giophore first recoils (Figs. 1, 2, and 4) and then collapses (Figs. 3 and 5). In some cases the subsporangial swelling appears to contract irregularly (Fig. 4), but in others it maintains its general shape even after it has been thrown to the surface of the medium (Fig. 3*b*, arrow).

It is not clear whether the liquid that constitutes the jet comes entirely from the subsporangial swelling. The appearance of some cylindrical jets, and Buller's observation (*2*) that the orange protoplasmic ring from the base of the subsporangial swelling may sometimes be found among discharged sporangia adhering to a target, suggest that the liquid may come, at least in part, from the stipe of the sporangiophore. However, two lines of evidence suggest that the liquid comes mostly from the subsporangial swelling. First, it is difficult to see how a tapered jet could be formed by the stipe, and second, the crimp apparent in the stipes of some sporangiophores during early stages of discharge (Figs. 2 and 4) would seem to prevent a rapid movement of liquid up the stipe. Moreover, the volume of the subsporangial swelling is more than adequate to accommodate all of the liquid in even a long jet. The volume of the subsporangial swelling in Fig. 3, for example, is approximately 0.08 mm^3, but the total volume of the jet and visible droplets is less than 0.06 mm^3.

The photographs not only confirm Buller's deduction (*2*) that the sporangium of *Pilobolus* is propelled by a jet of cell sap which breaks into droplets, but they also reveal unsuspected details of the behavior of the jet, the sporangium, and the sporangiophore during discharge.

ROBERT M. PAGE
Department of Biological Sciences,
Stanford University,
Stanford, California

References and Notes

1. R. M. Page, *Science* **138**, 1238 (1962).
2. A. H. R. Buller, *Researches on Fungi* (Longmans, Green, New York, 1934; Hafner, New York, 1958), vol. 6.
3. C. T. Ingold, *Spore Discharge in Land Plants* (Clarendon Press, Oxford, 1939); *Dispersal in Fungi* (Clarendon Press, Oxford, 1953).
4. R. M. Page and D. Kennedy, *Mycologia* **56**, 363 (1964).
5. This equipment was designed and constructed by M. O. Roberts to whom I am also indebted for advice and assistance.
6. I am grateful to H. E. Edgerton for suggesting the use of this unit and for other counsel.
7. E. G. Pringsheim and V. Czurda, *Jahrb. Wiss. Botan.* **66**, 863 (1927).
8. This study was begun in the laboratory of J. R. Raper, Harvard University. It is a pleasure to acknowledge his hospitality.

11 September 1964

Ionizing Radiation: Effect on Genetic Transcription

Abstract. *Cells of* Escherichia coli *grown on maltose can be induced by the addition of thiomethyl galactoside to produce β-galactosidase. If cells are irradiated shortly after induction, the transcription of the DNA ceases, and the enzyme produced by the messenger RNA is observed to reach a maximum. From these data the calculated half-life of unstable messenger RNA is given over a temperature range from 8.1 minutes at 10°C to 0.7 minute at 45°C. The kinetics of cessation of transcription give information on both messenger RNA decay and rate of transcription. Arrhenius graphs for both these rates are given, and the activation energies measured are 11,000 calories per mole for decay and 22,000 calories per mole for transcription. This relation to temperature is characteristic of enzymatic behavior.*

It has been suggested that one important action of ionizing radiation is concerned with the transcription of the genetic message from DNA to RNA (*1*). Billen and Lichtstein (*2*) studied the effect of ionizing radiation on formic hydrogen lyase and Clayton and Adler (*3*) showed that induced catalase synthesis in *Rhodopseudomonas spheriodes* is inhibited by low doses of rays, giving experimental support to the idea. Pollard and Vogler (*4*), using *Escherichia coli* cells in which the process of induction was dependent on both permease induction as well as the measured enzyme induction, showed that there is some sensitivity to radiation. Novelli (*5*) found a reduced sensitivity as compared with colony formation, but still a considerable sensitivity.

