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Circadian Rhythms in DNA Synthesis and Mitosis in Normal Mice and in Mice Bearing the Lewis Lung Carcinoma*

E. ROBERT BURNS, LAWRENCE E. SCHEVING and TIEN-HU TSAI

Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72201, *U.S.A.*

Abstract—*Circadian rhythms in the incorporation of* ^{3}H-*Tdr into chemically isolated DNA (DNA specific activity or DNA-SA) from spleen, thymus, duodenum, bone marrow and liver were altered by the presence of the Lewis Lung Carcinoma (LLC) in* BDF_1 *mice standardized to a* 12:12 *light–dark cycle (light* 0600–1800, *CST). In general, the longer the LLC was present in the host the more pronounced the changes were in the normal circadian pattern of DNA-SA in the host's organs. Different ages* (6, 10 *or* 14-day *old tumors) of LLC caused different changes in* (1) *phasing,* (2) *waveform and* (3) *over-all level of DNA-SA in all organs examined. The circadian rhythm in the mitotic index in the corneal epithelium, however, remained unperturbed by the presence of a* 6-, 10- *or* 14-day-*old LLC. The LLC itself exhibited significant high-frequency fluctuation in the amount of DNA-SA during the circadian period.*

INTRODUCTION

CHRONOBIOLOGICAL evaluation of DNA synthesis and mitosis has revealed the existence of biological rhythms in these and many other physiological variables [1]. Knowledge and use of biological rhythmicity have provided a better understanding of many biological phenomena and have significantly increased the results obtained in many therapeutic situations [2–5]. Chronobiological investigation of DNA synthesis and mitosis in the tumor-bearing host has provided a better understanding of the effects the tumor (Ehrlich Ascites Carcinoma) has on the host [6–8]. The study reported here continues our chronobiological investigation of DNA synthesis and mitosis in normal and tumor bearing mice. In this investigation, rhythms in DNA-SA and/or mitosis were studied in control mice and in mice bearing 6-, 10- and 14-day-old subcutaneous Lewis Lung Carcinomas (LLC) as well as in the LLC itself.

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MATERIALS AND METHODS

Male BDF_1 mice were used in this experiment. The mice were kept on a light–dark cycle (light from 0600 to 1800 CST) for 2 weeks prior to and throughout the experiment. Food and water were available *ad libitum*. The mice were 8 weeks old at the beginning of the experiment. Four separate groups of 80 mice each were utilized. One group served as non-tumor-bearing controls. The second group (LLC-6) was bearing a 6-day-old subcutaneous implant of Lewis Lung Carcinoma at the time of sacrifice. The third (LLC-10) and fourth (LLC-14) groups of 80 mice each were bearing 10-day-old and 14-day-old implants of LLC, respectively, at the time of sacrifice.

The LLC used in this experiment was obtained originally from Dr. W. D. De Wys of Northwestern University and has been transplanted by trocar in our laboratory at 2-week intervals. To inoculate the 3 different groups of mice with a known number of LLC cells, the following procedure was used: large subcutaneous LLC tumors were minced and then mildly homogenized in a motor driven, glass-teflon, loose-fitting homogenizer in Minimal Essential Medium—Earle's base (BBL, Cockeysville, MD) buffered to pH 7.2

with $NaHCO_3$. The homogenate was strained through a stainless-steel wire mesh screen and a cell count (viable cells only) was made using a hemocytometer and a solution of 0.5% eosin in saline. The counted cell suspension was kept in a stoppered flask in crushed ice during the injection procedure. The different groups of mice were injected subcutaneously between the scapulae with an LLC cell suspension between 1130 and 1215 CST: the LLC-14 mice received 3.2×10^6 LLC cells 14 days prior to the day of sacrifice; the LLC-10 group received 3.5×10^6 LLC cells 10 days prior to their death; and the LLC-6 group received 3.1×10^6 LLC cells 6 days prior to death. Thus, each group received approximately the same number of LLC cells.

On the day of sacrifice, the mice in each group were subdivided into smaller groups of 7–9 mice each; and one subgroup was killed at each of the following time points: 0800a (i.e., 0800 on the first day), 1100, 1400, 1700, 2000, 2300, 0200, 0500 and 0800b (0800 on the second day). One-half hour prior to being killed, each mouse received an intraperitoneal injection of 10 μCi of tritiated thymidine (H^3-TDR; specific activity of 15.2 Ci/M mole; Schwarz/Mann) in 0.1 ml saline. The mice were killed by cervical dislocation, and the abdominal and thoracic cavities were opened and the entire mouse was fixed in 10% phosphate-buffered formalin.

Organs were dissected out of the formalin fixed mice, the DNA was chemically isolated, and the amount of ^{3}H-TdR incorporated into DNA was measured via liquid scintillation counting and expressed as CPM/μg DNA (DNA-SA). This procedure has produced consistent results in rhythms in DNA-SA from a variety of tissues repeated from experiment to experiment and even from year to year [1, 9]. The corneas were removed, stained and counted; and the results were expressed as mitoses/1000 cells (mitotic index). The details of these technical procedures have been published [6]. During the dissection of the LLC, the tumor mass was bisected, the necrotic-hemorrhagic areas were identified, and a piece of non-necrotic, gray-white tumor was removed for analysis.

