growth temperature range showed an inability to grow above 33°C. Thus, the psychrophilic transductants resembled psychrophilic mutants in the shift of their growth temperature maxima and minima to that characteristic of wild-type psychrophilic pseudomonads of the soil. Cotransduction of tryptophan independence shown here may be trivial to the causal basis for psychrophily. However, this observation indicates that the involvement of many scattered loci as contributing to psychrophily is unlikely. From these and the mutagenesis experiments, it would seem that the growth temperature range may be prescribed by a limited number of genetic loci or a closely linked constellation of characters.

Hence, of fundamental importance is the relationship of these findings to causal factors responsible for the growth temperature range of these organisms. Although it has been repeatedly shown that psychrophiles have temperature-sensitive enzymes limiting growth much above 32° to 33°C (*8*), it seems unlikely that either mutagenesis by ultraviolet irradiation or transduction would change the total enzymic makeup of the cell sufficiently to promote psychrophily, if psychrophily were dependent on a whole array of temperature-sensitive enzymes. Consequently, it may be that, throughout the evolution of these species, mutation toward thermal sensitivity may have occurred and been maintained in the absence of counterselection at warm temperatures not prevalent in the usual environment of psychrophilic bacteria. Thus, psychrophily and perhaps the delineation of the growth temperature range of mesophilic bacteria may reflect the temperature response of the products of a limited number of genetic loci whose primary function is the regulation of cell division in response to temperature fluctuations.

RONALD H. OLSEN
ELEANOR S. METCALF
Department of Microbiology, University of Michigan, Ann Arbor

References and Notes

1. G. A. O'Donovan and J. L. Ingraham, *Proc. Nat. Acad. Sci. U.S.* **54**, 451 (1965).
2. Y. Azuma, S. B. Newton, L. D. Witter, *J. Dairy Sci.* **45**, 1529 (1962).
3. R. H. Olsen, E. S. Metcalf, J. K. Todd, *J. Virol.* **2**, 357 (1968).
4. R. H. Olsen and J. J. Jezeski, *J. Bacteriol.* **89**, 429 (1963); J. L. Ingraham, *ibid.* **76**, 75 (1958).
5. We have used bacteriophage PX4 to transduce isoleucine plus valine (ilv) or leucine (leu) independence in addition to tryptophan (try) independence and psychrophily (psy), referred to in this paper. The transduction frequency for ilv or leu independence was approximately the same as for try independence or psy. However, cotransduction of ilv and psy or leu and psy was less than 1 percent when tested as described for try and psy cotransduction. Some (two- to fivefold) stimulation in the transduction of ilv after irradiation of phage suspension sufficient to cause a 100-fold decrease in phage titer occurred. However, leu, try, or psy transduction frequencies decreased in parallel fashion with the number of surviving lytic phage particles as the ultraviolet exposure time was lengthened.
6. B. W. Holloway and P. Van De Putte, *Nature* **217**, 460 (1968); A. M. Chakrabarty, C. F. Gunsalus, I. C. Gunsalus, *Proc. Nat. Acad. Sci. U.S.* **60**, 168 (1968).
7. H. J. Vogel and D. M. Bonner, *J. Biol. Chem.* **218**, 97 (1956).
8. J. L. Ingraham and J. L. Stokes, *Bacteriol. Rev.* **23**, 97 (1959); R. Y. Morita, *Ann. Rev. Oceanogr. Mar. Biol.* **4**, 105 (1966).
9. Supported by NIH grant AI-07533 and NSF grant GB-693.

25 July 1968; revised 30 September 1968

Dictyostelium discoideum: A Gamma-Ray Resistant Organism

Abstract. *The vegetative cells of the slime mold* Dictyostelium discoideum *are extremely resistant to cobalt-60 gamma rays. The survival curve has a shoulder at low doses and a 10 percent survival dose of 300 kilorads in air. Dose fractionation experiments indicate that repair of sublethal damage is an important factor in this resistance.*

The cellular slime mold *Dictyostelium discoideum* is an excellent model system for studying the molecular and cellular mechanisms associated with development and differentiation (*1*).

