In other words, the fraction of proliferating cells within the total cell system can be obtained by extrapolation of the linear function to the ordinate; and the cell cycle time can be estimated by extrapolation to the abscissa. Furthermore, as stated above, for $t = t_{np}$, the fraction of labelled cells is equal to 1. Thus, the intersection of the linear function with the 100% level will yield t_{np}. These properties of equation III are shown graphically in Figure 2. Since, in continuous labelling experiments, the entrance of labelled cells into the non-proliferating compartment does not start immediately at $t = 0$ as assumed above, but only after $t_{g_2}+t_m$, the linear function with the properties of equation III can be obtained by shifting all experimental data by the value of $t_{g_2}+t_m$ to the left.

For the sake of simplicity, in the above calculations variations of cell cycle parameters were disregarded. Considering such variations, equation III remains valid for the observation period $(t_c - t_s)_{max} \leq t \leq t_{np\,min}$, if t_c is replaced by the arithmetic mean of t_c. Accordingly, the intersections of the equation will then yield the mean values of the cell cycle parameters.

Experimental results. The theory outlined above was applied to mouse granulocytopoiesis. ♂ Balb/c mice (24–28 g) were injected with ^{3}H-TdR (1 µCi/g body weight) at 8-h intervals to ensure that all cells produced within the experimental period were labelled. Previous experiments had shown that significant numbers of labelled granulocytes do not appear in the circulation up to 50 h after the first injection of ^{3}H-TdR (= minimum transit time through the non-dividing marrow pool). Hence, animals were killed at 10, 20, 30, 40 and 50 h, femoral marrow was removed and processed for autoradiography. The percentage of labelled granulocytic marrow cells was scored after adequate exposure time and Giemsa staining by evaluating 500 cells irrespective of their stage of maturation.

The results obtained are shown in Figure 3. Each point on the graph represents the mean value of 6 animals. A linear increase of the percentage of labelled granulocytic marrow cells was observed between 10 and 50 h, confirming the theory developed above. Extrapolation of the experimental curve to the abscissa and to the 100% level gave values for t_c of 14 h and for t_{np} of 66 h, respectively. The relative size of the proliferating pool, obtained from the ordinate at $t = 0$, was 18%. From the initial part of a PLM-experiment, $t_{g_2}+t_m$ for marrow granulocytopoiesis was estimated to be 2 h. In correcting for $t_{g_2}+t_m$ in the way described above, the final estimates were $t_c = 16$ h, $t_{np} = 64$ h and relative size of proliferating cell pool = 20% (dotted line, Figure 3).

Discussion. The method introduced here for determination of various kinetic parameters of proliferating cell systems is generally applicable under steady state conditions as long as the minimum transit time through the non-proliferating cell compartment considerably exceeds the value of $t_c - t_s$. Compared to the PLM-method, this alternative approach shows a number of advantages. Because of the linear relationship described by equation III, only 2 samples taken from the cell system under investigation during continuous availability of ^{3}H-TdR are needed to obtain estimates for the cycle time, the proliferating fraction and the transit time through the non-proliferating pool. If, in addition, the percentage of labelled cells 1 h after injection of ^{3}H-TdR is determined (flash labelling index), calculation of DNA synthesis time is also possible. Thus, 3 biopsies may give as much information as a complete PLM experiment requiring between 10 and 20 tissue samples. Moreover, in some sit uations, where PLM experiments fail to give clearcut results, the continuous labelling method remains applicable. Whenever second waves of labelled mitoses are not observed after flash labelling, it seems worthwile to investigate the cell system in question under continuous availability of ^{3}H-TdR in order to find out whether a linear increase of the percentage of labelled cells can be demonstrated, which allows application of the theory developed above. Early reutilization of labelled DNA, a process which may lead to a failure of PLM experiments, does not interfere with the continuous labelling method. Thus, in mouse granulocytopoiesis, a plateau curve of labelled mitoses at 100% was observed after a single injection of ^{3}H-TdR due to tracer reutilization, and information about cell cycle parameters was restricted to $t_{g_2}+t_m$ in that experiment[4]. By a continuous labelling experiment, however, it was possible to obtain information about the cell cycle time of the proliferating cells and the proliferating cell fraction in this renewal system, as demonstrated in the experimental part of this communication.

[4] D. GERECKE, unpublished observation.

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