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DNA and Protein Synthesis in Normal and Transformed Lymphocytes Exposed to Abrin

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BENNETT, C. E., A. B. GLASSMAN AND M. WITTEN. *DNA and protein synthesis in normal and transformed lymphocytes exposed to abrin.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 185–188, 1982.—The dose dependent effects of abrin, a toxic D-galactose binding plant lectin, on ^{3}H-TdR and ^{14}C-leucine uptake are studied in normal and Epstein Barr Virus (EBV) transformed lymphocyte cultures. Results show that while abrin is highly toxic to both the DNA and protein synthesis in EBV lymphocytes, some toxicity to the normal cells is also seen. It is postulated that lymphocyte DNA synthesis is affected by ribosomal shutdown induced by the abrin.

Human peripheral lymphocytes Virus transformed lymphocytes Abrin Tritiated thymidine
C-14 leucine DNA synthesis Protein synthesis

THE effects of abrin, a lectin derivative (glycoprotein) of *Abrus precatorius*, on various cell culture systems have been documented by a number of researchers [1–7, 9–15]. Recently, we described a series of simultaneous ^{3}H-TdR and ^{14}C-leucine measurements made on a set of normal and Epstein Barr Virus (EBV) transformed human lymphocyte cultures [16–18]. In these experiments, lymphocytes were isolated from the peripheral blood of healthy volunteers by Ficoll-Hypaque separation. An EBV producing, human lymphoma cell line, suppled from Baylor University, was also used at the same concentration. Both cell types were cultured in RPMI 1640 media plus 15% fetal bovine serum without antibiotics at densitites of 10^{5}, 10^{6}, 10^{7} cells/ml. Each set of cultures was subsequently exposed to 16 dose levels of abrin from 0 ng/ml to 50 ng/ml. Cultures were pulsed with 1 μCi/ml ^{3}H-TdR or 1 μCi/ml ^{14}C-leucine and processed at 0, 24, 48, 72, and 96 hour intervals. Cell viability was assessed by trypan blue exclusion. All experiments were performed in triplicate.

Because of the number of independent variables involved in this experiment, i.e., dose, time, cell density, an analysis of variance (ANOVA) was performed in an effort to ascertain which of the just mentioned independent variables seriously contributes to the toxic effects of abrin on DNA and protein synthesis in the two lymphocyte cell cultures. The analysis of variance was performed using BMDP2V (Biomedical Data Processing software) for an analysis of variance on a completely randomized factorial experiment with repeated measures. BMDP2V uses raw CPM as data for its calculations. The results of the ANOVA analysis are illustrated in Table 1 and Table 2.

Table 1 illustrates the ANOVA for a completely randomized factorial design having DNA and protein synthesis as the dependent variables (^{3}H-TdR CPM and ^{14}C-leucine CPM in normal lymphocytes). In normal cells, one can see that the only variable having a significant contribution to the variance in the ^{3}H-TdR uptake, and consequently the DNA synthesis rate, is the dose of abrin ($p<0.05$). This data adds justification to the current hypothesis that abrin does, in fact, effect DNA synthesis in normal lymphocytes. The mechanism for this effect is most probably through the action of abrin on lymphocyte protein synthesis. (This relationship has been documented in Hela and RBC systems. It is currently the subject of investigation in EBV systems in our laboratory.) Data supporting the decreased lymphocyte protein synthesis in normal lymphocytes is also illustrated in Table 1. Here one can see that the initial dose of abrin (denoted A_0), cell density, and time all have a significant