Vegetative amoeboid cells of *Dictyostelium discoideum* NC-4, a stable haploid strain (*2*), were grown in an aerated liquid suspension of *Escherichia coli* B/r (10^{10} cell/ml) in tris-saline solution (TSS) (0.01*M* NaCl; 0.01*M* KCl; 0.04*M* tris, *p*H 7) at 23°C (*3*). The cell doubling time was 2.5 hours. At 10^6 cell/ml (exponential growth phase), the suspensions were cooled to 0°C and irradiated with Co^{60} gamma rays (Gammacell-200) at a dose rate of 13.1 krad/min with aeration. Irradiating in the presence or absence of the bacteria did not substantially alter the results. After irradiation, the cells were either immediately diluted in TSS for plating or held for further treatment. The nutrient plates (*4*) were spread with 0.1 ml of the diluted *D. discoideum* suspension along with 0.1 ml of an unirradiated *E. coli* B/r suspension (5×10^9 cell/ml). As each deposited viable cell multiplied, it consumed the surrounding bacteria and after many divisions gave a large clear colony or "plaque" in the bacterial lawn. The plates were incubated at 23°C and counted at 2, 3, 4, and 5 days, after which no new plaques appeared. For dose fractionation experiments, irradiated suspensions were aerated at 23°C for various times in the presence of *E. coli* (10^{10} cell/ml, generally irradiated with *D. discoideum*) and then irradiated again.

Figure 1 ("0 hr" curve) gives the percent survival of plaque-forming ability as a function of unfractionated gamma ray dose. A threshold dose of about 200 krad is needed before survival swings sharply downward, becoming exponential at higher doses (*5*) (Fig. 1). These cells are extremely gamma-ray resistant compared to most other organisms (*6–10*) and in fact rank with some of the most radiation-resistant organisms known, including *Micrococcus radiodurans* (*11*), some protozoa (*12*), and a Hartmannellid amoeba (*13*). Although a shoulder exists on the survival curve, the initial slope is not zero, possibly indicating a heterogeneous population with respect to extent of shoulder (*6*). This may be the consequence of irradiating a non-

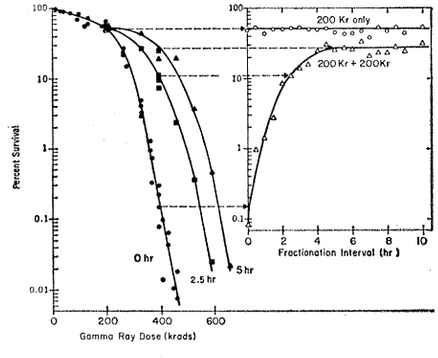

Fig. 1. Percentage survival of colony-forming ability of *Dictyostelium discoideum* vegetative cells as a function of total gamma ray dose in kilorads. Zero-hour curve (—●—): unfractionated doses; 2.5-hour (—■—) and 5-hour (—▲—) curves: obtained by initially irradiating with 200 krad, then incubating the suspension for 2.5 or 5 hours before the remainder of the dose was given. Inset: Survival as a function of time of incubation at 23°C with aeration before plating after a single dose of 200 krad (—○—); and recovery of survival as a function of incubation time between an initial dose of 200 krad and a later dose of 200 krad (—△—).

synchronous population. The rate of inactivation at high doses is not particularly unusual and compares favorably with many other organisms not exhibiting such an extensive shoulder at low dose.

Growth and plating of the *D. discoideum* cells on *E. coli* B_{s-1} (more radiation-sensitive than *E. coli* B/r) did not alter the survival curve as it might have if repair enzymes of *E. coli* B/r had been ingested intact into the slime mold cells.

Hemocytometer counts of aerated liquid suspensions after irradiation indicated an absolute cessation of cell division followed by a sharp return to an almost normal rate of division. The duration of the division lag was linear with dose up to at least 120 krad where it was 6.3 hours. This division delay did not depend on whether the slime mold cells were grown on unirradiated or irradiated bacteria (up to 800 krad). Growth of control *D. discoideum* cells occurred normally in liquid suspensions of bacteria given doses of 800 krad, with only a small effect for doses as high as 3.2 Mrads.

Irradiation in the presence of nitrogen rather than air increased the resistance of *D. discoideum* even further, giving a 10 percent survival dose of 500 krad (*14*).

The presence of an extensive low dose shoulder and the well-defined division lag followed by resumed growth are strong indications of a mechanism for repairing sublethal damage (*6, 8, 9, 12*). Dose fractionation experiments further elaborate such mechanisms. During an incubation period of 2.5 or 5 hours between doses (Fig. 1) the cells recover the ability to cope with additional radiation and the shoulder of the survival curve reappears, in the sense that the 5-hour curve superimposes on the 0-hour curve if translated to 100 percent survival and zero dose. This response is typical of dose fractionation experiments with organisms capable of repairing sublethal damage (*6, 8, 9, 12*). The recovery is virtually complete by 5 hours for this dose combination (Fig. 1, inset). No change in survival after a single dose of 200 krad is observed up to 10 hours of incubation. For the fractionated doses, once recovery has reached its maximum at 5 hours, it does not change further up to at least 10 hours. An effect from sensitivity changes due to progression through the cell-division cycle is not observed as has been shown for some other systems (*6, 8*). Other fractionation survival curves done after initial doses of 120 krad and 275 krad were analogous.

