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Reduced Muscle Creatine in Paralyzed Young E-Low Rats.*

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Olcott¹ first observed that muscular dystrophy occurred in the suckling young of vitamin E-low rats. Knowlton and Hines² noted a slight drop in muscle creatine in 4- to 6-months-old E-deficient rats with no visible signs of paralysis. Goettsch and Brown³ demonstrated a loss in skeletal muscle creatine in rabbits suffering from nutritional muscular dystrophy. Later Ni,⁴ using the same diet, noted in guinea pigs a corresponding creatine loss in the skeletal musculature accompanying the muscle degeneration. Morgulis⁵ reported a marked increase of creatine in the urine of rabbits held on a dystrophic diet. These findings suggested the probability that more significant changes in muscle creatine would be found in suckling young rats when extensive paralysis was evident.

Experimental. Each member of a group of young E-low females with a history of a resorption gestation was given a single dose of 1.0 g of wheat germ oil on the day following copulation. The litters resulting from these pregnancies were reduced to 6 young each. On the day of parturition one-half of the mothers were given 2.0 g of wheat germ oil by stomach tube. All mothers received Diet 808.†

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¹ Oleott, H. S., *J. Nutr.*, 1938, **15**, 221.

² Knowlton, G. C., and Hines, H. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 665.

³ Goettsch, M., and Brown, E. F., *J. Biol. Chem.*, 1932, **97**, 549.

⁴ Ni, T. G., *Chinese J. Physiol.*, 1936, **10**, 199.

⁵ Morgulis, S., *Nutritional Muscular Dystrophy*, Paris, Hermann and Cie, 1938.

† Diet 808:	casein (commercial)	27
	cornstarch (cooked)	30
	lard	22
	cod liver oil	2
	brewers' yeast	15
	salts No. 185	4

(Ingredients except cod liver oil were allowed to stand for 2 weeks at room temperature in order that the rancidity of the lard would destroy the vitamin E in the diet. The cod liver oil was added just before feeding.)

One-half of the members of each litter were transferred to a foster mother (3 of the young of the mother destined to receive wheat germ oil were given to an untreated mother, and vice versa). At the end of the lactation period the young suckled by the treated mothers appeared normal and served as controls. Half of each group were sacrificed on day 21; the remainder were sacrificed on day 45. The fresh muscle creatine content of the gastrocnemius and soleus muscles was determined by the Rose-Helmer-Chanutin technic⁶ (Table I).

Parts of these same muscles were saved for microscopic examination. There was a marked drop in the muscle creatine in the E-low animals as contrasted with that in the normal group (average values 152 mg and 242 mg respectively). This loss of 37% of the creatine content was paralleled by extensive muscle degeneration. The paralyzed young which were allowed to survive until the 45th day recovered spontaneously although in all cases the initial paralysis was of the same severity as that affecting their littermates sacrificed at day 21. When autopsied they presented the outward appearance of normal rats. The skeletal muscle creatine was determined and found to be normal, average values being 245 mg as compared with 246 mg for the control group. Histologically, also, the muscles of these rats appeared normal (paper in press). Thus a regeneration of muscle elements had occurred spontaneously without the addition of any vitamin E to the diet.

Since the animals in the E-low group in the first part of this experiment had just been weaned and were partially or completely paralyzed, they may have found it difficult to procure a sustaining quantity of diet. Hence the sudden change in muscle creatine might have been due to a semi-starvation, as suggested by studies on starvation by Mendel and Rose⁷ and Myers and Fine.⁸ The following experiment was devised to check the possible influence of starvation upon muscle creatine. Three litters, a total of 16 young, from normal mothers on stock diet, were used. Nine young remained with their mothers continuously throughout the period of the experiment. The remaining 7 rats were subjected to a semi-starvation regimen from day 5 to day 21. This regimen consisted of removing the young from their mothers 8 hours per day from day 5 to day 10; 12 hours daily from day 11 to day 15; 16 hours daily from day 16 to day 21. These animals were kept in a well ventilated incubator held at 37°C. The

⁶ Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

⁷ Mendel, L. B., and Rose, W. C., *J. Biol. Chem.*, 1911-12, **10**, 255.

⁸ Myers, V. C., and Fine, M. S., *J. Biol. Chem.*, 1913, **15**, 283.

TABLE I.
Skeletal Muscle Creatine Values in Young E-low Rats.

	No. of animals	Age at autopsy, days	Body wt at autopsy, g	Previous history	Condition at autopsy	Avg creatine value mg/100 g tissue
Diet 808 Mother given 2.0 g wheat germ oil	10	21	41	normal	normal	242
Diet 808 Mother E-low	9	21	38	,,	paralyzed	152
Diet 808 Mother given 2.0 g wheat germ oil	4	45	115	,,	normal	246
Diet 808 Mother E-low	5	45	108	paralyzed from day 21-25	normal (spontaneous recovery)	245
Stock diet Stock diet (semi-starvation)	9	21	54	normal	normal	218
	7	21	37	semi-starvation from day 5-21	undernourished	221

rats were given water by stomach tube once every 4 hours during the early stages of their incubator life, in order to prevent excessive dehydration. After day 15 this treatment was unnecessary. The average weaning weight of the experimental group was 37 g as compared with the littermate control group with 54 g. The 2 average creatine values are practically identical, being 221 mg and 218 mg respectively. It would appear from this study that semi-starvation for the last 16 days of lactation does not create a loss in muscle creatine. Therefore the drop in creatine value in the E-low paralyzed group was not occasioned or influenced by a semi-starvation which might conceivably have occurred.

Summary. A marked loss in skeletal muscle creatine occurs during the paralysis of suckling rats from mothers held on an E-low diet. These muscles showed extensive degeneration. There was a subsequent rise to normal creatine values with spontaneous recovery from the paralysis. Such muscles showed histological normality. The lowered muscle creatine was not produced by inanition.

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Failure to Produce Abdominal Neoplasms in Rats Receiving Wheat Germ Oil Extracted in Various Ways.*

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Rowntree and coworkers¹⁻⁴ reported the production of abdominal neoplasms in rats of the Wistar, Buffalo and Yale albino strains held for varying periods and on different intake of a crude ether extracted

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¹ Rowntree, L. G., Lansbury, J., and Steinberg, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 424.

² Dorrance, G. M., and Ciccone, E. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 427.

³ Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

⁴ Rowntree, L. G., Steinberg, A., and Brown, W. R., *Trans. Am. Phys.*, 1938, **53**, 199.