samples of the fatal tourniquet and hemorrhagic cases were all vaso-excitor. This, in general, is the initial reaction to fluid loss.[4] On the other hand, the blood samples taken during the late periods of the fatal shock syndrome were all vaso-depressor.

The effects of known substances were compared with those of the shock depressor blood, noted above. The criteria were diminished responsiveness to epinephrine, loss of vasomotion with the dilator phase persisting and hyperemia.

Normal rats were given intravenous injections of histamine (0.5-3.0 mg/100 g rat), adenylic acid (0.2 mg/100 g), diphosphopyridine nucleotide (3 mg/100 g), Padutin Niphanoid (Winthrop) (5-10 mg/100 g), leukotaxin (1-10 mg/100 g), physostigmine and acetylcholine. All lowered the blood pressure and induced peripheral hyperemia but none gave all the effects of the vaso-depressor serum. For example, Padutin, a kallikrein, slowed the vasomotion, but had no effect on the epinephrine reactivity, while adenylic acid increased the vasomotion.

The vaso-depressor effect of shock plasma or serum was not destroyed by incubation with histaminase (Winthrop T360-N) for 24 hours. That the vaso-depressor was probably not a choline derivative was indicated by the fact that atropinized test rats still gave the vaso-depressor response.

In conclusion, the presence of vaso-excitor substances was demonstrated in the blood of animals during the early period of shock treatment involving fluid loss. Subsequently, when a hypo-reactive state of the capillary circulation occurred, the blood was found to contain vaso-depressor substances having properties which differed from those of a number of known tissue extracts.

[4] Zweifach, B. W., Lowenstein, B. E., and Chambers, R., *Am. J. Physiol.*, in press.

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Rancid Fat in Experimental Diets.

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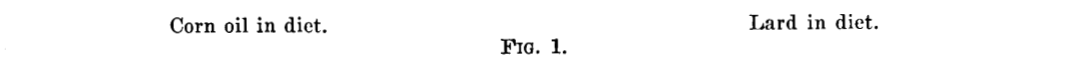
The deleterious effects caused by the ingestion of rancid fats have been reviewed recently by Burr and Barnes.[1] The possible effect of small amounts of rancid fat in the diet on the toxic action of a drug has not been sufficiently emphasized by nutrition investigators. The seriousness of the presence of oxidized fat in the diet was observed in this laboratory when it was found that rats, fed an experimental diet in which corn oil was replaced by lard, developed a humped back and partial paralysis, followed by death. The condition was first noticed in rats fed a diet containing cadmium as a toxic agent; however, it later developed in the control animals of this same series. We observed this abnormal condition in rats fed 6% lard as well as in those fed 25% lard in the diet. In order to interpret our previous data from the cadmium experiment, it was thought advisable to determine what effect, if any, the lard had on the toxicity results.

Experimental. Twenty pairs of albino rats, 21 days of age and equally divided between the sexes, were placed on the following diet: Casein 18%, cornstarch 60%, brewer's yeast 5%, whole dried liver 5%, salt mixture (U.S.P. XII No. 2) 4%, cod liver oil 2%, and lard* or corn oil 6%. Dietary mixtures were prepared in sufficient quantities to last

[1] Burr, G. O., and Barnes, R. H., *Physiol. Rev.*, 1943, **23**, 256.

* The lard used in this experiment was obtained from the Beltsville Experiment Station of the U. S. Department of Agriculture. It was stored at 35°F and was used as needed within a year.

Littermates one year old.



Corn oil in diet. Lard in diet.

FIG. 1.

approximately six weeks and were stored in covered tin buckets in a refrigerator at 35°F. The rats were kept in individual cages and fed and weighed once weekly. The initial weights of the littermates on the lard and corn oil diets respectively varied in no instance more than 2 g.

Results. The marked difference in appearance between the rats in the 2 groups is shown in Fig. 1. The humped back and the roughened coat of the rat on the lard is in sharp contrast to the sleek appearance of the rat on corn oil. The marked emaciation is characteristic of the animals in the lard group. During the rapid growth period of the first 3 months, there was only a slight difference in the growth rates of the 2 groups and no outward symptoms of deficiency in the rats on the lard. Moreover, they did not show the marked symptoms until they had been on the experimental diet for about a year. Before death they developed a partial paralysis, particularly of the hind legs. An animal was able to lift itself with great difficulty up to a food cup, and once with its head in the cup it hesitated to return to a normal position in the cage. The condition was a progressive type of paralysis and had not developed to the extreme stage in all animals at the time of termination of the experiment.

