Loss of Contractility of the Uterus and Partial Atrophy of the Uterus and Ovaries in Albino Rats Fed Choline-deficient Diets[1]

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The importance of choline as a dietary constituent has been recognized since the discovery that choline deficiency caused an accumulation of fat in the liver of rats fed diets low in casein and high in fat content (*2, 3*). It has also been demonstrated that such a deficiency causes a hemorrhagic condition of the kidneys (*6*) and of other organs (*4, 5*). In addition, it has been reported that dietary choline may be essential for biosynthesis of adequate amounts of acetylcholine (*9*), for normal contraction of skeletal muscle (*7, 8, 10*), and for normal contraction of cardiac muscle (*1*). The results obtained in the present investigation show that choline deficiency caused a complete loss of contractility of the uterus and partial atrophy of the uterus and ovaries in mature albino rats.

Two pregnant albino rats of the Wistar strain obtained from Carnsworth Farms were placed at parturition on a choline-deficient diet for 39 days; two others were placed on the same diet supplemented with choline. The

TABLE 1

FORMULA OF THE CHOLINE-DEFICIENT DIET

Casein (Labco vitamin free) extracted with alcohol		10%
Lard		20%
Salt mixture (Hubbell, Mendel, and Wakeman)		4%
Sucrose		66%
Thiamine chloride	400 μ	(per 100 gm of diet)
Riboflavin	200 μ	
Pyridoxin	200 μ	
Calcium pantothenate	1.5 mg	
Nicotinic acid	2.5 mg	
Vitamin A	80 I.U.	every second day in corn oil
Vitamin D	1.5 I.U.	
Vitamin E (α-tocopherol)	1.0 mg	

litter of one died on the first day; those of the other three were living at the end of the experiment. Of the lactating rats, No. 1 received 30 mg, and No. 3 a supplement of 200 mg, of choline every second day. Rat No. 2 and the nonlactating rat, No. 4, were given no choline supplements. The choline-deficient diet employed, that of Hegstead (1941), is represented in Table 1. Because of considerable loss of weight within the first four days, an additional supplement of 1 gm of dried brewer's yeast and 5 gm of extracted casein was supplied every second day to all the rats.

Weekly records were kept of food intake and weight gain and of differences observed between rats with and without choline. At the termination of the experiment the adults and some members of the three litters were killed with ether and autopsied immediately.

The most marked difference noted prior to the termination of the experiment was the development of a yellow, oily condition of the hair of rats which received the choline supplement. This appeared within 14 days in the rat receiving the 200-mg supplement of choline and a few days later in her litter. In the rat receiving the 30-mg supplement a similar appearance was noted on the 27th day and shortly after in her litter. By the 36th day it was marked in both adults and their litters and was so extreme in those receiving the 200-mg supplement that skin of persons handling these rats became oily. The hair of the deficient rats remained normal, and, on transfer to a stock diet, the hair of litters which had been fed the high fat diet supplemented with choline resumed its normal color and consistency. No other consistent differences were noted in adults or litters during the experiment.

At autopsy the outstanding differences between the rats fed diets with and without choline were the complete lack of contractility of the uterus and a partial atrophy of the uterus and ovaries in the choline-deficient rats. The uterus of each of the rats which received choline contracted as soon as it was exposed to air and on mechanical stimulation, whereas those of rats deprived of choline showed no contractility at any time, regardless of any stimulation. The horns of the uterus were small in diameter, greatly elongated, and pale in color; the ovaries were smaller in size and their ova negligible in the choline-deficient as compared with rats given choline. The dimensions of the uterus and of the ovaries are represented in Table 2.

TABLE 2

Rat No.	Diet	Horn of uterus		Ovary	
		Length (mm)	Diam. (mm)	Length (mm)	Diam. (mm)
1	Adeq.	23	3.5	5	4
2	Def.	27	2.0	3	3
3	Adeq.	24	3.0	5	4
4	Def.	37	3.0	4	4

In the deficient adults the liver was pale and glistened with fat, and the kidneys were obviously hemorrhagic. A similar condition was noted in the liver but not in the kidneys of members of their litters. No paralysis such as that reported by Sure (*10*) occurred in litters of rats fed on the choline-deficient diet.

