

While males and females did not differ notably in the weight of fat, relative to the total weight the sex difference was marked. The percentage of fat was estimated as 23.7 for the females and 16.8 for the males: on the basis of these figures, the females were approximately half again as fat as the males. Again, since female subcutaneous fat thicknesses were generally greater, but total fat was not notably different, it follows that the sex difference in the proportion of outer and inner fat is considerable. Women carry more fat on and less in their smaller frames.

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#### References

1. A. R. Behnke, *Ann. N.Y. Acad. Sci.* 56, 1095 (1953).
2. S. M. Garn, *Human Biol.* 26, 59 (1954); *Science* 125, 550 (1957).
3. S. M. Garn, S. Selby, R. W. Young, *Arch. Dermatol. and Syphilol.* 70, 601 (1954).
4. S. M. Garn and R. V. Harper, *Human Biol.* 27, 39 (1955); S. M. Garn and L. C. Clark, Jr., *J. Appl. Physiol.* 8, 135 (1955).
5. C. C. Peters and W. R. Van Voorhis, *Statistical Procedures and Their Mathematical Bases* (McGraw-Hill, New York, 1940).

8 March 1957

### Chemical Protection against X-radiation Death in Primates: a Preliminary Report

The ability of a wide range of biochemically active compounds to offer protection to the animal organism against x-radiation death has prompted a great deal of investigation in this direction within the past few years. Such compounds as  $\beta$ -mercaptoethylamine, 2,3-dimercaptopropanol, S2,  $\beta$ -aminoethylisothiuronium  $\cdot$  Br  $\cdot$  HBr (AET), and numerous others have shown a remarkable degree of protection in mice (1).

Outstanding among these compounds is AET. This drug is known to provide 100-percent survival at 30 days against a dose of whole body x-radiation which is 100-percent lethal in untreated mice. It has also been shown to offer more effective protection to mice than does  $\beta$ -mercaptoethylamine on an equimolar basis (2).

Considering the increased interest in the prevention of radiation death and the high degree of protection afforded the lower animals by AET, it seemed mandatory that further studies should be carried out in primates. This is a preliminary report (3) of work in progress to determine the protective effect of this drug in the monkey.

AET in doses ranging from 100 to 400 mg/kg of body weight has been given intraperitoneally to *Macaca mulatta* monkeys prior to the administration of a dose

of whole-body x-radiation. At dose levels above 250 mg/kg, the toxicity of the drug is prohibitive when it is administered intraperitoneally as a single dose. However, the monkey can readily withstand 250 mg/kg in a single dose, if lower doses are administered over a period of a few days, and the doses are gradually increased from 100 to 250 mg/kg. A Westinghouse Quadrocondex 240-kv therapy machine with 1.0 mm Al plus 1.0 mm Cu filters was used for radiating the monkeys. At 240 kv, 15 ma, and a half-value layer (HVL) of 2.0 mm Cu, the machine delivers 13.25 r/min at 100-cm target distance. The animals were secured in a wooden chair which was rotated 4 times per minute in the x-ray beam.

Paterson (4), using *M. mulatta*, has found that 100 percent of the animals die as a result of 600 r of whole-body x-radiation administered in a single dose. The dose level of 650 r employed in this experiment, therefore, appears to be well above the  $LD_{100}$  and has resulted in the death of 100 percent of the untreated radiated control monkeys in this laboratory.

One animal was injected intraperitoneally on successive days with the following doses of AET: 100, 150, and 200 mg/kg of body weight. Three days after the 200-mg injection, the animal was given 250 mg/kg and was immediately radiated with 650 r of whole-body x-radiation. This animal is surviving at 280 days postirradiation and is apparently normal. A second monkey was injected intraperitoneally with 100 mg of AET per kilogram of body weight and 4 days later was given 150 mg/kg. Two days after the 150-mg injection, the animal received 200 mg/kg and was immediately radiated with 650 r of whole body x-radiation. This animal was surviving and apparently normal at 124 days when it was sacrificed for histological examination.

Peripheral blood studies of the two animals were indicative of the protective ability of the drug. By the fourth day postirradiation in both animals, the number of circulating blood cells was greatly reduced and remained at a low level until the 18th day. On the 18th day postirradiation, the circulating reticulocytes showed a dramatic steep increase in number, with an increase also in the number of circulating leucocytes. The increase in reticulocytes was followed in 4 to 6 days by a return of the hematocrit toward normal. The influx of reticulocytes began to subside by the 30th to 32nd days, and the entire peripheral blood picture had returned to normal by 65 days. In neither case did the peripheral blood picture reach the low levels observed in unprotected control animals that were irradiated at the same dose level.

