tion curve relating peak area to the percentage of brucite in the sample. This curve was constructed from data obtained by the differential thermal analysis of artificial standards comprising mixtures of pure brucite and Iceland spar calcite. The results indicate between 4 and 5 percent of $Mg(OH)_2$ by weight. It appears to be the first recorded occurrence of this mineral in recent carbonate sediments. The distribution of brucite in natural, organic, high-magnesian calcites is currently under investigation with the use of the electron probe microanalyzer (*11*).

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1. Essentially, the dolomite problem involves the discrepancy between the existence of vast quantities of dolomite in ancient sedimentary carbonate rocks and the absence of any comparable sediments forming at the present time. This mineral has not been synthesized at temperatures and pressures of the marine environment, and the mechanism of dolomitization of pre-existing calcite sediments is very poorly understood. Ingerson maintains that the "problem of the origin of dolomite is one of the most fascinating as well as one of the most important in all of geochemistry or in sedimentary petrology." Reviews have been provided by P. Sonnenfeld, *Bull. Can. Petrol. Geol.* **12**, 101 (1964); E. Ingerson, *Geochim. Cosmochim. Acta* **26**, 815 (1962); and R. W. Fairbridge, *Soc. Econ. Paleontologists Mineralogists Spec. Publ. 5* (1957), p. 125.
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11. R. F. Schmalz and J. W. Kaufman, in preparation. We are indebted to Dr. R. F. Schmalz for stimulating discussion and for several samples of magnesian calcites.
12. The investigations described in this report and in the report by R. F. Schmalz (*Science*, this issue, p. 993) were carried out independently. Our experiments were completed in July 1964, and the manuscript was prepared in August 1964. The results summarized by Schmalz were obtained by experiments conducted over a period of several years at the several laboratories mentioned in the text, and they were prepared for publication during the spring of 1965. Both manuscripts were submitted for publication at the same time.

24 May 1965

Inhibition of L1210 Tumor Growth by Thymus DNA

Abstract. *Growth of L1210 leukemia cells which had been previously incubated with thymus DNA was inhibited. Leukemia-cell DNA did not affect tumor growth under similar conditions. Pretreatment of the thymus DNA with deoxyribonuclease suppressed the DNA-induced inhibition. Both ribonuclease-treated DNA and untreated DNA inhibited tumor growth.*

Penetration of mammalian cells by DNA has been observed in a number of laboratories (*1*). The genetic transformation of mammalian cells by mammalian DNA has been reported to occur in tissue culture (*2*, *3*). This report describes work undertaken to determine any effects of DNA on the growth of tumor cells in vivo.

Thymus DNA was isolated by the method of Szybalska and Szybalski (*2*) from male $B6D2F_1$ mice weighing 20 to 25 g; DNA was isolated in the same way from L1210 leukemia cells which had been maintained in the ascitic form. Previously established methods were followed whenever DNA was treated with deoxyribonuclease (*4*) and ribonuclease (*2*). Leukemia cells were washed at 3°C with a phosphate-buffered saline (*2*) until free of red blood cells. Cell viability was determined by eosin staining. The cells were suspended at a concentration of 2 to 8 $\times$ 10^6 viable cells per milliliter in 4 ml of phosphate-buffered saline containing $5.5 \times 10^{-3}M$ glucose and $2 \times 10^{-4}M$ spermine. The cell suspension was then shaken very slowly for 1 hour at 37°C. When DNA was added to the incubation medium, it was present at a final concentration of 2 to 8 $\times$ 10^2 μg/ml, as determined spectrophotometrically (*5*). The cells were centrifuged after incubation and were then suspended in fresh phosphate-buffered saline. About 250,000 viable leukemia cells in 0.1 ml were injected subcutaneously into the lower left ventral regions of the $B6D2F_1$ mice.

Tumors were scored as positive when they were first detected by palpation as tiny nodules. They were often verified by a second observer and were rechecked each day. The following conventions were adopted in order to measure the degree of inhibition of tumor growth by DNA. The "reciprocal of the latent period" (RLP) was obtained by dividing 100 by the day on which the tumor first appeared (*6*). Similarly, the "reciprocal of the survival period" (RSP) was obtained by dividing 100 by the day on which the animal died. Thus, an animal that did not develop a tumor had an RLP of zero and an RSP of zero.

In the experiment illustrated in Fig. 1, 59 mice were injected with leukemia cells that had previously been incubated with thymus DNA; 50 control mice were injected with cells incubated without DNA. Mice injected with DNA-treated cells developed tumors later, and survived longer, than mice injected with saline-treated cells. Approximately 19 percent of the mice survived free of tumor growth after injections of the DNA-treated cells, whereas none of the control mice survived after injections of the saline-treated cells.

Other data (Table 1) demonstrate that thymus DNA significantly depressed both the mean RLP and the mean RSP and thus delayed both the growth of tumor and the onset of death of the animals. Pretreatment of the thymus DNA with deoxyribonuclease eliminated the DNA-induced effects. We have subsequently found that ribonuclease treatment of thymus DNA does not interfere with the tumor inhibitory action of the DNA. As shown in Table 1, leukemia DNA did not affect the growth of the tumor cells.

These results indicate that native DNA per se was the biologically active substance in the thymus DNA preparations, since treatment of these preparations with deoxyribonuclease

Table 1. The effect of DNA on L1210 tumor growth. The values reported are means ± the standard error of the mean. Percentage changes from the control values are given in parentheses.