The results indicate that choline deficiency in mature albino rats caused a loss of contractility of the uterus and a partial atrophy of the uterus and ovaries, in addition to accumulation of fat in the liver and hemorrhages in the kidneys characteristic of choline-deficient animals. Choline supplements prevented such abnormalities, facilitated the mobilization of fat from the liver, as indicated

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by the relatively low fat content of the latter, and led to the elimination of excess fat by skin glands, as indicated by the oily condition of the skin in rats supplied with choline.

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Calcium Deficiency in Serpentine Soils as Revealed by Adsorbent Technique

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The Conejo soil series near Morgan Hill, California, is derived from outwash material of a serpentine rock area. The soil is deep, and rich in clay. The pH of the surface is 7.2. Conejo clay contains about 5.5 milliequivalents of exchangeable calcium/100 gm of soil. In comparison with many productive soils, this value is not low.

Conejo soil was brought to Berkeley and placed in pots containing 1,600 gm of soil. In the check pots romaine lettuce made little growth. Additions of nitrogen, phosphorus, and potash increased plant growth significantly, seemingly supporting the idea of Gordon and Lipman (*1*) that serpentine soils are unproductive because of deficiencies in nitrate, phosphate, and potassium.

After two to four weeks the lettuce plants on the fertilized soil developed a rosette disease. The young, inner leaves curled and became extremely stunted. Intense light enhanced these symptoms. Rosette, in various degrees, was also observed on serpentine soils of the Henecke and Venado series and on Maxwell and Dublin soils.

Following a suggestion by Robinson, *et al.* (*4*) that chromium and nickel, and perhaps cobalt, are the dominant causes of infertility in serpentine soils, normal lettuce plants grown on a productive soil and abnormal plants (rosette) from Conejo soil were analyzed spectrographically by A. P. Vanselow at the University of California Citrus Experiment Station. No consistent differences in Cr, Ni, and Co content could be observed. Adding these elements to good Yolo soil or spraying normal lettuce leaves with the soluble salts did not produce rosette disease.

After having initiated a great variety of treatments, we observed that gypsum and, to some extent, lime would combat the disease. Since serpentine soils are notoriously high in magnesium, these observations seemed to agree with Loew's (*2*) old theory of the Ca-Mg-proportions.

The question whether rosette disease is conditioned by magnesium excess or calcium deficiency, either absolute or relative, was elucidated with the aid of adsorbent technique, using cation amberlites as ionic carriers. Amberlites saturated with Ca, Mg, Sr, and K were added to fertilized Conejo soils and Yolo soils, the latter being a productive soil. The results were as follows:

(1) Ca-amberlite added to Yolo soil did not retard the excellent growth of the plants. Apparently the amberlite anion itself, in the amounts used, had no injurious effect on lettuce plants. Ca-amberlite added to Conejo soil overcame the disease and produced normal plants, comparable in weight and appearance with the plants grown on Yolo soil.

(2) Mg-amberlite added to Yolo soil produced characteristic rosette disease symptoms. When Mg-amberlite was added to Conejo soil, the extent of the disease was enhanced.

Experiments 1 and 2 rule out Cr, Ni, and Co as disease-producing agents.

(3) K-amberlite incorporated in Yolo soil produced lettuce rosette, and it emphasized the disease in Conejo soil. This experiment rules out magnesium as the primary factor. In the case of Yolo soil, it also eliminates the absolute level of exchangeable calcium (Moser's hypothesis, *3*) as the causal element.

Experiments 1, 2, and 3 suggest that the degree of saturation of exchangeable calcium is a prime contributing factor in the origin of lettuce rosette.

(4) Sr-amberlite added to Yolo soil in the same amount as Mg- and K-amberlite did not produce the disease. In Conejo soil it improved plant growth slightly but did not appreciably reduce the disease. The behavior of Sr-amberlite suggests a partial substitution of Sr for Ca in the growth of romaine lettuce.

While these experiments established the cause of rosette disease with the aid of soil studies and adsorbed ion technique, the crucial aspects could also be demonstrated with water culture experiments. Thus, the disease was produced in nutrient solutions as a result of calcium deficiency, in absence of significant amounts of cobalt, chromium, and nickel.

Similar results have been obtained with barley plants, which exhibit their own specific symptoms. A more detailed report will be published elsewhere.

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