RESULTS

Lewis lung carcinoma (Fig. 1)

When the various organs were being removed from the formalin-fixed mice, it was concluded that there was not enough viable LLC tumor in the LLC-6 group to warrant an analysis of DNA synthesis. Therefore, Fig. 1 contains only the chronobiological profiles of DNA-SA for the LLC-10 (solid line) and LLC-14 (dashed line) tumors.

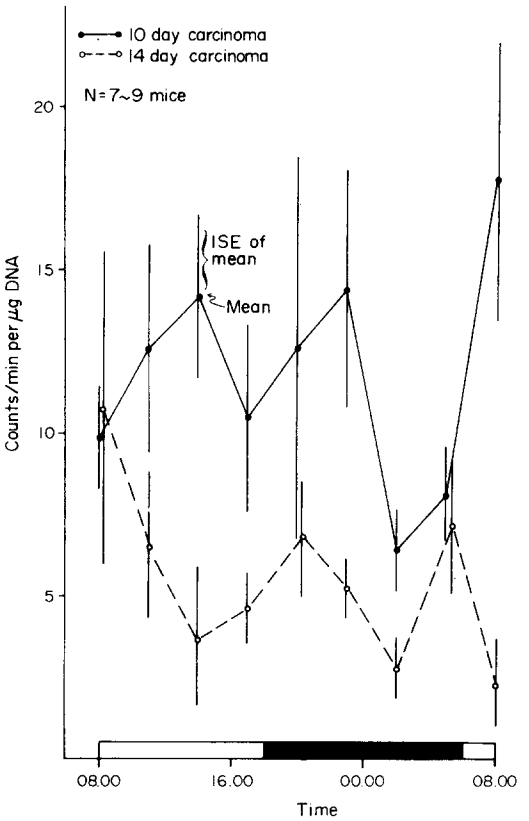

Fig. 1. Chronogram of DNA-SA in 10- and 14-day-old Lewis lung carcinomas.

The DNA-SA profile for the LLC-10 tumor demonstrated trough values during the latter part of the nocturnal period (0200 and 0500) and higher values during the diurnal period and the early nocturnal period. The difference between the highest point (0800b) and the lowest point (0200) was statistically significant ($0.01 < P < 0.005$). The DNA-SA profile for the LLC-14 tumour was characterized by an over-all decrease in DNA-SA. The difference between the high and low points was not statistically significant ($0.2 < P < 0.15$), probably because of the large standard error at 0800a. The per cent difference between 0800a and 0800b, however, was 320%. A statistical comparison between the data at 1100 and 0800b indicated a significant difference between these two points ($P < 0.05$). A statistical comparison between the 24-hr means of the LLC-10 and LLC-14 tumors was significant ($P < 0.0005$) indicating a depression of growth as the tumor aged. However, if single time-

point sampling of this system was used, no statistically significant differences would be recorded between the LLC-10 and LLC-14 tumors at 0800a, 2000 and 0500.

Spleen (Figs. 2 and 3)

The entire spleen was removed from the formalin-fixed mice, blotted dry and weighed to the nearest 0.1 mg. The spleen weights from the non-tumour-bearing mice (solid circles and line, Fig. 2) averaged 78.7 ± 1.3 mg with a high value of 83.5 ± 3.2 mg at 0800a and a low value of 72.3 ± 2.1 mg at 1000. The difference between the high and low points was statistically significant ($0.01 < P < 0.005$). The spleen weights from the LLC-6 mice (open circles, dotted line, Fig. 2)

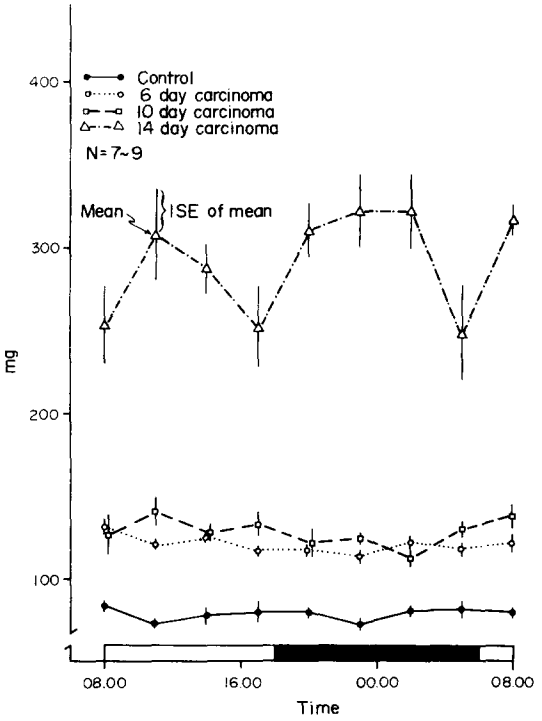

Fig. 2. Chronogram of spleen weights in control or non-tumor-bearing mice compared to spleen weights from LLC-6, LLC-10 and LLC-14 mice.

showed statistically significant fluctuation ($0.025 < P < 0.0125$) between the high point of 131.7 ± 6.0 mg at 0800a and the low point of 113.7 ± 4.1 mg at 2300. Likewise the spleen weights from the LLC-10 mice (open squares, dashed line, Fig. 2) showed statistically significant fluctuation ($0.025 < P < 0{,}0125$) between the high point of 141.1 ± 9.2 mg at 1100 and the low point of 113.3 ± 6.6 mg at 0200. A comparison of the 24 hr means of the spleen weights for the LLC-6 (121.0 ± 1.7 mg) and the LLC-10 mice (128.8 ± 2.8 mg) resulted in a significant difference between these groups ($0.025 < P < 0.0125$).