The capacity to repair ionizing radiation damage is an important feature of the radiation response of vegetative cells of *D. discoideum*, and a knowledge of the repair processes will be essential in order to understand their metabolic capacities.

R. A. DEERING

Biophysics Department,
Pennsylvania State University,
University Park 16802

References and Notes

1. J. T. Bonner, *The Cellular Slime Molds*, (Princeton Univ. Press, Princeton, N.J., ed. 2, 1967).
2. Kindly supplied by M. Sussman, Dept. of Biology, Brandeis University; M. Sussman and R. R. Sussman, *J. Gen. Microbiol.* **28**, 417 (1962).
3. H. R. Hohl and K. B. Raper, *J. Bacteriol.* **85**, 191 (1963); R. L. Dehaan, *J. Embryol. Exp. Morphol.* **7**, 335 (1959).
4. Per liter: Bacto-peptone, 10 g; dextrose, 2 g; KH_2PO_4, 1.45 g; $Na_2HPO_4 \cdot 12\ H_2O$, 0.96 g; agar, 20 g. Modified from (*1*), p. 83.
5. This resistance was first found in preliminary experiments in this laboratory by J. O. Freim, Jr.
6. M. M. Elkind and G. F. Whitmore, *The Radiobiology of Cultured Mammalian Cells* (Gordon and Breach, New York, 1967).
7. H. I. Adler, *Advan. Radiat. Biol.* **2**, 167 (1966).
8. S. Bacchetti, R. Elli, R. Falchetti, F. Mauro, A. Sacchi, *Int. J. Radiat. Biol.* **10**, 213 (1966).
9. D. R. Davies, *Radiat. Res.* **29**, 222 (1966).
10. R. A. Deering, *ibid.* **34**, 87 (1966).
11. C. J. Dean and P. Alexander, *Nature* **196**, 1324 (1962).
12. J. Calkins, *Radiat. Res.* **26**, 124 (1965).
13. J. B. Little and R. S. Chang, *ibid.* **35**, 132 (1968).
14. Experiments by A. Khoury, this laboratory.
15. I thank B. Thompson and M. Staufer for technical assistance. Supported by Biomedical Sciences support grant SO5-FR-07082-02.

9 August 1968; revised 21 October 1968

Morphine Tolerance, Physical Dependence, and Synthesis of Brain 5-Hydroxytryptamine

Abstract. *Tolerance and physical dependence development to morphine in mice can be prevented by concomitant administration of cycloheximide. The fact that the rate of synthesis of brain 5-hydroxytryptamine (5HT) increases with tolerance to morphine suggests that the protein involved may be associated with 5HT synthesis. Inhibition of this synthesis with* p*-chlorophenylalanine markedly decreases tolerance and physical dependence development to morphine.*

The development of tolerance with chronic administration of morphine is generally accompanied by the development of physical dependence. Indeed, the intimate relation between physical dependence and tolerance has led many investigators to believe that a common underlying or closely related mechanism operates within the matrix of the central nervous system (*1*). It is important to distinguish between central receptor tolerance, which might be linked to physical dependence and nonrelated mechanisms that prevent access of morphine to the locus of action (*2*).

It has been proposed that tolerance and physical dependence to a pharmacologic agent are the consequences of drug alterations of the steady-state level of the receptor enzyme, and that tolerance and dependence may be the consequence of repression and derepression of enzyme synthesis (*3*). If tolerance to morphine at the central receptor is linked to enzyme synthesis, it should be possible to prevent tolerance development with inhibitors of protein synthesis, and such studies have been reported (*4*). Since the effects of protein inhibitors are widespread, the task remains to select the possible reactions or enzymes that might be involved in interaction with morphine. Alterations in demethylating enzyme activity have been proposed (*5*), but numerous arguments have been invoked to challenge this concept (*2*). As an alternative, it appears logical to consider those reactions or enzymes associated with the biogenic amines in the central nervous system.

The relation of catecholamines to morphine has been extensively studied (*6*) and, to a lesser degree, that of acetylcholine (*7*). While changes in the concentrations of these substances in the brain occur with development of morphine tolerance, the changes have not been uniform or dramatic and do not occur consistently from species to species. The change in amount of 5-hydroxytryptamine (serotonin or 5HT) in the brain after repeated morphinization has been only cursorily examined. Several laboratories have reported that levels of 5HT in the brain remain unchanged after long-term morphinization (*8*). Since such measurements reflect essentially the steady-state level of brain 5HT resulting from equal