The process of induction of an enzyme has been thoroughly studied (*5–10*), particularly by Pardee and Prestidge (*6*), Boezi and Cowie (*7*), Nakada and Magasanik (*8*), Levinthal, Keynan, and Higa (*9*), and Kepes (*10*). Their work, which supports the well known suggestion by Monod, Jacob, and Gros (*11, 12*), indicates that the transcription of the genetic message is repressed by something which can be acted on by a small molecule, the inducer, to remove repression and permit the formation of messenger RNA which then acts to make the enzyme. The messenger RNA undergoes decay, by a process which is still not clear. Very elegant measurements by Kepes (*10*) show that for the messenger RNA for β-galactosidase the half-life is 1.02 minutes at 37°C and 2.05 minutes at 25°C. The time of onset of the enzyme activity after induction was about 3 minutes.

If the process of transcription is indeed sensitive to ionizing radiation, then the irradiation of cells which have just been induced should show development of the enzyme to the extent of formation of new messenger RNA for a few minutes until transcription stops and should show the formation of the enzyme while the messenger RNA is decaying. This pattern was found by Clayton and Adler (*3*). Our experiments now amplify and extend their work, and also permit some measurements of the half-life of the messenger RNA, which are in agreement with the work of Kepes. The experimental procedure is as follows. Cells of either *Escherichia coli* B or *E. coli* 15 Thy^-Leu^- are grown in minimal medium with maltose as a carbon source. This does not repress the formation of enzyme, nor does it induce it. When the cells are at a concentration between 5×10^7 and 1.0×10^8 per milliliter they are induced by the addition of 1 ml of thiomethyl galactoside (TMG) (0.2 g/100 ml) to 20 ml cells. The concentration of cells at irradiation must be kept relatively low. At higher concentrations the cells are much less sensitive for reasons not yet wholly clear, but partly the cause is

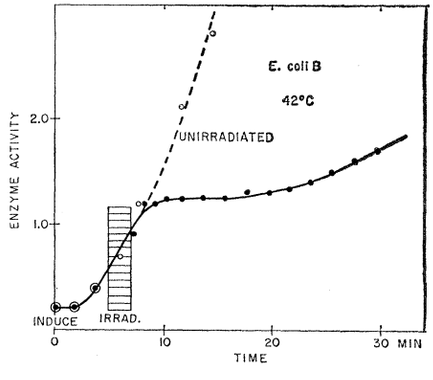

Fig. 1. A culture of *E. coli*, strain B, previously grown on maltose was induced with TMG at time zero. It was then allowed to grow at 42°C and irradiated in a Co^{60} source for 2¼ minutes, with a dose of 13,500 r. The amount of β-galactosidase in 1 ml of the culture was measured at various times for this culture and an unirradiated control. The production of enzyme continues for a short while after irradiation and then ceases. From the kinetics of cessation of production the half-life of messenger RNA can be found. Later, presumably as new DNA synthesis starts, the supply of enzyme begins to increase.

Table 1. Rate of decay of messenger RNA (k_1) and rate of transcription of DNA; a, molecules of messenger per cell per minute at different temperatures.

Temp. (°C)	Temp. ($10^{-3} \times 1/K°$)	Half-life (min)	Decay constant, k_1 (min^{-1})	a (molecules per cell)
10	3.45	8.1	0.085	9.1×10^{-3}
17	3.45	5.7	.12	6.8×10^{-3}
20	3.42	3.8	.18	1.8×10^{-2}
25	3.35	1.9*	.36	0.28
30	3.31†	2.7‡	.26	.63
37	3.23	0.80†	.86	4.2
42	3.18	0.80	.86	2.9
45	3.14	0.70	1.0	13.5

* Compare 2.05, Kepes (*10*). † Compare Kepes, 1.05 (*10*) and at 33.5°C, 1.4 minutes, Levinthal *et al.* (*9*), *B. subtilis*. ‡ Compare 2.5, Nakada and Magasanik (*8*).

