ing 0.5 μc of C^{14} (Nuclear) and crystallized bovine serum albumin as indicated (Armour). The *dl*-carnitine used was a gift from International Chemicals, Inc., Chicago, and was supplied as the hydrochloride. All flasks were incubated at 37°C for 30 min in air, after which 0.2 ml of 50-percent citric acid was added from a side arm to insure complete liberation of $C^{14}O_2$ in the medium. The $C^{14}O_2$ was trapped by alkali having filter paper immersed in the center well. The plating as $BaCO_3$ and the calculations employed were the same as those previously reported (*6*). The dry weight of muscle aliquots used in each flask was approximately 25 mg. The experiments reported are representative of six experiments performed in a similar fashion.

2 September 1958

Acrolein for the Control of Water Weeds and Disease-Carrying Water Snails

Abstract. Injection of the biocide acrolein into irrigation canals killed submersed weeds, thereby reducing flow resistance and increasing capacity. In a 20-mile canal, 150 gallons raised throughput from 311 to 552 ft³/sec. Acrolein also effectively eradicated aquatic snails; it promises to become a useful tool in the battle against *Schistosoma* blood flukes.

Irrigation is a blessing, for it turns deserts into productive lands, but there are certain drawbacks which must be overcome before the full benefits of irrigation can be realized. First, there is the menace of aquatic weeds, which, when left uncontrolled, soon render an irrigation system inoperative. Then, there is a menace to health, as several vectors of human and animal diseases are aquatic in habit. Various new irrigation systems in the tropics, for instance, introduce aquatic snails which are alternate hosts for a trematode worm (the blood fluke) which causes schistosomiasis (*1*).

Although ditch-bank weeds are difficult to control, submersed aquatic weeds constitute an even more difficult problem. It is the submersed weeds which cause the most trouble from the standpoint of reduced water flow in irrigation ditches (*2*). In the western United States alone farmers spend millions of dollars every year for the control of these weeds —commonly called "moss"—in canals and drains (*3*). Various mechanical methods—such as draining and drying, hand-cleaning, chaining, and dredging—for the control of submersed aquatic weeds in irrigation channels have been used extensively. In many situations, however, these methods have proved relatively inefficient or cumbersome, time-consuming, and expensive (*4*). Among chemical methods, use of copper sulfate was found to be effective on algae, but it was not generally effective on plants that were rooted in the bottom of the channel (*4*). Aromatic solvents such as crude xylenes gave effective control of submersed aquatic weeds but could be used economically only in smaller channels (up to 50 to 70 ft³ of water flow per second). Large amounts of solvent and emulsifier are required, and even when as much as 10 gal of solvent per cubic foot of water flow per second is applied in the channel, such an application is effective only for distances up to 5 mi without "booster" applications (*4*).

We have now developed a method, involving the use of a technical product the active ingredient of which is acrolein (CH_2:CH · CHO), which will control submersed aquatic weeds economically even in large canals (with water flow of 300 ft³/sec and over). Submersed weeds have been controlled as far as 15 to 20 mi below the point of application through the use of only 1 to 1.5 gal of acrolein per cubic foot of water flow per second over a period of 30 to 45 min. In less than 1 week after treatment the water-carrying capacity of such a large canal, 60 ft wide, nearly doubled, and the beneficial effect lasted for as long as 8 weeks before retreatment became necessary. Figure 1 shows the results of the water-weed control by acrolein in a large canal in Kern County, California (*5*). The major weed was the pond weed, *Potamogeton crispus*, but the chemical controlled all other submersed vegetation as well. After treatment the dead vegetation disintegrated and hence did not clog gates, weirs, and pumps, as happens after chaining (dragging). When deposited on the land, such masses of organic matter could be beneficial. Treated water, when used for irrigation, did not harm crops. Further studies, on possible acrolein residues in crops and on the toxicity of treated water with respect to farm animals, are being made.

Acrolein is a potent irritant and lachrymator, but in the hands of a skilled operator with proper application equipment it can be applied safely and without irritation or discomfort.

Acrolein readily forms a true solution in water and travels down the canal as a chemical wave. The location of the acrolein wave in the canal can be detected easily with a drop of potassium permanganate solution in a test tube. There is no loss of the chemical due to breaking of emulsions, as there is with aromatic solvents.

Acrolein has been found highly effective against water snails in the canals treated. Against adults of the pond snail *Lymnaea bulimoides,* and also against the local planorboid snail, *Helisoma* (Planorbis) *trivolvis,* it was found to be twice as effective, in laboratory tests in ½-gal jars, as sodium pentachlorophenate. The latter is commonly used for the control of bilharziasis (*6*). Against snail egg masses it was found to be more effective than copper sulfate, another agent commonly used as an aquatic molluscicide.

Acrolein is a potent sulfhydryl reagent and has been observed to destroy isolated enzyme systems. For instance, against urease it is nearly four times as toxic on a molar basis as ethyl maleimide, a common —SH reagent. Turgor in cells of the leaves of the water plant *Elodea densa* was maintained for a few hours after a dip in 1000 parts of acrolein per million, even though microscopic examination showed the cell interior to be destroyed. This observation leads to the conclusion that acrolein destroys —SH enzymes in the cytoplasm of the cell, unlike aromatic solvents, which act primarily on the plasma membrane.

Dosages required to kill *Elodea* leaf cells under laboratory conditions are small: 0.5 parts per million (ppm) for

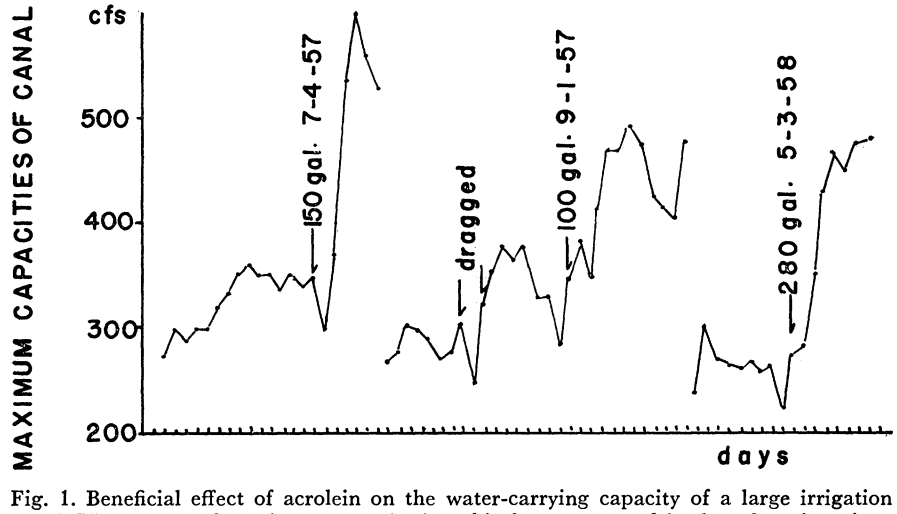

Fig. 1. Beneficial effect of acrolein on the water-carrying capacity of a large irrigation canal. The computed maximum capacity in cubic feet per second is plotted against time. The horizontal units are single days. The three trials reported refer to the same canal. The amount of acrolein and the date of treatment are indicated on each graph. The increase in flow is due to destruction of submersed water weeds by the chemical.

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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