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# The Intravascular Lifespan of Monocytes

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*With the technical assistance of Maureen Bell*

ALTHOUGH MONOCYTES have been observed to persist for days or weeks in tissue culture[2] and for periods up to 59 days after migration into a rabbit ear chamber,[5] no direct measurements of their lifespan in the circulation have been made. The macrophages or large mononuclear cells occurring in inflammatory lesions of the skin have recently been shown by Volkman and Gowans[12] to be derived from precursors originating in the bone marrow and spleen which are transported to the inflamed site as monocytes in the blood stream. Further experiments[11] have indicated that the monocyte lingers at the site of production for about 24 hours after the last period of DNA synthesis before emerging into the circulation, whence it quickly escapes into the inflamed tissue. These observations would set a maximum intravascular sojourn for this cell of about 5 days.

The present study was undertaken to determine the intravascular lifespan of monocytes in the intact animal. It is based on a principle first enunciated by Osgood[7] that if an animal is continuously infused with a material which will permanently lable all dividing cells, the survival of the unlabeled portion of a population of cells gives a measure of their life span. The rate of decline of the unlabeled population will be independent of the production of new cells and can be determined from the absolute number of unlabeled cells, but if the total population of the cells under observation remains constant the decline in per cent of unlabeled cells suffices to determine their disappearance rate.

## Method

### *Identification of Monocytes*

The monocyte was identified as a mononuclear cell in the peripheral blood containing finely divided peroxidase granules distributed unevenly through the cytoplasm (Fig. 1). The peroxidase staining separates monocytes from lymphocytes which are invariably peroxidase negative. To confirm this the thoracic duct was canulated in two rats by the method of Bollman et al.[3] and the effluent lymphocytes were examined after staining for peroxidase. No positive cells were noted among 4000 cells counted. The reverse proposition that all monocytes are peroxidase positive cannot be proved in the absence of any absolute criterion for recognizing all monocytes. To approach this problem differential white cell counts were performed on the same blood. stained for peroxidase and counterstained with Wright-Giemsa stain on the one hand, and stained with Wright stain only on the other. With

