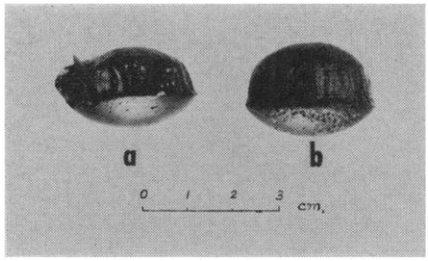

Fig. 2. The partially spalled "indicator" core of an australite (*a*) from Charlotte Waters, Northern Territory, Australia. Ring waves on the remnant unspalled portion are clearly visible. A spalled core with the aerothermal shell completely removed is shown on the right (*b*), from Hampton Hills, Kalgoorlie, Western Australia.

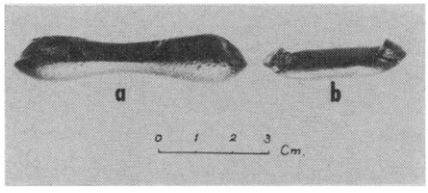

Fig. 3. (*a*) A spalled dumbbell with the aerothermal shell completely removed, and (*b*) an "indicator" dumbbell with remnant aerothermal stress shell. Both from Hampton Hills.

take place. Complete spalling of this aerothermal stress shell, formed by secondary reheating and aerodynamic ablation, results in a core as shown in Fig. 2*b*. Similarly, an indicator of a partly spalled dumbbell (Fig. 3*b*) shows how a spalled dumbbell-shaped australite (Fig. 3*a*) was derived. The spalled australite teardrop (Fig. 1*b*) and the spalled moldavite (Fig. 1*a*) are similarly derived, both having a history of aerodynamic heating and ablation.

Formation of a core as a result of the spalling off of the aerothermal stress layer of the anterior and girdle areas of ablated australites and other Australasian tektite specimens has been experimentally demonstrated by Chapman (*3*), using artificially ablated specimens of tektite glass. There is no uncertainty concerning the derivation of these spalled tektites, although evidence of primary aerodynamic ablation has been completely removed.

Suess (*4*) described in detail the sculpture of moldavites and interpreted the various highly polished intricate patterns of surface pits and grooves as being of aerodynamic origin. Although the detailed mode of formation of such sculptures is not well known in all cases, I have observed identical sculptures in philippinites, indochinites, and Thailand tektites where the sculptures were formed subsequent to rounding of the specimen by transport in terrestrial bodies of waters. The similar intricate sculpture on moldavites which I have examined also has a late history—that is, the sculpture was formed after the moldavites landed on Earth. It is considered to be of corrosion origin, in agreement with the independent study of Rost (*5*).

The large, teardrop-shaped moldavite provides the first tangible evidence that moldavites also suffered aerodynamic heating of the anterior face, and perhaps ablation, during a second melting on entry into Earth's atmosphere. The moldavite must have entered the atmosphere as a cold glass body, probably at velocities greater than 5 km/sec; no ablation occurs when tektites enter at less than 5 km/sec (*6*).

Chapman and Larson (*6*, p. 4340) discussed in detail the difficulty involved for terrestrial impact as an origin of tektites from the point of view of aerodynamics, and pointed out that, for molten blobs of fused earth to fly through the atmosphere unmolested by aerodynamic forces at exit angles as shallow as 12 degrees, a relatively high vacuum of less than 2×10^{-7} atm must exist over an area of 500 km radius. The moldavite from Slavice was aerodynamically heated and perhaps ablated, requiring the presence of atmosphere over the Bohemian and Moravian tektite-strewn field which is less than 400 km from the Ries crater of southern Germany. This is strong indication that moldavites could not have derived from the Ries. This conclusion agrees with the chemical evidence that moldavites are not related to Ries glass or to the Ries crater (*7*, p. 90).

This report is intended not only to record evidence favoring the cosmic origin of moldavites, but also to call attention to the need for finding better-preserved moldavites that will provide more useful aerodynamic information regarding their place of origin.

