C. PROCEDURE

1. The sample must be absolutely clear. If not, filter through a specified paper at a temperature of at least 10°C. above the melting point of the fat. The sample should not be held melted longer than necessary since darkening may occur.
2. Turn on the spectrophotometer and allow at least a 20-minute warm-up period before standardizing or making any measurement.
3. Set the wavelength scale to the desired wavelength.
4. Recheck the zero reading of the instrument and, with a cuvette filled with CCl_4 in the instrument, set the 100% transmission point exactly.
5. Fill a cuvette with the standardizing nickel sulfate solution and read the transmittance at 400 mμ. Repeat 3 and 4 at 470, 510, 525, 550, and 700 millimicrons. The readings must fall within the limits prescribed, or the instrument should be adjusted to give the correct response.
6. Fill a cuvette with the sample using a sufficient amount of oil to insure a full column in the light beam.
7. Place the filled tube in the instrument and read the optical density to the nearest 0.001 at 525 millimicrons.

REPORTING:

1. Report the optical density multiplied by 1,000.

(Red color = density × factor)
Factors: up to .085 density
red = density × 42
above .085 density
red = 23.4 density + 1.52

Special instrument scales for reading red colors directly may be used.

NOTE: The above report, which was prepared by a subcommittee, was approved by a majority vote of the Oil Color Committee and submitted to the Uniform Methods Committee for action. That committee recommended the spectrophotometric method for vegetable oil color measurement at the convention of the A.O.C.S. in New Orleans, and by unanimous vote of the Society it was adopted as a tentative method.

G. WORTHEN AGEE, chairman.

Nutrition in Relation to the Glyceride Oils*

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WHEN viewed in the light of their industrial and physiological importance, the oils and fats have received far less research consideration than they deserve. A scientist, who has worked with oils and fats, seldom has any difficulty in seeing opportunities for research on their nutritive properties that he would like to see explored. But an executive, who is responsible for managing an industrial enterprise, or a layman, who simply wants to make a contribution to the betterment of mankind, may have more difficulty in gaining a comparable degree of enthusiasm. There is constant need to focus convincing arguments upon specific problems that offer promise.

First of all, I would urge the basic principle that those who work toward the solution of practical problems in agriculture, in the food industry, and in other areas where fats and oils are used, could approach many of their respective tasks more efficiently if chemists could discover the reactions by which the various types of fats are formed. For example, no one knows how linolenic, oleic, ricinoleic, or eleostearic acids arise from simpler intermediates. In an empirical way it is known that carbohydrates are converted to fats in both plants and animals, but little is known of the intermediate steps. Economic gains from breeding farm crops with higher oil yields can be seen readily, as in the case of soybeans and corn. The geneticist could work more efficiently if the reactions he is seeking to accomplish were known.

The mechanism by which carbohydrates are converted to fats in the animal body is of frequent concern to scientists who are interested in food. Those who are dealing with human health are especially interested in the changes because the key to several medical probelms lies at the crucial point of carbohydrates-to-fat conversion in the human body. For example, there is much evidence to support the current concept of physicians and bio-chemists that in the diabetic patient most of the acute or chronic damage to the body is caused by the accumulation of intermediate fragments of fatty acids. At one time the injuries were thought to be associated almost solely with the high sugar content in the tissues of diabetic patients, but current evidence points chiefly toward the fats.

Additional illustrations of the importance of gaining further knowledge of fat metabolism come from observations on nutrients other than fats. Several acute and chronic diseases are characterized by an initial disturbance in fat deposits. The first evidence that something is wrong may be seen in the form of abnormal deposits of glyceride fats within the cells. Several vitamin deficiencies, for example, are characterized by such a change. The chemist who attempts to work with the physician or veterinarian in gaining a clue to the mechanisms that have become disturbed when the fat droplets appear in the kidneys and liver, scarcely has enough information about the origin or transport of fat to make a helpful suggestion.

ONE may well ask, "Why should a food scientist be concerned with this problem?" There are numerous answers, but perhaps one will suffice. Several years ago Wendell Griffith of St. Louis University,

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H. C. Best of the University of Toronto, and others observed that a deficiency of one of the common ingredients of crude fats, choline, resulted in a loss of growth capacity in experimental animals and within a fairly short time there were structural changes in the liver and kidneys, followed by hemorrhages and ultimately death. Massive deposits of fat and swelling were followed by deposition of scar tissue, or cirrhosis.

Meanwhile many investigators were exploring the chemical and biological properties of choline, and it came to be regarded as a member of the vitamin B complex as well as an ingredient of many crude fats and proteins.

