None of the second generation swine on 625 μc/day survived to produce a third generation. There have been 69 cases of hematopoietic disorders in swine ingesting ^{90}Sr—55 in the F_1 and F_2 animals and 14 in the original dams; there were two such disorders in the original control dams. Bone tumors have been seen in seven animals of those groups that received 125 and 625 μc/day. Progress reports on these facets of the experiment have been published previously (*7*, *8*).

Our purpose in this report is to emphasize the fact that, despite the high levels of ^{90}Sr fed and the abundant evidence of hematopoietic and carcinogenic effects, we have seen no evidence of an effect on fetal or neonatal mortality. From these studies we would infer that effects on fetal or neonatal mortality are not to be anticipated in human populations when mothers are exposed to ^{90}Sr, because ^{90}Sr intake levels high enough to affect fetal or neonatal mortality would not permit maternal survival long enough for the bearing of young. Since ^{90}Sr is a predominantly bone-seeking radionuclide, the effects will be manifest in bone and hematopoietic tissue in later life and will not affect fetal or neonatal mortality.

WILLIAM J. CLARKE
RAY F. PALMER
EDWIN B. HOWARD
PATRICIA L. HACKETT
Biology Department,
Pacific Northwest Laboratories,
Battelle Memorial Institute,
Richland, Washington 99352
JOHN M. THOMAS
Biology and *Mathematics Departments,*
Pacific Northwest Laboratories,
Battelle Memorial Institute

References and Notes

1. V. G. Horstman, W. J. Clarke, P. L. Hackett, M. E. Kerr, R. L. Persing, L. K. Bustad, in *Hanford Biological Research Annual Report for 1959, HW-65500* (Hanford Atomic Products Operation, Richland, Washington, 1960), p. 59.
2. R. O. McClellan and L. K. Bustad, *Ann. N.Y. Acad. Sci.* **111**, 793 (1964).
3. R. O. McClellan, *Health Phys.* **12**, 1362 (1966).
4. R. F. Palmer, J. M. Thomas, C. R. Watson, *Annual Report for 1968 in the Biological Sciences, BNWL-1050* (Battelle Memorial Inst., Pacific Northwest Laboratories, Richland, Washington, 1970); *Health Phys.*, in press.
5. *Recommendations of the International Commission on Radiological Protection,* ICRP Publ. 9 (Pergamon, New York, 1966).
6. *Report of U.N. Scientific Committee on the Effects of Atomic Radiation,* General Assembly Official Records, 19th Session, Supplement 14(A-5814), United Nations, New York (1964).
7. E. B. Howard, W. J. Clarke, M. T. Karagianes, R. F. Palmer, *Radiat. Res.* **39**, 594 (1969).
8. E. B. Howard and W. J. Clarke, *J. Nat. Cancer Inst.*, in press.
9. Supported under contract AT(45-1)-1830 with the AEC.

18 May 1970

Gibberellic Acid: A Growth Factor in the Unicellular Alga Gymnodinium breve

Abstract. *Gibberellic acid stimulates growth in the unicellular alga* Gymnodinium breve *(dinoflagellate). The maximum effect was obtained with 10^{-7} molar gibberellic acid, whereas concentrations greater than 5×10^{-7} mole per liter were inhibitory. The effect of the compound is observed as a marked shortening of the lag period, which is normally 6 to 8 days after innoculation.*

Gibberellic acid (GA) is a plant hormone which influences the activity of almost any plant tissue or organ. Responses include changes in growth rates and development of excised embryo, coleoptile, or primary leaf (*1*). The excised endosperm of cereals respond very quickly (1 day) to small quantities of GA. As little as 3×10^{-10} mole of GA per liter can cause release of reducing sugars from barley endosperm resulting from increased formation and secretion of α-amylase by aleurone cells surrounding the endosperm (*2*). At least part of the increased α-amylase activity is the result of de novo synthesis of the enzyme molecule (*3*). This synthesis is associated with synthesis of RNA after the addition of GA (*4*).

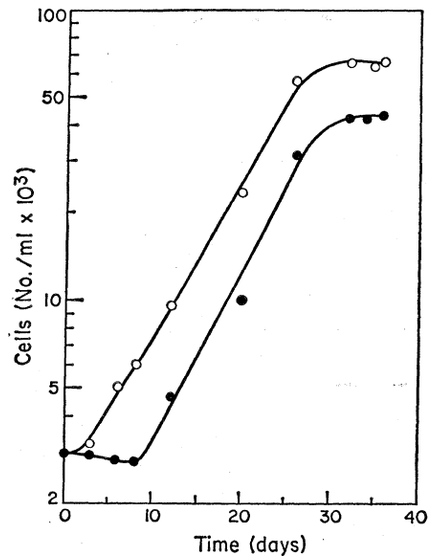

Fig. 1. Growth curves of *Gymnodinium breve* without GA (●) and with $10^{-7}M$ GA (○).