The spleen weights from the LLC-14 mice (open triangles, dot-dash line, Fig. 2) fluctuated significantly in a trimodal fashion with sharp troughs occurring at 0800a, 1700 and 0500, and three peaks; one peak occurred during the mid-light period, one occurred during the mid-dark period and lasted for 6 hr, and one occurred at 0800b. Since the 0800b value was in the peak range whereas the 0800a value was in the trough range, the impression given is that the peaks and troughs would have occurred earlier during the second 24-hr span, if this period has been included in the experimental design. It also is possible that the fluctuation is not circadian, but perhaps ultradian. The high point (322.6 ± 22.6 mg) occurred at 0200 and the low point (248.7 ± 28.8 mg) occurred at 0500. These values were statistically significant ($0.05 < P < 0.025$).

All 24-hr means of spleen weight from the LLC-6, LLC-10 and LLC-14 mice were significantly greater ($P < 0.0005$) than the control over-all mean. The longer the LLC was present in the host, the greater the spleen weight was i.e., over-all means were 78.7 ± 1.3 mg for the control mice and 121 ± 1.7 mg, 128.8 ± 2.8 mg and 291.8 ± 10.6 mg for the LLC-6, LLC-10 and LLC-14 mice, respectively.

Figure 3 is a display of the changes observed in DNA-SA in the spleens of control, non-tumor-bearing mice (solid circles and line), LLC-6 mice (open circles, dotted line), LLC-10 mice (closed squares, dashed line) and LLC-14 mice (open squares, dashed line). The circadian rhythm in the control mice reached a peak early in the nocturnal period and remained high until after 0200. Trough levels occurred during the majority of the diurnal phase. The difference between the peak and trough values was significant ($0.0024 < P < 0.01$). The phasing of this rhythm in DNA-SA in the spleen from the control animals confirms a similar finding by Burns *et al.* [6]. A rhythm in the uptake of ^{3}H-TdR into DNA of the rat spleen with very similar phasing has been reported [1].

The presence of LLC for 6 days (open circles, dotted line) did not markedly alter the phasing or the over-all level of this rhythm. There were no statistically significant differences between the control and the LLC-6 mice at any time point. The phasing of the rhythm in the LLC-10 mice (closed squares, dashed line) was somewhat similar (except for the 0800a point which was high) to that observed for the LLC-6 and control mice, but the over-

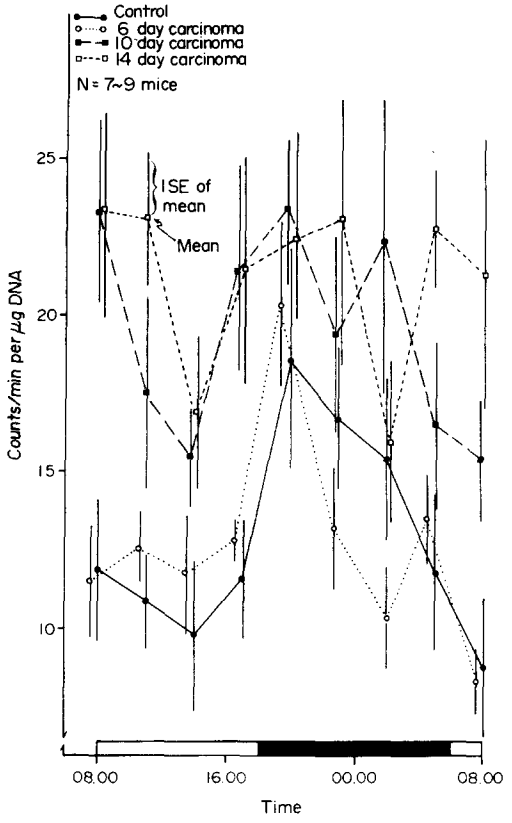

Fig. 3. Chronogram of DNA-SA in spleen in control or non-tumour bearing mice compared to DNA-SA rate in LLC-6, LLC-10 and LLC-14 mice.

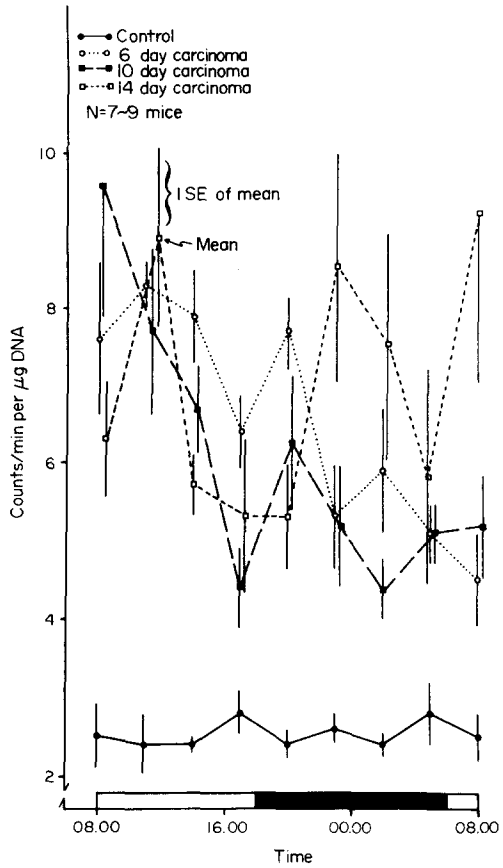

Fig. 4. Chronogram of DNA-SA in liver of control or non-tumor bearing mice compared to that in LLC-6, LLC-10 or LLC-14 mice.