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References and Notes

1. C. Fenner, *Trans. Roy. Soc. S. Australia* **59**, 125 (1935).
2. G. Baker, *Mem. Natl. Museum Victoria* **23**, (1959).
3. D. R. Chapman, *Geochim. Cosmochim. Acta* **28**, 841 (1964).
4. F. E. Suess, *Jahrb. Geol. Reichsanstalt Wien* **50**, 193 (1900).
5. R. Rost, verbal communication (1964).
6. D. R. Chapman and H. K. Larson, *J. Geophys. Res.* **68**, 4305 (1963).
7. E. C. T. Chao, in *Tektites*, J. A. O'Keefe, Ed. (Univ. of Chicago Press, Chicago, 1963), pp. 51–94.
8. I am grateful to D. R. Chapman and H. K. Larson of Ames Research Center, NASA, Moffett Field, Calif., for use of casts of the australite indicator button and the large spalled teardrop, and to Carleton Moore for the loan of australites obtained by H. H. Nininger from Hampton Hills, Kalgoorlie, Australia. I particularly thank Vladimír Bouška of Charles University, Prague, who planned and guided my field trips to the Bohemian and Moravian basins, and Jaromír Šofr of Třebíč for contributing the moldavite teardrop described.

31 August 1964

β-Galactosidase: Inactivation of Its Messenger RNA by Ultraviolet Irradiation

Abstract. *A brief exposure of* Escherichia coli *cells to an inducer for β-galactosidase results in the production of a messenger RNA which subsequently expresses itself as β-galactosidase. Ultraviolet irradiation of cells after exposure to the inducer results in a decrease in the amount of β-galactosidase formed. The messenger RNA formed during the brief exposure to inducer is evidently inactivated by the ultraviolet radiation. The decay of messenger RNA activity in irradiated cells has the same kinetics as that observed in unirradiated cells.*

Ultraviolet irradiation prevents the formation of induced enzymes in yeast (*1*) and *Escherichia coli* (*2*). The spectra of the active substances for the inhibition of both systems have maxima at 2600 Å (*2*, *3*). This result suggests that nucleic acids are damaged by ultraviolet irradiation but does not distinguish between DNA and RNA as the affected compound. We have taken advantage of the fact that *E. coli* cells, exposed briefly to an inducer for β-galactosidase, form messenger RNA (mRNA) that functions even after the inducer is withdrawn (*4*, *5*). Measurements of β-galactosidase activity in cells irradiated after the inducer is withdrawn, but before mRNA has decayed, yield evidence that mRNA is inactivated by ultraviolet radiation.

The action of ultraviolet radiation on cells that had been exposed to inducer was studied by the method described by Kepes (*4*). The general plan of the experiment—with its interpretation of the attendant molecular events—is shown in Fig. 1. During the 20-second exposure to inducer isopropyl-

thio-β-D-thiogalactoside, the mRNA of β-galactosidase is formed. Dilution of the cells lowers the inducer concentration to less than that effective in mRNA production. Before the addition of chloramphenicol, the mRNA expresses itself in β-galactosidase production, and during this time the mRNA decays with a half-life of about 1 minute. Thus, after 6 minutes most of the β-galactosidase resulting from the brief exposure to the inducer has been formed. Ultraviolet irradiation in the period after administration of the pulse inhibits the formation of the β-galactosidase. The details of the experiment follow.

