These results provide an explanation for the changes in the dextro- and levocardiograms produced by temperature. It is demonstrated that alterations in the rate of recovery at an isolated region of the surface of the heart result from changes in temperature. When a large part of a single ventricle is similarly heated or cooled, the rate of recovery would be changed over the entire region, and this would be reflected in an alteration in the record of electrical activity from this ventricle, *i. e.*, by a change in duration of the dextro- or levocardiogram.

It should be pointed out that the monophasic records as derived in experiments of this type contain some conducted effects from portions of the ventricles at a distance from the electrodes. In the experiments reported here these conducted effects were unaffected by heating and cooling, since this treatment was restricted to the immediate area of the electrode. The changes recorded in the monophasic action potentials could, therefore, have been produced only by alterations of the action potential derived from the immediate vicinity of the active electrode.

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Effect of Pro-Oxidants upon Reproduction in Rats.

F. E. DEATHERAGE, K. P. MCCONNELL AND H. A. MATTILL.

From the Biochemical Laboratory, State University of Iowa.

Vitamin E is readily destroyed by oxidation in the presence of autoxidizing fats and this destruction can be prevented by suitable antioxidants or stabilizers. This oxidation is responsible for the production of muscle dystrophy in herbivora on certain diets containing cod liver oil. To insure the complete absence of vitamin E from diets designed to produce a deficiency in rats, the mixed rations are often allowed to stand at room temperature some days before use. Whether pro-oxidants can also accomplish the destruction of vitamin E in the tissues, or otherwise interfere with the normal progress of reproduction, is uncertain.

Waddell and Steenbock[1] believed that the "antivitamins" produced in a diet treated with ethereal ferric chloride damaged the reproductive capacity of female rats, because 4 to 6 weeks elapsed

[1] Waddell, J., and Steenbock, H., *J. Nutrition*, 1931, **4**, 79.

(on normal food) before the animals recovered from the effects of this diet. More recent observations,[2, 3] however, indicate that even highly pro-oxygenic substances can be fed without immediate derangement of reproduction, provided the vitamin and pro-oxidant do not meet in the alimentary tract.

According to Kudrjashov and his coworkers,[4] variously prepared decomposition products, in the unsaponifiable fraction of fats undergoing rancidity, when fed to pregnant rats (and rabbits) on normal diets tended to liquidate the pregnancy by causing the death of implanted embryos or by interfering with implantation. Subcutaneously administered concentrates were effective in one-tenth the amount given by mouth and the feeding of large amounts of rancid fats also interrupted pregnancy. Rats were most susceptible immediately after implantation, on the 6th to 9th days, and the placental sign appeared at the 8th to 10th day instead of at the normal time. Ovaries and uterus were undamaged. From numerous observations, some of which are contradictory and not too well controlled, Kudrjashov concluded that vitamin E and the decomposition products of fat are not antagonistic *in vivo,* that these products, presumably higher aldehydes and ketones, do not destroy the vitamin E of the tissues but have a direct toxic action on the fetus.

More recently[5] he produced the same results by feeding or injecting ethereal-ferric chloride treated wheat germ oil. If, after a resorption so produced, females were immediately transferred to a vitamin E deficient diet and mated, litters were born normally, indicating again that tissue vitamin E had not been destroyed, and that embryonic death was due to direct toxic action of the products of rancidity.

The work whose contrary results are reported here, had been under way during 2 years, as opportunity offered, when Carruthers[6] described the effects of heptaldehyde on mice. The daily feeding of 40-50 mg of heptaldehyde to mice, beginning from the first to the fourth day after insemination and continuing until birth or resorption, was followed by a resorption in more than half the animals. Resorptions could also be produced by daily intraperitoneal injections of 0.02 to 0.06 cc of heptaldehyde, dissolved in the

2 Weber, J., Irwin, M. H., and Steenbock, H., *Am. J. Physiol.*, 1939, **125**, 593.

3 Shimotori, N., Emerson, G. A., and Evans, H. M., *J. Nutrition*, 1940, **19**, 547.

4 Kudrjashov, A. B., *Arch. exp. Path. Pharm.*, 1933, **169**, 275; Kudrjashov, B. A., and Benjajeva, H., *ibid.*, 1934, **175**, 489; Kudrjashov, B. A., and Agatov, P. A., *Ginekol. i. Akush.*, 1935, **6**, 1.

5 Kudrjashov, B. A., *Bull. biol. med. expt., U.R.S.S.*, 1938 **6**, 220; through *Chem. Abs.*, 1939, **33**, 3428.

6 Carruthers, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 336.

ethyl esters of lard fat acids, and beginning as late as the 13th day of gestation. In many cases the placental sign appeared early.