At the Alabama Institute of Technology, W. D. Salmon and his associates, R. W. Engel and D. H. Copeland, recently hit upon another discovery of wide interest regarding choline. Their animals showed the expected deposition of fat and scar tissue when injured by a choline deficiency, but when they were continued through a longer period, a high incidence of tumors or cancer tissue developed. This report of carcinogenesis subsequent to distortion of fat deposits, has been confirmed during the year by another laboratory and has been extended to include another type of animal.

To avoid misunderstanding may I emphasize that these findings have dealt only with experimental animals; they should not be interpreted as having a direct significance in human physiology except in this sense, research men now have a new pathway charted by which to explore both the early stages of fat deposition and the origin of cancer under carefully controlled conditions. As a further item of interest in relation to the cancer problem and its association with unknown areas of fat metabolism, one should also note the findings of Applebaum, Rusch, Bauman, and others in which they observed an increased incidence of cancer in rats when there had been a high intake of fat and a high intake of total calories. These reports in no way imply that a normal use of fat will increase the risk of cancer, but they offer interesting and significant areas to explore.

A further and recent discovery is highly significant from the point of view of choline and fat metabolism. Dr. C. H. Best of Toronto reported recently at the New York Academy of Medicine a most interesting sequence of experimental findings. Young growing rats, after being subjected to a choline deficiency for only a few days, were then given a generous and adequate mixed diet through succeeding months. They showed the usual fatty deposits in the liver and kidneys within 24 hours, but something else was set going that did not become evident at once. After a few months' feeding on a diet regarded as generous in all respects, the animals, which had been injured apparently in a transitory way, began to show a rising blood pressure and enlarged hearts. When examined by a pathologist, the tissues of the animals showed the general types of lesions associated with hardening of arteries and hypertension, despite their relative youth on a chronological basis.

Again this evidence does not mean that human arteriosclerosis, hypertension, and kidney failure result from choline deficiencies or abnormal metabolism of fats, but research men exploring these areas of human health and disease, have a new and controllable method of study and they have identified one specific causative agent—there may be others.

RETURNING to some of the more practical aspects of fats in human nutrition, the nutritive quality of butter fat compared with other plant and animal fats, with or without hydrogenation and blending, can scarcely escape consideration. Past disagreements in this area are not new to our audience, I am sure. Possibly some individuals, however, have not seen the excellent review by J. A. B. Smith in the fall issue of the British Journal of Nutrition [2, 190-201 (1948)]. In summary, there is almost complete agreement among research men, including those who, like Dr. Smith, work in dairy research laboratories, that the nutritive quality of the glyceride portion of butter fat is not significantly different from that of other common edible fats, when measured in human or rat tests on a mixed diet. Such a conclusion does not imply that under highly restricted conditions, all fats will permit the same response. It is obvious that they do not and that research should be continued at a so-called pure research level to define the differences. (The heat of the arguments and possibly the desire on the part of non-scientists to gain political or economic advantages have been more intensive than the support of research to find accurate objective answers on which competent scientists can agree.)

A considerable amount of research with small animals has dealt with the apparently unique role of certain fatty acids. These are concerned primarily with the essential requirement of linoleic or closely related Δ-9 fatty acids with a cis-configuration. In animal tests an advantage for such acids can be demonstrated, but there is no adequate evidence by which to appraise their value in human feeding. This situation seriously needs consideration. One of the stumbling blocks at present is the lack of satisfactory micro methods of analysis for the unsaturated fatty acids.

Perhaps the most recent arrival in the fatty acid group with specific nutritive claims advanced for it, is vaccenic acid, the delta-11, trans-isomer of oleic acid. The early findings in regard to the unique role of vaccenic acid have not been confirmed. In a recent paper Jansen, too, reports that with a purer product he cannot confirm his earlier finding.

On the negative side, Harris and his associates have shown that an excessive intake of the hydroxy fatty acids can cause marked injury and hemorrhages in rats as a result of vitamin K deficiency.

It is probable that indirect effects, analogous to Harris's findings for vitamin K, and favorable effects reported in some instances for biotin, vitamin B_1, vitamin B_6, etc., account for many of the difficulties encountered in studies of the nutritive qualities of fats.

In the cases where apparent differences between individual glycerides are not great enough to be significant in the mixed diets of our human population, I do not see how one can justify leaving these areas of research unexplored. There is often an expressed concern regarding fat products on the part of the public, the medical profession, legislative leaders, and civic-minded individuals. Even minor differences should be understood and clarified for use by the food industry and by the medical profession.