all 24-hr mean in the LLC-10 mice was significantly greater ($P<0.0005$) than both the control and the LLC-6 over-all means (see also Fig. 9). There was no statistically significant difference between the 24-hr means from the LLC-10 and LLC-14 groups. However, the rhythm in DNA-SA in the spleen in the LLC-14 group demonstrated a change in phasing in comparison to the rhythms obtained from the control, LLC-6 and LLC-10 groups. In the data obtained from the LLC-14 group during the 24-hr observation period, the rhythm was trimodal with peak activity occurring around the transition from dark to light, at mid-dark, and again at the transition of light to dark. Trough activity occurred at mid-light and again at mid-dark.

Liver (Fig. 4)

The amount of ^{3}H-TdR incorporated into DNA isolated from the livers of the control mice (solid circles and line) was very low and did not reveal a statistically significant fluctuation. The presence of LLC resulted in a statistically significant increase in DNA-SA in the liver in the other three groups ($P<0.0005$ in each case; see also Fig. 9). There were no staistically significant differences between the over-all means of the LLC-6 (6.5 ± 0.5), LLC-10 (6.1 ± 0.6) and LLC-14 (6.9 ± 0.5) groups. However, there were statistically significant differences between the peak and trough values for each of these groups ($P<0.0005$ for LLC-6, $0.01<P<0.005$ for LLC-10, and $0.05<P<0.025$ for LLC-14). The rhythmic variations in DNA-SA in the liver for the LLC-6 and LLC-10 groups were basically unimodal, i.e., one area of peak values occurred during early to mid-light phase and one area of trough values occurred during the mid- to late-dark phase. Such phasing and the over-all increase in DNA-SA in the liver is very similar to the results reported for mice bearing an 8-day-old Ehrlich ascites carcinoma [8]. The rhythm obtained from the LLC-14 group was basically bimodal, i.e., trough levels occurred around the time of transition from light to dark and again at the time of transition from dark to light; this finding was very similar to that observed for DNA synthesis in the spleen in LLC-14 mice.

Thymus (Fig. 5)

The rhythm in DNA-SA in the thymus in the control animals (solid circles and line) had

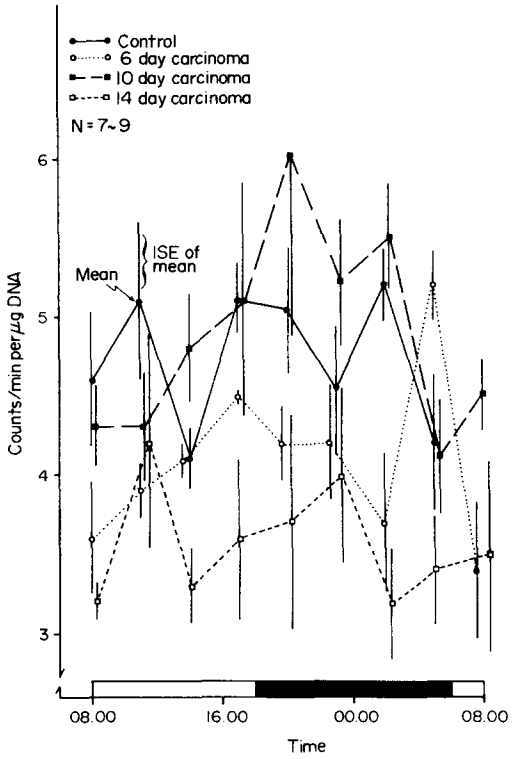

Fig. 5. Chronogram of DNA-SA in thymus in control or non-tumor-bearing mice compared to that in LLC-6, LLC-10 or LLC-14 mice.

a peak at 0200 and a trough at 1400; this difference was statistically significant ($P < 0.0005$). The phasing of this rhythm was in some respects similar to previously published data on the thymus [7]. The differences seen in the phasing and over-all level of DNA-SA in the thymus between the present study and the 1976 study may be due to the difference in the age of the mice used; in the younger mice used by Pauly *et al.* [7], the rhythm was more prominent and the level of DNA-SA was higher. The presence of the LLC-6 tumor altered this rhythmic pattern such that the peak (open circles, dotted line) occurred at 0500 and the trough at 0800 ($0.0025 < P < 0.0005$). The presence of an LLC-10 tumor caused the rhythm in DNA-SA in the thymus (closed squares, dashed line) to reach a peak at 2000 and to reach a trough at 0500 ($0.10 < P < 0.05$). The LLC-14 (open squares, dashed line) resulted in a bimodal rhythmic pattern with peak values occurring at 1100 and 2300, and trough values at 0800, 1400 and 0200 (P at least < 0.005 in all cases). A comparison of the over-all 24-hr means of the three LLC rhythms with the control rhythm (Fig. 9), revealed that DNA-SA in the thymus from the LLC-6 and LLC-14 mice was statistically significantly depressed ($P < 0.05$ and < 0.0005, respectively), whereas the presence of an LLC-10 tumor did not cause any statistically significant stimulation or suppression in the thymus.