Our own observations on about 75 rats can be summarized briefly by saying that it has not been possible, without inflicting systemic damage or severe intoxication, to interrupt pregnancy in animals on an adequate diet (Purina dog chow) by administration of products of fat oxidation, subcutaneously, intraperitoneally, or by mouth, or by prolonged feeding of this diet after treatment with ethereal ferric chloride or other agencies designed to promote oxidative rancidity. Among the oxidation products administered by mouth were: 200-450 mg heptaldehyde in methyl oleate or olive oil daily for 18 to 21 days during pregnancy (1 cc of heptaldehyde daily for several days proved highly toxic); 0.5 cc biacetyl in cod liver oil for a like period. Many more substances when tested by subcutaneous (S) or intraperitoneal (I) injection failed to interrupt pregnancy: 50 mg of heptaldehyde in methyl oleate (I) daily for 20 days; 1 cc (S) of heptaldehyde or pelargonic aldehyde on the 6th, 7th and 8th days of pregnancy; (very deep and severe lesions appeared at the site of injection); 0.5 cc to 1 cc doses (S) of irradiated unsaponifiable matter of cod liver oil, irradiated rancid lard or cod liver oil, their unsaponifiable matter, and the highly prooxygenic volatile products obtained during the oxidation of oleic acid or of aerated lard,[7] given daily between the 4th and 8th day of pregnancy, sometimes earlier or later. None of these measures prevented the gestation from being completed, unless the doses were so large or so often repeated as to cause the death of the animal. The mortality of the young was high.

The presence of 0.75 to 2% of heptaldehyde in the stock diet over long periods did not interrupt gestation; some of these animals were later subjected to the tests described above. More surprising was the capacity of ferric chloride-ether treated dog chow (first ground to powder) to maintain reproduction; this diet was begun with some young animals, was continued for many days (over 100) and was even successful in the second generation. The presence of additional cod liver oil during the spontaneous evaporation of the ether had no apparent effect.

One explanation for the lack of agreement between these results and those of the Russian investigators would appear to reside in differences in body stores; some of the variability revealed by their protocols suggests that their animals had been on a borderline diet, with a resulting confusion such as has occurred before in the history

[7] Deatherage, F. E., and Mattill, H. A., *Ind. Eng. Chem.*, 1939, **31**, 1425.

of vitamin E.[8] The effect of these pro-oxidants may not be entirely nil in the rat, but it has not been confirmed in our normally nourished animals.

The innocuous character of non-toxic doses of heptaldehyde with respect to reproduction in rats recalls similar observations of the Wisconsin investigators.[2] The effectiveness of small doses in abolishing reproduction in mice, even when pregnancy is far advanced, indicates a much greater susceptibility in these small animals than in rats. Effective total injections in mice[6] ranged from 0.04 to 0.38 cc, whereas 3 cc and, exceptionally, even 5 cc failed to interfere with reproduction in rats if they survived. Carruthers[9] has confirmed the resistance of rats to this treatment. On the other hand, Bryan and Mason[10] in a reëxamination of the vitamin E requirements of the mouse found male mice fertile even after 400 days on an E-deficient diet that produces sterility in male rats at 100 to 150 days.

The reproductive behavior of rats on ferric chloride-treated dog chow is further evidence of the inadequacy of this treatment completely to rid a diet of its vitamin E content. For a coupled reaction requiring the simultaneous oxidation of fat, intimate contact of the fat and ferric chloride must be secured for a sufficient length of time; aqueous ferric chloride was not effective[5] and possibly the character of the diet might provide certain physical barriers that would prevent adequate contact.

Summary. It has not been possible to interfere with the process of reproduction in female rats on an adequate diet by administering various oxidation products of fats, by mouth, subcutaneously, or intraperitoneally. These products included rancid animal fats, their volatile oxidation products with extremely high peroxide content, the unsaponifiable portion of irradiated fats and some aldehydes. Mortality of the young was very great. Unlike mice, rats were not susceptible to the damaging effect of heptaldehyde on reproduction; unless the mothers succumbed to systemic intoxication they bore litters even with serious lesions at the site of injection. An adequate stock diet (Purina dog chow, finely ground) treated with ethereal ferric chloride supported reproduction in the second generation, indicating that the coupled oxidation of tocopherol in the presence of rancid fats requires adequate contact for an adequate time.

[8] Mattill, H. A., *Am. J. Physiol.*, 1927, **79**, 305; Evans, H. M., *J. Nutrition*, 1928, **1**, 1.

[9] Carruthers, C., personal communication.

[10] Bryan, W. L., and Mason, K. E., *Am. J. Physiol.*, 1940, **131**, 263.