Hypersegmentation of Nuclear Lobes of Megakaryocytes in Rats with Folic Acid Deficiency

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HIGASHI, O., HONDA, Y., KARITA, M. and ARAKAWA, Ts. *Hypersegmentation of Nuclear Lobes of Megakaryocytes in Rats with Folic Acid Deficiency*. Tohoku J. exp. Med., 1971, 104 (2), 177-182 — The incidence in megakaryocytes with hypersegmentation of nuclear lobes was increased in the bone marrow of rats when fed on a diet deficient in folic acid with an addition of sulfaguanidine for a period of 2-3 weeks. — folic acid deficiency; hypersegmentation of nuclear lobes of megakaryocytes

During the bone marrow study on a child with homocystinuria complicated with folic acid deficiency of mild degree, one of us had found that there was an increase in the incidence of megakaryocytes with nuclear lobes of 4 or more (Higashi *et al.* 1971).

It has been well known that hypersegmentation of neutrophil nuclei is a sign of folic acid deficiency in humans (Herbert 1962).

The aim of present investigation was to examine a relationship, if any, between the number of nuclear lobes of megakaryocytes and folic acid deficiency in rats.

METHODS AND MATERIALS

White male rats of the Wistar strain, weighing 38-52 g, were divided into two groups.

The control group, consisting of 10 rats (Nos. 11 to 20), was fed on a control diet with a composition shown in Table 1.

The experimental group, consisting of 10 rats (No. 1 to 10), was fed on a diet devoid of folic acid from the control diet.

In both groups, 1 g of sulfaguanidine was added to 100 g of the diet described above.

All the rats were housed in individual cages with wire screen bottoms. The water was given *ad libitum*, but the amount of the diet was limited to 10 g per day per rat.

Estimation of urinary formiminoglutamic acid (FIGLU): Twenty-four hour urine specimens were collected in bottles containing 5 ml of 1N HCl. The urine specimens were adjusted to pH 6.2 with 1N KOH just prior to the FIGLU estimation according to the method described by Tabor and Wyngarden (1959).

Histidine monohydrochloride, 0.5 g per kg of body weight, was given intraperitoneally as a 10% solution dissolved in saline, on the 13th and 20th days of the experimental feeding.

TABLE 1. *Composition of a control diet for rats*

Ingredients	%
Casein (free from riboflavin)	20.0
Corn starch	40.0
Sucrose	27.0
Oil (soybean oil 80% and cod liver oil 20%)	8.0
Salt mixture*	4.0
Vitamin mixture†	0.85
Cholin chloride	0.15

* Salt mixture: CaCO_3 , 29.29%, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ 0.43%, KH_2PO_4 34.31%, NaCl 25.06%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 9.98%, $\text{Fe}(\text{C}_6\text{H}_5\text{O}_7) \cdot 6\text{H}_2\text{O}$ 0.623%, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.156%, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.121%, ZnCl_2 0.02%, KI 0.0005%, and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 0.0025%.

† Vitamin mixture: Thiamin hydrochloride 0.059%, riboflavin 0.059%, Ca pantothenate 0.235%, niacin 0.294%, pyridoxine hydrochloride 0.029%, menadione 0.006%, biotin 0.001%, folic acid 0.0588%, inositol 1.176%, ascorbic acid 0.588%, hydroxocobalamin 0.0002%, and corn starch 97.494%.

Examination of nuclear segmentation (a karyogram) of megakaryocytes: The bone marrow was aspirated from tibia or femur, which was removed and cracked immediately after the animal was killed, into a glass capillary tube containing an appropriate amount of 1% sequestrene- Na_2 (disodium ethylene-diamino-tetraacetate dihydrate) dissolved in 0.7% sodium chloride solution (Stefanini and Dameshek 1955). Smears were prepared by the cover-glass technic (Wintrobe 1961) and Wright-Giemsa stained. The degree of nuclear segmentation, a karyogram, of megakaryocytes was estimated by the method described previously (Higashi *et al.* 1971).

RESULTS AND DISCUSSION

The urinary excretion of FIGLU after a histidine loading was found to be increased in rats fed on the diet deficient in folic acid with an addition of sulfaguanidine for 2 weeks and this tendency was observed more exaggerated in rats put on the diet for 3 weeks (cf. Table 2).

These findings revealed that the rats were made definitely deficient in folic acid when fed on the diet deficient in folic acid supplemented with sulfaguanidine for a period of 3 weeks (Silverman and Pitney 1958).

The results of examination on nuclear shift of the bone marrow megakaryocytes were shown in Tables 3 and 4 and Fig. 1. The incidence of megakaryocytes with four or more nuclear lobes was higher in the folate deficient group than in the control group, *i.e.* a total of megakaryocytes with four or more lobes being 6 and 25 in the folate deficient group as compared with 0 and 5 in the control group on the 16th and 23rd day of the experimental feeding (cf. Tables 3 and 4, and Fig. 1). Also the lobe average of megakaryocytes showed a tendency to increase in the folate deficient group than in the control group.

