qualities of crackers produced with good hydrogenated shortenings.

Oat flour and oat flour extracts were found to have but a slight favorable effect on the keeping qualities of crackers (Triebold, 1938). The oat flour and extracts were added to the cracker sponge, in doughing up the sponge, or sprayed or dusted upon the baked crackers. A protective factor from 0 to 2, which for all practical purposes is negligible, was exerted by the various treatments.

Lundberg, Halvorson, and Burr (1944) found that NDGA (nordihydroguaiaretic acid) when added to a lard used in making pie crusts and soda crackers exerted some stabilizing effect on the resulting product (protective factor of approximately 2 in crackers and 10 in pie crusts). The lesser effectiveness of the NDGA in crackers was thought to be due to the alkalinity imparted by the baking soda since alkaline solutions of NDGA oxidize rapidly when exposed to air.

Higgins and Black (1944) studied the effect of several antioxidants added to lard used in the preparation of crackers. They found gum guaiac to be an effective antioxidant for lard with the stability carrying over into the baked product (protective factors of 2.5-7, depending upon concentration). Propylgallate exerted a stabilizing effect on the lard but practically none on the resulting crackers. The same was also true for the tocopherols and for a wheat germ oil derivative (an ethylene dichloride extract of wheat germ oil combined with citric acid).

Mixing, Fermentation and Baking. These manipulative procedures involved in the manufacture of baked goods likely play a role in the keeping quality of the resulting product. This has been referred to previously in the destruction of pro-oxidants and antioxidants present in a fat when baked into crackers. Mixing spreads the fat over a greater area and also in the presence of salt may cause the solution of certain fat components into the aqueous phase, thereby facilitating their oxidation later. Fermentation produces sugars and organic acids, and these may have an effect upon the stability of the shortening.

The temperature and length of baking time might be anticipated to have an effect on the stability of the fat in baked products. Apparently as long as there is sufficient moisture present so that the product does not scorch, the effect is not great. However, crackers with scorched spots or crackers that have been crisped by successive reheatings show a marked decrease in keeping quality.

Packaging. The possibility of the absorption of fat from a baked product by the package must not be overlooked. The lining of cracker packages with grease-proof paper has helped greatly. However, some baked products are packaged in cardboard boxes. In such packages the fat may be absorbed rapidly by the cardboard, thereby spread over a large surface, and consequently undergoes rapid oxidation. This emphasizes the need for proper packaging to insure a good keeping quality product.

Several patents have been taken out on the impregnation of cardboard packages and wrappers with antioxidants and these are used to some extent. In certain instances these have proved helpful in retarding spoilage of the products contained, while in other instances they have been ineffective.

The use of colored glass to cut out the blue and ultraviolet rays of light was suggested by Burr (1907) as a means of protecting dairy products from oxidative deterioration. This has led to the development and effective use of colored cellophanes for packaging food products that will be exposed to light when merchandized.

Summary. In summarizing, it would appear that effects on the stability of cereal products to oxidative deterioration by formula components as well as methods of processing, are not understood to the degree that they should be and that there is a great need for studies along these lines.

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Toxicity of Rancid Fats*

F. W. QUACKENBUSH
Purdue University, Lafayette, Ind.

THE scientific literature contains a number of reports of malnutrition resulting from rancid dietary fat. The symptoms include ophthalmia, gastric papillomatosis, and other digestive disturbances, reproductive failure, anemia, dermatitis, and cancer. In some cases the symptoms have been attributable to known deficiencies. Some others are not so readily explained even in terms of present knowledge. Whether rancid fats exert a direct toxic action is also uncertain.

First, I should like to discuss briefly some effects which oxidized fats are known to produce through the oxidation of dietary essentials, especially the vitamins. The five fat-soluble dietary essentials, linoleic acid, tocopherol, vitamins A, D and K, are susceptible, though not equally so, to oxidation. Tocopherol and vitamin A are the least resistant, and their oxidation is accelerated by the peroxides of linoleic acid. It has long been recognized that dry foods or food materials containing them lose their potency on continued exposure to air. Recently it has been shown (1, 2) that even when these materials are fed in fresh condition, they may lose potency in the digestive tract

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before absorption. Carotene fed in linoleic ester was found ineffective unless fed with tocopherol. Undoubtedly, the tocopherol itself was partially inactivated in protecting the carotene from oxidation. Water-soluble antioxidants such as hydroquinone were ineffective in the tract.

