r/sec. For sham exposures the silent shutter was operated but the x-ray machine voltage was turned down to an ineffective level. Each rat was observed separately in order to facilitate behavioral observations. Movements of the head and eyes in the animals with transected spinal cords were used as an index of behavioral arousal. Each animal was exposed to radiation at least twice over a period of about 4 hours. When both the head and the body were to be separately exposed in the same animal, each region was exposed at least twice. Half of the animals with transected spinal cords, which were exposed to both head-only and body-only radiation, received exposures of the head first; the other half were given exposures of the body first. After each experiment the spinal cord was removed and fixed in formalin for subsequent verification of the spinal transection.

In Table 1 the results of the first two trials are summarized. Most rats were exposed for 5 seconds or less; however, in some rats the body only was exposed for 30 seconds to 2 minutes. No desynchronization of the electroencephalogram occurred when only the body of spinal-transected rats was exposed. The failure to respond was in contrast to the large number of animals which showed desynchronization under the other three conditions of exposure. The results of those experiments in which the body only of intact rats was exposed corroborate Hunt and Kimeldorf's finding (*1*) that exposure of the body only to x-irradiation elicits behavioral arousal in rats. In our study behavioral arousal was observed in approximately 80 percent of all cases where electroencephalogram desynchronization occurred.

In Fig. 1*A*, the electroencephalogram shows an example of the response of a rat with a transected spinal cord to exposure of the whole body at 1 r/sec; desynchronization occurred within 1 second of the onset of exposure. No differences could be detected between the responses to exposure of the whole body and the responses to exposure of only the head in rats with transected spinal cords. Figure 1*B* shows the response to exposure of the head only at 1.5 r/sec. Figure 1*C* shows that no changes occurred in the electroencephalogram of rats with transected spinal cords when the body only was exposed for 45 seconds at 1.5 r/sec. With the spinal cord intact, exposure of the body only at 1.0 r/sec (Fig. 1*D*) caused desynchronization of the electroencephalogram within 1 second as did exposure of the whole body or only the head.

The fact that exposure of the body to short and long periods of irradiation failed to produce desynchronization of the electroencephalogram in rats with transected spinal cords establishes that immediate or "late" effects mediated by the circulatory system cannot be essential in the arousal response to x-irradiation. Sensory input through the vagi is also ruled out because the vagi were intact in all experiments. It is concluded that the arousal reaction to x-irradiation of the body only is mediated by the spinal cord, and that direct sensory or neural activation is responsible for the arousal reaction.

G. P. COOPER
D. J. KIMELDORF
Physiology-Psychology Branch, U.S. Naval Radiological Defense Laboratory, San Francisco, California 94135

References and Notes

1. E. L. Hunt and D. J. Kimeldorf, *Nature* **200**, 536 (1963).
2. J. Garcia, N. A. Buchwald, G. Bach-y-Rita, B. H. Feder, R. A. Koelling, *Science* **140**, 289 (1963).
3. This study was supported through funds provided by the Bureau of Medicine and Surgery, U.S. Navy.

20 November 1963

Actinomycin D: Effect on the Immune Response

Abstract. *Actinomycin D injected simultaneously with sheep erythrocytes in female rats caused a delay in the immune response but had no effect on the rate or maximum amount of hemagglutinin produced. The delay was roughly proportional to the concentration of the antibiotic administered, and was up to 2 days for 75 μg in a 200-gram female rat (sublethal dose for females). The dose effect in the delay in response is consistent with the time when actinomycin would no longer be available to bind with newly synthesized DNA and when messenger-RNA production could occur. Similar results were obtained with another antigen, the enzyme β-galactosidase, in male rats during the secondary response.*