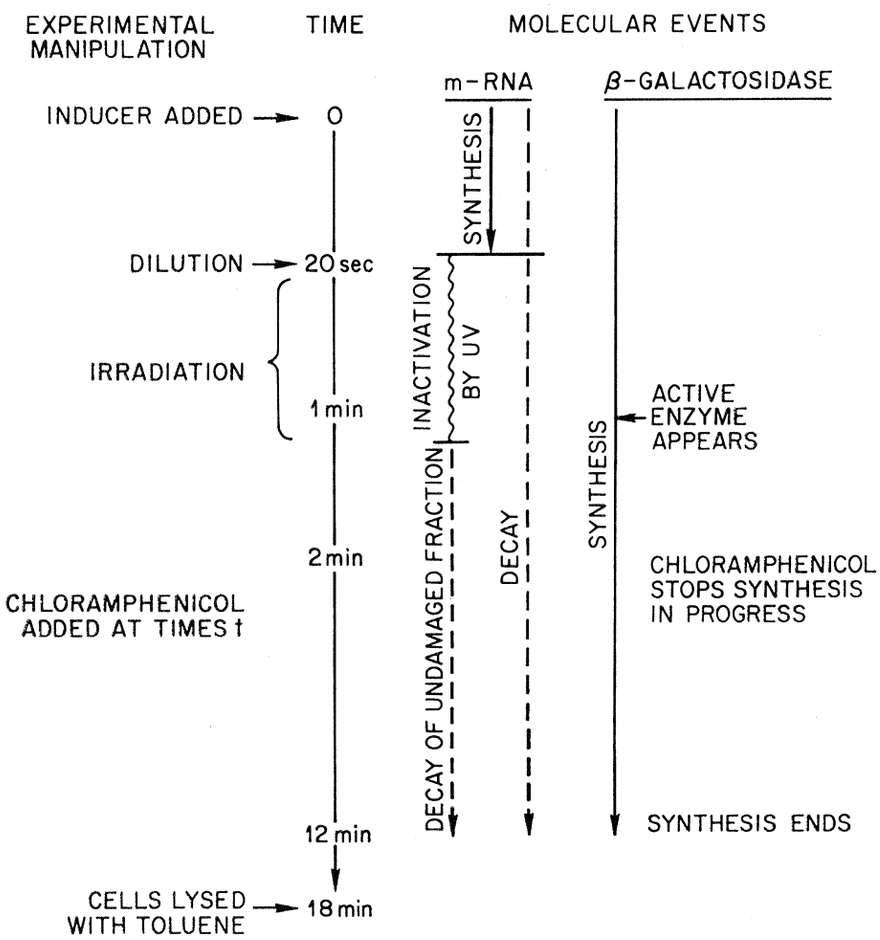

Fig. 1. Timetable of experimental procedure and presumed molecular events associated with the inactivation in *E. coli* B/r of β-galactosidase mRNA by ultraviolet irradiation.

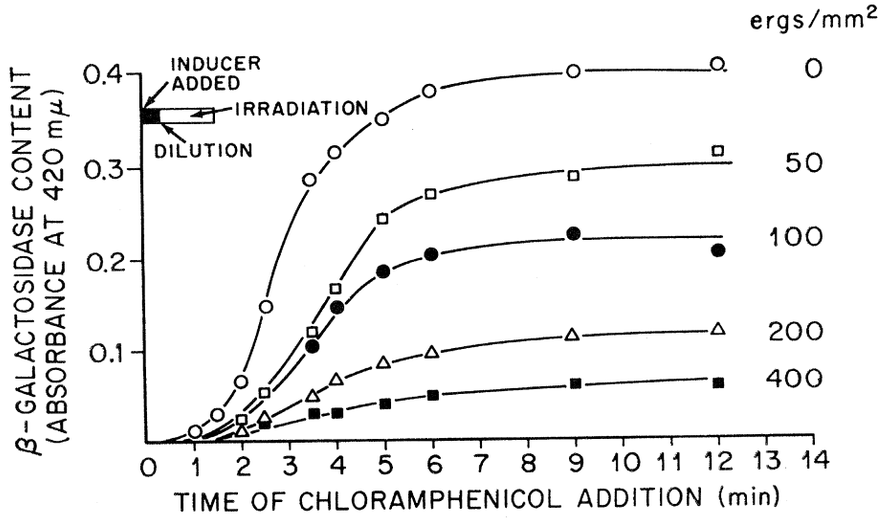

Fig. 2. Synthesis of β-galactosidase by *E. coli* B/r cells that were subjected to a brief pulse of inducer and then irradiated with ultraviolet radiation as indicated in Fig. 1.

