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Lactation Leucopenia in Rats on Purified Diets.*

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Recently long-term experiments have shown that purified diets for rats are equivalent to stock diets for growth[1,2] and reproduction[2] but not for lactation.[1,2,3] The criteria used for lactation have been the percentage of young weaned, their average weight at weaning, and the loss in weight of the lactating mother during the lactation period. In comparison with the 60-70% weaning reported by other investigators[1,3] working with purified diets, in this laboratory rats maintained throughout their entire life on a purified diet (836‡) have weaned 90% of their young.[2] Since this is equivalent to the lactation performance of stock animals in our rat colony, this criterion has been eliminated from our work. By placing adult stock animals with their litters on the purified diet at parturition it is possible to test dietary factors in 3 weeks for their effects on the weaning weight of the young and the weight loss of the mother.[2] We now wish to report an additional criterion for testing the adequacy of purified diets, namely, the occurrence of leucopenia in the lactating mother during this short period.

Procedure. Adult stock females with their litters were placed on the purified diet (836‡) at parturition or were continued on the stock diet[5] as controls. The litters were limited to 6 young, preferably 3 males and 3 females, which were weaned on the twenty-first day following parturition. Total and differential white blood counts were carried out by standard procedures at 2 different times, near the beginning of the lactation period (Day 5) and at the time of weaning (Day 21).

Results and Discussion. Table I shows typical results for rats maintained on the stock diet or on the purified diet with or without supplements. There was no change in total WBC, lymphocytes and monocytes, or granulocytes during the lactation period in animals maintained on the stock diet whereas a marked leucopenia occurred by the twenty-first day in animals maintained on the purified diet (836). All animals receiving the purified diet (without any supplementation) showed a significant decrease in granular and non-granular leucocytes from their original level (Day 5) and the drop to pathological levels of granulocytes (*i.e.* granulocytopenia, less than 750 cells per

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1 Vinson, L. J., and Cerecedo, L. R., *Federation Proc.*, 1943, **2**, 73; *Arch. Biochem.*, 1944, **3**, 389.

2 Nelson, M. M., and Evans, H. M., in preparation.

3 Richardson, L. R., and Hogan, A. G., *Federation Proc.*, 1945, **4**, 161.

4 Salts No. 4 of Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1944, **138**, 459.

‡ Basal diet 836 consists of alcohol-extracted casein 24%, sucrose 64%, hydrogenated vegetable oil (Crisco) 8%, and salts[4] 4%. Crystalline B vitamins are added per kilogram diet: thiamine HCl 5 mg, pyridoxine HCl 5 mg, riboflavin 10 mg, *p*-aminobenzoic acid 10 mg, nicotinic acid 20 mg, calcium pantothenate 50 mg, inositol 400 mg, and choline chloride 1 g. One cc of a fat-soluble vitamin mixture containing 6 mg alpha-tocopherol, 115 chick units vitamin D, 800 U.S.P. units vitamin A, and 650 mg corn oil (Mazola) is given weekly to each litter.

5 Wainwright, W. W., and Nelson, M. M., *Am. J. Orthodontics and Oral Surgery*, 1945, **31**, 406.

TABLE I.
Maternal Leucocytes During Lactation.

Experimental diet	Day of lactation (No. of rats)	Total* WBC	Lymphocytes and monocytes % Total*	PMN % Total*
Stock Diet I + lettuce	Day 5 (10)	13,095 (8,000-21,000)	70% 9,325 (5,200-14,175)	30% 3,770 (1,710-4,945)
	" 21 (10)	12,375 (7,350-17,300)	72% 9,035 (4,770-14,530)	28% 3,340 (2,350-4,720)
Purified Diet 836	" 5 (7)	11,845 (6,150-17,400)	72% 8,430 (4,670-10,265)	28% 3,415 (1,480-7,135)
	" 21 (11)	5,205 (2,625-9,600)	83% 4,195 (2,310-7,200)	17% 1,010 (215-2,730)
Purified Diet 836 + 1 g brewers' yeast daily	" 5 (8)	12,850 (8,500-19,000)	69% 9,025 (4,385-16,500)	31% 3,825 (1,700-6,690)
	" 21 (9)	7,200 (3,400-12,350)	79% 5,530 (3,400-10,375)	21% 1,670 (0-4,405)
Purified Diet 836 + 1 cc liver extr. daily	" 5 (8)	13,245 (10,800-17,000)	68% 9,000 (6,780-13,040)	32% 4,245 (1,860-7,515)
	" 21 (8)	8,005 (5,670-9,775)	74% 5,950 (3,995-8,435)	26% 2,055 (645-3,400)

* The range is given in parentheses below the average value.

mm^3) occurred in two-thirds of the group. Daily oral supplementation with 1 cc liver extract (Lederle 93% alcohol liver concentrate H-9381-B) resulted in some improvement but not in a return to the normal blood picture; 1 g brewers' yeast was somewhat less beneficial.

Preliminary studies were carried out with a folic acid concentrate (potency 5,000) kindly furnished by Dr. R. J. Williams, University of Texas. Oral supplements of this concentrate, equivalent to 150 μg "folic acid" (*i.e.* 7.5 μg daily) were given during the 3-week period to the lactating mothers without beneficial effects on any of the criteria for lactation. While the effects of "folic acid" concentrates on the other criteria for lactation are questionable,[3,6] there is no doubt concerning the curative effects of such concentrates or of crystalline vitamin Bc (total dose 100 μg) on the leucopenia and granulocytopenia of non-lactating rats maintained on purified diets with sulfa drugs[7] or even without their addition.[8] Either considerably higher levels of "folic acid" or additional factors must be necessary for a normal leucocyte picture during the strain of lactation.

Summary. Adult stock rats maintained on stock diets show no changes in the white blood cells during the 3-week lactation period. Maintenance on a purified diet during the same period results in a marked leucopenia and granulocytopenia by the twenty-first day. Daily supplements of brewers' yeast or liver extract result in improvement but not entire prevention of the blood dyscrasia.

[6] Cerecedo, L. R., and Vinson, L. J., *Arch. Biochem.*, 1944, **5**, 469.

[7] Sebrell, W. H., and Daft, F. S., *Pub. Health Rep.*, 1943, **58**, 1542.

[8] Kornberg, A., Daft, F. S., and Sebrell, W. H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 46.