Number of mice injected	Treatment of L1210 cells	Mean reciprocal of the latent period	Mean reciprocal of the survival period
40	Buffer-saline	14.8 ± 0.4	7.3 ± 0.2
40	Thymus DNA	9.7 ± 0.4 (− 34)*	5.6 ± 0.3 (− 23)*
20	DNase†	14.8 ± 0.9 (0)	6.9 ± 0.4 (− 6)
20	DNase-treated thymus DNA	15.0 ± 0.5 (+ 1)	7.0 ± 0.4 (− 4)
30	Leukemia DNA	14.7 ± 0.5 (− 1)	7.3 ± 0.2 (0)

* $p < 0.001$. † Deoxyribonuclease.

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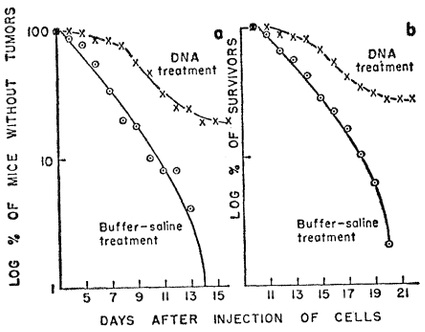

Fig. 1. (*a*) The delayed appearance of tumor nodules, and (*b*) the prolongation of life in mice given L1210 cells treated with thymus DNA.

abolished their inhibitory effect on tumor growth. The temperature denaturation curve for our DNA preparations was sharp and had a temperature midpoint at 86°C, as expected for native thymus DNA (*7*). Moreover, during the DNA isolation procedure, the DNA was extracted repeatedly in a chloroform-butanol mixture to remove contaminating protein, and ribonuclease was sometimes used to destroy any traces of RNA in the DNA preparation.

It is most unusual that a natural molecule such as thymus DNA could prevent the growth of the tumors in as many as 19 percent of the experimental animals. Even in those animals that did develop tumors after injection of DNA-treated cells, the DNA must have affected the vast majority of the cells for the following reasons. On the basis of two cell-generations per day for L1210 leukemia cells, tumors became palpable in about 14 cell-generations (7 days) in control animals and in about 20 cell-generations (10 days) in the experimental animals (Table 1). Let us assume that, when first detected, palpable tumors are composed of a relatively constant cell number. The number of cells which must have been altered by the DNA treatment can now be calculated. If $2^{14}N = 2^{20}(N - n)$, then $n = 98$ percent of N, where N is the total number of viable cells injected per animal, and n is the number of DNA-treated cells which do not form tumor.

Surprisingly, 98 percent of the cells are susceptible to the action of DNA. The average lag of 3 days in the appearance of tumors of DNA-treated cells in comparison with control cells can be accounted for by the survival of only 2 percent of the leukemia cells following DNA treatment. It is, of course, possible that DNA affected all the cells by temporarily delaying or permanently lengthening, the cell-generation time, but this would not readily explain how tumors were sometimes completely suppressed. More likely, any animals that survived free of tumors did so because most of the DNA-treated cells were no longer able to form tumors, and because the animals may have been injected with none or too few of the unaltered cells.

Apparently, thymus DNA did not merely destroy the leukemia cells during the incubation period, because cell viability, as determined by the eosin procedure, was unaffected by DNA. Eaton *et al.* (*4*) had previously demonstrated, by means of metabolic inhibitors and cell-dilution and cell-respiration techniques, that only nonviable ascites tumor cells were stained by eosin. In our experiments, only about 25 percent of the cells that had been incubated, with or without DNA, were permeable to eosin. Yet, the DNA-treated cells may have survived the incubation period only to succumb in the animal.

We strongly doubt that chromosomal genetic transformation would explain our results, since Szybalska and Szybalski (*2*) found that transformation for a specific genetic marker was less than 0.1 percent of the total number of mammalian cells exposed to DNA. However, the exogenous DNA may have acted either independently or together with some endogenous factor(s) in the cytoplasm or in the extrachromosomal portion of the nucleus in order to prevent the cells from forming tumor nodules.

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Focal Antibody Production by Transferred Spleen Cells in Irradiated Mice

Abstract. *Lethally irradiated mice were injected with small numbers of normal spleen cells and then immunized with sheep erythrocytes. Antibody activity was found in their spleens in localized areas whose number corresponded to the number of spleen cells injected. When sheep and pig erythrocytes were injected together, antibody against each was found in separate areas. Each area may consist of the progeny of a single precursor cell, restricted to forming a single antibody.*

Our understanding of lymphoid-cell differentiation would be advanced if the antibody-forming descendants of a single cell could be tested for their response to several antigens. In the absence of an ideal technique for studying antibody-forming cells in vitro, something may still be learned from their behavior under controlled conditions in vivo. We now report experiments in which normal (nonimmunized) lymphoid cells were injected into lethally irradiated syngeneic mice and then induced to make a primary response to foreign erythrocytes (*1*). By using small numbers of donor cells and studying the distribution of specific hemolysin in the recipient spleens, we have shown that the earliest production of antibody is localized in a few small highly active areas, whose number depends on the number of donor cells injected. These areas were found in the central white pulp of the spleen —that is, the normally lymphopoietic part—and not in the hemopoietic nodules (*2*), which do not contain lymphocytes or antibody (*3*). Lymphoid nodules of the type derived from phytohemagglutinin-stimulated donor cells (*4*) were not seen in our experiments.