Cultures of *E. coli* B/r in M63 medium (*6*) supplemented with 0.001 percent of casein hydrolyzate were grown to a concentration of 2×10^8 cells per milliliter. The bacteria were concentrated in growth medium to 2×10^9 cells/ml, and at zero time 5 μl of inducer was added to 100 μl of cells at 37°C. A high final concentration (0.4m*M*) of inducer was used so that permease induction was not required (*7*). At 20 seconds the cells and inducer were diluted 50-fold with warm medium and immediately irradiated (2650 Å) from a large quartz-prism monochromator. The average intensity through each sample was about 6 erg mm^{-2} sec^{-1}, and the exposures were between 8 and 65 seconds.

The cell suspensions were kept at 37°C, and at intervals 200-μl samples were removed and pipetted into test tubes containing 200 μl of a chloramphenicol solution (final concentration 50 μg/ml) in order to stop all protein synthesis. At 18 minutes the cells in the chloramphenicol were lysed with a drop of toluene. The amount of β-galactosidase formed before the addition of chloramphenicol was determined as follows: 400 μl of *o*-nitrophenyl-β-D-galactoside were added to give a final concentration of 2.66m*M*, and the tubes were incubated at 37°C for 4 hours. The reaction was stopped by adding 1.6 ml of Na_2CO_3 (final concentration 0.25*M*). The absorbancies measured at 420 mμ with a Beckman DU spectrophotometer were taken to be proportional to the amount of β-galactosidase formed before the addition of chloramphenicol. A correction was made for turbidity and basal concentration of the enzyme. Figure 2 shows the effect of various ultraviolet doses on β-galactosidase synthesis. A family of S-shaped curves was obtained. Kepes (*4*) calculated the half-life of mRNA from the differences between the maximum absorbance and the absorbancies at various times. Our data agree with his, and we find the same half-life, about 1 minute, for mRNA in the irradiated cells.

At the low doses used in these experiments, it seems unlikely that ribosomal RNA is affected, because Wacker *et al.* (*8*), using a cell-free amino acid incorporating system, found that a dose of 3.5×10^4 erg/mm^2, 100 times our maximum dose, was required to inactivate ribosomal function by 55 percent.

Soluble RNA is also very insensitive to ultraviolet radiation (*9*) as is the synthetic messenger polyuridylic acid which is assayed by its ability to stimulate the incorporation of phenylalanine in a cell-free system (*8, 10*). No natural messenger RNA is available for testing in vitro, nor do we know the photochemical events (dimer formation, photohydration, cross linking, or other) that are responsible for the ultraviolet-inactivation of mRNA.

The inhibition of what we have called mRNA is different from the inhibition of general protein synthesis as indicated by incorporation of H^3-leucine (Fig. 3) (*11*). The dose-effect curve for mRNA is exponential, whereas that for protein synthesis is not. Only small effects are seen at low doses; at higher doses the dose-effect curve for protein synthesis is approximately exponential. The $1/e$ doses (the doses that, in exponential portions of the dose-effect curves, leave 37 percent remaining activity) are 180 erg/mm^2 for β-galactosidase mRNA and 250 erg/mm^2 for protein synthesis. Thus the sensitivity of the β-galactosidase mRNA is greater than that of general protein synthesis. The 37 percent dose for colony formation is about 100 erg/mm^2.

Masters and Pardee (*12*) and Kameyama and Novelli (*13*) found that if cells were irradiated before induction (a situation in which one presumably measures not inactivation of mRNA but the DNA-directed synthesis of specific mRNA), β-galactosidase synthesis was more sensitive to ultraviolet than general protein synthesis. We have verified this result and have found that the $1/e$ dose for the inhibition of β-galactosidase synthesis for *E. coli* B/r cells irradiated prior to induction is 100 erg/mm^2 (*14*). RNA synthesis and protein synthesis continue when DNA synthesis is completely blocked by ultraviolet radiation (*15*). In the experiments dealing with general protein synthesis (Fig. 3), mRNA formation is occurring during the time when measurements are taken, but during the short interval so close to zero time this complication is minimized.

