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Cell population kinetics of the germinal centres of lymph nodes of BALB/c mice

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INTRODUCTION

There have been many studies of the kinetics of the lymphocyte populations in various haemopoietic organs. The fraction of labelled mitoses (FLM) technique has been used on the germinal centres of the spleen (Fliedner, Kesse, Cronkite & Robertson, 1964) and the lymphoid cells of the small intestines (Glaister, 1973). No cell cycle time (t_c) value could be derived directly from such studies since no second peak was obtained, and the generation time was therefore estimated from the grain count diminution rate of the germinal centre cells.

In an addition or as an alternative to FLM technique, some workers have used stathmokinetic methods (Osogoe & Awaya, 1958; Kawamura, 1960). On the basis of mitotic rates Kawamura distinguished four different areas in the lymph node and showed local variations in the turnover times.

This present study describes an attempt to estimate the duration of the cell cycle parameters in the germinal centres of mesenteric, axillary and inguinal lymph nodes using a Gilbert program for the analysis of FLM curves (Gilbert, 1972). An attempt has been made to obtain more precise stathmokinetic results by employing vincristine sulphate rather than the less effective agents colchicine or colcemid for arresting cells in metaphase.

MATERIALS AND METHODS

Female BALB/c mice aged between 12 and 14 weeks were used in these experiments. In all experiments it was necessary to exclude, after histological examination, lymph nodes which did not exhibit germinal centres.

Experiment 1: 35 mice were flash labelled by intraperitoneal injection of 1 μCi/g body weight of ^{3}H-TdR (specific activity 5 Ci/mmol, Radiochemical Centre, Amersham). Animals were killed at predetermined intervals until 20 hours after ^{3}H-TdR administration.

Experiment 2: Five animals were pulse labelled with ^{3}H-TdR in a dose of 1 μCi/g body weight given intraperitoneally and killed after 1 hour to measure the labelling and mitotic indices. In both experiments mesenteric, axillary and inguinal lymph nodes were removed and fixed in Carnoy's fluid for at least 6 hours. They were then placed in Cellosolve (2-ethoxy-ethanol) solution for about 2 weeks and then processed for autoradiography (Zaitoun, Lauder & Aherne, 1979). Individual readings for FLM curves were obtained by counting at least 100 metaphases and anaphases at each time period. The FLM curves were fitted by the Gilbert (1972) program using the University of Newcastle upon Tyne IBM 360/67 computer. The labelling and mitotic indices were determined from Experiment 2 by counting at least 2000 nuclei for each reading.

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Experiment 3: Ten animals were given 1 mg/kg body weight of vincristine sulphate (Oncovin, Eli Lilly and Co. Ltd., Basingstoke) by intraperitoneal injection. They were then killed at 15 minutes intervals after the injection and the lymph nodes were dissected out and fixed in formalin. Sections were cut and stained with haematoxylin. The mitotic index was measured in the germinal centres by counting at least 3000 nuclei for each reading. The mitotic index was then plotted against time after vincristine injection, and the line was fitted by linear regression analysis.

ABBREVIATIONS USED

To facilitate the reading of this article a list of the abbreviations used in the text, graphs and tables follows:

μCi	micro-curie	K_B	birth rate
FLM	fraction of labelled mitoses	r^2	linear correlation coefficient
G_1	period of post-mitotic (pre-synthetic) rest	t_C	cell cycle time
		$t_{C(a)}$	apparent cell cycle time
^{3}H-TdR	tritiated thymidine	t_{G_1}	duration of G_1 period
I_m	mitotic index	t_{G_2}	duration of G_2 period
I_P	growth fraction	t_S	duration of the DNA synthetic period
I_S	pulse (1 hour) labelling index		

RESULTS

FLM data

The FLM curves for the germinal centre of mesenteric, axillary and inguinal lymph nodes are shown in Figure 1. In each curve there was a well defined first peak. Although the computer fitted line does not show a clearly defined second peak in any of the FLM curves the points do start to rise again after about 8 hours. The maximal level is then reached at or just before 15 hours, and there is then a flattening off of the curve. The failure to obtain a clearly defined second peak is probably related to great variation in the G_1 phase in individual cells so that randomisation throughout the phases of the cell cycle occurs very quickly. It can be seen also that the line produced by the computer follows the data point very well with the exception of the tops of the first peaks where the data points are slightly lower than the computer peaks. The mean values for phase durations with their standard errors are given in Table 1. The lymph node germinal centre t_C values were found to be very similar in the three anatomical sites examined. The values for t_S, t_{G_1} and t_{G_2} (the durations of the synthetic, G_1 and G_2 periods respectively) were also similar.

