Summary

The ATPase activity is inhibited by Colchicin in concentrations of 10^{-3} m and 10^{-4} m at a rate of 40 to 20%, but in no way in the range of 10^{-7} m and 10^{-8} m, where Colchicin exerts its typical spindle-blocking action on the mitotic cell division. It is therefore no evidence, that Colchicin acts by influence on the energy yielding ATP-ATPase system, which was proposed to be responsible for the spindle contraction.

Heparinocytes and Hibernation

The most characteristic feature of the physiology of hibernation is the change of a mammal within certain limits from homoiothermy to poikilothermy (cf. for instance SUOMALAINEN[1]). Thus for instance the body temperature of the hedgehog in hibernation may fall to about 2° C. When the animal wakes out of hibernation, its body temperature rises in some hours from 2–5° C again to 30–35° C and the animal returns to homoiothermy.

Because of the low body temperature the metabolism of the animal is greatly reduced during hibernation. This appears in such phenomena as retardation of the heart rate (SUOMALAINEN and SARAJAS[2]). In the summer the heart of the hedgehog beats about 190 times per minute, during hibernation only about 20 times per minute. Since no thrombi occur in the circulatory system in spite of the reduced heart function, and blood circulation occurs without disturbance, there seemed to be reason to investigate to what extent changes appear during hibernation in the number and histologically observable heparin content of the heparin-secreting heparinocytes or Ehrlich's mast cells.

The fixation of the tissues was made with a modification of Holmgren and Wilander's method[3]. With the use of Holmgren and Wilander's basic lead acetate, harmful crystals may appear in the preparations. They are not formed if formalin and alcohol are added to the fixative. At the same time the general fixability and stainability are improved.

Relative heparinocyte content of the small intestine and bronchial branches in the hedgehog. Each figure represents the mean of a hundred unit areas used in the investigation.

	Normal hedgehog	Hibernating hedgehog	Woken from hibernation
Small intestine	4·3	24·3	14·1
	5·9	18·2	7·9
	4·2	11·9	—
	4·8	18·1	11·0
Bronchial branches . .	27·0	75·5	69·7
	39·2	70·2	74·5
	25·4	92·3	—
	30·5	79·3	72·1

The quantitative determination of heparinocytes was made by counting their number in the visual field of a microscope at ×450 magnification. From each preparation, 100 visual fields were chosen entirely at random. In these the number of heparinocytes was counted within the frame of the limits of depth of the fine adjustment. To facilitate counting, the visual field was divided into squares with the aid of a grid.

Heparinocytes are numerous in the small intestine and lungs round the bronchial branches in the hedgehog.

Their number is increased during hibernation, but is also large in animals just woken from hibernation (table).

Judging from the increase in the number of heparinocytes and their histological appearance, heparin secretion is greater than normal in hibernation, when the heart rate in the hedgehog is greatly reduced. Actual physiological determinations of the coagulation time of the blood of the hedgehog in hibernation are still incomplete.

A full report of this work will appear elsewhere.

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Zoological Laboratory, Helsinki University, Helsinki, Finland, April 14, 1951.

Zusammenfassung

Trotz der stark herabgesetzten Schlagfrequenz des Herzens stellen sich beim winterschlafenden Igel keine Thromben ein. Die Menge der Heparinozyten oder Ehrlichschen Mastzellen ist denn auch beim Igel während des Winterschlafs erhöht. Daraus, und aus dem histologischen Bild dieser Zellen, kann geschlossen werden, daß die Heparinsekretion im Winterschlaf zugenommen hat.

Study on the Growth of the Erythroblast in Normal and Bone-Marrow Erythroblastosis Conditions

During the last few years our knowledge of the biology of the bone-marrow cells has been considerably widened with particular regard to their differentiation and proliferation. However there is still a complete lack of information about the growth of the bone-marrow cells during the interkinetic period. The lack of information on this subject is due to the impossibility of studying directly the development of the living cell, by measuring at suitable intervals of time the increase in size which is of biometric interest.

We thought it might be interesting to collect some indirect data on the cytoplasmic growth through the observation of fixed and stained films. As it was not possible to relate the cytoplasmic growth to time, we studied the growth of the cytoplasm with respect to the nucleus, the latter being considered as a function of time. In other words, the aim was to investigate the extent to which the cytoplasmic diameter increased when the nuclear diameter increased a specified amount.