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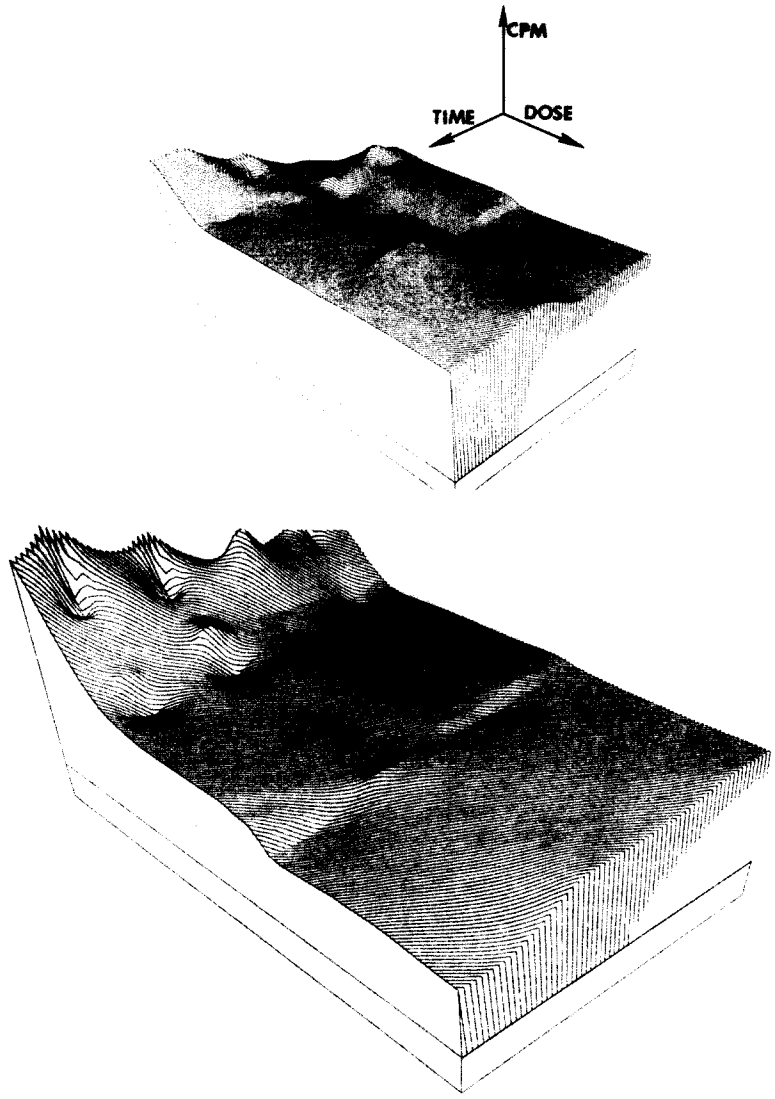

FIG. 1. Top: A three dimensional surface plot of the dose-time relationship for mean ^{3}H-TdR cpm in normal lymphocytes at a cell density of 10^5 cells/ml. The plots were drawn on a Calcomp plotter using the Harvard University SYMAP/SYMVU graphics software package. This set of software routines interpolates point data so as to produce a spacially continous surface which is then plotted according to user specified orientation. Plots of this type are available for other cell densities as well. Bottom: A three dimensional surface plot of the dose-time relationship for mean ^{14}C-leucine CPM in normal lymphocytes at a cell density of 10^5 cells/ml.

($p<0.05$) effect upon the rate of protein synthesis in normal lymphocytes. Figure 1 shows three-dimensional plots of mean CPM ^{3}H-TdR and ^{14}C-leucine (cell density=10^5 cells/ml) as a function of dose and time for normal lymphocytes.

For the EBV transformed lymphocytes at all levels of independent variable interaction, both DNA and protein synthesis mechanisms are significantly ($p<0.05$) diminished. This data is illustrated in Table 2. The link between DNA synthesis depression and ribosomal shutdown is discussed in [15]. At this point, it is also relevant to remark that abrin not only inhibits protein synthesis, and inhibits DNA synthesis, but it also damages the plasma membrane of cells subjected to abrin drug regimens (see [8] and references contained

