atmosphere, then raise pressure in the bomb to 50 ± 5 lb, close the valves, and disconnect the bomb assembly from the oxygen cylinder. A tire valve may be used to facilitate oxygen entry to the bomb. If a tire valve is used, pressure should be released through a brass stopcock. The stopcock is connected to a T-joint located between the tire valve and needle valve.

4. Immerse the bomb assembly in a bucket of water to check for leaks. Tighten any joints or seals that leak and adjust the pressure to 50 ± 5 lb.
5. A permanent connection between bomb assembly and recorder is desirable. If not permanently connected, connect bomb assembly to pressure recorder and open the valve so that pressure in the bomb will register on the recorder. Place the bomb in the boiling water bath.
6. Observe the pressure change and record the time indicated on the chart that the bomb was placed in the bath and the time at the end of the first 60-minute period in which the pressure drop is 2 psi. All times are read to the nearest 5 minutes.
7. Report to the nearest tenth of an hour the difference in times.

D. Notes and Cautions

1. Do not set oxygen regulator valve to more than 10 psi above the pressure to which the bomb is being filled to insure uniform composition of the atmosphere in the bomb and to protect the recorder.
2. The recorder or pressure gauge used for measuring the pressure in the bombs when filling with oxygen must be checked against an accurate gauge and correction made if necessary to assure accurate filling in the range specified.
3. Keep gasket and sealing surface of bomb clean at all times.
4. The stem attached to the top of the bomb should be cleaned at selected intervals or whenever there is evidence that the rate for filling and exhausting has increased. To clean, remove the top from the bomb, disconnect top from flexible tubing to the recorder and screw out the metal insert in the stem. Using a small test tube brush, brush inside of stem with a generous application of oven cleaner, allow to stand for ½ to 2 hr, rinse out thoroughly with water admitted through the top of the stem and the oxygen inlet, rinse with acetone in the same manner and dry by drawing air through the stem and oxygen inlet. Clean the insert and dry in a similar manner. Assemble stem, connect to the recorder, attach the bomb and check for leaks.
5. The filter pulp is weighed directly into the liner, or transferred to it, being careful not to compress it in any manner.

E. Precision

Collaborative studies have shown that the following 95% confidence limits may be expected.

	Oxygen bomb—Hours					
	5	10	20	40	80	100
Duplicate determinations on the same day by an analyst should not differ more than approximately	0.2	0.4	0.6	1.2	2.0	2.3
Single determinations in two different laboratories should not differ more than approximately	1.2	2.3	4.0	6.8	12.0	15.0

DAN L. HENRY, Chairman, and
E. M. SALLEE
Uniform Methods Committee