These preliminary studies indicate that AET in doses of 200 to 250 mg/kg of body weight is capable of protecting the primate from x-radiation death when it is administered prior to irradiation. Expansion of this study is in progress both with regard to the toxicity of the drug and to its radioprotective ability.

*Note added in proof.* Since this paper was submitted, four monkeys have reached 30-day survival after having received 150 mg of AET per kilogram of body weight in a single dose prior to administration of 650 r of whole-body x-radiation. Peripheral blood studies of these four animals bear out the findings up to 30 days described in this report.

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#### References and Notes

1. D. G. Doherty, W. T. Burnett, Jr., R. Shapira, *Radiation Research*, in press; R. Shapira, D. G. Doherty, W. T. Burnett, Jr., *ibid.*, in press.
2. D. G. Doherty and W. T. Burnett, Jr., *Proc. Soc. Exptl. Biol. Med.* 89, 312 (1955).
3. This work was supported by the U.S. Atomic Energy Commission under contract No. AT-(40-1)-1642. The AET used in this study was supplied by D. G. Doherty of the Biology Division of Oak Ridge National Laboratory. We wish to acknowledge the assistance of David S. Carroll and the staff of the department of radiology of the City of Memphis Hospitals in making these results possible.
4. E. Paterson, *J. Fac. Radiologists London* 5, 189 (1954).

25 February 1957

### Control of Certain Forms of Zooplankton in Mass Algal Cultures

The most common difficulty experienced in growing phytoplankton on a large scale, in tanks of several-thousand-liter capacity, is the invasion of the cultures by various forms of zooplankton. In our cultures (1), the common offenders are crustaceans, especially the members of the subclass Copepoda. Upon entering cultures of such forms as *Chlorella*, these pests rapidly multiply to such an extent that they consume most of the plant cells, rendering the cultures worthless.

We have tried a number of measures to prevent contamination with zooplankton of open-air algal cultures or to free the cultures from these animals after they become established. However, this was usually impossible to achieve because some eggs, juveniles, or adults were either left behind or quickly reintroduced. Other workers (2) have reported contamination in their open-air algal cultures and also that attempts to exterminate the undesirable forms were practically unsuccessful.

During our recent efforts to develop chemical methods for controlling enemies of oysters and clams (3), we found several substances that are highly toxic to many aquatic arthropods, such as green and mud crabs, prawns, shrimps, and so forth, which are direct or indirect enemies of shellfish (4). We decided to try some of these substances to exterminate the crustaceans so often prevalent in algal cultures. Those selected were O,O-dimethyl-S-(4-oxo-benzotriazino-3-methyl) phosphorodithioate, O,O-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate, O,O-diethyl-O-*p*-nitrophenyl thiophosphate,  $\gamma$ -benzene hexachloride, and 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane, which are used in the manufacture of insecticides, and are known under the trade names of Guthion, Dipterex, Parathion, Lindane and DDT, respectively.

The experiments were conducted as follows: To each vessel containing 1000 ml of phytoplankton culture, consisting largely of *Chlorella*, *Chlamydomonas*, and other common forms, and also containing large numbers of crustaceans, chiefly copepods, solutions of the aforementioned chemicals were added to create concentrations of 1.0, 0.1, 0.05, and 0.01 ppm. In a 1.0-ppm solution of Guthion, all crustaceans were killed within 2 hours at room temperature. With a similar concentration of Dipterex, Parathion, Lindane, or DDT, this was achieved at the end of 8, 20, 22 and 46 hours, respectively. Complete mortality was also caused by 0.05 ppm of Guthion and Lindane at the end of 20 and 53 hours, respectively, while no appreciable mortality occurred in solutions of 0.01 ppm.

An aim of these experiments was to find a chemical which, when added to the infested algal culture, would kill the invaders but soon lose its toxicity. Thus, it would exert no ill effect on the mollusks or other forms for which we use our algal cultures as food. For this, the stability of the compounds mentioned was tested in concentrations of 1.0 and 0.1 ppm in pure sea water and in dense *Chlorella* cultures. Both solutions of Guthion were as toxic to copepods at the end of 5 days as they were at the initial testing. Solutions of Lindane and DDT also showed no loss of toxicity.

Dipterex, on the other hand, indicated that it has the desirable characteristic of losing its toxicity soon after it is added to algal cultures. Under this condition, it lost its lethal power after 3 days, whereas, when it was dissolved in pure sea water, it was still lethal to the crustaceans even after 20 days. Since Dipterex is relatively unstable in strongly alkaline media, it is probable that it disintegrated rapidly in algal cultures because the photosynthetic activities increased the pH to a high level. It may be, however,

that the decrease in toxicity of the Dipterex was the result of adsorption of the chemical by the large quantities of particulate organic matter present in the cultures. Commercial TEPP, containing 40 percent of tetraethyl pyrophosphate, which we found highly toxic to copepods but which, in solution, completely detoxifies within a few days, may sometimes be substituted for Dipterex.