TABLE 1

ANALYSIS OF VARIANCE FOR NORMAL HUMAN LYMPHOCYTES IN SIMULTANEOUS ^{3}H-TdR AND ^{14}C-LEUCINE UPTAKE EXPERIMENTS

	DNA Synthesis (^{3}H-TdR)		Protein Synthesis (^{14}C-Leucine)	
Source of Contribution	F	p-value	F	p-value
[D]ose	3.5	0.0097*+	78.32	0.0000*+
[T]ime	1.11	0.3483	5.38	0.0016*+
[D']ensity	0.18	0.8319	10.46	0.0001*+
[D][T]	0.47	0.9303	10.09	0.0000*+
[D][D']	0.41	0.9101	2.14	0.0371*
[T][D']	0.36	0.9003	2.96	0.0098*+
[D][T][D']	0.48	0.9800	2.43	0.0008*+

An asterisk (*) indicates a significant source of contribution at the $p<0.05$ level (95% confidence), and a plus (+) indicates a significant contribution at the $p<0.01$ level (99% confidence). Thus, we may conclude (at a 99% confidence) that the variance in the DNA ^{3}H-TdR CPM is truly a function of dose in the normal lymphocyte population; further, it is truly a function of dose, time, and cell density in the EBV lymphocyte population.

TABLE 2

ANALYSIS OF VARIANCE FOR EPSTEIN BARR VIRUS TRANSFORMED LYMPHOCYTES IN SIMULTANEOUS ^{3}H-TdR AND ^{14}C-LEUCINE UPTAKE EXPERIMENTS

	DNA Synthesis ^{3}H-TdR)		Protein Synthesis (^{14}C-Leucine)	
Source of Contribution	F	p-value	F	p-value
[D]ose	4553.36	0.0000*+	7924.72	0.0000*+
[T]ime	635.08	0.0000*+	3554.19	0.0000*+
[D']ensity	74.40	0.0000*+	64.24	0.0000*+
[D][T]	931.65	0.0000*+	2274.66	0.0000*+
[D][D']	5.04	0.0000*+	25.75	0.0000*+
[T][D']	2.35	0.0352*	9.43	0.0000*+
[[D][[T][[D']	3.11	0.0000*+	5.80	0.0000*+

An asterisk (*) indicates a significant source of contribution at the $p<0.05$ level (95% confidence), and a plus (+) indicates a significant contribution at the $p<0.01$ (99% confidence) level.