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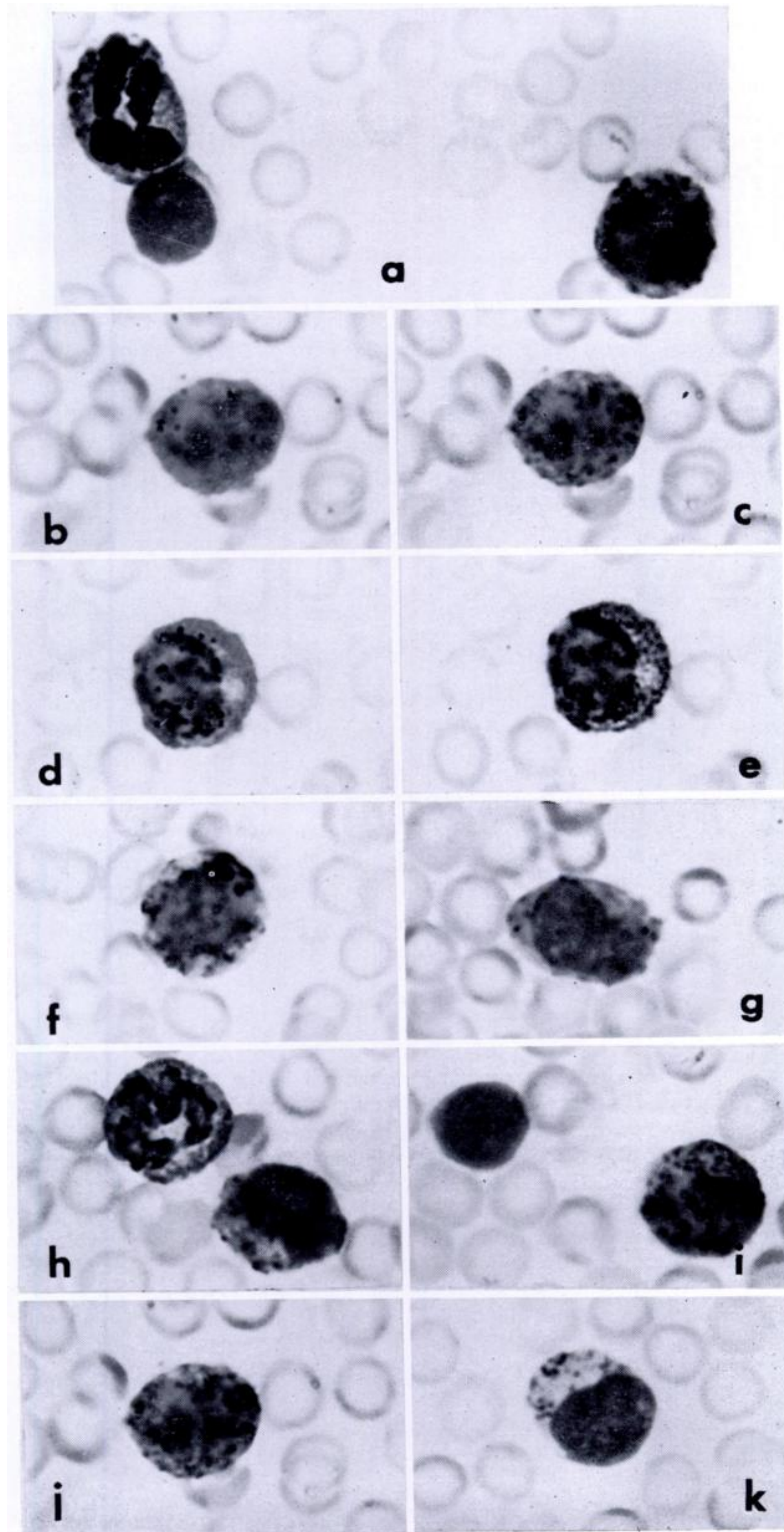


Fig. 1.—*(See figure legend on facing page)*

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human blood there was agreement within the error of sampling, whereas in the rat the former yielded a significantly higher percentage of monocytes than the latter, indicating that some rat cells which on the basis of morphology alone would be classified as lymphocytes were, in fact, monocytes.

The monocyte has a round or irregularly reniform nucleus with finely divided "lacy" chromatin. This distinguishes it from the eosinophil which, in the rat, has an annular nucleus and from the mature neutrophil which has a lobed nucleus in which the chromatin is dense and compacted into clumps. The type of peroxidase granule seen in the granulocytes is different from that of the monocyte in being larger, more uniform and more numerous. Staining is considerably denser in the eosinophils than in the neutrophils. Metamyelocytes and myelocytes are peroxidase positive mononuclear cells, but these were probably not numerous since the studies were performed on peripheral blood of uninfected animals, the peroxidase staining of myelocytes resembled that of neutrophils rather than that of monocytes, and no early granulocytes were recognized in ordinary Wright-stained preparations.

Ten female, inbred, hooded rats, weighing 162 to 195 Gm., obtained from the colony of the Cancer Research Unit, University of British Columbia,* were housed 5 to a cage and maintained on Buckerfield rat diet and water *ad libitum*. Tail blood was drawn for white blood counts and coverslip preparations.

Tritiated thymidine (obtained from the New England Nuclear Corporation), with a specific activity of 6.7 curies/mm was diluted with saline to a concentration of 40 μc. per ml. Each rat was injected intraperitoneally every 6 hours, ±15 minutes, with 1 ml. of solution for a total of 23 doses. The total dose to each rat was slightly less than 1 μc./Gm./day and the total dose over 6 days was about 6 μc./Gm. Intermittent intraperitoneal injections of tritiated thymidine will succeed in labeling all dividing cells only if the interval between injections is less than the period of DNA synthesis ("S") for the type of cell under consideration. Various mammalian cells have been examined and "S" has been found to vary between 5½ and 12 hours.[6,8] The present technic of 6-hourly injections is, therefore, likely to have labeled the majority of proliferating cells.