Duodenum (Fig. 6)

The rhythm in DNA-SA in the duodenum of the control animals (solid circles and line) demonstrated a trough at 1400 and a peak at 0500 ($0.0025 < P < 0.0005$). This rhythmic pattern was disrupted by the presence of the LLC. The LLC-6 (open circles, dotted line)

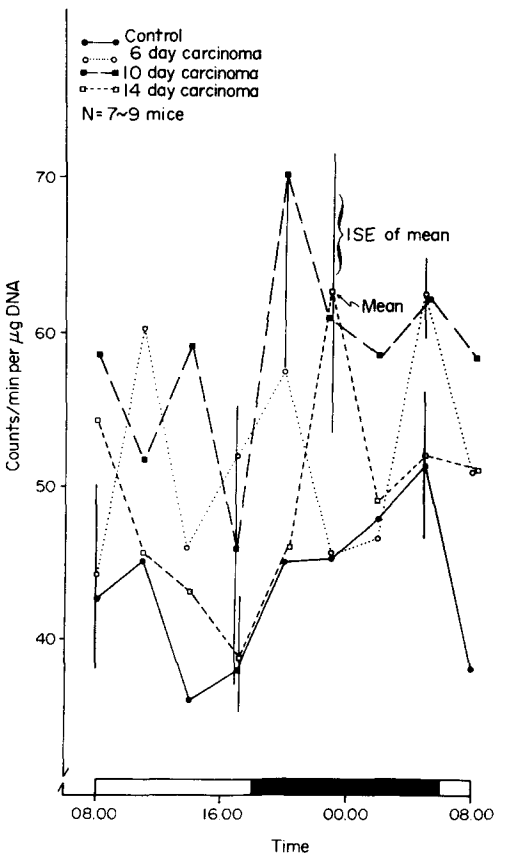

Fig. 6. Chronogram of DNA-SA in duodenum in control or non-tumor bearing mice compared to that in LLC-6, LLC-10 or LLC-14 mice.

changed the rhythm so that instead of one peak in activity per 24-hr period, there were 3 peaks (1100, 2000 and 0500) during one 24-hr period or 1 peak every 8 hr. The LLC-10 (solid squares, dashed line) resulted in a different pattern which demonstrated a trough at 1700 and a peak at 2000 ($0.0025 < P < 0.0005$). The LLC-14 (open squares, dashed line) resulted in a rhythmic pattern which was similar to that seen in the LLC-10 mice in that the trough occurred at 1700; the peak occurred not at 2000, however, but at 2300 ($P < 0.0005$). A statistical comparison (Fig. 10) of the 24-hr means of the three LLC groups with that of the non-tumor-bearing control

group revealed that the presence of the LLC significantly increased the amount of DNA-SA in the duodenum ($P<0.002$ for LLC-6, $P<0.0005$ for LLC-10 and $P<0.05$ for LLC-14).

Bone marrow (*Fig.* 7)

The rhythm in DNA-SA in the bone marrow from the non-tumor bearing mice (solid circles and line) reached peak levels beginning at the transition from light to dark and lasting until 0200 (actual peak). Trough levels occurred during the majority of the light period with the actual trough occurring at 0800a (and 0800b). The difference between the peak and trough values was statistically significant ($0.0025<P<0.0005$). The presence of LLC-6 (open circles, dotted line) altered the phasing of the control rhythm by extending the peak levels an additional 3 hr, from the transition of light to dark, to 0500 (actual peak). Trough levels occurred at 0800a (and 0800b). The difference between the peak and trough levels was statistically significant ($0.0025<P<0.0005$). The rhythm for the LLC-10 mice (solid squares, dashed line) had a sharper peak than the control rhythm, with peak levels occurring at 1700 and 2000 (actual peak). Trough levels occurred at the time of transition from dark to light and lasted until 1100; the actual trough was at 0800b. The difference between the peak and trough values again was statistically significant ($0.0025<P<0.0005$). In the LLC-14 mice (open squares, dashed line), the normal rhythmic pattern was radically disrupted. A very sharp peak was reached at 1100 and a secondary peak occurred at 2300. The trough occurred at 1400, but near-trough levels of activity occurred also at 2000, 0200, 0500 and 0800b. The difference between the peak and trough values was statistically significant ($P<0.0005$). A comparison of the over-all 24-hr means for the control groups and the LLC animals (Fig. 10) demonstrated that the presence of 6- and 10-day-old LLCs significantly stimulated DNA-SA in the bone marrow ($P<0.0005$), whereas the 14-day-old LLC had no effect on the over-all level of DNA-SA.