Grossly, the internal viscera of the animals on the diet with lard were small. The uteri were deep brown in color. Microscopically, the significant lesions were uterine pigmentation, tubular atrophy of the testes, and focal degeneration of the voluntary muscles. In the muscles, a moderate number of scattered individual fibers showed varying degrees of vacuolation and gray or tan colored pigmentation (in hematoxylin-eosin stained sections). Occasionally a small number of macrophages were present within a muscle fiber. More pigment was present in macrophages in the interstitial tissues. In the uterus, the pigment was present in muscle cells, in macrophages between the muscle cells, and even in the walls of blood vessels. The pigment had the same appearance as that of the voluntary muscles, and, like the latter, gave no reaction for iron when treated with acid ferrocyanide. The spinal cord and peripheral nerves were not examined.

No anemia was present in the lard group at 14 months. This is in contrast to the effect reported by György *et al.*[2] for a similar diet and confirmed by Burr and Barnes.[1] X-ray photographs revealed no abnormality of the bone structure of the spinal column despite the extreme curvature.

Rancidity of the Lard. The rancidity of the lard, although stored at 35°F, was established by organoleptic tests and the peroxide number was determined. The peroxide number (41.0 millimols per kilo) was high compared to that (2.0 millimols per kilo) of a sample of lard from the same batch but which had been stored at 20°F. By organoleptic test the latter lard was also not rancid. Peroxide numbers were not attempted on the diet stored at 35°F but organoleptic tests on the diet in the food cups were strongly positive for rancidity.

[2] György, P., Tomarelli, R., Ostergard, R. P., and Brown, J. B., *J. Exp. Med.*, 1942, **76**, 413.

Discussion. The significant pathological lesions point toward a vitamin E deficiency. Paralysis, with an accompanying emaciation, and failure to increase in weight in adult life are found in vitamin E-deficient rats. The appearance of the voluntary muscles is consistent with that described in the literature[3] as "nutritional myodegeneration" and attributable to vitamin E deficiency. There is some difference in appearance probably because of the slower development of the process in our rats. For example, there is very little coagulation necrosis or inflammatory reaction, and little formation of new muscle fibers, as seen in the less chronic forms. The process appears to have developed earlier in the leg and thigh muscles than in the body-wall muscles. The marked pigmentation of the musculature of the uterus is indicative of prolonged E-depletion.[3]

Although the basic diet is relatively low in vitamin E, it should be noted that the experimental diet included 5% yeast as well as only 6% lard. The early work of Evans and Burr[4] showed that 7.2% lard in the diet did not destroy the vitamin E supplied by wheat germ oil while 22% lard did destroy the vitamin E. Recently, Morris, Larsen, and Lippincott[5] reported that rats fed 50% unheated lard in the diet grew normally. The latter authors made no mention of any antioxidant in their lard. Yeast has been reported[2,6] to have an antioxidant action.

In other experiments we have found that fresh lard is nutritionally equivalent, in our colony of animals, to the vegetable oils which is in agreement with the observations of other laboratories.[7,8]

We wish to emphasize that although a diet may be stored in a refrigerator to prevent oxidation of fat, the storage temperature and length of storage are important. Dietary mixtures should be prepared as frequently as possible and should be stored at a relatively low temperature. We have found that lard did not become rancid within a year when stored at 20°F. Attention is called to the fact that prepared diets fed the animals will become rancid more rapidly than pure fats. Residues in feeding cups should be discarded.

Small amounts of rancid fat in the diet of animals on experiments of long duration may be very important. We found in our earlier experiment with cadmium that rancid fat increased the toxic symptoms. As has been pointed out by Burr and Barnes[1] the deleterious effect of rancid fat is not limited to a deficiency of vitamin E. End products of fat oxidation seem to have toxic actions of their own.

Summary and Conclusions. Studies on the deleterious effects of rancid lard in an experimental diet indicate that it is important to choose fats that will supply the necessary nutrients and not introduce other complicating factors. Rats on diets containing 6% rancid lard developed pathological lesions similar to those found in vitamin E deficiency. These changes were not present in rats receiving a diet in which corn oil replaced the lard. The observations are of particular importance in chronic toxicity studies in which animals are maintained on experimental diets for long periods of time.

[3] Mason, K. E., *Yale J. Biol. and Med.*, 1942, **14**, 605.

[4] Evans, H. M., and Burr, G. O., *J. A. M. A.*, 1927, **88**, 1462.

[5] Morris, H. P., Larsen, C. D., and Lippincott, S. W., *J. Nat. Cancer Inst.*, 1943, **4**, 285.

[6] Clausen, D. F., Barnes, R. H., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 176.

[7] Evans, H. M., and Lepkovsky, S., *J. Biol. Chem.*, 1932, **96**, 157.

[8] Hoagland, R., and Snider, G. G., *U. S. Dept. Agr. Tech. Bull.* No. 821, 1942.