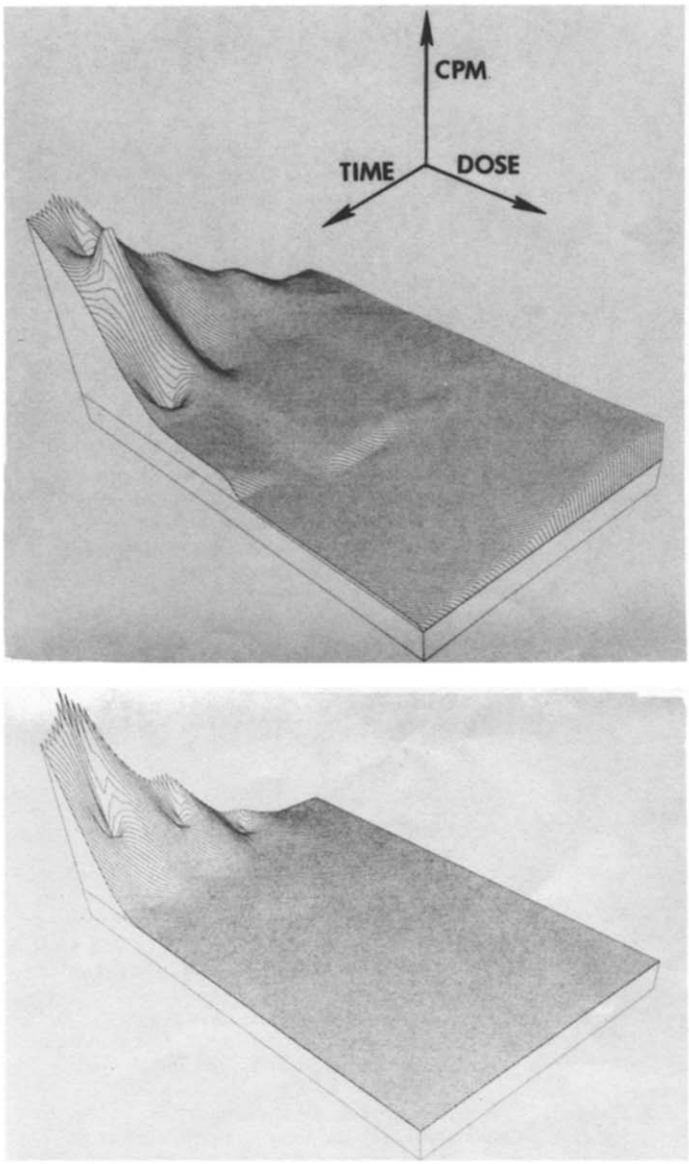

FIG. 2. Top: Dose-time relationship for mean ^{3}H-TdR CPM in transformed lymphocytes at a cell density of 10^{5} cells/ml. Bottom: Dose-time relationship for mean ^{14}C-leucine CPM in transformed lymphocytes at a cell density of 10^{5} cells/ml.

therein). Figure 2 illustrates the same data surfaces for transformed lymphocytes.

In summary, abrin has a statistically discernable dose dependent effect on both the DNA and protein synthesis rates in normal lymphocyte cell culture. From our experimental data, as well as the previous statistical analysis, it is clear that this effect is significantly less than abrin suppression of DNA and protein synthesis in the EBV lymphocyte culture. This relationship is studied, in more detail, in [19]. It is intriguing to speculate that this or similar biochemical selective activity in normal versus transformed cells systems could have application in the therapy of neoplasia. Consider, for example, Fig. 2, which illustrates a plot of ln ($t_{.5}$), where $t_{.5}$ represents the time it takes for the ^{3}H-TdR CPM in the EBV system to drop to half the initial ^{3}H-TdR CPM, versus A_0. From our experimental data one can demonstrate that, over all doses, the ^{3}H-TdR CPM never drops to half its initial rate in the normal lymphocyte culture. Hence, by making use of the curve in Fig. 3 we can ascertain (graphically) an optimal dose of abrin which will depress the ^{3}H-TdR CPM to 50% of the original CPM. If abrin were added at this optimal dose the EBV lymphocytes, in their depressed state, might be more susceptible to other cancer chemotherapeutic agents. One possible combined regimen would be with organo-platinum-II complex. This drug regimen is currently under investigation in our labs.

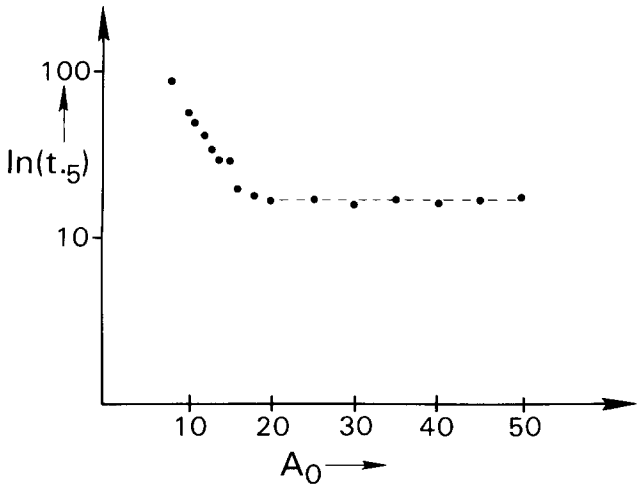

FIG. 3. A plot of ln ($t_{.5}$) versus A_0 where A_0 is the initial dose of abrin in nanograms/ml and $t_{.5}$ is the time it takes for the ^{3}H-TdR CPM in the EBV lymphocyte system to drop to one half the initial ^{3}H-TdR CPM.

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