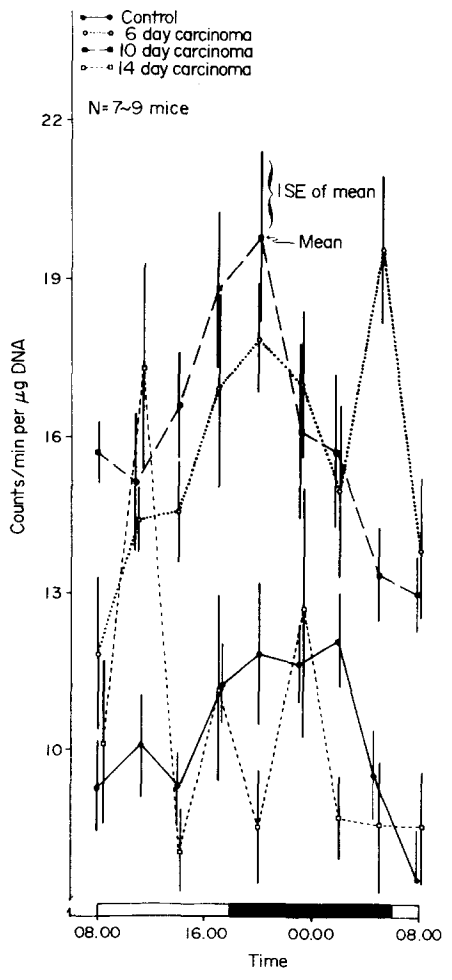

Fig. 7. *Chronogram of DNA-SA in bone marrow in control or non-tumor-bearing mice compared to that in LLC-6, LLC-10 or LLC-14 mice.*

Cornea (*Fig.* 8)

The circadian rhythm in the mitotic index in the corneal epithelium in the non-tumor-bearing animals was characterized by a peak in the early part of the diurnal period and a

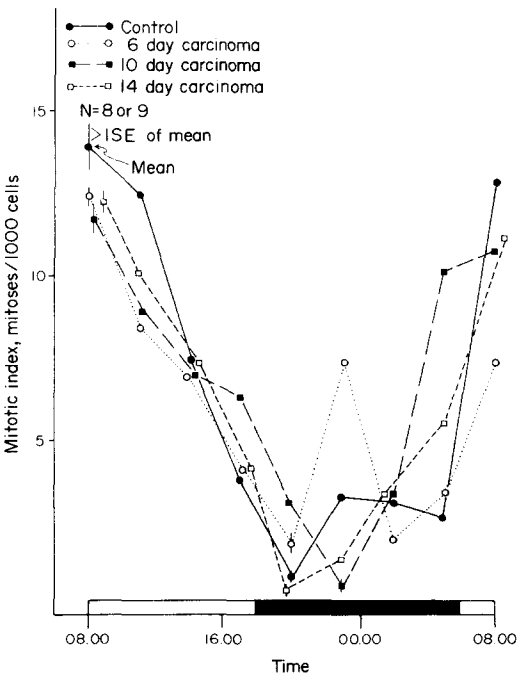

Fig. 8. *Chronogram of mitotic index (number of mitoses/1000 cells) in the corneal epithelium of control or non-tumor-bearing mice compared to that in LLC-6, LLC-10 and LLC-14 mice.*

trough in the early part of the nocturnal period. The general phasing of this rhythm was not altered by the presence of the LLC. In the LLC-6 group, the corneal mitotic index was higher than expected at one point, 2300; but in consideration of all of the other points determined for this group and from our extensive experience with this rhythm, this 2300 value was not considered to be of biological significance but rather as a probable technical error. The over-all level of mitotic activity in the corneal epithelium was not statistically significantly altered by the presence of the LLC.

DISCUSSION

The phasing, amplitude and amount of the standard error of the mean (S.E.) of the individual time points of the circadian rhythms in DNA-SA in the non-tumor bearing animals in such organs as the spleen, thymus, duodenum and bone marrow, and the circadian rhythm in the mitotic index in the corneal epithelium, confirm earlier chronobiological work on these variables [1, 6, 7]. These rhythms are synchronized to the light–dark cycle and therefore are predictable and reproducible.

The present results indicate that the circadian rhythms in DNA-SA in the host organs are significantly altered by the presence of the LLC. There were several kinds of alterations induced by the presence of the LLC: (1) phase changes, (2) waveform changes (3) changes in the 24-hr mean or over-all level of activity and (4) increase in the variation of the data obtained at each time point, i.e., larger S.E. were consistently found in the data obtained from the tumor-bearing mice. In general, the longer the LLC was present in the host the more pronounced were the alterations in the rhythm in DNA-SA. The first changes noted were usually changes in phasing, i.e., a different time for the occurrence of the peak and/or trough in the rhythm. During the later stage of LLC growth (LLC-14), the rhythms in DNA-SA in the spleen, liver, bone marrow and thymus demonstrated a trimodal instead of a normal unimodal pattern. In some organs (e.g., spleen), changes were not noticed until the later stages of LLC growth, whereas in others the changes were observed early in the course of growth of the LLC; in the duodenum, for example, a trimodal pattern was apparent on the sixth day of LLC growth.

