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Dietary Granulocytopenia in Rats Corrected by Crystalline *L. casei* Factor ("Folic Acid").

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Granulocytopenia and anemia are produced in rats by the administration of sulfonamides in purified diets.[1,2] These blood dyscrasias are corrected by liver concentrates containing "folic acid"[1,2,3] and by crystalline *L. casei* factor or vitamin B_c.[4] In the present studies granulocytopenia has been observed in a small percentage of rats fed a purified diet without sulfonamides. Crystalline *L. casei* factor was effective in the correction of this blood dyscrasia. The occurrence of granulocytopenia in rats fed stock rations has not been observed in this laboratory or reported by others.[5,6]

Methods. Albino rats of Wistar and Osborne and Mendel strains were fed an experimental diet at weaning. 171 rats were fed purified diet No. 956.* 14 rats were fed another purified diet No. 937.† 21 rats were fed a sulfasuxidine-containing purified diet No. 945. It consisted of essentially the same ingredients as diet No. 956 but contained sulfasuxidine as 1% of the diet in place of 1% of sucrose.

The technic for making total leucocyte and polymorphonuclear cell counts and hematocrit determinations was the same as in previous studies.[7] With rats fed purified diets No. 956 and No. 937, blood counts were made 30 to 50 days after starting the diet. At this time, 155 of these 185 rats were used in another experiment and they are not considered further in this study. On the other 30 rats, blood counts were repeated at irregular intervals. With rats fed the sulfasuxidine-containing diet No. 945, a single count was made 36 days after starting the diet.

Treatment was by the oral administration of 25 γ of crystalline *L. casei* factor[8‡] for each of 4 days. Before starting treatment to correct granulocytopenia, 1 to 4 repeat counts were made over a period of 1 to 4 weeks. In every case, counts were made on the day when treat-

[1] Spicer, S. S., Daft, F. S., Sebrell, W. H., and Asburn, L. L., *Pub. Health Rep.*, 1942, **57**, 1559.

[2] Kornberg, A., Daft, F. S., and Sebrell, W. H., *Science*, 1943, **98**, 20.

[3] Axelrod, A. E., Gross, P., Bosse, M. D., and Swingle, K. F., *J. Biol. Chem.*, 1943, **148**, 721.

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

[5] Scarborough, R. A., *Yale J. Biol. and Med.*, 1931, **3**, 267.

[6] Creskoff, A. J., Fitz-Hugh, T., Jr., and Farris, E. J., in *The Rat in Laboratory Investigation*, edited by J. G. Griffith and E. J. Farris, Philadelphia, J. B. Lippincott Co., 1942, p. 351.

* Diet No. 956 consisted of sucrose 67.88 g, casein (Labco) 18.0 g, Crisco 8.0 g, and salt mixture No. 550 (1) 4.0 g. Into this diet was incorporated 2 g of corn oil containing 16,000 units of vitamin A and 1,600 units of vitamin D (Natola), 0.12 g of ethyl laurate containing 12 mg of α-tocopherol, 1 mg of thiamine hydrochloride, 2 mg of riboflavin, 1 mg of pyridoxine hydrochloride, 4.0 mg of calcium pantothenate, 2 mg of niacin, 200 mg of choline chloride, .001 mg of biotin, and 0.4 mg of 2-methyl-1,4-naphthoquinone.

† Diet No. 937 consisted of anhydrous dextrose 71.76%, casein (Labco) 18%, cod liver oil 2%, cottonseed (Wesson) oil 3%, ferric citrate (iron 18.09%) 1.16%, copper sulfate ($5H_2O$) .08%, and Salt mixture No. 550 4%. Each rat received a daily supplement of 100 γ of thiamine hydrochloride, 200 γ of riboflavin, 100 γ of pyridoxine hydrochloride, 200 γ of calcium pantothenate, 1 mg of niacin, 10 mg of choline chloride, 0.5 γ of biotin (Merck), and 20 γ of 2-methyl-1,4-naphthoquinone.

[7] Kornberg, A., Tabor, H., and Sebrell, W. H., *Am. J. Phys.*, 1944, **142**, 604.

[8] Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., and Slobodkin, N. H., *Science*, 1944, **99**, 371.

‡ The crystalline material used in the present studies was furnished through the courtesy of E. L. R. Stokstad, B. L. Hutchings, and N. H. Slobodkin of Lederle Laboratories.

ment was started and on the day following the last treatment.

The term "granulocytopenia" is used in this report to indicate a total polymorphonuclear cell count of less than 500 cells per cu mm.

Results. Granulocytopenia was observed in 6 of 185 rats fed purified diets for 30 to 50 days. Thirty rats were continued on these diets (155 were used in another experiment). Two of the 30 rats developed granulocytopenia after 125 days. Average values for the entire group of 185 rats 30 to 50 days after starting the diet were as follows: polymorphonuclear leucocytes: 2,448 cells per cu mm; total leucocytes: 13,931 cells per cu mm; hematocrit: 41.5 vol.%. In 2 rats with granulocytopenia there was a very slight and gradual increase in cell count without treatment; 1 rat died before treatment was started and 5 rats in which granulocytopenia was persistent were treated. Crystalline *L. casei* factor administered to these 5 rats with persistent granulocytopenia resulted in a rapid and striking response in every case (Table I). A concomitant, though lesser increase in non-polymorphonuclear leucocytes was noted. A relapse to granulocytopenic levels approximately one month following the responses to treatment was found in 3 of the 5 rats. Two of these rats were treated again and responded as well as after the initial treatment. A second relapse occurred a month later in one of these rats. Weight loss or poor growth, and sluggish activity were noted in all the rats prior to treatment. Weight gains and a general clinical improvement were noted in some of the rats following treatment. An hematocrit value under 30 vol.% was found in only one of this series of 185 rats.