Preliminary studies (*1*) showed that an antigenic stimulus increases RNA metabolism in the spleen within the first 2 days. These findings suggested that to induce antibody synthesis, a particular messenger RNA might be required. Many reports have indicated that actinomycin D affects nucleic acid metabolism in vivo and inhibits DNA-dependent RNA polymerase reactions in vitro (*2*). These effects are apparently related to the capacity of the antibiotic to bind with the guanosine groups of DNA (*2*). The inhibition of induced protein synthesis occurs from the inhibition of the formation of new messenger RNA (*3*). The lack of effect of actinomycin D during the immune response was reported by Sterzl (*4*), who determined the amount of circulating antibody to *Brucella suis* antigen at a time when maximum titer to this antigen was established in control animals. Nathan *et al.* (*5*), using as a criterion the formation of sheep erythrocyte hemagglutinin, found that actinomycin D partially inhibited the immune response. In a review of the suppression of immunity, however, Schwartz and Andre (*6*) concluded that the antibiotic (actinomycin C) was ineffective. We now report the effect of actinomycin D on the immune response to two antigens: sheep erythrocytes and the enzyme, β-galactosidase. The results show that the drug delays the induction phase of antibody synthesis but does not affect the total antibody production. The implication of these results is that DNA-dependent messenger RNA is synthesized during the induction phase of antibody formation.

Sprague-Dawley female rats, 4 months of age, were used. Each rat was injected intraperitoneally with 1 ml of a 5-percent suspension of washed sheep erythrocytes (*7*). Actinomycin D (*8*) was dissolved in 1,2-propanediol and injected intraperitoneally. Animals were decapitated on various days after the antigen injection, and the serums were collected. Complement was inactivated by heating the serums at 60°C for 30 minutes. Hemagglutinin activity was assayed by adding washed erythrocytes to twofold serial dilutions of serum. The tubes were incubated for 1 hour at 37°C and for 3 to 4 hours at room temperature to allow the erythrocytes to settle. The serum titer was defined as the reciprocal of the

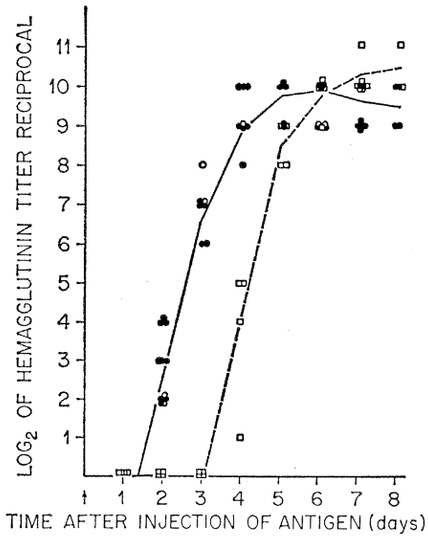

Fig. 1. Effect of actinomycin D on the primary antibody response of female rats to sheep erythrocytes when 75 μg of actinomycin and 1 ml of a 5-percent suspension of erythrocytes were simultaneously given intraperitoneally. Solid circles, control on sheep erythrocytes; open circles, control on 1,2-propanediol and sheep erythrocytes; squares, 75 μg of actinomycin and sheep erythrocytes.

final dilution of serum showing hemagglutination.

Circulating antibodies to sheep erythrocytes were detectable within 2 days and the maximum titer was observed in 5 days. The simultaneous injection of 75 μg of actinomycin D with antigen into a 200- to 225-g rat resulted in a 2-day delay in the appearance of antibody in the serum. These data are presented in Fig. 1. It can be seen that although there is a delay in response, both the slope of the response curve and the eventual maximum titer are similar to those of the untreated antigen-stimulated controls or to rats given antigen and 1,2-propanediol.

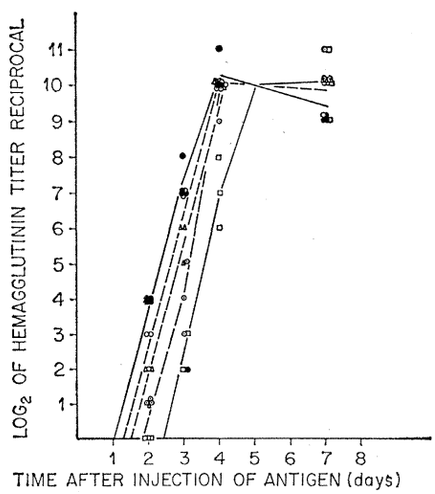

Fig. 2. Effect of dosage of actinomycin D on the response of female rats to sheep erythrocytes. