Comparative Nutritional Value of Diets Containing Rancid Fat, Neutral Fat, and No Fat

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IT has been previously reported from this laboratory[1] that the chemical condition of the fat of a ration was an important factor in determining the nutritional value of the ration. It was shown that when dogs were given a diet in which all the fat had been previously oxidized, they all succumbed to a disease which we have termed the oxidized fat syndrome. All the dogs lived in pens in the same room, and we succeeded in controlling all dietary factors other than fat as evidenced by the fact that control dogs maintained health. One control animal has been continued after the experiment was terminated and is in good health after two and one half years of the synthetic diet.

In searching for an explanation for these results several hypotheses present themselves.

First, it might be a direct toxic effect upon the animal.

Second, the oxidized fat may interfere with the utilization of one or more of the vitamins or call for a different balance between them. The symptoms, however, were not those characteristic of any one of the vitamin deficiencies, though it is conceivable that a disturbance in the metabolic balance which normally exists between these accessory substances would produce a clinical picture not like that of any of the avitaminoses.

A third possibility for the disastrous effects of long continued ingestion of slightly rancid fat is the effect of the oxidation on the fat itself. Burr and Burr[2] have shown that certain of the unsaturated linkages present in fat are essential for nutrition, and that the animal body is unable to synthesize them; that is, they must be fed preformed in the food. It is possible that the oxidation to which we subjected our fat, destroyed all such linkages and that since the rest of this ration was completely fat free our dogs were really suffering from a fat deficiency disease, even in the presence of 25 per cent fat.

In order to obtain more data we have transferred the problem from dogs to rats and have worked with a larger number of animals.

The following experiment was undertaken. Several litters of rats were divided into three groups. Each group was exactly comparable with respect to sex and litter. All groups were fed a basal diet which was completely fat free: Casein (fat free) 24 per cent, sucrose 72 per cent, salt mixture (Osborn & Mendel) 4 per cent. Vitamins were fed daily and separately from the rest of the food in order to insure complete consumption. The B vitamins were fed as whole dried brewer's yeast (ether extracted), and the fat soluble vitamins as non-saponifiable fraction of cod liver oil. Other studies in our laboratory showed that these vitamin supplements were adequate for normal growth and health.

To one group of rats, this basal diet was fed with 0.1 cc. of neutral fat (lard) added daily to the vitamin supplement, to the second group 0.1 cc. of slightly oxidized lard was added (peroxide value 15-20 millimols per kilo) and the third group was kept without any fat whatsoever.

For a brief time the animals grew normally but after about two weeks, all animals began losing weight. From all previous work we knew it was too early to expect falling off in weight, due to any alteration in the fat. The amount of yeast fed was 200 m.g.m. daily which is ample for the needs of the rat on diets containing liberal amounts of a good fat. However, Lepkowsky[3] has shown that the need for vitamin B is greater when the fat of a ration is reduced. We therefore divided each group into two parts and increased the amount of yeast from 200 to 700 m.g.m. in one-half of each group and left it at 200 m.g.m. in the other half. The animals receiving the higher yeast dosage immediately resumed their previous rate of growth, while those on the lower level of yeast grew at a retarded rate.

All work on dietary effects of fat is slow. Vitamin deficiences appear within a few weeks but disorders of fat metabolism take months for marked changes to become evident. However, when the average growth curves are plotted a consistent difference in growth is apparent from quite early in the experiment, although clinically no differences between the rats could be detected. The animals receiving oxidized fat were practically equal in their growth response to those receiving no fat. By the 50th day of this experiment, it was observed that the hind legs of the animals in both the fat free and the oxidized fat groups began to show a rough scaly dermatitis. We have not encountered the tail necrosis described by Burr and Burr. By the 120th day several animals in the oxidized fat group showed an edematous swelling of the lips with an increase in saliva to the point of drooling, edema and swelling of the forefeet with complete loss of hair over the regions of the abdomen and hind feet which showed similar dermatitis and swelling. These parts were apparently very tender, the animals cried out when touched and when left to themselves, rubbed and scratched themselves constantly, which may partly account for the loss of hair.

The edema was more marked in the oxidized fat group than in the fat free group where dry scaly dermatitis was a more prominent symptom. The control rats receiving fat remained in good condition.

The animals who were kept on the low yeast intake present some interesting material, although the picture was confused by the fact that the pathology was caused by two factors instead of one. Here, growth was seriously retarded in all groups, but the differences previously recorded were evident. Animals receiving a good fat were superior to those receiving oxidized fat and to those on the fat free ration, while rats receiving oxidized fat were practically identical with those receiving no fat, indicating again that oxidized fat did not supply the nutritionally essential ingredients which neutral fat supplied. On the 70th day the fat supplements were increased to some animals in each group. The results are interesting. The animals in the neutral fat group which did not receive the increase in fat died, while those receiving the increase continued to live and gained weight, slowly. Similarly the fat free group without any fat supplement died, but those who got additions of 0.2 cc. neutral fat managed to live and even to grow a little. However, increase in the oxidized fat supplements produced exactly the opposite results, increase in this supplement from 0.1 to 0.2 cc. cause death within

a short period, but the animals receiving 0.1 cc. of oxidized fat managed to stagger along another 70 days before death. In other words, more of the poor fat was harmful rather than beneficial.

These results can only be considered preliminary and there is much to be done to unravel this question.

At present we can only say that slightly oxidized fat does not have the same nutritional value as neutral fat. It may be further pointed out that the oxidized fat used in these experiments is only slightly rancid, the odor is hardly perceptible, it is therefore possible that much fat ordinarily consumed under conditions where storage facilities are not optimal may be as rancid as that used in these studies.

Bibliography

1. *Whipple, D. V. A Syndrome Produced in the Dog by the Inclusion of Oxidized Fat in the Diet. Proc. Soc. Exp. Biol. and Med.* 30, 319 (1932).

2. *Burr, G. O. and Burr, M. M. A New Deficiency Disease Produced by the Rigid Exclusion of Fat from the Diet. J. Biol. Chem.* 82, 345 (1929); *ibid* 86, 587 (1930); *ibid* 87, 1 (1932).

3. *Evans, H. M. and Lepkowsky, S. Sparing Action of Fat on Vitamin B₁. The Role Played by the Melting Point and the Degree of Unsaturation of Various Fats. J. Biol. Chem.* 96, 165 (1932).

Discussion of Paper

Mr. Andrews asked how the fat was oxidized.

Dr. Whipple replied that it was oxidized at 70° C. by blowing with air on a steam bath. Oxidation was measured by the peroxide value. It was considered that a fat with a peroxide value of 15-20 was oxidized.

Mr. Andrews commented that the peroxide value does not represent the true state of oxidation of a fat. Under the conditions described peroxide is forming and decomposing at the same time, and "peroxide value" is simply the measure of the difference between the two reactions.

Dr. Whipple stated that she had experimented with the Kreis method but had not found it satisfactory, and suggested that the Society furnish her with a method of determining oxidation.

Dr. Barbour inquired the constituents of the fat-free diet.

Dr. Whipple stated that casein was the protein constituent and sugar the carbohydrate.

Dr. Barbour asked if the "oxidized" fats could not have been tested for linolenic and linoleic acid deficiency.

Dr. Whipple replied that this had not been done.

Mr. Morison commented that animals deprived of Vitamine B, with which he had experimented, were comparable in appearance to those depicted by Dr. Whipple.

Dr. Whipple commented that her experiments indicated that to be effective vitamines must be accompanied by fat in the diet.

Food Research

The research work on the fundamentals of vegetable oil spoilage due to rancidity, which was vigorously prosecuted by the now defunct Mayonnaise Manufacturers Association at the Bureau of Chemistry and Soils at Washington, was brought to a sudden close when the Fellowship ended in June, 1932. This Fellowship sponsored by the M. M. A. and working under the guidance of Dr. F. C. Blanck, Chief of the Food Research Division of that Bureau, was very productive. Sixteen papers and articles dealing with rancidity and Mayonnaise spoilage, appearing in this journal and others, attest to the voluminous amount of work which was completed during the two year duration of the Fellowship. The research on rancidity and methods of retarding it were of particular importance, in that the results which have already been published are still regarded as real advances in that field.

The holder of the Fellowship, Dr. Lowell B. Kilgore, has continued the research upon oil spoilage and antioxidants by establishing his own laboratory in the Earle Building in Washington, under the name of Colloid Research Laboratories. The laboratory is maintained by a consulting service to manufacturers and users of edible oils and fats. In this way the valuable contributions which were made under the Fellowship plan are being discontinued.

Mr. Donald H. Wheeler, who was for a short time Associate Fellow with Dr. Kilgore, is now with the Industrial Alcohol Division of the Internal Revenue, at Baltimore, Maryland.

Imports of Foreign Fats and Oils,

Kind	Pounds
Animal oils and fats, edible	32,020
Whale oil	363,803
Cod oil	4,901,415
Cod-liver oil	6,518,378
Other fish oils	322,043
Tallow, inedible	126,455
Wool grease	1,007,676
Oleic acid or red oil	63,747
Stearic acid	1,228,259
Grease and oils, n. e. s. (value)	$9,449
Olive oil, edible	22,205,376
Corn oil	1,880,978
Peanut oil	388,663
Sunflower seed oil	5,620,645
Palm-kernel oil, edible	20,702
Other edible vegetable oils	9,180
Tung oil	23,704,406
Coconut oil	82,662,510
Palm oil	68,465,082
Sulphur oil or olive foots	14,099,448
Other olive oil, inedible	3,122,490
Palm-kernel oil	1,915,935
Carnauba wax	1,436,688
Other vegetable wax	829,392
Rapeseed (colza) oil	2,955,580
Linseed oil	17,041
Perilla oil	10,694,096
Other expressed oils, inedible	847,196
Glycerin, crude	1,977,364
Glycerin, refined	1,004,302

Exports of Domestic Fats and Oils,

Kind	Pounds
Oleo oil	9,255,326
Oleo stock	1,654,569
Tallow, edible	1,580,979
Lard	122,719,982
Lard, neutral	950,479
Oleo stearin	1,194,617
Neat's-foot oil	157,317
Other animal oils, inedible	283,749
Fish oils	449,951
Grease stearin	155,631
Oleic acid, or red oil	613,848
Stearic acid	100,749
Other animal greases and fats	12,114,713
Cottonseed oil, crude	2,096,441
Cottonseed oil, refined	3,282,573
Coconut oil, crude	6,261,436
Coconut oil, refined	230,844
Corn oil	312,712
Soybean oil	340,572
Cooking fats other than lard	704,653
Other edible vegetable oils and fats	68,811
Linseed oil	142,393
Other expressed oils and fats, inedible	189,023
Vegetable soap stock	3,612,705