related to a strong effect of dissolved oxygen and partly to the formation of peroxides of long life in the medium. A few minutes after induction (the time depends on the temperature) the cells are irradiated in a Co^{60} source, the dose being about 13,500 r which takes 2¼ minutes. In the meantime 1-ml samples were taken at about 2-minute intervals, both from the irradiated and a nonirradiated culture, and assayed for β-galactosidase activity. This was done by putting the 1-ml sample into 4 ml of ice-cold distilled water containing 1 drop of toluene and 1 drop of detergent (Sarkosyl, 2 percent). The samples were shaken vigorously at intervals for 1 hour, and then placed in a waterbath at 34°C. Then *o*-nitrophenyl-β-D-galactopyranoside was added to each tube, and the yellow color was allowed to develop until suitable for reading, the time of assay being recorded. The reaction was stopped with 0.5*M* sodium carbonate, and the percentage transmission was read on a Bausch and Lomb spectronic spectrophotometer at 420 mμ. A calibration curve with known relative amounts of enzyme is used to derive arbitrary units of enzyme activity.

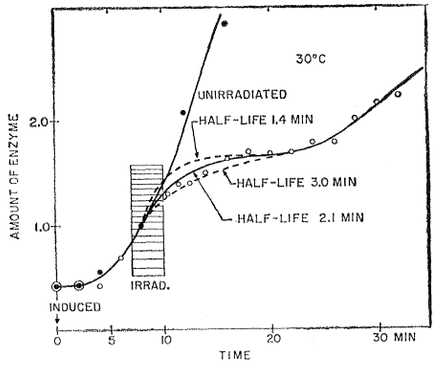

Fig. 2. Data similar to Fig. 1 with the temperature at 30°C. The three curves through the irradiated sample points are theoretical curves for three different half-lives of messenger RNA.

Figure 1 shows the results of one experiment. The formation of messenger RNA continues for about 2 minutes after the mid-time of the irradiation and then ceases. The decay of the messenger is indicated by the slowing down to a stop of the production of enzyme, and a reduced rate of synthesis is seen to follow later. This may be due to newly synthesized DNA. In Fig. 2 the same kind of data taken at 30°C is shown.

The presence of oxygen in the culture greatly increased the sensitivity of the process of transcription to the damaging effect of radiation. Cells irradiated at a concentration of 5×10^8 cells per milliliter, where respiration rapidly removes the dissolved oxygen, show a much reduced effect, in which there is no cessation of synthesis of enzyme, but a short linear period of synthesis, followed by a second steady increase. In our experiments the sensitivity was kept high by bubbling the oxygen through the cell suspension before irradiation, as well as by keeping the cell concentration low.

The kinetics of development of enzyme can be analyzed as follows. Denote enzyme produced by E, messenger RNA by R. Let a be the rate of formation of messenger RNA per minute, k_1 the decay constant per minute, and b the number of (arbitrary) enzyme units produced per minute per molecule of messenger RNA. Then

$$\frac{dE}{dt} = bR \qquad (1)$$

$$\frac{dR}{dt} = a - k_1R, \; t < t_0 \qquad (2)$$

$$\frac{dR}{dt} = -k_1R, \; t > t_0 \qquad (3)$$

from which it follows that

$$\frac{d^2E}{dt^2} = ba - k_1\frac{dE}{dt}, \; t < t_0 \qquad (4)$$

$$\ln \frac{E_0 - E}{E_0} = -k_1t, \; t > t_0 \qquad (5)$$

t_0 is the time at which transcription is assumed to stop because of the action of ionizing radiation and E_0 is the maximum amount of enzyme produced.

From Eq. 5 it follows that a graph of the amount of enzyme short of the plateau figure, $(E_0 - E)$, plotted against time, on a logarithmic scale (Fig. 3), should yield a straight line, and from it the half-life of the messenger RNA could be calculated. This is the method used by Kepes. The half-lives and decay constants derived in this way from a number of experiments are given in Table 1 together with data appropriate to other messenger decay studies. The agreement with Kepes is reasonable, and the consistency of all the data adds weight to the general idea that radiation has inhibited transcription in somewhat like the same way that the removal of inducer does, as shown by Kepes. The mechanism suggested for these experiments is totally different; only the result is the same.