At the present time it has been accepted (Fliedner *et al.* 1964, Chanarin 1969) that the number of nuclear lobes of neutrophils is determined by the character of granulopoiesis, and not by the development of increased nuclear segmentation of a cell with fewer lobes.

TABLE 2. *The urinary excretion of FIGLU after histidine loading in rats of experimental and control groups*

Group	No. of rats	FIGLU excreted (μ moles/24 hr urine)	
		13-14th experim. day	20-21st experim. day
Rats fed on a folate deficient diet	1	56.2	
	2	51.0	
	3	89.7	
	4	75.4	
	5	39.1	
	6	41.4	225.0
	7	4.2	204.8
	8	64.6	170.0
	9	19.3	184.8
	10	61.6	78.4
Rats fed on a control diet	11	2.2	
	12	2.2	
	13	1.8	
	14	1.1	
	15	2.1	
	16	n.d.	19.5
	17	2.7	1.7
	18	3.1	1.4
	19	1.8	2.1
	20	1.0	1.8

n.d.=not detectable

Fig. 1. Incidence of megakaryocytes with various numbers of nuclear lobes in the rat bone marrow.

(●) Rats fed on a folate deficient diet for 23 days.

(○) Rats fed on a control diet for 23 days.

TABLE 3. *The incidence (%) of bone marrow megakaryocytes with various obtained on the 16th day of the feeding with the*

Number of nuclear lobes of megakaryocytes	Folate deficient group					
	No. 1	No. 2	No. 3	No. 4	No. 5	Mean $\pm$ s.d.
I	87	87	81	95	91	88.2 $\pm$ 4.7
II	9	8	15	5	5	8.4 $\pm$ 3.7
III	3	4	1	0	3	2.2 $\pm$ 1.5
IV	1	1	3	0	1	1.2 $\pm$ 1.0
Lobe average	1.18	1.19	1.26	1.05	1.14	1.16 $\pm$ 0.07

TABLE 4. *Incidence (%) of bone marrow megakaryocytes with various obtained on the 23rd day of the feeding with the*

Number of nuclear lobes of megakaryocytes	Folate deficient group					
	No. 6	No. 7	No. 8	No. 9	No. 10	Mean $\pm$ s.d.
I	98	90	55	65	78	77.2 $\pm$ 15.7
II	2	4	15	18	11	10.0 $\pm$ 6.1
III	0	3	19	11	6	7.8 $\pm$ 6.7
IV	0	3	6	4	4	3.4 $\pm$ 2.0
V	0	0	5	2	1	1.6 $\pm$ 1.9
Lobe average	1.02	1.19	1.91	1.60	1.39	1.42 $\pm$ 0.31

As for the mechanism through which the nuclear lobulation of megakaryocytes is determined, Bond *et al.* (1962) carried out an investigation on the kinetics of megakaryocytes of the rat bone marrow using tritiated thymidine as a cell label and concluded that it was comparable to nuclear segmentation in neutrophilic granulocytes.

The results of our present investigation may, therefore, be explained reasonably in the following way: hypersegmentation of nuclear lobes of megakaryocytes was resulted from an experimentally induced folic acid deficiency by which an impaired DNA synthesis in primitive unrecognized cells (Cooney 1962), precursors of megakaryocytes, was brought about.

number of nuclear lobes in folate deficient and control rats. Specimens were folate deficient and the control diets, respectively

Control group					
No. 11	No. 12	No. 13	No. 14	No. 15	Mean $\pm$ s.d.
91	91	98	92	93	93.0 $\pm$ 2.6
6	9	2	6	7	6.0 $\pm$ 2.3
3	0	0	2	0	1.0 $\pm$ 1.3
0	0	0	0	0	0 $\pm$ 0
1.12	1.09	1.02	1.10	1.07	1.08 $\pm$ 0.08

number of nuclear lobes in folate deficient and control rats. Specimens were folate deficient and the control diet, respectively

Control group					
No. 16	No. 17	No. 18	No. 19	No. 20	Mean $\pm$ s.d.
90	88	77	96	82	86.6 $\pm$ 6.6
7	10	12	3	15	9.4 $\pm$ 4.1
2	2	9	1	1	3.0 $\pm$ 3.0
1	0	1	0	1	0.6 $\pm$ 0.5
0	0	1	0	1	0.4 $\pm$ 0.5
1.14	1.14	1.37	1.05	1.24	1.19 $\pm$ 0.11

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Fig. 2. Megakaryocytes from the bone marrow of the rat No. 8. A and B show the cells with a single-lobed nucleus; C and D show the cells with a five-lobed nucleus.