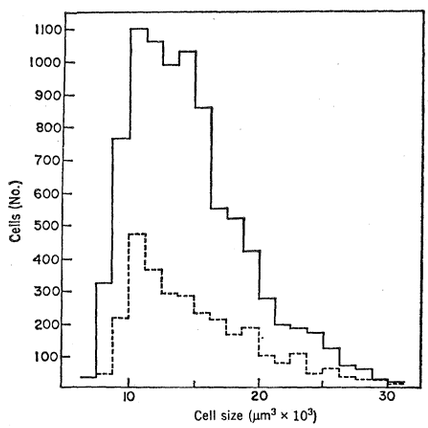

Fig. 2. Distribution of cell sizes of *Gymnodinium breve* recorded and plotted with automatic particle size distribution analyzer model J (Coulter Electronics Inc.). (Solid line) With $10^{-7}M$ GA; (dashed line) control. Histogram was plotted from cell sample after 28 days of growth.

There is little information on the effect of GA on either multicellular or unicellular algae. The cell size of *Euglena* is increased when GA is added at a concentration of 1000 mg/liter (*5*). In *Ulothrix subtilissima* 0.05 mg/liter gives a sevenfold increase in growth, whereas higher concentrations are inhibitory (*6*). In *Trichomonas foetus* 910 mg of GA per liter has an inhibitory effect, whereas lower concentrations have no effects (*7*).

We have found that GA has a marked effect on the growth of *Gymnodinium breve*, a toxigenic marine dinoflagellate. Periodically this organism increases in number and discolors coastal waters mainly along the eastern coast of the United States and the Gulf of Mexico (*8*), resulting in the phenomenon popularly known as Red Tide. There is ample evidence that an active compound produced by this organism is responsible for catastrophic mortalities of various marine animals (*9*), presumably through a neurotoxic effect (*10*).

While looking for optimum conditions for the growth of this organism, we found that GA (*11*) in low quantities induces rapid growth in axenic cultures. We used a synthetic medium containing inorganic salts, trace elements, and thiamin-HCl (1 mg/liter), biotin (0.5 μg/liter), and vitamin B_{12} (1 μg/liter). The cultures were grown at 18°C under continuous illumination.

The growth curve (Fig. 1) of this organism is normally characterized by a lag period of 6 to 8 days; in many instances cell numbers are even reduced

during this period. Addition of GA causes the lag phase to be shortened or to disappear completely. The final number of cells in the culture containing GA is doubled.

We calculated generation time from the slope of the logarithmic phase of the growth curve. Cell numbers were determined with a Coulter counter which also provided a histogram of cell sizes. The mean generation time of *G. breve* is 6.6 days. The mean generation time and cell size (Fig. 2) of experimental and control are not significantly different. Thus the main effect of GA appears to be elimination of the lag period and increase in the final yield of cells. The optimum concentration of GA causing this effect was 10^{-7} mole/liter, whereas higher concentrations (above 5×10^{-7} mole/liter) markedly inhibited the effect.

The use of *G. breve* as a homogenous unicellular cell population for studies on the mode of hormone action has several advantages over use of complicated multicellular plant systems with extensive morphological differentiation.

In nature *Gymnodinium breve* blooms periodically during the year, but blooms in the laboratory have been elusive. If GA can be used to effect algae blooms in the laboratory, we would suggest that the level of GA in seawater may be one of the causes for the bloom. A possible source of GA could be debris of higher plants washed to sea and marine algae, both of which contain GA in their tissues (*12*). The possibility exists that the effect of GA on *Gymnodinium breve* results from the same mechanism as that in higher plants (*13*); however, the mode of action is unknown.

ZVI PASTER*
BERNARD C. ABBOTT
Department of Biological Sciences, Allan Hancock Foundation, University of Southern California, Los Angeles 90007