Twenty of 21 rats fed the sulfasuxidine-containing purified diet No. 945 for 36 days had granulocytopenia. Only 1 rat had an hematocrit value under 30 vol.%.

Discussion. These data indicate that granulocytopenia, correctable by the *L. casei* factor, may occur in rats solely as the result of the ingestion of a highly purified diet. It is suggested that factors which may contribute to the infrequent appearance of this condition are the presence of body stores of the *L. casei* factor at weaning, contamination of the diet

TABLE I. Production of Dietary Granulocytopenia and Correction by Crystalline *L. casei* Factor ("Folic Acid.").

Rat No.	Diet	No. of days on diet	Values before treatment				Values after treatment*			
			Polymorphonuclear cells per cu mm	Total leucocytes per cu mm	Hematocrit vol. %	Wt gain in preceding 10 days, g	Polymorphonuclear cells per cu mm	Total leucocytes per cu mm	Hematocrit vol. %	Wt gain in 10 days, g
1	937	134	100	12,350	40	—15	5,300	27,750	42	0
		169†	50	4,750	38	— 3	16,600	34,550	43	—11
		203†	250	650			‡			
2	937	135	0	2,100	27	—39	3,500	9,000	27	—10
		167†	350	2,300	36		‡			
3	956	64	100	5,500	37	+11	2,800	10,300	39	+37
		126†	450	3,950	40	— 2	2,700	8,750	42	+29
4	956	43	450	3,150	43	—10	3,900	11,650	35	+17
5	956	33	450	2,700	36	+ 5	3,050	10,150	41	+24
6	937	30	200	6,350	40		‡			
7	956	30	400	7,050	41		§			
8	956	30	350	6,150	40	+ 5	‖			

* Treatment was by the oral administration of 25 γ of crystalline *L. casei* factor ("folic acid") for each of 4 days. Counts were made immediately before treatment and on the day following the last treatment. † Note relapse to granulocytopenic level. ‡ Died before treatment was started.
§ No treatment given. Values after 36 days on diet were 800 polymorphonuclear cells and 7,350 leucocytes per cu. mm.
‖ No treatment given. Values after 41 days on diet were 850 polymorphonuclear cells and 6,700 leucocytes per cu. mm.

and the low requirement of the rat for this vitamin. It also appears plausible from analogous studies on vitamin K deficiency[9-12] that intestinal synthesis of the *L. casei* factor, known to occur,[13] may be a significant factor.

It should be pointed out that the number of cases of granulocytopenia noted in this series of rats does not necessarily represent the maximal incidence. Observations were not made over a long period of time in most rats, and the purified diets used are not considered to be completely free from "folic acid."

The absence of severe anemias in this series of rats does not prove that the erythropoietic system was unaffected. It has been shown that when sulfasuxidine-fed rats with normal hematocrit values are subjected to repeated bleedings, red blood cell regeneration may be very inadequate.[7] Similar studies have not been carried out as yet in a large series of rats fed purified diets without sulfonamides.

Summary. 1. Granulocytopenia was observed in a small percentage of rats fed purified diets without sulfonamides. 2 Crystalline *L. casei* factor ("folic acid") administered to 5 rats with granulocytopenia corrected the blood dyscrasia in every case. One or more relapses occurred in 3 of the rats. Crystalline *L. casei* factor ("folic acid") corrected the granulocytopenia in relapse.

[9] Dam, H., and Glavind, J., *Z. f. Vitaminforschung*, 1939, **9**, 71.

[10] Greaves, J. D., *Am. J. Phys.*, 1939, **125**, 429.

[11] Kornberg, A., Daft, F. S., and Sebrell, W. H., *J. Biol. Chem.*, 1944, **155**, 193.

[12] Kornberg, A., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1944, **59**, 832.

[13] Mitchell, H. K., and Isbell, E. R., *The University of Texas Publication*, No. 4237, 1942, 125.

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A Heretofore Undescribed Phase-Variation in Salmonella.

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Andrews[1] discovered the phenomenon of *phase-variation* in bi-phasic Salmonella types. If a motile strain f.i. *S. typhi murium* (IV.V;i-1,2,3 . . .) is plated out on a moist agar plate, single colonies after 24 hours present either the i or the 1,2,3 phase. On further subculture either phase splits off the other phase in a certain percentage, generally not exceeding 5%. The graphic expression of this phase-variation reads: IV.V; i $\longleftrightarrow$ 1,2,3.

The two phases present in the two kinds of colonies are easily identified by known monophasic antisera or by antibody production. If a hitherto unknown phase appears and is not agglutinated by known antisera, it can be classified by immunization of rabbits with single colonies of this phase.

The unusual phase-variation which we are reporting differs from this pattern. A culture isolated from the blood stream of a severely sick elderly woman* showed culturally and serologically almost all the characteristics of *S. typhi murium*. The somatic antigen was IV.V, the flagellar antigen bi-phasic, one phase representing the 1,2,3 antigen. The other phase, however, was not the presumed i nor was it agglutinable by any one of our complete set of H-antisera. On occasion a trace of a 1,2,3 reaction was seen, suggesting that this phase, for simplification called X phase, may split off the 1,2,3 phase. Plated out from single colonies the X phase split off 1,2,3. The original 1,2,3 phase on the other hand split off

[1] Andrews, F. W., *J. Path. and Bact.*, 1922, **25**, 505.

* The patient died. Culture and clinical data were kindly submitted by Dr. Kantrowicz, Beth Moses Hospital, New York.