The changes in the over-all level (24-hr mean) of the DNA-SA in the organs studied are presented in the histograms in Figs. 9 and 10. In general, with the exception of the thymus, the presence of the LLC resulted in a stimulation of the over-all level of DNA-SA. The spleen did not demonstrate this stimulation until after the sixth day of LLC growth, although the spleen weight was increased by that time. The bone marrow showed increased levels of DNA-SA in the LLC-6 and LLC-10 groups, but not in the LLC-14 mice. Liver and duodenum had increased levels of DNA-SA during all stages of LLC growth. The thymus demonstrated an inhibition rather than stimulation of DNA-SA in response to the LLC. The stimulation of DNA-SA in the spleen and bone marrow and possibly in the duodenum may be a reflection of a B-cell type

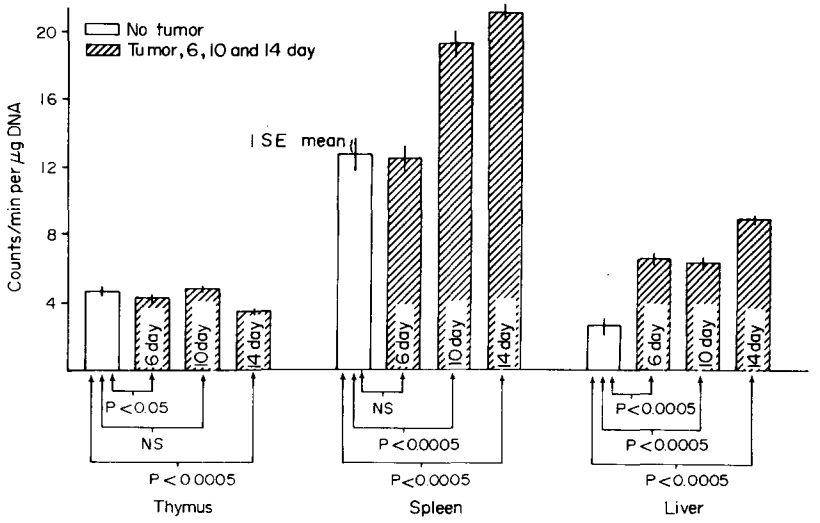

Fig. 9. Histogram of 24-hr *means of DNA-SA in thymus, spleen and liver in control or non-tumor-bearing mice compared to that in LLC-*6, *LLC-*10 *and LLC-*14 *mice.*

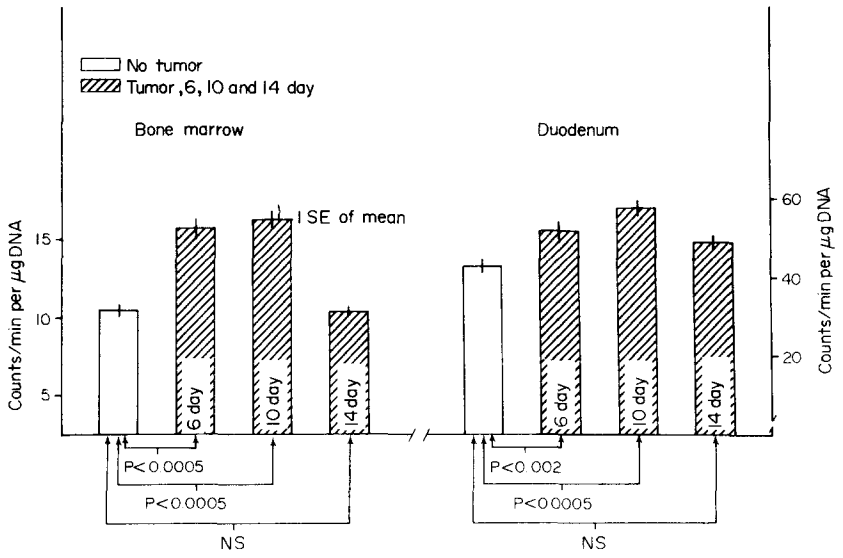

Fig. 10. Histogram of the 24-hr means of DNA-SA in bone marrow and duodenum in control or non-tumor-bearing mice compared to LLC-6, LLC-10 and LLC-14 mice.

of immune response, whereas the depression of DNA-SA in the thymus may be related to T-cell-specific immunosuppression elicited by the LLC [6].

The larger S.E. in DNA-SA obtained in the organs in the tumor-bearing mice (Figs. 3–7) indicates that in addition to the changes in the phasing, waveform and over-all 24-hr level in the rhythms in DNA-SA, there was an increase in the variation between measurements in the individual animals at each time point. This observation is in agreement with the changes observed in the phasing, waveform and over-all level of these rhythms, i.e., the presence of the LLC causes a significant amount of variation or disturbance in the physiology of the host. Since the LLC metastasizes early and widely it is possible that some of the variation recorded is due to the physical presence of viable LLC metastatic nodules.

The cell population kinetics of the LLC at different stages of growth have been studied by Simpson-Herren *et al.* [10], but without regard for possible chronobiological variation in DNA-SA in the LLC. In the current study the fluctuations in DNA-SA in the LLC itself over one 24-hr period indicate that the tumor, as well as the host tissue, is characterized by complex chronobiological variation. However, no evidence was obtained to suggest that this fluctuation is synchronized or predictable. Nevertheless, the possibility of encountering fluctuation of this magnitude within a very short time span (24 hr) is of importance to any investigator of tumor-cell kinetics, especially when one realizes that different cell-kinetic data (e.g., frequency of labelled mitoses method) can be obtained when the ^{3}H-TdR is injected at the time of maximal vs minimal DNA synthesis. This phenomenon has been reported in both normal [11, 12] and neoplastic cell populations [13].