The evaluation of the growth of the cytoplasm with respect to the nucleus was made by the slope (a) of the interpolating line of the cytoplasmic diameter with respect to the nuclear one.

In this study another value was also considered, i. e. the correlation coefficient of nucleo-cytoplasmic diameters (r).

By "cytoplasmic diameter" we mean the cubic root of the difference between cellular diameter and nuclear diameter, both cubed.

We made our researches on the basophil erythroblast including also proerythroblast – because the basophil

[1] P. SUOMALAINEN, Sitz.-Ber. Finn. Akad. Wiss. 1943, 163 (1944).
[2] P. SUOMALAINEN and S. SARAJAS, Ann. Zool. Soc. "Vanamo" *14*, 2 (1951).
[3] HJ. HOLMGREN and O. WILANDER, Z. mikr.-anat. Forsch. *42*, 242 (1937).

Conditions	Normal man	Normal child	Pernicious anaemia	Thalassaemia major	Thalassaemia minor	Congenital haemolytic jaundice	Idiopathic hypochromic anaemia	Ankylostomiasis anaemia	Gastric carcinoma anaemia	Post-haemorrhagic anaemia
Correlation coefficient (r)	0·670	0·703	0·689	0·802	0·744	0·772	0·689	0·760	0·803	0·695
	0·580	0·726	0·720	0·779	0·725	0·721	0·794	0·651	0·761	0·769
	0·585	0·579	0·575	0·785	0·682	0·796	0·795	0·680	0·799	0·794
	0·721	0·547	0·745	0·788	0·775	0·826	0·760	0·854	0·736	0·805
	0·659	0·706	0·680	0·709	0·794	0·784	0·773	0·786	0·804	0·785
	Significance[1]		–	+	+	+	+	–	++	+
Slope (a)	0·716	0·763	1·011	0·925	0·888	0·857	0·741	0·730	0·906	0·646
	0·614	0·729	0·942	0·950	0·725	0·775	0·980	0·633	0·846	0·798
	0·642	0·557	0·835	0·945	0·757	0·925	0·885	0·735	0·994	0·939
	0·675	0·705	1·007	1·015	0·938	0·985	0·848	0·938	0·813	0·854
	0·734	0·586	0·865	0·747	0·876	0·778	0·852	0·959	1·008	0·870
	Significance[1]		++	+	+	+	+	–	++	–

[1] Results of significance test between normal average value and in conditions under examination ones: — no significance, + significance 5%, ++ significance 1%.

one is the cell of erythroblastic differentiation which proliferates most actively, and in which therefore the phenomena under examination would be more evident.

We studied the normal bone-marrow both of man and child, in order to gather normal data for comparison with pathological cases; and also the bone-marrow in the following pathological conditions: pernicious anaemia in relapse, thalassaemia major and minor, congenital haemolytic jaundice, idiopathic hypochromic anaemia, gastric carcinoma anaemia, ankylostomiasis anaemia and post-haemorrhagic anaemia. We examined 5 cases of each pathological condition; in each case we measured the nuclear and cytoplasmic diameters of 300 basophil erythroblasts.

Results were submitted to statistical analysis, adopting PEARSON's χ^2, STUDENT-FISHER t test, COCHRAM-COX modification, according to the standard deviation of the results of the different conditions under examination.

We shall now explain the system of calculation.

The cytoplasm diameter (y) has been inferred for every pair of observations in the following way:

$$y = \sqrt[3]{u^3 - x^3},$$

where u = cell diameter and x = nucleus diameter.

The correlation between two variables y and x has been measured by the correlation coefficient of BRAVAIS PEARSON, i. e.

$$r = \frac{\sum_{i=1}^{s}\sum_{j=1}^{v} f_{ij}(x_i - \bar{x})(y_j - \bar{y})}{N\,\sigma_x\,\sigma_y}$$

where for every x_i $(i = 1, 2, 3, \ldots, s)$ we take y_j $(j = 1, 2, 3, \ldots, v)$; f_{ji} is the frequence of the observation in which x and y have respectively the values x_i and y_j.