Several questions in regard to the utilization of highly unsaturated fatty acids have arisen from studies of vitamin E deficiency. Peculiar yellow-to-brown deposits of complex fatty material are observed in the muscle tissues of animals subjected to a chronic deficiency of the vitamin. The metabolic breakdown shows some resemblance to the human condition in "muscular dystrophy." It has not been possible, however, to define the basic chemical changes that become disorganized in either case. The relationship between the two disorders may be casual, rather than direct, but there are indications of a common chemical disturbance.

The over-all rate of oxidative processes in the animal body, as measured by oxygen uptake, becomes accelerated as a result of prolonged vitamin E deficiency, much as it does when there is a prolonged deficiency of vitamin C. This relationship of regulating normal biological oxidation piques the curiosity of an oil chemist, because, though apparently unrelated in chemical structure, the two vitamins are used commercially as fat antioxidants.

ANOTHER nutritive relationship of the glyceride fats of rather wide interest is the sparing action in the requirement for certain vitamins. In the case of vitamins B_1 and B_6, for example, the requirement is markedly less when fats replace carbohydrates in the diet isocalorically.

A few laboratories have made remarkable progress within the last two years in charting the steps by which fatty acids are broken down in the animal body to yield energy. Low molecular weight fragments apparently enter into the general channels of intermediate metabolism, where they overlap with both proteins and carbohydrates. It is a pleasure to note that in tomorrow's program some of these men will have an opportunity to present their work. This area of study is one of the brightest chapters in recent fat research.

From the point of view of practical human feeding under differing environmental conditions there has been progress. For example, with human subjects in aviation chambers at low temperatures, Keeton and H. H. Mitchell and their associates found the greatest degree of resistance against physical and mental impairment from extreme cold when the subjects were fed, at frequent intervals, a diet high in fat content. To almost everyone's surprise high protein diets came out a poor third. When the feeding intervals were more normally spaced, greatest efficiency was achieved with diets high in carbohydrate. The latter has practical advantage also in feeding men under special conditions: a diet relatively high in carbohydrate, but not necessarily free from fats or protein, can impart to the aviator an increased tolerance of anoxia, or altitude exposure in parallel with increased tolerance of cold.

There is relatively little data from which one can evaluate the practical gains in physiological efficiency that might be accomplished during periods of hard work and severe climatic exposure, by differing fat ratios in a diet. One observes under natural conditions a general practice of consuming diets high in fat, as among natives in arctic areas, but this result may be a practical matter of natural selection on the basis of a limited food supply rather than a result of experience in achieving maximum physiological efficiency.

THE work at Pennsylvania State College with experimental animals is of wide interest because of its implication that maximum physiological efficiency might be achieved with a fat intake somewhere in the range of 40% of the total caloric value. It should be a relatively simple matter to obtain comparable evidence with human subjects working in different types of environment and with varying caloric distribution between carbohydrates, fats, and proteins. These problems are not just academic. For example, the problem of supplying food to isolated or distant population groups is always important, but it will take on added significance if we should have to feed scattered groups in the arctic areas in wartime. There would be a great advantage in the tonnage requirement of diets relatively high in fat. Not only is the weight to-energy ratio two and one-fourth times more favorable from fat, but there is a further gain in that fats can be transported with a minimum of water and in small volume. Again, an individual would have a great advantage in carrying an emergency ration with a maximum acceptable content of fat. Offsetting this advantage, however, is the antiketogenic value of carbohydrates, permitting an individual to burn his own surplus of fat for short periods.

Quite aside from commercial considerations, with world conditions as they are, problems of this kind merit investigation. Many of you know the army's experience with pemmican and the related unsolved hazard of acceptability, but these preliminary discouraging reports should not stand in the way of attempts to prepare foods that might combine physiological and military efficiency without sacrificing the basic requirement of providing a ration that men will enjoy eating.

In the foregoing I have tried to point out a few areas of research on the nutritive quality of fats and oils that may justify attention on the part of research men. At the moment they may have only theoretical significance in the field of human health and physiological efficiency. But reliable scientific information has had a record of becoming useful in almost every area of human endeavor, including industry. Hence, as a matter of broad policy, there is sound reason to encourage and support research that gives humanity new, fundamental information in such areas as the following:

(1) The chemistry of the origin of fats and oils as a biological process;
(2) The utilization of fats in the cellular phases of metabolism in the animal body; and
(3) The best use of fats in practical situations for the purpose of achieving greater economy or physical and physiological efficiency.