strate ^{125}I-PBI plasma activity in greater detail for the 2-h sampling periods for each patient, the second day samples from patient A and the first day samples from patient B; and the data points have been connected by smooth curves. The 4-h sampling period data, for patient A only, has similarly been recorded in Figure 3. Radioactivity of the 4-h samples taken on the third day in patient B was insufficient for a reliable representation of the curve.

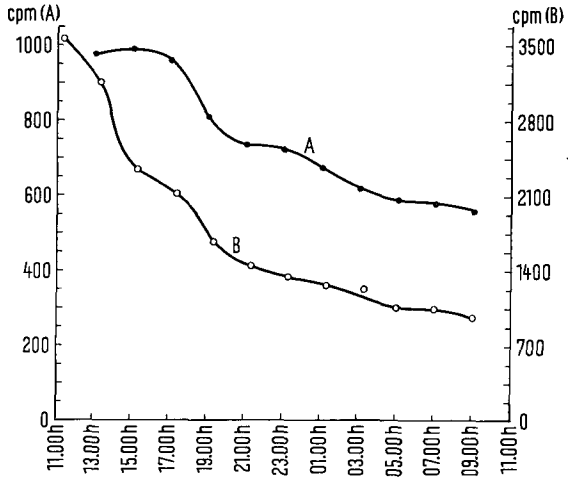

Fig. 2. ^{125}I-PBI activity as in Figure 1, but magnified and abbreviated to illustrate only the 2-h sampling period data in patients A and B.

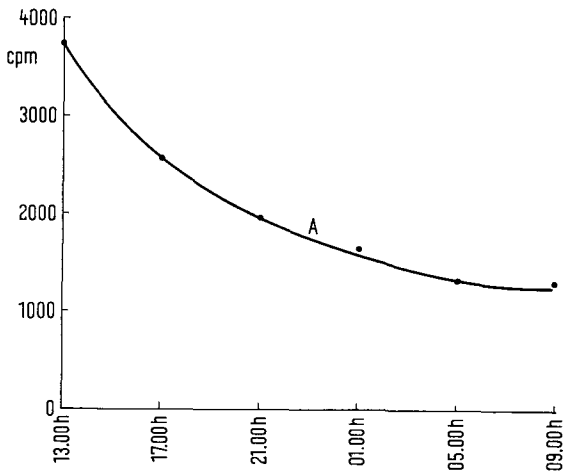

Fig. 3. ^{125}I-PBI activity as in Figure 1, but for only the 4-h sampling period data in patient A.

Results. The overall character of curves A and B in Figure 1 is grossly exponential, as expected. The important result is illustrated in the amplified curves in Figures 2 and 3. Both curves A and B in Figure 2 exhibit relatively smooth and well-defined oscillatory variations of several percent about an approximately exponential decay function, while the curve A in Figure 3 shows no significant variations. This difference is further emphasized by comparing curves A only in Figures 2 and 3, since they represent data taken from the same subject. The data suggests that the pronounced variations exhibited on the second day of sampling (every 2 h) in subject A were unobservable on the first day because the 4-h sampling period was too great.

Conclusions. Oscillations in the rate of disappearance of labeled-T3 have been observed in this experiment. A 4-h sampling period is insufficient to demonstrate this behavior; and sampling every 2 h appears to be an approximate upper limit on the sampling period. A more detailed knowledge of the character of these oscillations may be obtained by sampling more often than every 2 h during time intervals when the variations are largest. Curve A in Figure 2 suggests that sampling every hour during the mid-day would be desirable.

The results about T3 reported here are not necessarily incompatible with reports that thyroxine (T4) concentration may not be diurnal, as discussed in the introduction. Although T3 and T4 are almost the same species, to within a single atom, their dynamics of distribution, binding and metabolism are sufficiently different[6] for one to be observedly diurnal and the other not. Another possibility is that diurnal variations may exist in T4 disappearance which are too small to measure by currently available techniques.

Résumé. Des oscillations dans la réduction du taux de triiodothyronine (marquée ^{125}I) ont été observées dans le plasma humain. On a trouvé qu'une période d'échantillonage de 4 h était insuffisante pour révéler ces oscillations. Une période de 2 h constituerait une limite supérieure adéquate.

J. J. DiStefano, G. Scuncio[7] and I. Baschieri[7]

Biocybernetics Research Laboratory, School of Engineering and Applied Science, University of California, Los Angeles (California 90024, USA), 4 July 1969.

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The Generation Time of Human White Blood Cells taken from a Mother and her Daughter

As described by Painter and Drew[1] the duration of S, G_2 plus mitosis, G_1 and of the generation time (T) can be estimated by plotting percentage of labelled metaphase cells between ^{3}H-thymidine pulse labelling and harvest.

In the experiments presented, leukocyte samples from blood of a 44-year old female donor and her 8-year old daughter were cultured as described by Herzog and Steffenson[2] and labelled as described by German[3]. The results are presented as mitotic index curves illustrated in

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Figure 1a. These data are based on about 1000 mitotic figures for each donor. Mitotic figures were considered labelled if they contained more than 5 grains[4].

