Research on the Proliferation Activity of Erythroblasts from Pernicious Anaemia in Relapse under Low Atmospheric Pressure

In a previous note, published in this same journal[1], we reported the results of an experimental research, aimed at investigating the development of proliferation activity of the erythroblast from normal bone-marrow (normoblast) living under low atmospheric pressure. Such a research was carried out on bone-marrow cultured *in vitro* and directly submitted to various pressure levels. It proved that, under such a condition, erythropoietic proliferation decreases with the decrease of tension level and that a lineal relation does exist, at least as regards pressure values included between 760 → 160 mm Hg; viz: proliferation activity decreases with an intensity which is a function of the vacuum degree. It also proved that the decrease rates of the basophile and of the polychromatophile erythroblasts are different; the former cell showing more reaction than the latter.

In the present note, we report the results of a similar research on bone-marrow erythroblast from pernicious anemia in relapse (megaloblast), keeping in mind that megaloblast is endowed with some biological peculiarities differing from those of normoblast[2]. For this research, we used the same technical procedures followed in our previous investigation[3]. Particularly, the culture medium was prepared using the plasma from the subjects who had supplied bone-marrow, i.e., in this case, plasma of patients suffering from pernicious anemia in relapse. This plasma was added with an optimum dose of colchicine, in order to investigate the proliferation activity according to the ASTALDI-MAURI stathmocinetic test[4]. Cultures were put into a special apparatus, previously described[3], where depressions from 760 to 160 mm Hg were obtained and maintained.

Table I (see on page 262) reports the average stathmocinetic indices of the cases studied, obtained under different pressure levels, respectively relating to the basophile and polychromatophile megaloblasts and normoblasts. These indices show the ratio of proliferation obtained under the different conditions, and they are expressed as percentages in respect to the proliferation activity of the basophile at 760 mm Hg; such an activity being conventionally assumed = 100.

In order to establish the significance of the results, they were submitted to the analysis of variation with three criteria, viz: subjects—differentiation phase—degree of pressure. The results are reported in Table II.

Moreover, the stathmocinetic indices reached under the different pressure levels were interpolated, in order to establish the quantitative relation between the lowering of pressure and the decrease in megaloblast proliferation activity. The results reported in the diagram reveal the following functions:

$$f(xi) = 33{\cdot}9 + 0{\cdot}134(xi-M) + 0{\cdot}0001\,(xi-M)^2;$$

for the basophile, and

$$f(xi) = 20{\cdot}7 + 0{\cdot}134(xi-M) + 0{\cdot}0001\,(xi-M)^2;$$

for the polychromatophile.

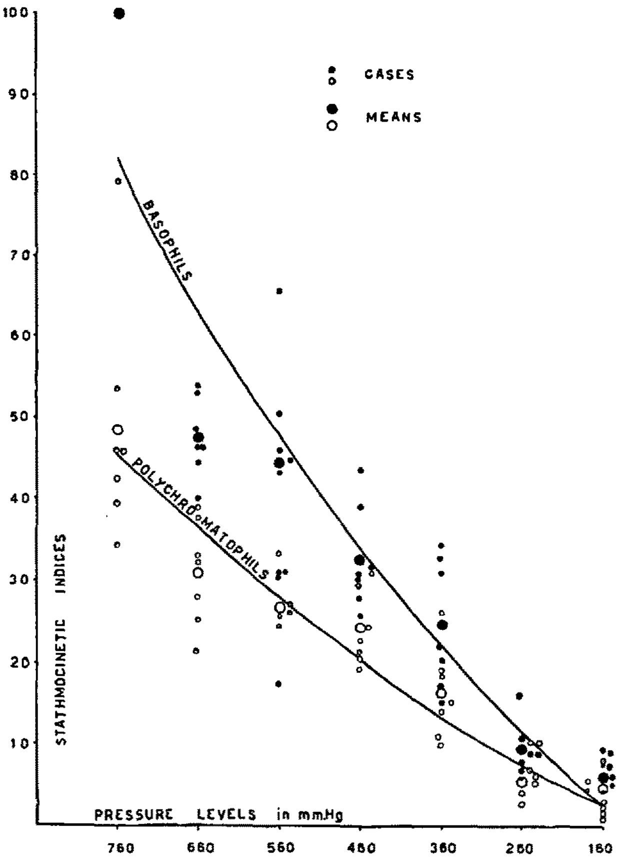
[1] G. ASTALDI, E. BERNARDELLI, and G. REBAUDO, Exper. *8*, 117 (1952).

[2] P. JONES, Arch. Path. *35*, 752 (1943). – G. ASTALDI, C. MAURI, and A. ALLEGRI, Haematol. *34*, 1091 (1950).

[3] G. ASTALDI, E. BERNARDELLI, and G. REBAUDO, Le Sang *23*, 293 (1952).

[4] G. ASTALDI and C. MAURI, Haematol. *33*, 583 (1949); Le Sang *21*, 378 (1950).

Finally, the indices of megaloblast proliferation activity were compared with those of the normoblast by statistical method (using STUDENT-FISHER t^2 and SNEDECOR'S F). The results are reported in Table III.



The stathmocinetic index in basophils at 760 mm Hg is considered equal to 100.

The results of our investigations show that:

(1) The decrease of pressure levels causes a decrease in the proliferation activity of megaloblasts.