## *Preparation of Cells*

The blood films were dried in air. They were fixed for 1 minute in 10 per cent neutral formalin and 96 per cent ethanol (1:3) and then washed with 0.01 molar phosphate buffer at pH 7.0, dried in air and washed successively with ether and 0.01 molar phosphate buffer. They were then stained by the method of Rytomaa.[9]

Coverslips were attached to glass slides with the cell side outwards, coated with Kodak NTB 2 emulsion, placed in light-tight boxes with calcium chloride as a dehydrating agent, and stored for 28 days in a lead castle at 4 C. They were developed in Kodak D 19 developer and fixed with Kodak acid fixer. They were then stained through the emulsion with Wright-Giemsa stain.

## *Counting Procedure*

For each individual coverslip an estimate of the background was made. A cell was considered labeled if the number of grains superimposed upon it would have occurred by chance no more than once in 20 cells. In none of the preparations did the background amount to more than 1 grain per cell.

*Supplied through the courtesy of Dr. R. L. Noble.

**Fig. 1.**—*a:* Neutrophile and monocyte, peroxidase positive, and lymphocyte, peroxidase negative. *b* and *d:* Same monocytes focused to show overlying silver grains. *c* and *e:* Monocytes focused to show peroxidase granules. *f* and *g:* Monocytes. *h:* Neutrophile and monocyte. *i:* Lymphocyte and monocyte. *j* and *k:* Monocytes. Stained for peroxidase by method of Rytomaa and with Wright-Giemsa (X 1200).

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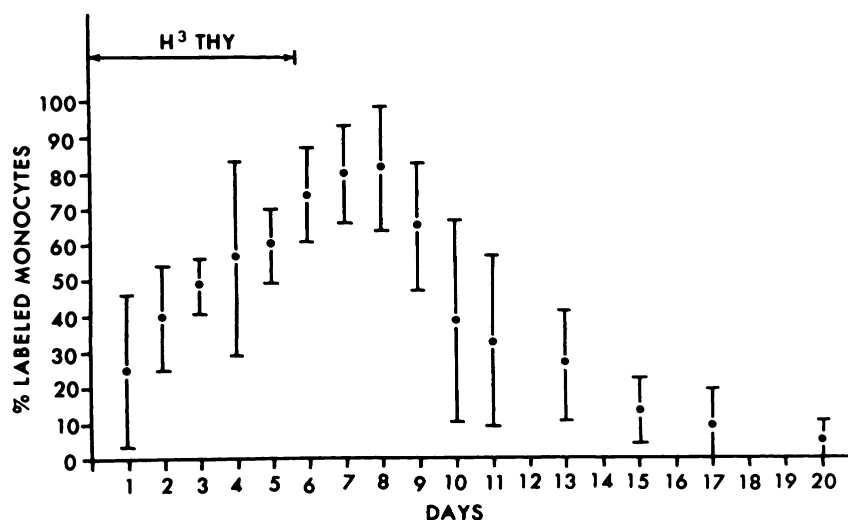


**Fig. 2.—The rise and fall of the per cent of labeled cells plotted on arithmetic coordinates.** Each vertical line represents 2 standard deviations on either side of the mean.

One hundred monocytes were identified from each of the 10 rats on each day that the rats were bled, so that 1000 monocytes were examined for each point on the curves. The grain count over each monocyte was recorded.