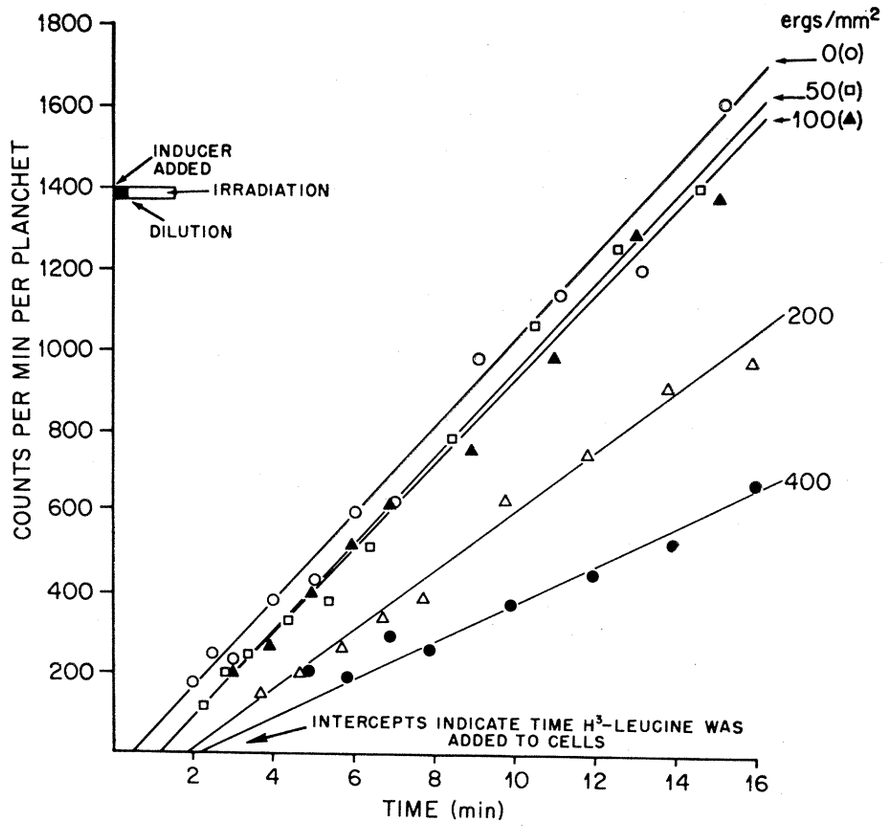

Fig. 3. Incorporation of H^3-leucine into ultraviolet-irradiated (2650 Å) cultures of *E. coli*. The procedure through the irradiation step was outlined in Fig. 1. Immediately after irradiation 200 μl of a cell suspension were added to 5 μl of H^3-leucine (5 c/mmole, 0.54 mc/ml). The cells were incubated at 37°C and 10-μl samples were removed at intervals and pipetted onto stainless steel planchets into drops of 0.01*M* leucine. The cells were fixed with a drop of 2 percent formalin, and the planchets were dried at 65°C. The acid-soluble materials were washed out of the cells (*11*), and after drying the planchets were counted in a gas-flow counter.

Nakada and Magasanik (*5*) showed that 5-fluorouracil is incorporated into the mRNA fraction of *E. coli*. When this base analog is present with the inducer, β-galactosidase synthesis is inhibited, but a protein biologically related to the enzyme is produced. If 5-fluorouracil is added after the removal of the inducer, no effect on β-galactosidase production is observed. Kepes (*4*) found a linear relation between the time the cells were with inducer and the total amount of enzyme produced during and after contact with the inducer. This relation is not changed if 5-fluorouracil is added after removal of the inducer (*4*). These data may be interpreted as indicating that, at the end of a 20-second pulse of inducer, the amount of noncompleted mRNA present on the DNA template is negligible compared with the amount which is completely formed. This is evidence that we are inactivating mRNA in our experiments rather than producing lesions, for example, thymine dimers, in DNA (*16*) which would prevent the completion of mRNA molecules that were on the DNA template at the time of dilution of the inducer.

