24 hours will destroy all cells. A 2-hour exposure to a dose of 5 ppm is equally effective. It has been found that the product of minimum concentration and minimum time of exposure is a constant. This constant is the effective dose; under laboratory conditions, this is of the order of 10 ppm-hours for *Elodea*. The dosage rule also holds under field conditions, but the dose applied is usually larger, for obvious reasons. There also appears to be a temperature factor. Preliminary tests indicate a Q_{10} of 2.

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A Substance in Liver Which Inhibits Thymine Biosynthesis by Bone Marrow

Abstract. A substance was extracted from rabbit liver which inhibited the incorporation of formate into bone-marrow thymine in vitro. In view of the important role of thymine biosynthesis in cell division, it is suggested that the inhibitor present in liver is a naturally occurring mitosis inhibitor.

Some of the factors which control cell division have been considered in a recent review (*1*). It was pointed out that duplication of deoxyribonucleic acid (DNA) must occur in the cycle of cell division. It is obvious that in certain tissues there must exist some mechanism for control of mitotic rate. The presence of a mitotic inhibitor was suggested by the recent finding that the injection of liver homogenates from adult animals inhibited the mitotic rate of regenerating rat liver (*2*). In view of the importance of DNA in cell division it seemed possible that there might exist in tissues naturally occurring inhibitors of DNA biosynthesis which would then be mitosis inhibitors. This report presents experimental evidence for the occurrence of such an inhibitor (*3*).

The experimental procedure consisted of incubating rabbit bone-marrow homogenates or cell suspensions with sodium formate-C^{14} and measuring the incorporation of the formate into ribonucleic acid (RNA) and DNA. The effect of various rabbit-liver preparations on the incorporation of the radioformate into nucleic acids was then determined. Bone marrow from normal rabbits was homogenized with 4 volumes of Robinson's medium (*4*), or a suspension of marrow cells was prepared with the same relative quantity of buffer. One milliliter of the marrow preparation was incubated with the liver fraction for 20 minutes in a Dubnoff incubator at 37°C, 8 μc of sodium formate-C^{14} was added, and the incubation was continued for 2½ hours. The final volume of incubation mixture was 4 ml. The incubation mixture was then fractionated by a combined Schneider, Schmidt-Thannhauser procedure (*5*), and the incorporation of the formate into RNA and DNA was measured.

In the first experiments it was found that under these conditions bone-marrow homogenates actively incorporated formate into DNA, whereas liver homogenates did not. It was further found that when liver homogenates were added to the bone-marrow homogenates there was marked inhibition of DNA biosynthesis.

Efforts were then made to concentrate the factor in liver which inhibited DNA biosynthesis by bone marrow. Rabbit liver was homogenized with Robinson's medium, heated to boiling, and filtered. The filtrate was batch-treated with Dowex-1 exchange resin. The filtrate was then lyophilized, and the dry powder was extracted with 95-percent ethanol. The ethanol was evaporated, and the powder was dissolved in Robinson's medium and assayed for inhibition of bone-marrow nucleic acid biosynthesis. Typical results are given in Table 1. Six milligrams of this alcohol-soluble material quite markedly inhibited the incorporation of formate into DNA by marrow homogenates.

The next experiments were designed to determine whether the inhibition in DNA biosynthesis was due to inhibition in thymine biosynthesis. One milliliter of marrow cells was incubated with 10 μmole of deoxyuridine, 10 μmole of adenosinetriphosphate, and 50 μmole of sodium succinate. Other details of incubation were as previously described. At the termination of the incubation the reaction mixture was made 2*N* with perchloric acid and heated on a steam bath for 30 minutes. This treatment extracted DNA and hydrolyzed all thymine nucleotides (free thymine nucleotides and DNA thymidylic acid) to free thymine. After neutralization of the perchloric acid with KOH, carrier thymine was added and acetol osazone (*6*) formed and was counted. The results are given in Table 2. It may be seen that the liver fraction strongly inhibited the biosynthesis of thymine by bone marrow.

Table 1. Effect of liver fraction on the incorporation of formate-C^{14} into nucleic acids by bone-marrow homogenates.

Addition	Counts	
	RNA	DNA
None	272	398
Liver fraction (6 mg)	177	78
Inhibition (%)	35	80

Table 2. Effect of liver fraction on the incorporation of formate-C^{14} into thymine by bone-marrow cells.

Addition	Thymine counts
None	5105
Liver fraction (6 mg)	366
Inhibition (%)	92

These experiments demonstrate the presence of a substance in liver which inhibits thymine biosynthesis by bone-marrow cells. In view of the requirement for thymine biosynthesis in cell division it appears that this substance may be a naturally occurring mitosis inhibitor.

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