During these experiments, our large culture tank, containing approximately 6000 liters of phytoplankton, became heavily infested with copepods. This gave us an opportunity to try the control measure on a large scale. A solution of Dipterex was added to the tank to make a concentration of 1.0 ppm. By the end of the second day, no living crustaceans remained in the tank.

Since our phytoplankton cultures are grown principally to provide food for mollusks and their larvae, it was necessary to determine the effect of the chemicals on the algal cultures themselves and also on the mollusks. Experiments showed that the algal cultures were not unfavorably affected even when the concentration of the chemicals in them was as high as 1.0 ppm.

In feeding the mollusks, one volume of our algal cultures is usually diluted with about 50 volumes of sea water. Therefore, the strongest concentration of the chemicals to which the mollusks or their larvae would be exposed would be approximately 1 part in 50 million. However, to be certain that exposure to the chemicals was relatively safe for the mollusks, we tried the same concentrations as those used to exterminate crustaceans.

Adult oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*, were kept for 2 days at room temperature in solutions of 1.0 and 0.1 ppm of Guthion, Dipterex, Parathion, Lindane, and DDT. In all these solutions the oysters behaved normally. Later, the oysters were returned to running water and kept under observation. Their behavior remained normal.

The mussels pumped continuously in both concentrations of Dipterex, Lindane, and DDT. However, in a 1.0-ppm solution of Guthion and Parathion, they pumped less actively than they did in 0.1 ppm. When they were returned to sea water, however, all were equally active and normal in their behavior.

In another series of experiments, several species of adult and juvenile bivalves, including *Crassostrea virginica*, *C. rhizophora*, *Ostrea edulis*, *Venus mercenaria* and *Mytilus edulis*, were kept for 1 month in running water to which a Dipterex-treated culture of *Chlorella* was continuously added. The ratio of the volume of *Chlorella* culture to sea water was approximately 1/50. During the period of exposure, all groups of mol-

lusks behaved normally, fed well, grew, and showed no unusual mortality.

The effects of the chemicals on the survival and growth of oyster larvae were ascertained by subjecting young, straight-hinged larvae, 2 days after fertilization, to solutions of 1 part of a chemical in 1, 20, and 40 million parts of sea water, thus creating concentrations of 1.0, 0.05, and 0.025 ppm. Observations on larvae subjected to these concentrations were continued for 14 days (5).

Larvae kept in 1.0 ppm of DDT all died within 4 days; in 0.05 ppm, the growth was almost completely stopped, and even in 0.025 ppm, the larvae did not grow well.

Larvae kept in concentrations of 1.0 ppm of Dipterex, Parathion, or Lindane did not suffer greater mortality than did the controls, but their growth was retarded. At the two lower concentrations, however, the larvae did not differ significantly in size from the controls.

The most promising results were obtained with Guthion. Even at a concentration of 1.0 ppm, it caused no appreciable mortality or retardation of growth of oyster larvae. Larvae kept in 0.05 ppm of Guthion actually grew faster than did those in untreated cultures.

The results of these experiments indicate that chemical control of crustaceans should greatly simplify maintenance of open-air cultures of phytoplankton. Some of the chemicals should be especially helpful in controlling crustaceans in large tanks and in small natural bodies of water intended for use as controlled sources of foods in our work on utilization of salt-water ponds for shellfish culture (6). It is believed that modifications of this method can be effectively employed to help grow *Chlorella* as a source of protein to be utilized in agriculture or by human beings, and also to control a copepod of the genus *Mytilicola*, which is a dangerous intestinal parasite affecting several species of oysters and mussels.

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#### References and Notes

1. V. L. Loosanoff, *Ecology* 32, 4 (1951).
2. J. S. Burlew, *Carnegie Inst. Wash. Publ.* 600 (1953).
3. We are indebted to Thaddeus Parr of Chemagro Corporation and to John Les Veaux of Niagara Chemical Division, Food Machinery and Chemical Corporation, for cooperating with us in certain aspects of these studies and for providing us with the necessary chemicals.
4. V. L. Loosanoff, J. E. Hanks, A. E. Ganaros, *Natl. Fisherman* 37, 11 (1956).
5. We are indebted to H. C. Davis of our laboratory for testing the effects of the chemicals on oyster larvae.
6. V. L. Loosanoff, *Ecology* 37, 3 (1956).

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18 February 1957