niere und eine Zunahme von Katecholamin im Herzen beobachtet. LINÉT, WIDHALM und HERTTING[20] berichteten über Katecholamingehalt bei Thiaminmangeltauben, wobei sie auch Untersuchungen am Gehirn durchgeführt haben. Aber es gibt noch keine Berichte über genauere Analysen des Katecholamingehalts in den anderen Organen bei Thiaminmangeltieren. Der Mechanismus des erhöhten Katecholamingehaltes in Organgeweben unter Thiaminmangel bleibt noch unverständlich und bedarf weiterer Klärung.

Aus den dargelegten Beobachtungen dürfen folgende Schlüsse gezogen werden: (1) Bei Ratten unter Thiaminmangel wurde ein erhöhter Katecholamingehalt im Vorhof, Ventrikel, ferner in der Grosshirnrinde und Milz festgestellt, jedoch nicht im Hirnstamm und den Nebennieren. (2) Die verstärkte Reaktion des Herzens unter Thiaminmangel auf Tyramin ist höchstwahrscheinlich durch den hohen Katecholamingehalt bedingt, der durch den Thiaminmangel hervorgerufen wurde. (3) Der erhöhte Katecholamingehalt bei Thiaminmangelratten ist nicht durch die Zunahme von Brenztraubensäure im Gewebe entstanden.

Summary. (1) The increased tissue catecholamine level of the thiamine deficient rat was shown in the atrium, the ventricle, the brain cortex and in the spleen but not in the brain stem and the adrenal gland. (2) The increased response to tyramine of the thiamine deficient heart is most probably due to the high catecholamine level caused by thiamine deficiency. (3) The increased catecholamine content in the thiamine deficient rat does not result from the increased pyruvic acid.

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The Influence of Partial Synchronization of Cell Growth on the Inhibition of L1210 Cell Viability by Mouse Thymus DNA

Several laboratories have reported inhibition of cell viability and tumor growth by exogenous DNA[1-9]. Only specific kinds of DNA were found to be capable of acting against certain cell lines. The lethal effects of DNA could be reduced or even prevented by altering the incubation conditions[3,4,10]. The present investigation suggests that the life cycle of the L1210 cell is another variable which regulates the inhibition of cell viability by mouse thymus DNA.

DBA_2 mouse thymus DNA was isolated and treated with RNase following the method of SZYBALSKA and SZYBALSKI[11]. In some cases H^3-DNA was prepared, after mice had been injected each with 25 μcuries of H^3-thymidine[12].

L1210 leukemia cells were grown in spinner flasks containing medium RPMI No. 1634 + 5% fetal calf serum. Partial synchronization of the L1210 culture was induced by lowering the cell population to $1.5–2.5 \times 10^5$ cells/ml and then incubating the culture for 6 h at 37 °C with $10^{-6}M$ 5-fluorodeoxyuridine (FUDR). After incubation with FUDR, $10^{-4}M$ thymidine was added to the culture[13]. Cells were removed either from partially synchronized cultures at 6.33, 10.5 and 14 h or from asynchronous cultures. The cells were washed and incubated at 37 °C in 4 ml of phosphate-buffered saline (pH 7.5) containing 5.5 mM glucose and 0.2 mM spermine tetrahydrochloride[11]. The cell concentration was around 1.2×10^6 cells/ml. The cells were treated for 30 min with or without DNA (approximately 58 μg of DNA/10^6 cells) and then washed and reincubated in DNA-free, phosphate-buffered saline + glucose. Cell counts were taken at various time intervals, and cell viability was determined by means of trypan blue staining[3].

Uptake of DNA was measured by exposing cells to H^3-DNA for 30 min. Aliquots were removed at 0 and 30 min, and were washed thrice[12]. Radioactivity was determined by means of a Packard liquid scintillation counter.

L1210 DNA was isolated from partially synchronized cells by the method of SCHNEIDER[14] and was analyzed by means of the diphenylamine test[14].

Figure 1a demonstrates that the DNA content of the partially synchronized L1210 cells nearly doubled 2 h after addition of thymidine to a FUDR-treated culture. Figure 1b represents the growth curves of partially synchronized cultures. One treatment, first with FUDR and then with thymidine, induced at least 2 cycles of partial synchrony. Each cycle was about 15 h long, compared to the normal doubling time of 14 h. If the S phase (length of time for DNA synthesis) occurred 6–8 h after addition of FUDR, and the M phase (length of mitosis) lasted from 11–15 h, then the G_2 phase (length of time between DNA synthesis and mitosis) must have taken place at 8–11 h.

Figure 2a demonstrates the inhibition of cell viability caused by mouse thymus DNA at various phases of cell growth. The degree of inhibition was measured as the % change between the number of viable DNA-treated cells and the number of viable control cells at 90 and 120 min after removal of the DNA. In each group data obtained at both of these times were combined, since within this time interval there was hardly any change in

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the degree of inhibition. Previous studies with asynchronous cells had demonstrated that significant inhibition of cell viability by DNA occurred 90 min after reincubation of the cells in DNA-free medium[1,3,5]. In the present study cells obtained at the G_2 phase of partially synchronized cultures were inhibited by DNA almost 2 times more than cells obtained from asynchronous cultures. Moreover, the exogenous DNA had a markedly smaller inhibitory effect on cells obtained during the S and M phases of cell growth than on cells obtained during the G_2 and asynchronous phases.