From the figures one can also derive two other sets of numbers. The first is the time taken to halt transcription. This is measured from the start of irradiation to the time at which the irradiated culture deviates from the control. When the full radiation sensitivity is operating the time is remarkably short, often less than 1 minute and rarely exceeding 5. It seems to depend more on the conditions of irradiation, such as good oxygenation, than on the temperature.

The second numbers are the rates of transcription at different temperatures. From Eq. 4 we see that when the rate of formation of enzyme $(dE/dt)m$ is

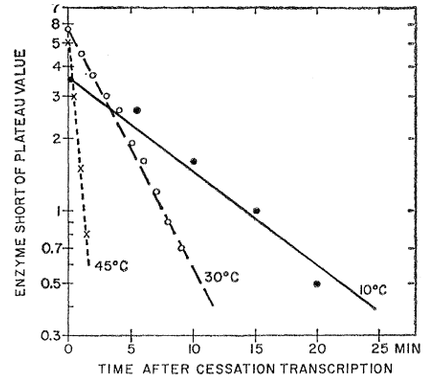

Fig. 3. The amount of enzyme short of the plateau value is plotted against time on a semilogarithmic scale. The amounts are read off the curves through data similar to Figs. 1 and 2. The decay character is clearly visible, and the half-life of messenger RNA can be found for various temperatures.

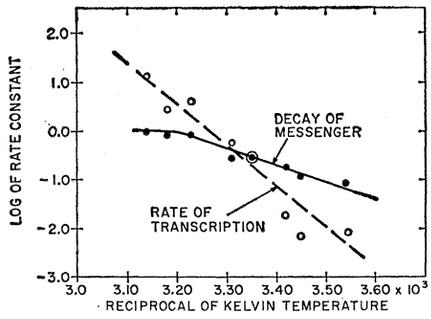

Fig. 4. The logarithm of the decay constant for messenger RNA and the rate constant for transcription of message are plotted against the reciprocal of the absolute temperature. The activation energy for decay is 11,000 cal/mole, while that for transcription is 22,000 cal/mole.

constant, so that $(d^2E/dt^2) = 0$, we have $ab = k_1(dE/dt))m$. Thus we can find the quantity ab dependent on the measurement of E being in arbitrary units. If we follow the suggestion that the basal amount of enzyme in a fully repressed culture corresponds to one molecule of messenger RNA per cell, we can make the arbitrary units indicative of the numbers of transcribed molecules per unit of time. This has been done by measuring the number of cells in each experiment, and also by measuring the amount of enzyme from a known number of fully repressed cells with exactly the same assay procedure. We designate these units as "basal units" and hope that one basal unit is equivalent to one molecule of messenger RNA.

In Table 1 we give numbers found in this way for different temperatures. In Fig. 4 we show the Arrhenius graphs [in which the relation $\ln(\text{rate}) = -(\Delta H^*/RT)$ is used, where ΔH^* is the energy of activation, R the gas constant and T the absolute temperature] for the decay of messenger and also for the rate of transcription. For the decay of messenger, ΔH^* is found to be 11,000 calories per mole, while that of the rate of transcription is 22,000 calories per mole. Both are in a reasonable range for enzyme action; although the transcription value is rather high.

The variation of the decay constant with temperature is further evidence that the decay is some kind of enzymatic degradation rather than physical inactivation analogous to thermal inactivation. Although the points do lie sufficiently on the line, the individual variations in decay constant can be as high as a factor of two. This suggests that the metabolic state, which is altered by, for example, good aeration, may also affect the decay, so that temperature is only one of several variables of importance.