The loss of dietary essentials through oxidation by rancid fat is not confined to those which are fat-soluble. Some of the water-soluble vitamins are known to be inactivated as well. Biotin has been shown to be destroyed by oxidized fat (3), and ascorbic acid may be oxidized to some extent in performing its synergistic role with antioxidants of the phenolic type (4). Not nearly enough work has been done to establish the effects of rancid fat on other B-vitamins, either directly or through an influence on the microflora which produce vitamins in the intestinal tract.

So much for effects on known dietary essentials. The symptoms which result from the individual deficiencies are well known, and many of them have been observed in animals on rancid diets.

Secondly, I should like to review briefly some of the symptoms which have been observed on rancid diets but which were not readily attributable to known deficiencies. One of these is anemia. Gyorgi et al. (5) fed rats a synthetic diet containing thiamin, riboflavin, pyridoxine, pantothenic acid, and 16% of crude linoleic acid. The rats lost weight, developed an anemia of the secondary type, and a leucopenia. The condition was prevented by the addition of yeast to the diet.

Burr and Barnes (6) fed a synthetic diet containing lard, cod liver oil, and wheat germ oil and found that rats lost weight and died. Even when the cod liver oil was fed separately from the rancid lard diet the animals lost weight and became anemic, and their white and red blood cell count fell. Vitamin A deficiency was ruled out since the livers of these animals contained normal amounts. Yeast prevented the anemia. Since yeast also prevented the development of rancidity in the diet, its effect was attributed to its antioxidant activity. It was suggested that the rancid fat exerted a direct toxic action. A study of the relationship to certain B-vitamins might be worthwhile in this connection.

Dermatitis is another symptom sometimes reported to occur on rancid diets. Whipple (7) fed four dogs a diet containing 25% rancid lard and produced an "oxidized fat syndrome." The symptoms were loss of hair, skin lesions, emaciation, intestinal hemorrhage, and death. Three dogs on fresh fat all remained normal. Since the dermal symptoms resembled those of linoleic acid deficiency in rats, the probable cause was believed to be destruction of the unsaturated fat linkages. Similar symptoms were subsequently (8) produced in rats on a low fat diet. Ether-extracted yeast was fed as a separate supplement. The rats which were fed .10 cc. daily of oxidized lard (per. no. 15 to 20) lost weight, lost hair, and developed scaly legs and later edematous swelling of lips and paws. The symptoms were similar to those later described as acrodynia. Increasing the lard supplement to .20 cc. did not cure but hastened death. On the other hand, increasing the yeast supplement caused the animals to resume their previous rate of growth.

In our studies (9) on acrodynia, several highly oxidized fats (per. no. 300 to 400) were tested for curative properties. The only B-vitamins contained in the basal diet were thiamin and riboflavin. Ten mg. daily of linoleic ester effected permanent cures. Twenty mg. of oxidized wheat germ oil or corn oil likewise effected prompt cures; however, many of these were followed by relapse and death two or three weeks later. Increased supplements of rancid fat intensified the symptoms and hastened death. In view of these observations and the comparatively low degree of oxidation of the lard used by Whipple, it seems doubtful that linoleic deficiency caused the death of her dogs and rats. Apparently the rancid lard exerted some other effects.

Some attention has been given to reproductive failure as one of the symptoms caused by rancid dietary fat. Kudrjashov (10) reported a direct toxic effect on the fetus during pregnancy and in some cases the prevention of implantation of the fertilized egg. Pregnant rats fed rancid fats resorbed their fetuses on the 6th to 9th day, not because of vitamin E deficiency but because of toxic decomposition products in the fat. These were believed to be higher aldehydes and ketones. Others (11) have reported degenerative changes in the testicles of rats which were fed 10 to 25% rancid fat in addition to a diet containing ample vitamin E. On the other hand, Mattill and his coworkers (12) found that rancid fats and their degradation products did not interrupt pregnancy in rats. Litters were born normally unless the doses were large enough to produce systemic intoxication in the mother. However, mortality of the young was high.

Finally, mention should be made of the alleged carcinogenic properties of rancid fats. Roffo (13) has reported that olive oil or animal fats which had been oxidized by heating tended to produce cancer when fed to rats. He pointed out that the spectral characteristics of the heated fats resembled those of carcinogenic phenathrene derivatives. Lavik and Bauman (14) found that high fat diets increased tumor formation on the skin of rats which had been painted with methyl cholanthrene. The action of fat was increased by heating one hour at 300° C. Prolonged heating had no further effect. The carcinogenic effect of fats was found to be at least partly due to an increased caloric intake. Apparently it was not related to rancidity since fats rancidified by treatment with ultraviolet light or copper oleate were not more carcinogenic than fresh fats.

In summary, it is evident that one of the chief adverse effects of rancid fat is the destruction of vitamins and other dietary essentials. However, some symptoms which have been observed and confirmed are not readily explained in such terms. These include certain types of anemia, dermatitis, and reproductive failure. It is probable that further work will provide additional specific instances in which rancid fat exerts its effect through inactivation of dietary essentials. However, until such experimental evidence provides the full explanation, it must be assumed that rancid fats are able to exert a direct toxic effect.

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A Volumetric and a Weighing Method for Measuring Semi-Micro Oil Samples*

RUSSELL O. SINNHUBER
Seafoods Laboratory, Oregon Agricultural Experiment Station
and
J. G. RUGGLES
Bioproducts, Oregon Ltd.
Astoria, Oregon

DURING several years of spectrophotometric vitamin A assay and plant testing of the liver reduction process two methods for measuring semi-micro oil samples have been developed which have served almost all laboratory and plant requirements. The volumetric procedure described below enables the analyst rapidly to test vitamin A bearing oils for routine control work. The volumetric method also has been of value in conducting vitamin A stability tests on oils during storage. The weighing procedure described has been used as an accurate and rapid method in the quantitative estimation of vitamin A in fish liver oils and concentrates.

Volumetric Method: This procedure is suitable for routine use in vitamin A liver processing plants. Blood pipettes commonly in use for red and white cell counts are employed. The red cell type holds approximately 9 mg. of oil and the white cell type approximately 26 mg. of oil. The pipettes are standardized by weighing them filled to the mark below the bulb with the oil commonly being analyzed. In the assaying procedure the pipette is filled to the mark with the oil, and the tip is wiped carefully with a cleansing tissue or towel. The filled pipette is then attached to a siphon containing the desired solvent, and the sample is washed thoroughly into a volumetric flask. The pipette may then be cleaned with petroleum ether and dried with suction. Acetone should not be used since it may dissolve the mixing bead.

Weighing Method: In vitamin A assay, a simple method of weighing small oil samples is that of using a micro cover glass. The square cover slip, which has been cut into halves or thirds by using the edge of a carborundum pencil or stone, is suitable as long as it will easily slip through the neck of the volumetric flask. The cut cover slip will weigh approximately 100 mg. With the aid of a small glass rod, samples of oil from 10 to 30 mgs. may be transferred to the slip and weighed. With forceps the slip with oil is dropped into a volumetric flask, which contains a small amount of suitable solvent. The flask is swirled for a few seconds until all visible oil is dissolved then made up to volume and mixed. This technique eliminates the washing error that may be inherent in some oil weighing methods since fish liver oils frequently vary as regards their solubility, e.g. in isopropanol. The error involved in using the cover slip procedure is roughly plus 0.04% in 100 ml. and is of little concern in the present vitamin A assay.

* * *

THE foregoing note by Sinnhuber and Ruggles was shown before publication to the members of the Vitamin Committee of the American Oil Chemists' Society, and two of them made further suggestions.

For field use, when samples of 100 to 500 mg. are needed, we have satisfactorily employed a calibrated 0.5 cc. tuberculin syringe. The conventional weighing of a sample of a vitamin A oil in a volumetric flask can be somewhat simplified by adjusting the length of brass jack chain (used to secure the stopper to the volumetric flask used for the primary dilution) so that the tare is always within + 100 mg. of an even gram. This permits the tare to be obtained on a chainomatic balance without the use of the weight on the notched beam and simplifies somewhat the manipulation and arithmetic involved. A small card in the balance case giving flask numbers and tare weights also facilitates the weighing of the sample.

T. D. SANFORD
F. E. Booth Company, Inc.
San Francisco 11, California

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A useful and accurate method for weighing semi-micro samples is the following, which was suggested by Dr. K. C. D. Hickman in 1935, and has since been used for rapid tests as well as exact control work:

The carrier for the oil is a light gauge nichrome wire about three inches long bearing a loop on one end and on the other a helix consisting of five or six turns $\frac{1}{8}$" in diameter and spaced about $\frac{1}{64}$" apart. The coil is dipped into the oil sample to pick up a 20 to 40 mg. drop. To be weighed, the carrier is then hung by the loop on a balance. The drop of oil is rinsed off into the volumetric flask, or the whole carrier may be dropped in before the flask is filled with solvent. Very rapid weighings can be made with the use of a Roller-Smith 125 mg. torsion balance with counterweight.

NORRIS EMBREE
Distillation Products, Inc.
Rochester 13, New York

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