are not identical and, if all the bands represent potentially dangerous antigens, then mixed antigens for desensitization treatment are probably needed when sensitizing insects are not definitely known. Variation in migration rates due to the more complex mixtures of proteins in sac and whole-insect extracts make species comparisons difficult with these patterns.

ROD O'CONNOR
WM. ROSENBROOK, JR.
ROBERT ERICKSON
Department of Chemistry,
Montana State College, Bozeman 59715

References and Notes

1. T. W. Richey, *Medical World News* (19 July 1963).
2. O. M. Jensen, *Acta Pathol. Microbiol. Scand.* **54**, 9 (1962); R. O'Connor, R. A. Stier, W. Rosenbrook, Jr., R. Erickson, *Ann. Allergy* **22**, 385 (1964).
3. E. L. Foubert and R. A. Stier, *J. Allergy* **14**, 347 (1956).
4. M. H. Loveless, *J. Immunol.* **89**, 204 (1962).
5. A. W. Benton, R. A. Morse, J. D. Stewart, *Science* **142**, 228 (1963); D. J. Palmer, *Bee World* **42**, 225 (1951).
6. R. O'Connor, W. Rosenbrook, Jr., R. Erickson, *Science* **139**, 420 (1963).
7. C. E. Gaillard, R. Schellin, R. A. Mayers, *Ann. Allergy* **21**, 69 (1963).
8. M. H. Loveless, *Federation Proc.* **21**, No. 2 (1962).
9. L. Ornstein and B. J. Davies, *Disc Electrophoresis* (Distillation Products Industries, Rochester, N.Y., 1962).
10. F. Huneeus-Cox, *Science* **143**, 1036 (1964).
11. W. Rosenbrook, Jr., and R. O'Connor, *Can. J. Biochem.* **42**, 1005 (1964).
12. J. R. Whittaker and A. S. West, *Can. J. Zool.* **40**, 655 (1962).
13. Supported in part by NIH grant GM-09388. Field collections of insects were supported by a grant from the Hollister-Stier Laboratories, Spokane, Wash. We thank Gordon Lillis and his science students in Delano Joint Union High School, Delano, Calif., for supplying us with large numbers of *Polistes apachus* wasps. A Gelman d-c electrophoresis power supply (model 38200, Gelman Instrument Company, Ann Arbor, Mich.) was used.

16 July 1964

Skin Tumorigenesis by 7,12-Dimethylbenz(a)anthracene: Inhibition by Actinomycin D

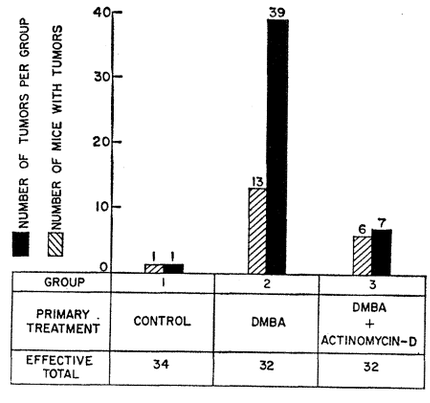
Abstract. *Topical application of actinomycin D during a short period of time immediately before and after a single application of 7, 12-dimethylbenz*(a)*anthracene markedly inhibits subsequent tumor formation. The results indicate that the initial events of carcinogenesis are dependent on DNA-dependent RNA synthesis.*

Experimental clarification of the initial changes during chemical carcinogenesis presents numerous problems. A major problem is the relatively long interval between exposure to a carcinogen and appearance of the tumors. Others are largely due to the variability in systemic transport, distribution, and metabolism of the "active" carcinogen. A system uniquely suited to the study of relevant early events in carcinogenesis is the two-stage system of skin tumorigenesis described by Mottram (*1*) and Berenblum and Shubik (*2*). In this system, a single minimal dose of carcinogen, topically applied to mouse skin, "initiates" the tumorigenic process. The "initiation" is essentially irreversible (*2*). The "initiated" centers in the skin are then "promoted" to the visible papilloma stage by repeated application of a "promoting" agent, such as croton oil, which alone is noncarcinogenic or weakly carcinogenic. Since most of the carcinogen administered by a single low dose disappears rapidly from the skin (*3*), the system allows for confinement of biochemical studies of carcinogen-induced alterations to a short time period. Also, the carcinogen is applied directly to the susceptible tissue and problems of variable transport, metabolism, and distribution of systemically administered carcinogens are minimized. Finally, the susceptible tissue is observable grossly and continuously and can be treated directly with agents of known biological activity.