## Results

The white blood counts remained relatively constant during the period of the experiment, averaging between 20,000 and 25,000/cu. mm. The absolute monocyte count varied from 960/cu. mm. to 2140/cu. mm., representing, on the average, about 6.5 per cent of the total white count. There was considerable variation from rat to rat and from day to day in the same rat. This was probably due to variations in the number of cells temporarily sequestered in the small vessels. The percentage of monocytes is slightly higher than that usually recorded for rats[1] and may be due to the ability of the monocyte, as opposed to the lymphocyte, to adhere to surfaces. Although there were variations in the proportion of monocytes, there was no trend toward a lower absolute monocyte count at the end of the experiment than at the beginning. No mitotic cells were observed among 16,000 monocytes inspected.

The rise and fall in the per cent of unlabeled cells is shown in Figure 2, plotted on linear coordinates as a function of time. The vertical lines at each point indicate two standard deviations on either side of the mean. It is obvious that the observations do not show a straight line relationship between the numbers of surviving cells and time. When the decline in the per cent of unlabeled cells is plotted on semi-log paper (Fig. 3), however, the relationship up to Day 8 is evidently linear, with a coefficient of correlation of 0.985. The mean half-life of the cells in the circulation is thus seen to be 3.1 days.

Since it appears that the per cent of labeled cells reached a peak between the 7th and 8th day, the rate of disappearance of labeled cells was calculated, taking the labeled population on Day 8 as 100 per cent. It will be seen that the

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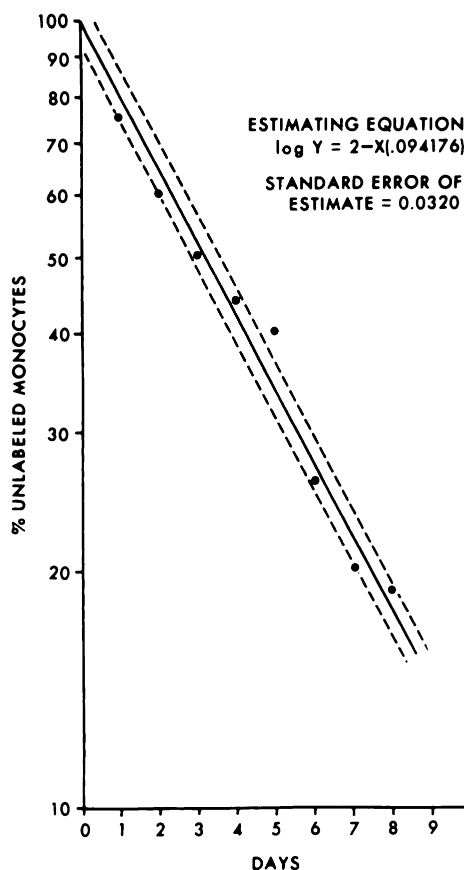


**Fig. 3.—Semilogarithmic plot of per cent unlabeled monocytes as a function of time.** Broken lines represent one standard error of estimate on either side of the line of regression.

decline in the count of labeled cells is also of exponential type with a slope almost the same as the decline in unlabeled cells already observed (Fig. 4), giving a half life of 2.8 days.

The mean grain count (Fig. 5) rose progressively for 3 days, after which it leveled off and remained constant until the 7th day and then declined slowly toward the base line. For any one day the distribution of the grain counts (Fig. 6) was consistent with a Poisson distribution.

The turnover rate of monocytes was calculated from the following equation:

$$\text{monocyte turnover rate} = \frac{\text{absolute monocyte count per cu. mm.} \times 1000 \times BV \times .693}{T\frac{1}{2}}$$

Substituting observed values for the mean absolute monocyte count and T½ and an estimated value for blood volume,[1] the turnover rate is $3.6 \times 10^6$ monocytes per day.