Stathmokinetic data

The stathmokinetic data for the germinal centres are shown in Figure 2. A good degree of linearity was obtained with linear correlation coefficient (r^2) values above 0·94 to a maximum of 0·96 for each line. The slope of the line will be closer to the real value of rate of entry into mitosis. The stathmokinetic data are summarized in Table 2. From the slope of the line a direct reading for the birth rate (K_B) was obtained. It has been assumed that the population of the germinal centres of the lymph node are in a steady state condition (Hanna, 1964). On this assumption the apparent cell cycle time $t_{C(a)}$ is equal to the reciprocal of K_B and so $t_{C(a)}$ was found to be

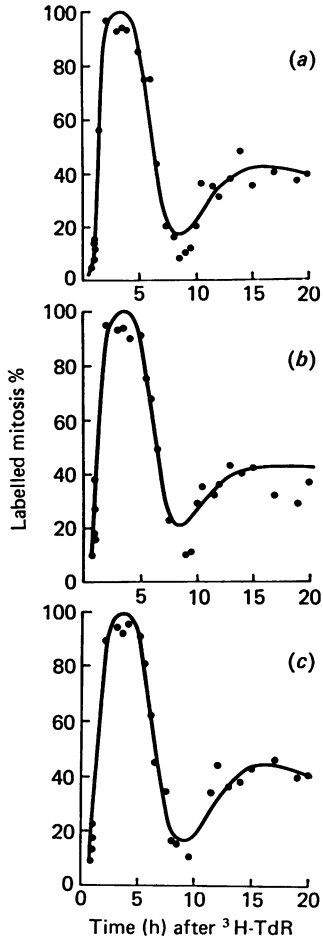

Fig. 1. The FLM curves for the germinal centres of mesenteric (*a*), axillary (*b*) and inguinal (*c*) lymph nodes of BALB/c mice. The lines were fitted by the Gilbert program.

Table 1. *Cell-cycle phase durations as measured by FLM technique in the germinal centres of lymph nodes of BALB/c mice* (*Duration in hours* ± S.E.)

	t_C	t_{G_1}	t_S	t_{G_2}
Mesenteric lymph node	12·28±0·24	6·05±0·24	4·79±0·06	1·45±0·03
Axillary lymph node	12·12±0·26	5·90±0·25	4·86±0·08	1·36±0·03
Inguinal lymph node	12·63±0·16	6·14±0·16	4·98±0·04	1·51±0·02

17·45 hours, 16·65 hours and 15·28 hours for the germinal centres of the mesenteric, axillary and inguinal lymph nodes respectively.

Labelling and mitotic indices (I_s *and* I_m)

Table 3 shows the mean values of I_s (1 hour pulse labelling index) and I_m (for the mitotic index) for groups of four animals. Only small differences between the three anatomical sites are apparent.

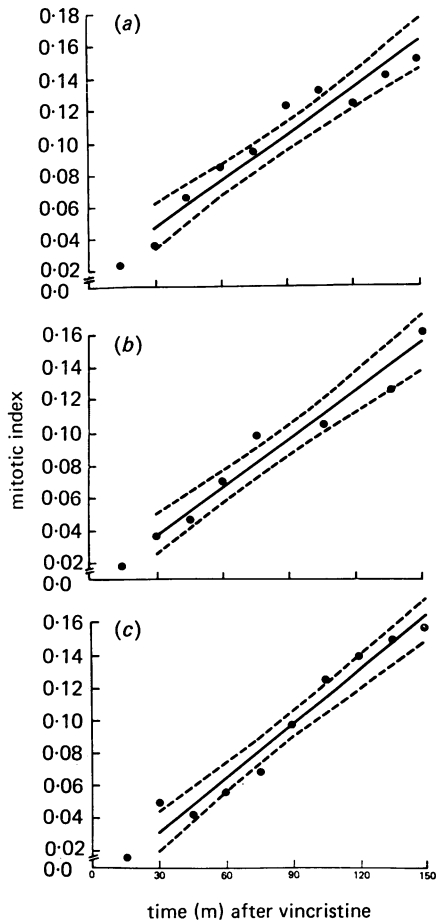

Fig. 2. Stathmokinetic experiment showing the mitotic indices of the germinal centres of mesenteric (*a*), axillary (*b*) and inguinal (*c*) lymph nodes of BALB/c mice. The dotted lines indicate the 95 % confidence limits.

Table 2. *Stathmokinetic data and coefficient of determination for the germinal centres of lymph nodes of BALB/c mice*

	r^2	Rate of entry into mitosis K_B (cells/ 1000 cells/hour)	Apparent cell cycle time $t_{C(a)}$ (hour)
Mesenteric lymph node	0·94	57	17·45
Axillary lymph node	0·96	60	16·65
Inguinal lymph node	0·96	65	15·28

Table 3. *Mitotic and labelling indices, growth fraction, cell birth rate and apparent cell cycle time in the germinal centres of lymph nodes of BALB/c mice*

	I_m	I_S	I_P	K_B (cells/1000 cells/hour)	$t_{C(a)}$ (hour)
Mesenteric lymph node	0·0164±0·0012	0·2318±0·0119	0·59	48	20·79
Axillary lymph node	0·0170±0·0014	0·2554±0·0134	0·64	53	18·94
Inguinal lymph node	0·0166±0·0009	0·2665±0·0155	0·68	54	18·59

Growth fraction (I_P)

I_P was obtained from the equation

$$I_P = \frac{I_{s\ \text{experimental}}}{I_{s\ \text{theoretical}}},$$

where $I_{s\ \text{theoretical}} = t_S/t_C$.