In this study, we examined the effect of actinomycin D, an inhibitor of DNA-dependent RNA synthesis (*4*), on "initiation" of skin tumorigenesis by 7,12-dimethylbenz(*a*)anthracene (DMBA). Experimental procedures and results are shown in Fig. 1. Groups of 35 mice were treated once with 12 μg of DMBA alone or with DMBA and actinomycin D. Control groups received acetone in place of DMBA. One week after the treatments and weekly thereafter the mice were treated with croton oil. The animals were examined weekly for 14 weeks for the presence of skin tumors. The results show a marked inhibitory effect of actinomycin D on skin tumorigenesis. When actinomycin D was applied six times during a 20-hour period before and after treatment with DMBA (group 3), the total number of tumors was decreased by 82 percent as compared with the controls treated with DMBA (group 2). The control group given acetone only (group 1) had one mouse with one tumor, and the other control (not shown in Fig. 1) given acetone with actinomycin D (group 4) had none.

The reason for the incompleteness of the inhibition with actinomycin D is not known. We do not know the degree of effectiveness or duration of drug action of this agent in blocking RNA and protein synthesis in the skin. It is possible that adjustment of dosage and time of administration of carcinogen or inhibitor may yield complete inhibition.

No grossly visible skin damage was observed in any of the groups during the 1st week of the experiment. At 2 weeks the groups that received actinomycin D (groups 3 and 4) followed by croton oil showed visible skin damage; at 3 weeks the skin damage was less apparent; at 4 to 5 weeks it was healed. Studies designed to detect



GROUP	1	2	3
PRIMARY TREATMENT	CONTROL	DMBA	DMBA + ACTINOMYCIN-D
EFFECTIVE TOTAL	34	32	32

Fig. 1. Effect of actinomycin D on skin tumorigenesis. Four groups of 35 male Swiss mice were treated as follows: The hair was removed from the back of each mouse with electric clippers one day prior to the start of the experiment. A single dose of 12 μg of DMBA in 0.2 ml of acetone was applied topically to the entire back of each mouse in groups 2 and 3. Acetone only was applied to the mice in group 1. In group 3, 14 μg of actinomycin D in 0.2 ml of acetone was applied at 2, 5, and 8 hours before and 2, 5, and 8 hours after the application of DMBA. Groups 1 and 2 were given acetone according to the schedule of group 3. Group 4, not shown, received actinomycin D with no DMBA. One week after the treatments and weekly thereafter, all the mice received topical applications of 0.2 ml of 1 percent croton oil in acetone. The tumor yield shown is that at 14 weeks and represents only tumors persisting for a minimum of 2 weeks. Essentially the same tumor yields were noted at 12 and 13 weeks. The effective totals represent the number of survivors at the time the first tumor was observed. Control group 4, which received no DMBA, had an effective total of 27 with no tumors in the group.

possible chemical interaction between actinomycin D and DMBA were negative. Thus the presence of 13.9 μg of actinomycin D per milliliter did not increase the solubility of DMBA in 0.01M NaCl, 0.001M NaH_2PO_4 (pH 7.4), and equimolar amounts of DMBA and actinomycin D in 25 percent acetone did not affect the absorption spectra of either between 325 and 500 mμ. Actinomycin D does not exert its action by stimulating the removal of DMBA from the skin. Thus, in the presence or absence of actinomycin D, more than 90 percent of DMBA labeled with carbon-14 disappeared from the skin 24 hours after application.

Our results suggest that the early biochemical events of skin tumorigenesis by DMBA are dependent on DNA-directed RNA synthesis. Thus the carcinogenic activity of DMBA is dependent on the presence of genetic activity. Whether the dependence is related directly to the RNA synthesized or to subsequently synthesized protein is unknown.

A single administration of methylcholanthrene, another carcinogenic polycyclic hydrocarbon, increases the amount of activity of several liver microsomal enzyme systems (*5*) and the benzopyrene hydroxylase activity in several rat tissues other than liver (*6*). Mechanism studies have shown that these effects are prevented by actinomycin D and puromycin (*7*). Furthermore methylcholanthrene increases the incorporation of amino acid into the liver microsomes (*8*), the amount of RNA in the nuclei of liver cells, and the "messenger RNA activity" of the nuclear RNA as measured by its activity in stimulating the incorporation of phenylalanine-C^{14} in an *Escherichia coli* system (*9*).

These findings (*5–9*) have led to the hypothesis (*7–9*) that early events in chemical carcinogenesis are alterations in the expression of specific genic information, that is, in the normal "gene-action systems," a term used by Waddington (*10*). Monod and Jacob (*11*) previously made a similar suggestion on theoretical grounds derived from their classical studies on the gene-action systems of microorganisms.