Lymphocytes were labeled at a much slower rate than monocytes, and after

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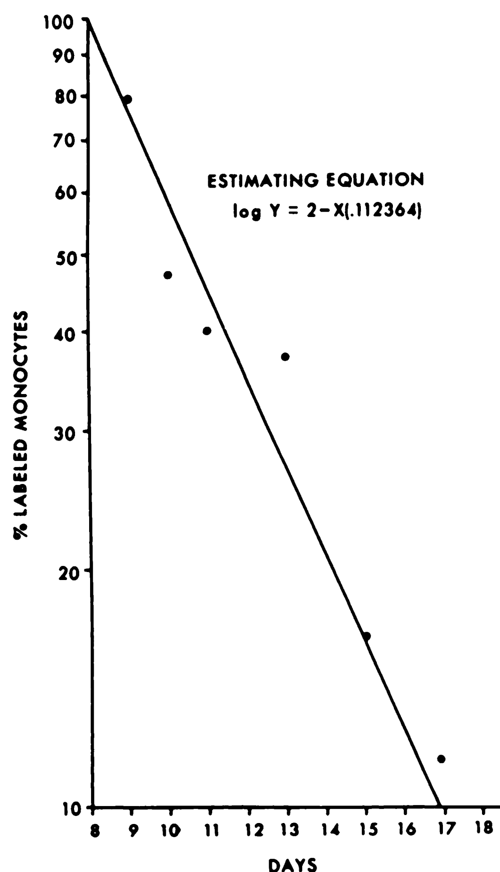


**Fig. 4.—Semilogarithic plot of per cent labeled monocytes as a function of time.** The population of labeled cells on day 8 is taken as 100 per cent.

24 hours of injections only 3.3 per cent of circulating lymphocytes were labeled.

## Discussion

The identification of monocytes and their separation from other mononuclear cells and especially from lymphocytes presents some problems. The peroxidase reaction sharply distinguishes monocytes from lymphocytes, since the latter are never positive in the circulating blood. In the absence of other absolute criteria for recognizing monocytes, it cannot categorically be stated that all monocytes show a positive reaction. It appears from comparative counts, however, that most cells which fulfill the usual morphologic criteria for monocytes are peroxidase positive. Whether the peroxidase granules are manufactured in the cell and indicate a specific genetic constitution, or are derived from granulocytic debris engulfed by the monocyte as Undritz[10] affirms and are thus an index of phagocytic ability, is uncertain but irrelevant to the present study. There is no reason to think that any considerable number of the circulating peroxidase positive mononuclear cells were myelocytes or metamyelo-

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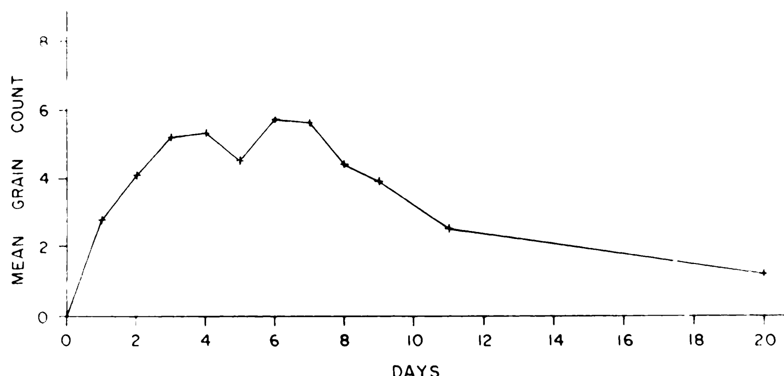