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References and Notes

1. P. A. Swenson and A. C. Giese, *J. Cell. Comp. Physiol.* **36**, 369 (1950).
2. G. W. Rushizky, M. Riley, L. S. Prestidge, A. B. Pardee, *Biochim. Biophys. Acta* **45**, 70 (1960).
3. P. A. Swenson, *Proc. Natl. Acad. Sci. U.S.* **36**, 699 (1950).
4. A. Kepes, *Biochim. Biophys. Acta* **76**, 293 (1963).
5. D. Nakada and B. Magasanik, *J. Mol. Biol.* **8**, 105 (1964).
6. A. B. Pardee and L. S. Prestidge, *Biochim. Biophys. Acta* **49**, 77 (1961).
7. L. A. Herzenberger, *ibid.* **31**, 525 (1959); A. B. Pardee and L. S. Prestidge, *ibid.* **76**, 614 (1963).
8. A. Wacker, D. Jacherts, B. Jacherts, *Angew. Chem.* **74**, 653 (1962).

9. P. A. Swenson and S. Nishimura, *Photochem. Photobiol.*, in press.
10. L. Grossman, *Proc. Natl. Acad. Sci. U.S.* **48**, 1609 (1962).
11. R. P. van Tubergen and R. B. Setlow, *Biophys. J.* **1**, 589 (1961).
12. M. Masters and A. B. Pardee, *Biochim. Biophys. Acta* **56**, 609 (1962).
13. T. Kameyama and G. D. Novelli, *Arch. Biochem. Biophys.* **97**, 529 (1962).
14. P. A. Swenson, unpublished data.
15. P. Hanawalt and R. Setlow, *Biochim. Biophys. Acta* **41**, 283 (1960).
16. R. B. Setlow, P. A. Swenson, W. L. Carrier, *Science* **142**, 1464 (1963).

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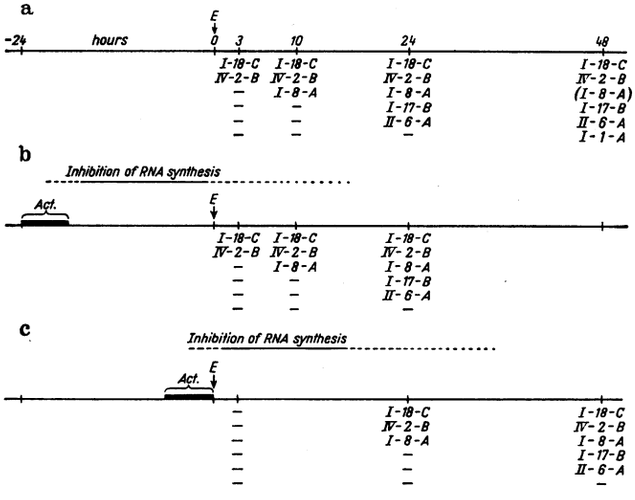
Actinomycin and Puromycin: Effects on Sequential Gene Activation by Ecdysone

Abstract. *A temporary inhibition of RNA synthesis leads to a corresponding delay in the formation of all puffs which are stimulated by ecdysone in the salivary gland chromosomes of* Chironomus tentans. *Inhibition of protein synthesis does not influence the induction of those puffs which appear shortly after injection of ecdysone. Puffs which develop after a longer period are delayed in appearance. It is concluded that early reacting genes are involved in those processes leading to the sequential activation of the puffs which appear later.*