(2) The more intense the decrease in pressure level, the lower the proliferation activity.

(3) Such a phenomenon occurs both in basophile and polychromatophile megaloblast, but the response of the basophile megaloblast to pressure variations is significantly different from that of the polychromatophile megaloblast. Particularly, in the latter an increased vacuum causes a heavier reduction of proliferation than in the former.

(4) The response of pernicious anemia erythroblasts (megaloblasts) to pressure variations is significantly different from that of normal bone-marrow erythroblasts (normoblasts). Particularly, in the latter an increased vacuum causes a heavier reduction of proliferation than in the former.

To summarize, we may conclude that hypoxia, when acting directly on bone-marrow surviving *in vitro*, acts as an inhibiting factor on erythroblast proliferation activity—irrespective of whether erythroblast is a normoblast or a megaloblast, but that these two cellular types react in a quantitatively different manner. Thus, it remains to be established whether the different biology of the two cellular types—normoblasts and megaloblasts—, or whether only the greater proliferation activity of megaloblasts[1] is the cause of the greater

[1] G. ASTALDI, C. MAURI, and A. ALLEGRI, Haematol. *34*, 1091 (1950).

reaction to hypoxia detected in the pernicious bone-marrow. This problem might be solved by experiments on bone-marrow with higher proliferation activity than normal marrow, but containing normoblasts and not megaloblasts, such as the marrow in thalassemia major[1].

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[1] G. ASTALDI and P. TOLENTINO, J. Clin. Pathol. *5*, 140 (1952).

Resumé

Les auteurs ont étudié l'action de l'hypoxie sur la prolifération des mégaloblastes dans l'anémie pernicieuse et ont mis en évidence un effet inhibiteur. Le phénomène est analogue à celui qui a été démontré pour l'érythroblaste de la moelle osseuse normale, mais il en diffère en ce sens que la sensibilité négative du mégaloblaste est plus grande que celle du normoblaste. Il n'est pas encore établi s'il s'agit d'une action spécifique en rapport avec la biologie différente des deux cellules ou simplement liée à leur différent degré d'activité prolifératrice, plus élevé pour le mégaloblaste que pour le normoblaste.

Table I
Averages of Stathmocinetic Indices Under Different Pressure Levels

Pressure Levels mm Hg	Proliferation			
	Mean $m \pm \sigma_m$	Dispersion $\sigma \pm \sigma_\sigma$	Mean $m \pm \sigma_m$	Dispersion $\sigma \pm \sigma_\sigma$
	Basophile normoblasts		Polychromatophile normoblasts	
760	100 ± 0	0 ± 0	64·6 ± 2·51	7·94 ± 1·77
660	88·4 ± 3·13	9·90 ± 2·21	53·3 ± 2·25	7·13 ± 1·59
560	83·3 ± 2·68	8·47 ± 1·89	44·3 ± 2·36	7·48 ± 1·67
460	52·8 ± 4·22	13·34 ± 2·98	28·1 ± 2·76	8·75 ± 1·95
360	43·9 ± 2·28	7·21 ± 1·61	24·2 ± 1·77	5·61 ± 1·25
260	35·1 ± 1·29	4·08 ± 0·91	18·7 ± 1·39	4·42 ± 0·98
160	27·2 ± 1·86	5·88 ± 1·31	14·0 ± 1·21	3·83 ± 0·85
	Basophile megaloblasts		Polychromatophile megaloblasts	
760	100 ± 0	0 ± 0	48·8 ± 5·26	13·9 ± 3·71
660	47·6 ± 1·74	4·6 ± 1·22	30·9 ± 2·15	5·7 ± 1·52
560	44·6 ± 4·20	11·1 ± 2·97	26·7 ± 1·78	4·7 ± 1·25
460	32·9 ± 2·15	5·7 ± 1·52	24·2 ± 1·55	4·1 ± 1·09
360	24·8 ± 3·33	8·8 ± 2·35	16 ± 1.89	5 ± 1·34
260	9·3 ± 1·21	3·2 ± 0·85	5·9 ± 0·90	2·4 ± 0·64
160	6·2 ± 0·64	1·7 ± 0·45	5 ± 0·95	2·5 ± 0·66

Table II
Results of Variation Analysis with Three Different Criteria

Sources	Degrees of freedom	Sum of the squares	Mean square	F ratio (SNEDEGOR)	F significative	
					5%	1%
Totale	98	64 220·3				
Between pressures	6	39 822·5	6 637·1	26·1	2·36	3·35
Between differentiation phases	1	5 999·9	5 999·9	23·6	4·11	7·39
Between cases	6	521·5	86·9	0·3	2·36	3·35
Interaction pressures-phases	6	5 894	982·3	3·9	2·36	3·35
Interaction phases-cases	6	337	56·2	0·2	2·36	3.35
Interaction pressures-cases	36	2 239·4	62·2	0·2	1·73	2·18
Residue (experimental error, etc.)	37	8 406	254·2			

Table III
Significance of the Differences Between Averages

Between basophile and polychromatophile megaloblasts				Between megaloblasts and normoblasts							
				Basophile				Polychromatophile			
t^2	F		Judgment	t^2	F		Judgment	t^2	F		Judgment
	0·1	0·5			0·01	0·05			0·01	0·05	
7·1	6·91	3·94	+	12·9	6·82	3·92	+	14·4	6·82	3·92	+