Summary

Myosin α is insoluble in KAc or NaAc 0·5 m at neutral p_H values and can be easily separated from myosin β under these conditions. The yield in myosin β is 80 to 90% of the total protein content of our solutions (WEBER-EDSALL myosin solutions prepared after 20 minutes extraction).

Experimental Investigations of the Proliferative Activity of Erythroblasts in their Different Stages of Maturation

The estimation of the proliferative activity of bone marrow cells is commonly based on the mitotic index and on FIESCHI's karyologic curve. These two elements have only a relative value. The former, in fact, is the result of two factors: the duration of the kinetic period and the duration of the medium interkinetic one. The latter does not only depend on the number of the cells entering into mitosis but also on the duration of each mitotic stage. We ought therefore to gain a more precise knowledge about the manner of reproduction of bone marrow cells; we are for instance at present unable to state whether the reproductive activity of leucæmic cells is higher than the reproductive activity of corresponding normal hæmopoietic cells.

In regard also to the erythroblasts of different stages of maturation, it is not yet established whether more basophilic or more polychromatophilic erythroblasts are reproduced. The small degree of reproductiveness of orthochromatic erythroblasts may, on the contrary, be assumed with considerable probability.

In order to reach a more exact estimation of the proliferative activity of bone marrow cells, it seemed justified to us to work out a method of investigation that is based upon the karyoclasic action of colchicine. It is known that this substance, in suitable concentrations, stops the mitotic process at the beginning of the metaphase (stathmokinesis). Thus, a progressive accumulation of mitotic divisions is formed in the examined tissue and it is to be presumed that the greater the number of these mitotic shapes is, the higher the reproduction must be. If it is so, it should be possible to estimate the proliferative activity of the tissue examined by the number of mitoses after the colchicinic check.

As to the techniques followed we refer to the publication *in extenso*. Here we shall merely mention that the observations were carried out on cultures of normal human bone marrow *in vitro*. The cultures were prepared in hanging drops with a medium composed of 1 cm^3 of human plasma, 0·4 cm^3 of chicken plasma (the plasmas were treated with Liquemine Roche), 0·4 cm^3 of embryonic extract, and 0·2 cm^3 of colchicine solution. The colchinine concentrations varied from 1:1 million to 1:40 millions. Control cultures were made in which the colchizine solution was replaced by 0·2 cm^3 of Tyrode. In the course of 36 hours, smears were made of the cultures every 2 hours. The preparations were stained with May-Grünwald-Giemsa and the following data were determined: (1) The total erythroblastic mitotic index, (2) the separate mitotic indices for basophilic, polychromatophilic and orthochromatic erythroblasts, (3) PONTONI's maturation curves.

The concentrations of 1:1 million appeared particularly suitable to the purpose, as they completely stop the evolution of all erythroblastic mitoses. With colchicine solutions in a greater dilution a certain percentage of mitoses on the contrary can evolve more or less slowly beyond the metaphase up to the accomplishment of the whole mitotic cycle.

The global mitotic index was, in the beginning, 12 to 20‰. In control cultures (without colchicine) the global mitotic coefficients are inferior to the initial values during the first hours, whilst the initial or slightly higher values are reached at about the 16th hour. In the cultures containing 1:1 million colchicine the mitotic index is lower during the first 4—6 hours. During the next hours it reaches progressively higher values, until it attains, within the 14th—20th hour, the highest values of 40—100‰. The lower values may be observed in those cultures where during the first 24 hours there is already a striking tendency of the erythroblasts to become mature. The highest values are reached in those cultures where such a phenomenon is not observed. We can therefore state that there is an antagonism between maturation and global reproductive activity.

Let us now examine erythroblasts in their single stages of maturation. As to orthochromatic erythroblasts, no mitosis is observed, except in rare cases. We can therefore state that orthochromatic erythroblasts may be reproduced as an exception; their mitotic reproduction has therefore no significant influence on the proliferative activity of the erythroblastic tissue as a whole.