The labelled mitotic indices for the 2 donors as presented in Figure 1a are quite similar in their shape and frequency. The generation time and other parameters of cell life cycle, as derived from the above graphs, are given in the Table. The generation times are almost identical in the 2 individuals. The only marked difference is a longer G_2 period found in the adult donor. The increase, however, in the G_2 period was compensated by a decrease in S. Because of the uncertainties involved in culturing, these differences are probably not inherent in the cells themselves. In general then the results seem to indicate that age of the individual has little if any effect on the duration of the cell cycle of leukocytes grown in vitro.

Results by KIKUCHI and SANDBERG[5] show that, using the same method of estimation, the duration of S is in the range of 16–20 h and the duration of G_2 is about 3 h. These estimates were based on growing leukocytes taken from 3 normal males and 3 normal females, their ages ranging from 20–45 years. GERMAN[3] used a slightly different method of culturing. The estimates of S and G_2 based on the mitotic index from his data were 21 h and 5 h respectively. These latter estimates are close to our results described above, which seems to indicate that duration of S does not vary significantly from one individual to another.

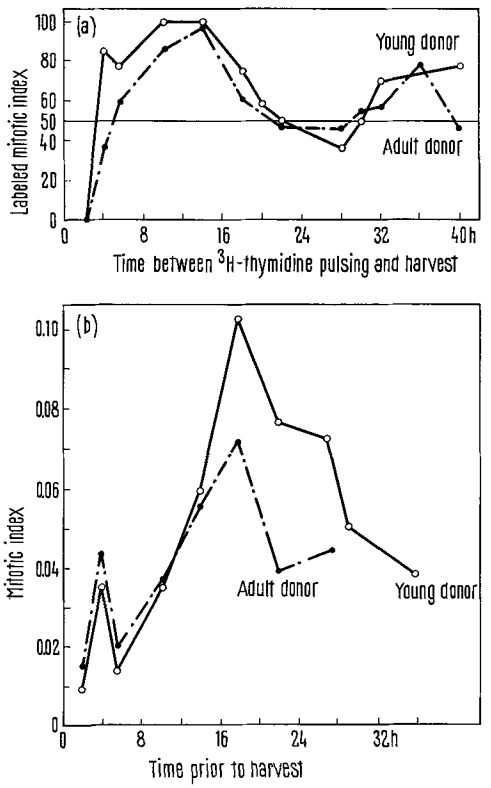

Fig. 1. (a) The labelled mitotic index as a function of the time between ^{3}H-thymidine pulsing and harvest. (b) Mitotic indices with respect to the times at which the medium was replaced.

The duration of the cell life cycle phases

Cell life cycle phase / Donor	G_2 + prophase	S	G_1 + metaphase	T
Adult	5	16	5	26
Young	3	19	5	27

The duration of the generation time (T) was estimated on different levels of Figure 1a.

The duration of mitosis was also estimated and compared to the results given in the literature. The method used was based on the mathematical treatment for exponentially multiplying cultures which are partially synchronized, as given by STANNERS and TILL[4]. They derived the following formula:

$$M = \frac{1}{1\,n\,2} \int_0^T M(t)\,\mathrm{d}t$$

where: M, duration of mitosis; T, generation time; $M(t)$, mitotic index at time t.

Computations for each donor using the T value found above and mitotic index curves shown in Figure 1b provided the following estimates: M (young donor) = 2.2 h, M (adult donor) = 1.5 h. These values are comparable to the results obtained by BOSE et al.[6], where the duration of M for human lipogenic sarcoma cell line was between 1–2 h.

In the course of the present experiments it was found that by replacing the media 18 h prior to the harvest, the mitotic index was significantly higher than in cultures which had the medium changed at other times. This observation is clearly indicated by the shape of the curves in Figure 1b. All cultures were harvested at the same time, thus precluding a pre-existing state for synchrony in the starting blood sample.

It appears that prolonged culturing of leukocytes increases the time in G_1, S, and G_2. Fresh medium causes an acceleration of these phases. The time taken in division is unaffected, thus an accumulation of mitoses is obtained by the increase of cells going into mitosis. The decrease in mitotic index after the 18th h probably results from the fact that most of the cells (70–85%) have already undergone mitosis and are in early interphase. The use of fresh media 18 h prior to harvest provides a method for accumulating cells in mitosis.

Zusammenfassung. Die Leukozyten einer 44jährigen Frau und ihrer 8jährigen Tochter wurden in vitro kultiviert und mit H^3-Thymidin markiert. Die Auswertung von je 1000 Mitosen ergab für die Zellen beider Personen eine Generationsdauer T von 26 h respektive 27 h, und eine Mitosedauer von 1,5, respektive 2,2 h.

RITA HERZOG[7]

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[7] Since a portion of this paper constituted part of my doctoral dissertation at Cornell University, I wish to express my thanks to Professor CYRIL COMAR under whom it was done. I wish also to thank Prof. D. STEFFENSON for helpful suggestions in preparing the manuscript.