Molting in insects is induced by the hormone ecdysone from the prothoracic glands (*1*). An early effect of this hormone is to change the activity of specific gene loci. This has been found in studies on the puffing phenomenon in salivary gland chromosomes of *Chironomus tentans* (*2*). After injection of ecdysone into last-instar larvae, two puffs (I-18-C and IV-2-B) appear within the next 15 to 30 and 30 to 60 minutes, respectively. In normal, as well as in experimentally induced metamorphosis, the appearance of these puffs is followed by a sequential activation of other loci (*2, 3*). The order in which puffs appear at some of these loci after an injection of ecdysone is shown in Fig. 1*a*. Changes in the puffing pattern during metamorphosis, comparable to those found in *C. tentans*, occur in other insects as well. That they are induced by or depend on the presence of ecdysone has been shown in *Acricotopus* and in *Drosophila* (*4*). In *Chironomus tentans*, the size of the two early induced puffs is controlled by the concentration of ecdysone. They differ, however, in their reaction thresholds and in their reactivities against different hormone concentrations (*3*). Puffing of some of the later-reacting loci is also dependent on the presence of ecdysone. These loci, however, react only 2 or 3 days after ecdysone injections, even when very large amounts are injected; thus they cannot be influenced by changes of hormone concentrations (*2, 3*). These results suggest that the early puffs may be controlled more directly by ecdysone than are the later ones. To determine whether early-reacting genes participate in the chain of processes which lead to an activation of the puffs which appear later, we temporarily inhibited RNA and protein synthesis.

The synthesis of RNA was inhibited by incubating *C. tentans* larvae in a culture medium containing 0.2 μg of actinomycin C_1 per milliliter (*5*). After 6 hours they were transferred to a medium lacking actinomycin. At various intervals, some larvae were killed, and the salivary glands were explanted and incubated for 30 minutes in a sucrose medium containing tritiated uridine (1.29 c/m*M*; 1.0 μc/ml) (*6*). The glands then were squashed and tested for RNA synthesis by means of autoradiography (*7*).

After 4 to 6 hours of treatment with actinomycin, incorporation of H^3-uridine had strikingly decreased. Seven to eight hours after beginning the actinomycin treatment (that is, 1 to 2 hours after the transfer of the larvae into a fresh medium) RNA synthesis could no longer be detected by our method (Fig. 2). Fifteen to twenty hours after trans-



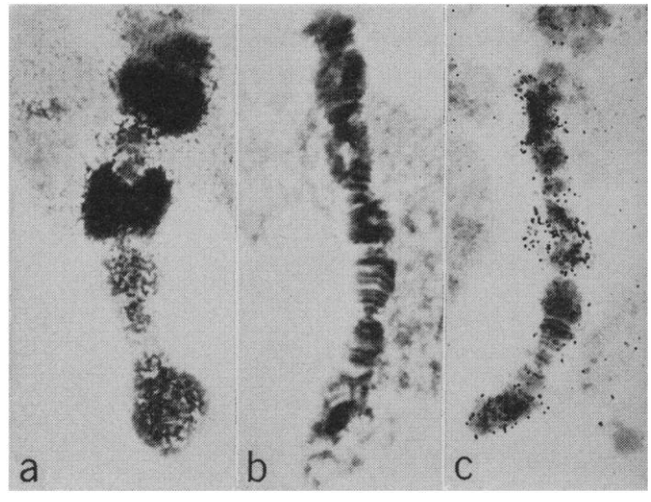


Fig. 1 (left). Puff-formation at representative loci of salivary gland chromosomes of *Chironomus tentans* 3, 10, 24, and 48 hours after injections of ecdysone (*E*). In *b* and *c* larvae were pretreated with actinomycin for 6 hours (*Act.*). The symbols indicate the chromosome regions in which the puffs are located. Their exact locations and details of their behavior in normal and experimentally induced metamorphosis have been published (*2, 3*). The number of animals used in *b* and *c* were 28 and 25, respectively. Fig. 2 (right). Autoradiographs of the IVth chromosome of *C. tentans* after incubation of the salivary glands in a medium containing H^3-uridine for 30 minutes. For *b* and *c*, the larvae were treated for 6 hours with actinomycin 10 hours and 20 hours, respectively, before the salivary glands were incubated with H^3-uridine.