**Fig. 5.—Changes in the mean grain count above background of labeled cells with time.**

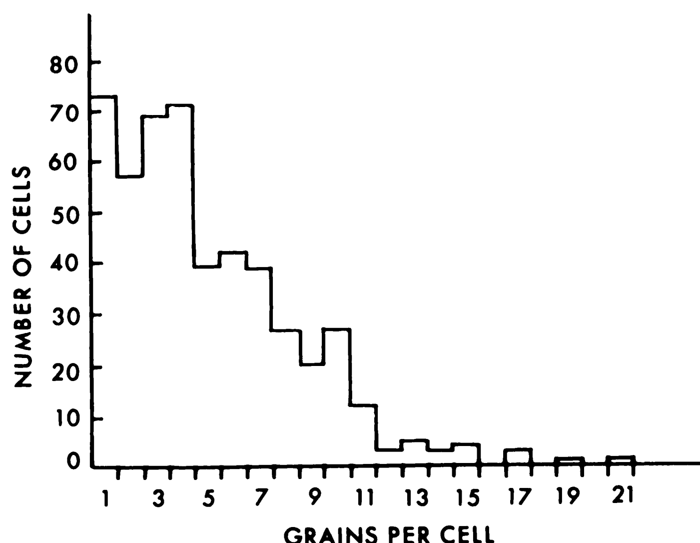


**Fig. 6.—Grain counts above background on day 3 showing a curve consistent with a Poisson distribution.**

cytes. The animals showed no evidence of infection during life or at autopsy, and the size and distribution of the granules were not characteristic of marrow myelocytes.

In interpreting this data it is necessary to assume that we are dealing with a steady-state renewal system and that labeling does not disturb this state. It will be seen that monocytes left the circulation at approximately the same rate whether labeled or unlabeled. This is interpreted to mean that the labeling process and consequent irradiation of the cell did not materially affect its longevity. It also indicates that the demand for monocytes was the same in the last half of the experiment as in the first half and shows that, in this respect, the animals can be considered as being in a steady state. This is further borne out by the relatively constant levels of monocytes and total white count during the procedure.

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The fact that 25 per cent of circulating monocytes were labeled within 1 day of beginning injections of tritiated thymidine shows that the monocyte precursors are actively proliferating cells, having manufactured DNA at some time in the preceding 24 hours. Since in the same interval only 3.3 per cent of the lymphocytes were labeled, it is unlikely that they are the immediate precursors of the monocyte, although these data do not eliminate this possibility since it has been demonstrated that the lymphocytic population is not homogeneous[4] and a small group of cells morphologically identical with lymphocytes could be turning over more rapidly than the general lymphocyte population and constitute a precursor pool for monocytes. The work of Volkman and Gowans,[12] which indicates that the monocytes are produced only in the bone marrow and spleen, supports the view that they are not derived from lymph node lymphocytes, although it does not eliminate the possibility that they are descended from bone marrow lymphocytes which may constitute a distinct population.

If monocytes had a finite lifespan and left the circulation as a consequence of senescence, the decline in the population of unlabeled cells should be a linear function of time. It is not possible to fit a linear curve to the data. The curve of best fit is an exponential where the log of the unlabeled monocyte count is a negative function of time. This shows that the sojourn of the monocyte in the circulating blood is a matter of chance and that monocytes are transformed or destroyed or leave the circulation at random.

The figure of 3.1 days for the lifespan of circulating monocytes may be compared to the observations of Volkman and Gowans,[11] who found that 18 to 21 hours after the intravenous administration of tritiated thymidine 60 per cent of the macrophages in an inflammatory lesion were labeled. Five days after tritiated thymidine only 2 per cent were labeled. Assuming that the labeled cells disappeared exponentially from the circulation, this suggests a half-life of about 1 day. Although this is substantially less than our figure, the methods are not quite comparable and the differences may not be significant.

Where a population of continuously dividing cells is exposed to label at regular intervals smaller than the interval of DNA synthesis ("S"), the mean grain count will rise asymptotically with succeeding generations. If the number of counts incorporated with each "S" is 2A, each daughter cell will receive A counts. As the number of divisions increases, the number of counts on each cell will approach 2A as a limit. These relations are expressed in the equation:

$$y = 2A\left(1 - 2^{\frac{-t}{T}}\right)$$

where y = mean grain count
2A = number of counts taken up in each "S"
t = time after the beginning of labeling
T = generation time

It follows from the equation that $y$ will equal $A$ only when $\frac{t}{T} = 1$—that is, when $t$ = the generation time.