The fact that very reasonable figures for the decay of messenger over a wide range of temperature, there being good agreement with good measurements made quite differently, are obtained by assuming that radiation acts to stop transcription forces consideration of this as a hypothesis. Such a hypothesis is supported by experiments of Pollard and Achey (*13*) in which it was found that in the presence of oxygen the DNA of *E. coli* is degraded to the extent of 50 percent, but no more, and also that there is a reduction in the synthesis of DNA after irradiation. If it were supposed, as a hypothesis, that the 50 percent degradation took place in one strand of DNA, which is the strand which is transcribed, then a natural explanation of a great reduction of transcription is at once available. In addition, if it is supposed that the new synthesis of DNA is temporarily halted by radiation, then no new DNA is available to be transcribed, and so there is a stoppage. It has been suggested, for quite different reasons, by Champe and Benzer (*14*), Bautz (*15*), and McCarthy and Bolton (*16*), that only one of the two strands of DNA is transcribed. The DNA is presumably in a different physical state while the process of transcription is going on. It would appear that the two effects of radiation mentioned above are sufficient to stop the process of transcription. Since this type of inhibition of transcription, regardless of the hypotheses advanced to explain it, is not specific at all, it should be possible to exploit it to make a general study of a wide variety of messenger RNA half-lives and also, possibly, of rates of transcription.

ERNEST C. POLLARD
Biophysics Department, Pennsylvania State University, University Park

References and Notes

1. E. Pollard, *Am. Naturalist* **44**, 71 (1960).
2. D. Billen and H. C. Lichstein, *J. Bacteriol.* **63**, 533 (1952).
3. R. K. Clayton and H. I. Adler, *Biochim. Biophys. Acta* **56**, 257 (1962).
4. E. Pollard and C. Vogler, *Radiation Res.* **15**, 109 (1961).
5. G. D. Novelli, T. Kameyama, J. M. Eisenstadt, *Cold Spring Harbor Symp. Quan. Biol.* **26**, 133 (1961).
6. A. B. Pardee and L. S. Prestidge, *Biochim. Biophys. Acta* **49**, 77 (1961).
7. J. A. Boezi and D. B. Cowie, *Biophys. J.* **1**, 639 (1961).
8. D. Nakada and B. Magasanik, *J. Mol. Biol.* **8**, 105 (1964).
9. C. Levinthal, A. Keynan, A. Higa, *Proc. Natl. Acad. Sci. U.S.* **48**, 1631 (1962).
10. A. Kepes, *Biochim. Biophys. Acta* **76**, 293 (1963).
11. J. Monod, F. Jacob, F. Gros, *Biochem. Soc. Symp.* **21**, 21 (1961).
12. F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).
13. E. Pollard and P. M. Achey, *Science* **146**, 71 (1964).
14. S. P. Champe and S. Benzer, *Proc. Natl. Acad. Sci. U.S.* **48**, 532 (1962).
15. E. K. F. Bautz, *ibid.* **49**, 68 (1963).
16. B. J. McCarthy and E. T. Bolton, *J. Mol. Biol.* **8**, 184 (1964).
17. We thank Dr. R. Wax for many useful suggestions, Dr. S. Person for constant discussion and skepticism, and Mrs. Kathy Kolacz for technical assistance.
18. Supported by NASA Contract NsG 324.

29 September 1964

Ceratocystis Infection in Sweet Potato: Its Effect on Proteins, Isozymes, and Acquired Immunity

Abstract. *Changes take place in the protein and isozyme patterns of tissue adjacent to cut surfaces of sweet potato roots infected by the fungus* Ceratocystis fimbriata. *Chromatography and gel electrophoresis of extracts from sections cut at known distances from a plane of infection showed that inoculation with a pathogenic or nonpathogenic isolate produced similar changes in several proteins and enzymes. Inoculation of a susceptible variety of sweet potato with the nonpathogenic isolate induced in a thin layer of tissue around the site of inoculation an acquired immunity to subsequent inoculation with the pathogen.*

Changes in protein metabolism have been associated with black rot infection of sweet potatoes; the protein content (*1*), polyphenol oxidase activity (*2*), and peroxidase activity (*3*) increase in the tissue adjacent to the site of infection. This suggests that more detailed analysis of the tissue adjacent to sites of infection would contribute to the understanding of the physiology of the diseased plant. We have used chromatography and gel electrophoresis in studies of resistant and susceptible sweet potatoes interacting with pathogenic and nonpathogenic isolates of the fungus *Ceratocystis fimbriata* (E. H.) Ell.

Resistant (Sunnyside) and suscepti-