The values are shown in Table 3, and once again there was little difference between the three anatomical sites. In general the I_P values in all the germinal centres of the lymph nodes were high.

The cell birth rate, K_B and apparent cell cycle time $t_{C(a)}$ deduced from labelling data

The cell birth rate (K_B) values were obtained using the ratio I_P/t_C. The calculation was based on labelling data (probably the most reliable) (Table 3). It can be seen that once again the values are similar in the three anatomical sites and are in good agreement with those obtained from the stathmokinetic data (Table 2).

The $t_{C(a)}$ values obtained from the ratio $1/K_B$ are also shown in Table 3. The figures for all three lymph node sites are similar, and there is also reasonably good agreement with the $t_{C(a)}$ values derived from the stathmokinetic experiment.

DISCUSSION

Like Fliedner *et al.* (1964) and Glaister (1973) the present author was unable to demonstrate any clear second peak in the FLM experiments.

The advantages of using the Gilbert program and computer analysis are that one can obtain values for the durations of the various cell cycle phases as well as some indication of the errors involved. The values for t_C were similar in all three anatomical sites, namely between 12 and 13 hours, and this is in good agreement with Glaister (1973) who estimated t_C at 10–14 hours, and Fliedner *et al.* (1964) whose value was 13·5 hours. Both these previous estimates of t_C were obtained by the grain count diminution method. Our t_S value was about 5 hours, and this is also in good agreement with Fliedner *et al.* (1964) who obtained a value of 4·5 hours. Some other workers found a longer time of 8–9 hours (Glaister, 1973), while others thought it might be as short as 3·5 hours (Cottier *et al.* 1971). The t_{G_2} value was very short, again in agreement with Fliedner *et al.* (1964). The value for t_{G_1} may appear rather long when compared with those for t_S and t_{G_2}, but it is in good agreement with Fliedner *et al.* who estimated 6·4 hours. Difficulties can arise in estimating t_{G_1} because of (*a*) very weak labelling of germinal centre cells; (*b*) migration of lymphocytes from the germinal centres and additions to them from

the bone marrow and thymus (Everett & Tyler, 1970); (*c*) differences in the labelled mitosis of large, medium and small lymphocytes (Fliedner *et al.* 1964); (*d*) and finally and most serious the heterogeneity of the germinal centre populations especially in relation to cell cycle time parameters. One or more of these factors could account for over-estimation of t_{G_1}. Indeed, all of our values and those of previous studies should be assessed in the knowledge that it is impossible to quantify the effects of such variables as cell migration.

It is apparent from $t_{C(a)}$, K_B and r^2 values that the stathmokinetic data were satisfactory. The $t_{C(a)}$ and K_B values for the germinal centres of the mesenteric, inguinal and axillary lymph nodes were similar, and comparable with the data provided by ^{3}H-TdR techniques. In relation to previously published stathmokinetic data on lymph nodes (Ito, 1959; Osogoe & Awaya, 1958; Kawamura, 1960) the present data compare very well. The present study may indeed be the most reliable because more readings were undertaken than in the studies cited. Most workers have regarded the lymphatic tissue in a particular lymphatic organ as homogeneous: Kawamura, however, showed variations between different areas in a lymph node in respect of the mitotic index and the turnover time. Unlike the present author, Kawamura was unable to estimate $t_{C(a)}$ and K_B. In addition, all previous workers used colchicine as their stathmokinetic agent, but in the present work vincristine was used because Dyson (1973), in his studies on the alveolar wall and jejunal crypt cells in A/jax mice, found that vincristine and vinblastine were far more efficient than colcemid (a derivative of colchicine) in that with the former drugs metaphase arrest began at once, lasted longer and the metaphase accumulation curves were more linear. The present study has indicated good correlation between the stathmokinetic data and the data obtained by using the much more arduous FLM technique.

The Gilbert program has enabled the author to conclude that there is very little difference between the cell cycle characteristics of lymphoid cells in the germinal centres of lymph nodes in three widely dispersed anatomical sites. In all the sites studied the cells were proliferating rapidly with a short cell cycle time, high labelling and mitotic indices and a relatively high growth fraction. In the past the similarity of the cell kinetics in different lymph glands has usually been assumed: the present results indicate that this assumption is justified.

SUMMARY

The cell population kinetics of the germinal centres of mesenteric, axillary and inguinal lymph nodes have been studied using ^{3}H-TdR and stathmokinetic techniques. The FLM computed by the Gilbert program showed an ill-defined second peak. The durations of the cell cycle phases were similar in the three anatomical sites. The stathmokinetic data showed only slight variation in the K_B and $t_{C(a)}$ values between the different groups of lymph nodes. The I_m, I_S and I_P values indicated that the number of proliferating cells of the germinal centre was very high. When the results obtained with ^{3}H-TdR were compared with those obtained stathmokinetically, good agreement was generally found.

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