With regard to the mitotic coefficient of basophilic and polychromatophilic erythroblasts, the former is generally at the beginning but slightly superior to the latter. In colchicine cultures with checking doses, the mitotic coefficient of basophilic erythroblasts within the 15th—20th hour usually shows values of which some amount to 200‰; this is observed as well when the transplanted marrow shows a striking maturative tendency as when, on the other hand, it remains indifferent. Polychromatophilic erythroblasts reach mitotic coefficients of about 45—90‰. Also in this case there is no significant difference between marrows showing a clear maturative tendency and marrows indifferent in this respect. *We can therefore state that the proliferative activity of basophilic erythroblasts is decidedly superior to that of the polychromatophilic erythroblasts: during cellular maturation the proliferative activity is then progressively prevented* (reduction of cells entering in mitosis and extension of the kinetic period). Moreover, the mitotic activity of the single reproductive cells (basophilic and polychromatophilic erythroblasts) does not show any variation in regard to the maturative activity. Therefore, if there is an antagonism between maturation and global proliferative activity, this appears only for the increased number of the erythroblasts of those stages which are unsuitable (or less suitable) for reproduction.

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Istituto di Clinica medica dell'Università di Pavia, September 15, 1947.

Zusammenfassung

Die Proliferationsintensität der Knochenmarkzellen ist noch nicht genau bekannt. Die Verfasser haben an menschlichen Knochenmarkkulturen die stathmokinetische Kolchizinmethode (DUSTIN) angewendet. Hiermit sollte ein Test für die Proliferationsaktivität der Markzellen gefunden werden.

Nach Kolchizinblockade zeigt sich ein Erythroblasten/Mitosen-Index von 40–100%. Dieser Index ist als Vergleichswert für die Erythroblasten-Proliferationsintensität in pathologischen Zuständen zu benutzen. Versuche in dieser Richtung sind im Gang.

Die basophilen Erythroblasten vermehren sich viel stärker als die polychromatophilen. (Der Mitosenindex beträgt 200% gegenüber 45–90%.) Die orthochromatischen Erythroblasten zeigen fast keine Mitosen.

Restitution of the Reduced Heat Tolerance of Thyroidectomized Animals with Thermothyrine

In previous papers[1,2] it could be shown that the heat tolerance of thyroidectomized animals—just as that of animals fed with methylthiouracil[1,3]—is reduced. The body temperature of thyroidectomized guinea pigs, when placed in a thermostat at 34–35 °C, rises more quickly and higher than that of normal controls. The quantitative measurement of the heat tolerance was carried out with the help of "time-temperature-areas", that is the area inclosed in a co-ordinate system by the hyperthermic body temperature curve and the horizontal axis measured planimetrically and expressed in mm².

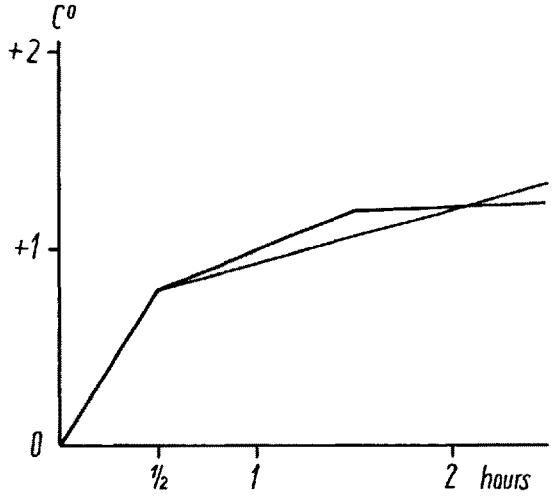

Fig. 1.