The rise in the mean grain count observed in this experiment corresponds

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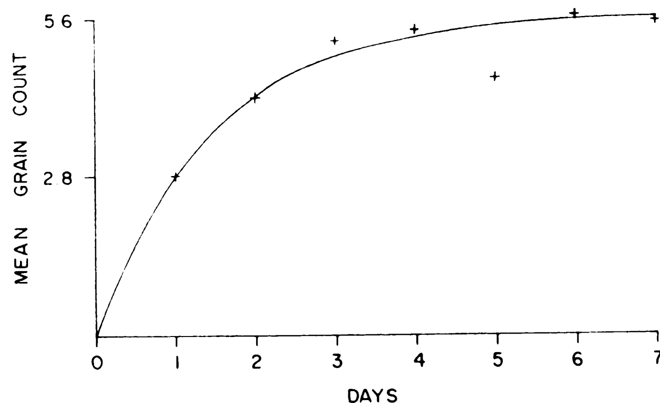


**Fig. 7.—The rise in mean grain count above background observed over the first 7 days (+) compared with the expected curve (solid line).**

closely to the expected curve (Fig. 7). The plateau is reached at 5.6 grains per cell and this value, therefore, corresponds to 2A. The value of A becomes 2.8 grains per cell, the level reached in 24 hours. We conclude that the generation time of the monocyte precursors averages about 24 hours. This being so, there would appear to be at least 3, and possibly 4, generations interposed between the earliest precursor and the circulating mature monocyte.

The proportion of labeled cells rose slightly for 3 days after the cessation of labeling, probably indicating that the precursor pool was fully labeled. If this were the only reason for the continued rise, however, the mean grain count should have begun to fall the day after cessation of labeling, whereas it continued at its plateau level for 2 days. This may mean the existence of a small storage pool where mature monocytes are temporarily sequestrated.

## Summary

Monocytes, defined as peroxidase positive mononuclear cells in the peripheral blood of healthy rats, were labeled by frequent intermittent injections of tritiated thymidine. About 25 per cent of the monocytes were labeled within 1 day and 82 per cent in 8 days. Both labeled and unlabeled monocytes disappeared from the circulation in accordance with an exponential function with a half-time of about 3 days. Mean grain counts increased asymptotically toward a limit reached in 4 or 5 days. The monocyte turnover rate in the rat is in the neighbourhood of $3.6 \times 10^6$ cells per day.

It is concluded that monocytes leave the circulation at random and not as a consequence of senescence. It is probable that they are the product of a cell lineage consisting of about 3 generations from the primitive precursor to the mature form, and that the average generation time is about 24 hours. Because of the rapid appearance of large numbers of labeled cells, it is unlikely that they are derived from lymphocytes which acquire label much more slowly.

## Summario in Interlingua

Monocytos—definite como cellulas mononucleari positive pro peroxidase in le sanguine peripheric de rattos normal—esseva marcate per frequente

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injectiones intermittente de thymidina a tritium. Circa 25 pro cento del monocytos esseva marcate intra 1 die e 82 pro cento intra 8 dies. Le monocytos tanto marcate como etiam nonmarcate dispareva ab le circulation secundo un function exponential con un periodo de medie valor de circa 3 dies. Le numerationes granular medie montava asymptoticamente verso un limite attingite intra 4 o 5 dies. Le transition de monocytos in le ratton affice circa $3{,}6 \times 10^6$ cellulas per die.

Es concludite que monocytos quita le circulation aleatorimente e non in consequentia de lor senescentia. Il es probabile que illos es le producto de un lineage cellular consistente de circa 3 generationes inter le precursor primitive e le forma matur e que le tempore medie de generation amonta a circa 24 horas. Viste le rapide apparition de grande numeros de cellulas marcate, il non es probabile que illos es derivate ab lymphocytos le quales acquire le marca multo plus lentemente.

## ACKNOWLEDGMENTS

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