Figure 2b illustrates that the different degrees of DNA-induced inhibition of cell viability did not result from commensurate differences of uptake of DNA. ADAMS et al.[15] similarly reported that the uptake of salmon sperm DNA by HeLa cells was independent of the cell cycle.

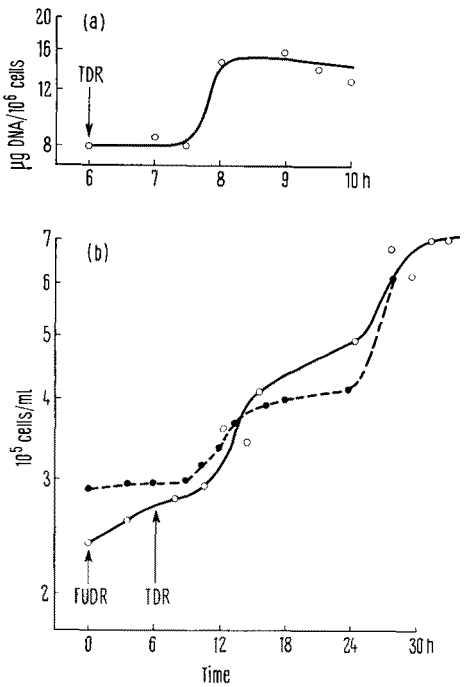

Fig. 1. Partial synchronization of L1210 cells. (a) The effects of thymidine (TDR) on the DNA content of L1210 cells which were preincubated with FUDR for 6 h. Each point represents the mean of 2 determinations. (b) Growth curves of 2 typical cultures of partially synchronized cells.

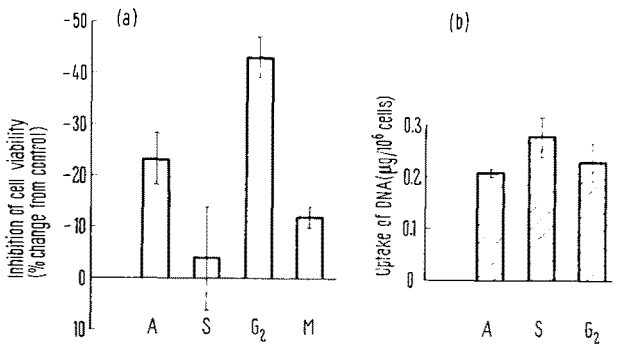

Fig. 2. Influence of partial synchronization of cell growth on the interaction of L1210 cells with mouse thymus DNA. (a) Inhibition of cell viability. Data represent the mean (± standard error) of 15 determinations for the asynchronous (A) phase, of 10 determinations each for the S and G_2 phases, and of 4 determinations for the M phase. (b) Uptake of DNA by L1210 cells. Data represent the mean (± standard error) of 4 determinations each for the A and G_2 phases, and of 3 determinations for the S phase.

Recent investigations in this laboratory indicated that the destruction of L1210 cells by BDF_1 or DBA_2 mouse thymus DNA was preceded by a stimulation of DNA, RNA and protein synthesis[16]. The newly formed macromolecules were shown to be responsible for causing cell death. Furthermore, at least some of the newly formed RNA was probably transcribed by thymus DNA. Evidence was also reported, that any one of a critical number of genetic sites could induce cell destruction if incorporated into the L1210 cell[12]. However, those experiments did not indicate whether the active genetic site integrated with the host DNA. Our present data correlate a twofold increase in inhibition of cell viability by thymus DNA with a twofold increase in host DNA. This evidence suggests that the host genome may be a target for the exogenous DNA during the process of DNA-induced cell destruction. BASILICO and MARIN[17] have demonstrated that synchronized cells treated with polyoma virus at the end of the G_2 phase are twice as likely to be transformed as cells treated prior to this phase. Their results were not due to a proportionate increase in uptake of polyoma virus. They suggested that the genome of the host cell was the target for polyoma virus, in agreement with the more direct evidence of AXELROD et al.[18].

Our data demonstrated little inhibition of cell viability during the S and M phases. This evidence suggests that some substance which is either activated or produced during these phases interferes with the inhibitory action of the exogenous DNA. It is also conceivable that a different substance is produced during the G_2 phase, which renders the cell highly susceptible to treatment with exogenous DNA. Other experiments have indicated that DNA does not inhibit the viability of cells which have been preincubated in DNA-free, phosphate-buffered saline[10]. Furthermore, DNA does not inhibit cells incubated in a growth medium[3]. All of these studies suggest that endogenous substances do indeed regulate the inhibitory action of mouse thymus DNA[19].

Résumé. Nous avons trouvé que le cycle de la vie de la cellule L1210 règle les effets inhibitoires du DNA du thymus de la souris sur la viabilité cellulaire. Les cellules obtenues à la phase G_2 des cultures partiellement synchronisées étaient inhibitées presque 2 fois plus que les cellules obtenues des cultures asynchroniques. Le DNA n'avait guère d'effet toxique sur les cellules obtenues aux phases S et M. Les fluctuations dans la viabilité cellulaire n'étaient pas causées par des changements proportionnels dans l'absorption du DNA.

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