References and Notes

1. L. S. Dure and W. A. Jensen, *Bot. Gaz.* **118**, 254 (1951); V. Raghaven and J. G. Torrey, *Plant Physiol.* **39**, 691 (1964); D. Boothby and S. T. C. Wright, *Nature* **196**, 389 (1962); F. C. Humphries and A. W. Wheeler, *J. Exp. Bot.* **11**, 81 (1960).
2. L. Paleg, *Plant Physiol.* **35**, 293 (1960); A. M. McLeod and A. S. Millar, *J. Inst. Brew.* **68**, 322 (1962); M. J. Chrispeels and J. E. Varner, *Plant Physiol.* **42**, 398, 1008 (1967).
3. P. Filner and J. E. Varner, *Proc. Nat. Acad. Sci. U.S.* **58**, 1520 (1967).
4. J. E. Varner and G. R. Chandra, *ibid.* **52**, 100 (1964).
5. D. N. Griffin, *Proc. Okla. Acad. Sci.* **38**, 14 (1958).
6. H. Conrad, P. Saltmann, R. Reepey, *Nature* **184**, 556 (1959).
7. C. Petroff, *Naturwissenschaften* **49**, 287 (1962).
8. G. Gunter, R. H. Williams, C. C. Danis, F. G. Smith, *Ecol. Monogr.* **18**, 309 (1948); W. B. Willson and S. M. Ray, *Ecology* **37**, 388 (1956).
9. S. M. Ray and D. V. Aldrich, *Science* **148**, 1748 (1965).
10. Experiments done in our laboratory with nerve-muscle preparations show a clear neurotoxic effect.
11. Gibberellic acid, potassium salt (Calbiochem, Los Angeles, California).
12. J. Kato, W. K. Purves, B. O. Phinney, *Nature* **196**, 687 (1962); J. A. Mowat, *ibid.* **200**, 453 (1963).
13. L. G. Paleg, *Annu. Rev. Plant Physiol.* **16**, 291 (1965).

* Present address: Department of Zoology, Tel-Aviv University, Tel-Aviv, Israel.

18 February 1970; revised 27 April 1970

Cell Surface Coatings and Membrane Potentials of Malignant and Nonmalignant Cells

Abstract. *A positive surface potential indicating a cell coating is common to malignant cells, lymphocytes, and normal and malignant trophoblastic cells. This characteristic was not generally found for other normal cell types tested by microelectrode penetration.*

Studies of electromobility show that malignant cells and trophoblastic cells of the placenta possess a highly charged surface constituent (*1*). This predominate surface negativity is attributed primarily to sialic acid moieties of mucopolysaccharides on the cell exterior. Treatment of the sialic acid groups with neuraminidase causes reduction of the characteristically high electromobility rates of these cells to near the rates for normal cells (*2*). Lymphocytes also possess a high negative surface charge (*1*, *3*). The distortion of normal cell interaction due to a similar surface charge on malignant cells, trophoblast, and lymphocytes may mediate the lack of immune rejection of trophoblastic cells during implantation in pregnancy and of malignant cells in cancer. On the other hand, the lack of such a coating and charge characteristic on transplanted cells of normal organs would permit the expected cellular reaction and rejection.

We have studied the electrophysiological properties of representative malignant cells and compared them to those of normal cells. The finding of an electrically charged sialomucin cell coating (similar to placental fibrinoid) on the surface of cancer cells and normal and malignant trophoblastic cells suggested an investigation to determine whether these surface characteristics could be detected by penetration of single cells and bioelectrical recordings (*4*).

The various types of cells under investigation were cultured on glass cover slips and studied by microscopic observation in special holding chambers used to maintain the test cells during experimentation. The cells were immersed in tissue culture medium kept at 36°C and adjusted to the appropriate *p*H with a variable mixture of air and carbon dioxide above the medium. Microelectrodes were used to impale single cells for electrical recordings (*5*) and were found acceptable when impedance was 20 megohms or above, a value indicating an electrode tip diameter under 0.5 μm (*6*).

We first studied transmembrane potential of the malignant trophoblastic cell (BeWo) in vitro (Fig. 1). This cell line, derived from a malignant tumor of the placenta following normal pregnancy, was transplanted and maintained in the hamster cheek pouch (*7*) and later established in continuous cell culture (*8*). It performs all of the normal functions of the placental trophoblast thus far tested (*9*).

Impalement of the malignant trophoblast revealed potentials which demonstrated a distinct positive deflection preceding the normal transmembrane potential (prepotential) (Fig. 1, right). A surface constituent producing this positive deflection was therefore implicated. The magnitude of the positive prepotential ranged between 1 and 8 mv and was dependent on several factors, most notably *p*H. The higher magnitudes (5 to 8 mv) were recorded at *p*H 8, lower values (1 to 2 mv) between *p*H 7 and *p*H 6. All malignant cells studied—cervical cancer (HeLa), human laryngeal carcinoma (HEp2 line), choriocarcinoma (BeWo line), rat sarcoma (256 and L cells)—uniformly demonstrated this characteristic. These properties were also found in normal human trophoblast and lymphocytes, whereas normal kidney, embryonic heart, and lung cells rarely gave prepotential records (Table 1).

Thus the prepotential appears to be