From the averages y' corresponding to every x is plotted as a straight line, the equation of which is

$$Y = \bar{y} + a\,(X - \bar{x})$$

where $\bar{y}$ is the average of all y_j, $\bar{x}$ the average of all x_i, and

$$a = r\,\frac{\sigma_y}{\sigma_x};$$

σ_y being the standard deviation of y and σ_x the standard deviation of x_i, that is

$$\sigma_y = \sqrt{\frac{\sum_{j=1}^{v} f_j (y_j - \bar{y})^2}{v-1}}, \qquad \sigma_x = \sqrt{\frac{\sum_{i=1}^{s} f_i (x_i - \bar{x})^2}{s-1}}$$

It is to be noted that $v = s$.

In order to calculate the average of r a substitution of variable has been made (FISHER), that is

$$z = \frac{1}{2}\ln\frac{1+r}{1-r};$$

from which

$$z'_e = \frac{(n_1-3)\,z_1 + (n_2-3)\,z_2 + \cdots + (n_2-3)\,z_k}{(n_1-3) + (n_2-3) + \cdots + (n_k-3)},$$

$n_1, n_2, \ldots, n_k$ being the respective numbers of the observations of every sample. Being in our case

$$n_1 = n_2 = \cdots = n_k,$$

$$z'_e = \frac{z_1 + z_2 + \cdots + z_k}{k}.$$

Therefore the sufficient estimate, for every group of samples, of the parameter r is $r'_e = th\,z'_e$, where th is the symbol of hyperbolic tangent. The parameter t of STUDENT (for the comparison of z'_e and the comparison of average a) is given by

$$t = \frac{(M_1 - M_2)}{{}_1\sigma_2}\sqrt{\frac{n_1 n_2}{n_1 + n_2}};$$

where M_1 and M_2 are the respective averages of the groups compared, n_1 and n_2 the respective numbers of the observations, and,

$${}_1\sigma_2 = \sqrt{\frac{\sigma_1^2(n_1-1) + \sigma_2^2(n_2-1)}{n_1 + n_2 - 2}}$$

where σ_1 and σ_2 are the respective standard deviations of the two groups.

For the synthetic valuation (FISHER) of several experimental series we have used the χ^2 test of PEARSON:

$$\chi^2 = \Sigma\, \chi_i^2 = -2 \cdot 2{,}3026\, \Sigma \log P_i;$$

P_i being the respective probabilities of finding a value of t equal or higher than the calculated one.

We have used the modification of COCHRAM and COX of test t when the variances of the two samples were significatively different.

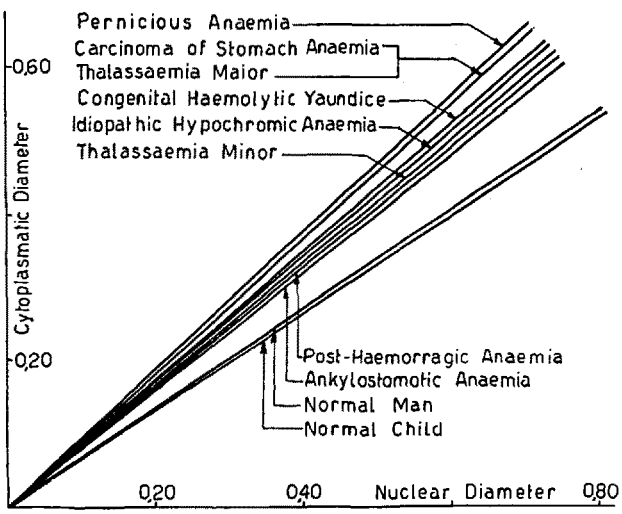
M_1, M_2, S_1, S_2 being the respective averages and sums of the squares of the deviations of the two samples, we calculate

$$\frac{(M_1 - M_2)}{\sqrt{\frac{S_1}{n_1(n_1-1)} + \frac{S_2}{n_2(n_2-1)}}}$$

and compare the result with the level 5% (or 1%) given by

$$\frac{\frac{S_1}{n_1(n_1-1)}\sqrt{t_{0,05}} + \frac{S_2}{n_2(n_2-1)}\sqrt{t'_{0,05}}}{\frac{S_1}{n_1(n_1-1)} + \frac{S_2}{n_2(n_2-1)}}$$

where n_1 and n_2 are the respective numbers of observations of the first and of the second sample, and $t_{0,05}$ and $t'_{0,05}$ the respective 5% significant t of the first sample (with degrees of freedom $= n_1 - 1$) and of the second sample (with degrees of freedom $= n_2 - 1$).