The size of this area depends on the degree of elevation of body temperature and the duration of hyperthermia, i.e. both factors characterizing heat tolerance. Time-temperature areas of thyroidectomized overheated animals are significantly greater than those of the controls, i.e. their heat tolerance is diminished. The fact that administration of thyroxine does not improve the disturbed heat tolerance in thyroidectomized guinea pigs (on the contrary: the height and duration of the rise of body temperature increase still further) suggested that lack of thermothyrine A ("cooling hormone" of the thyroid) must be the cause of this phenomenon. (Thermothyrine A, as MANSFELD[4] demonstrated, is a thyroxine-antagonistic hormone of the thyroid gland. It is poured out when danger of hyperthermia arises and is able to decrease the O_2-consumption and heat production of the organism below the level of basal metabolism.)

In the experiments reported in the following I tried to correct the failure of heat tolerance of thyroidectomized animals by administering thermothyrine. I did not use crystallized thermothyrine[1], but a fraction of the hydrolysed thyroid gland free of thyroxine but containing both thermothyrines A and B, called "thermothyrine total". (This preparation was produced and placed at my disposal by Mrs. A. MANSFELD-OPPENHEIM. I should like to express in this place also my best thanks for her kindness.) 1 cm³ of the solution corresponded to 5 g thyroid gland.

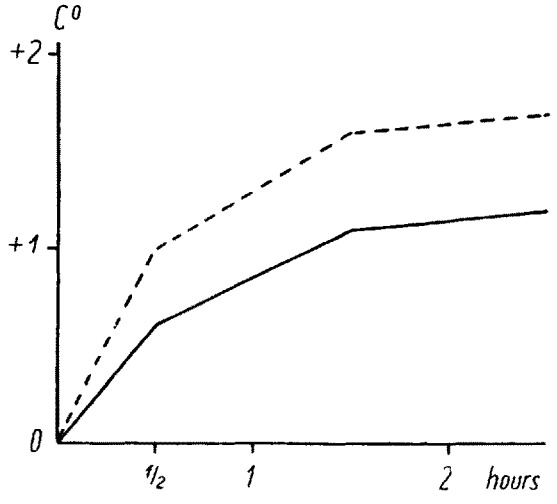

Fig. 2.

Pairs of guinea pigs (of the same sex and of equal weight) were chosen in preliminary experiments, which, when placed in a thermostat at 34–35 °C, have shown equal time-temperature areas, i.e. equal heat tolerance. (Fig. 1 shows an example of the body temperature curves and time-temperature areas of two paired guinea pigs in a two-and-a-half-hours experiment.) Afterwards, one animal of each pair was thyroidectomized. Overheating experiments 6–14 days after the operation in an environment of 34–35 °C proved that the heat tolerance of the thyroidectomized animals deteriorated. (Fig. 2 shows an example: the body temperature of the

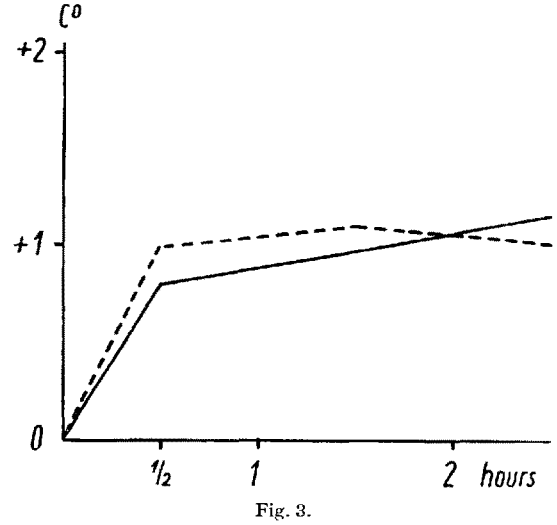

Fig. 3.

[1] B. BERDE, Exper. *2*, 498 (1946).
[2] B. BERDE, Hungarica Acta Physiologica *1*, 52 (1947).
[3] B. BERDE, Nature *159*, 748 (1947); Hungarica Acta Physiologica *1*, 62 (1947); Exper. *3*, 245 (1947).
[4] G. MANSFELD, Die Hormone der Schilddrüse und ihre Wirkungen. B. Schwabe, Basel 1943.

[1] A. MANSFELD, Nature *157*, 491 (1946); Schweiz. med. Wschr. *76*, 439 (1946).