Transitory contact with a carcinogen may induce the synthesis of RNA and protein molecules that alter cellular environments in a specific manner. The alteration may be permanent and represent "initiation." As a result of appropriate interlocking of gene-action systems, the new environments may inactivate progressively those genes necessary for normal tissue function and activate the gene-action systems that characterize the developing tumor cell or the preneoplastic state. In certain systems, such as the one we used, the progression may require a promoting agent. In others, it may not. If a carcinogen-specific alteration in the expression of genic information is indeed the nature of "initiation," then a blocking of all gene expression, that is, DNA-dependent RNA synthesis, would prevent this process. We interpret our results to support the hypothesis that alterations in gene-action systems are the early biochemical events of carcinogenesis.

HARRY V. GELBOIN
MICHAEL KLEIN
Carcinogenesis Studies Branch,
National Cancer Institute,
Bethesda, Maryland

References and Notes

1. J. C. Mottram, *J. Pathol. Bacteriol.* **56**, 181 (1944).
2. I. Berenblum and P. Shubik, *Brit. J. Cancer.* **1**, 383 (1947); ——— *ibid.* **3**, 109 (1949); ——— *Brit. Med. Bull.* **4**, 373 (1947).
3. H. Falk and P. Kotin, *Clin. Pharmacol. Therap.* **4**, 88 (1963).
4. E. Reich, R. M. Franklin, A. J. Shatkin, E. L. Tatum, *Proc. Natl. Acad. Sci. U.S.* **48**, 1238 (1962).
5. A. H. Conney, E. C. Miller, J. A. Miller, *Cancer Res.* **16**, 450 (1956);———*J. Biol. Chem* **228**, 753 (1957); A. H. Conney, J. R. Gillette, J. K. Inscoe, E. R. Trams, H. S. Posner, *Science* **130**, 1478 (1959).
6. L. W. Wattenberg and J. L. Leong, *J. Histochem. Cytochem.* **10**, 412 (1962).
7. H. V. Gelboin and Blackburn, *Cancer Res.* **24**, 356 (1964).
8. H. V. Gelboin and L. Sokoloff, *Science* **134**, 611 (1961); H. V. Gelboin and N. Blackburn, *Biochim. Biophys. Acta* **72**, 657 (1960); H. V. Gelboin, *ibid.*, in press.
9. L. Loeb and H. V. Gelboin, *Nature* **199**, 809 (1963); H. V. Gelboin and L. Loeb, in preparation.
10. Waddington's term to denote the "whole series of biochemical processes which lead from a gene to the phenotypic character by which it is recognized."
11. J. Monod and F. Jacob, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 389 (1961).
12. We thank D. Morgan and H. Waters for valuable technical assistance.

20 July 1964

Staphylococcus aureus UC-18: Agent of Nosocomial Infections

Abstract. *A new strain of* Staphylococcus aureus, *implicated in severe "hospital-acquired" infections, has been recognized and identified. This strain is characterized by lysis with a recently isolated bacteriophage, UC-18. Resistance to penicillin, streptomycin, and tetracycline combined with widespread prevalence in the hospital environment make* S. aureus *UC-18 a significant contributor to endemic staphylococcal disease in hospitals.*

Recognition and identification of a new strain of *Staphylococcus aureus* implicated in severe "hospital-acquired" infections is of profound epidemiologic significance. Isolates that cannot be typed at routine test dilution of the standard international set of phages are encountered frequently, the percentage varying with the source of the isolates. A higher percentage is obtained from the environment than from clinical material. In an effort to further define these isolates that cannot be typed, phages at concentrations 1000 times the routine test dilution were included in the daily typing procedure during 1961 at Peter Bent Brigham Hospital.

A new phage pattern soon became apparent in isolates recovered from wound infections, burns, and blood cultures, as well as from volume-air samples and settling plates in the hospital wards. These could not be typed at routine test dilution, with inhibition by group III phages 47/53/54/75/77/42B /83A/83B in varying combinations at 1000 times the routine test dilution.

Staphylococcus aureus 80/81 strains had never predominated in our surgical-wound infections. In a study of 250 surgical procedures, strains 80/81 were recovered only twice from wounds. Since in each case the patient himself had been a carrier before surgery, it is possible that the source of infection was endogenous.

Isolates that could not be typed at routine test dilutions, but were inhibited by group III phages, were seen so consistently that they were saved for future phage isolations.

In June 1963 Altemeier and others (*1*) reported the isolation of a new phage, UC-18, which lysed *S. aureus* strains recovered from cases of enterocolitis. We secured this phage and its propagating strain from Dr. Hill at the University of Cincinnati; it was added to the 28 phages routinely used in our daily typing. In addition to the phages of the international set, the following phages are used at Peter Bent Brigham Hospital: 83A, 70, 73, 42B, 83B, 47C, 82, and UC-18.

Of the 114 isolates saved over a period of a year and characterized by