The figure shows the slopes of the interpolating lines of the cytoplasmic diameter against the nuclear one. Each straight line indicates the average value in one condition studied. The straight lines are made to start from the origin of cartesian axis, i. e. as if the known terms of their equation were null: this was done to make more evident the slope of the straight lines.

Results, reported in the table and in the figure, show that:

(1) The average correlation coefficients (r) of thalassaemia major and minor, congenital haemolitic jaundice, idiopathic hypochromic anaemia, gastric carcinoma anaemia and post-haemorrhagic anaemia show a significant increase as compared with the normal one. On the contrary there is no significant difference between the normal average correlation coefficient (r) and the corresponding ones of pernicious anaemia and ankylostomiasis anaemia.

(2) The average slopes (a) of pernicious anaemia, thalassaemia major and minor, congenital haemolitic jaundice, idiopathic hypochromic anaemia and gastric carcinoma anaemia show a significant increase when compared with the normal one. On the contrary no significant difference is to be found between the normal average slope and the corresponding ones of ankylostomiasis and post-haemorrhagic anaemia.

These results taken as a whole show us that both dimensional nucleo-cytoplasmic correlation and growth of erythroblast are disharmonious not only when the erythropoiesis is induced in an abnormal erythroblastic type, such as the megaloblast of pernicious anaemia, but also when an active erythropoiesis occurs through normoblastic differentiation. For instance, in the case of thalassaemia major and minor, of congenital haemolitic jaundice, of idiopathic hypochromic anaemia, of gastric carcinoma anaemia. Instead in other erythropathies, although the erythropoiesis is altered, an abnormal growth of the erythroblast is not sufficiently demonstrated, as, for instance, in the case of post-haemorrhagic anaemia.

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Department of Internal Medicine, University of Pavia, January 15, 1951.

Riassunto

Sono state condotte ricerche sulla auxologia dell'eritroblasto basofilo del midollo osseo umano normale e di varie eritroblastosi. In particolare si è indagato, durante il periodo dell'intercinesi cellulare, sulla quantità di cui cresce il citoplasma rispetto al crescere di una quantità unitaria del nucleo, valutata in base al coefficiente angolare dell'interpolante lineare del diametro citoplasmatico rispetto a quello nucleare. È stato inoltre indagato sulla correlazione dimensionale nucleo-citoplasmatica.

Le ricerche hanno dimostrato anomalie auxologiche dell'eritroblasto non solo quando l'eritropoiesi segue una linea eritropoietica abnorme, quale ad esempio quella megaloblastica dell'anemia perniciosa, ma anche in molte eritroblastosi dove l'eritropoiesi si svolge lungo la differenziazione normoblastica.

Effect of the Reticulo-Endothelial Blockade by Thorotrast on the Development of Normal Heterohemagglutinins in Fowl

The origin of normal antibodies has been the subject of much controversy. LANDSTEINER[1] and HIRSZFELD *et al.*[2] developed the theory of "serogenesis". According to them, the main factors in the development of normal antibodies are genetic inheritance and age. On the other hand, there are some evidences which suggest that "normal" immune body production may be due to antigenic stimuli and may be from heterologous antibodies. E. g. FORSSMAN[3] and BAILEY[4] demonstrated that intake of certain foods or some infections may provoke production of antisheep hemolysins in rabbits. Furthermore,

[1] K. LANDSTEINER, *The Specifity of Serological reactions* (Harvard U. Press, 1945).

[2] H. HIRSZFELD, L. HIRSZFELD, H. BROKMAN, W. HALBER, and M. MAYZNER, J. Immunol. *9*, 571 (1924); Ergebn. d. Hyg. *8*, 367 (1926); Z. Immunitätsforsch. *53*, 391 (1927).

[3] J. FORSSMAN, Acta path. microbiol. Scand. *23*, 45 (1946).

[4] H. H. BAILEY, Amer. J. Hyg. *8*, 398 (1928).