The circadian rhythm in the mitotic index in the corneal epithelium was not altered in any manner by the presence of the LLC. This is a curious phenomenon in consideration of the alterations in the rhythm in DNA synthesis observed in a variety of organs in the LLC-bearing mice. The rhythm in the mitotic index in the corneal epithelium also was not changed in any manner by the presence of an 8-day-old Ehrlich ascites carcinoma [6] or by changing the meal schedule of the mice; the altering of lighting regimens, however, will phase-shift this rhythm. The circadian rhythm in the mitotic index in the corneal epithelium apparently is strongly synchronized by the lighting regimen and cannot be altered either by the presence of a neoplasm (Ehrlich ascites carcinoma or LLC) or by a radical change in the feeding schedule in animals kept on a 12-hr light/12-hr dark cycle.

The data presented indicate the fundamental role that chronobiological investigation plays in any analysis of DNA-SA in normal and neoplastic tissues and in the study of the interaction between tumor and host. A tremendous variety of erroneous conclusions can be obtained if an experimenter is unaware of the significant rhythms in the host and in the tumor, and how these rhythms are changed when the host and tumor interact, especially if only single time-point sampling is used to

compare tumor-bearing host to the non-tumor bearing control animals. For example, in Figs. 3–7 if single time point sampling were employed (i.e., only 0800, or only 2000, or only 1400, etc.) one can find examples of the LLC having no significant effect, or a significant stimulation or a significnat inhibition of DNA-SA depending on the single time point selected to compare with the controls or with the data obtained at a different time point (perhaps a different laboratory working with preceisely the same system).

By discerning the chronobiological nature of the host, the tumor and the tumor–host interaction, a better resolution or understanding of normal and neoplastic growth will be obtained, and this will lead to more accurately designed and therefore more efficient cancer therapeutic regimens [2].

REFERENCES

1. L. E. Scheving and J. E. Pauly, Cellular mechanisms involving biorhythms with emphasis on those rhythms associated with the S and M stages of the cell cycle. *Int. J. Chronobiol.* **1,** 269 (1972).
2. F. Halberg, B. D. Gupta, E. Haus, E. Halberg, A. C. Deka, W. Nelson, R. B. Southern, G. Cornelissen, J. K. Lee, D. J. Lakatua, L. E. Scheving and E. R. Burns, Steps toward a cancer chronopolytherapy. In *XIVth International Congress on Therapeutics*, p. 151. L'Expansion Scientifique Française, Montpellier, France (1977).
3. E. Haus, F. Halberg, L. E. Scheving, S. S. Cardoso, J. F. W. Kühl, R. Sothern, R. Shiotsuka, D. S. Hwang and J. E. Pauly, Increased tolerance of leukemic mice to arabinosyl cytosine with schedule adjusted to circadian system. *Science* **177,** 80 (1972).
4. J. F. W. Kühl, E. Haus, F. Halberg, L. E. Scheving, J. E. Pauly, S. S. Cardoso and G. Rosene, Experimental chronotherapy with ara-C: comparison of murine ara-C tolerance on differently timed treatment schedules. *Chronobiologia* **1,** 316 (1974).
5. L. E. Scheving, E. R. Burns, J. E. Pauly, F. Halberg and E. Haus, Survival and cure of leukemic mice after circadian optimization of treatment with cyclophosphamide and 1-β-D-arabinofuranosylcytosine. *Cancer Res.* **37,** 3648 (1977).
6. E. R. Burns, L. E. Scheving, J. E. Pauly and T. H. Tsai, Effect of altered lighting regimens, time-limited feeding and presence of Ehrlich ascites carcinoma on the circadian rhythm in DNA synthesis of mouse spleen. *Cancer Res.* **36,** 1539 (1976).
7. J. E. Pauly, L. E. Scheving, E. R. Burns and T. H. Tsai, Circadian rhythm in DNA synthesis of mouse thymus: effect of altered lighting regimens, restricted feeding and presence of Ehrlich ascites tumor. *Anat. Rec.* **184,** 275 (1976).
8. L. E. Scheving, E. R. Burns and J. E. Pauly, Coincidence in timing between the S and M stages of the cell cycle in liver parenchymal cells in mice bearing an 8-day Ehrlich ascites tumor (EAT). In *Proceedings of the* XII *International Conference* (International Society of Chronobiology), p. 427. Il Ponte, Milano (1977).
9. L. E. Scheving, E. R. Burns, J. E. Pauly and T. H. Tsai, Circadian variation in cell division of the mouse alimentary tract, bone marrow and corneal epithelium. *Anat. Rec.* **191,** 479 (1978).
10. L. Simpson-Herren, A. H. Sanford and S. P. Holmquist, Cell population kinetics of transplanted and metastatic Lewis Lung Carcinoma. *Cell Tiss. Kinet.* **7,** 349 (1974).
11. E. R. Burns and L. E. Scheving, Circadian influence on the waveform of the frequency of labeled mitoses in mouse corneal epithelium. *Cell Tiss. Kinet.* **8,** 61 (1975).

12. E. R. Burns, L. E. Scheving, D. F. Fawcett, W. M. Gibbs and R. E. Galatzan, Circadian influence on the frequency of labeled mitoses method in the stratified squamous epithelium of the mouse esophagus and tongue. *Anat. Rec.* **184,** 265 (1976).
13. E. R. Burns, L. E. Scheving, W. M. Gibbs, D. F. Fawcett and R. E. Galatzan, Circadian influence on the waveform of the frequency of labeled mitoses curve. In *Proceedings of the* XII *International Conference* (International Society of Chronobiology), p. 595. Il Ponte, Milano (1977).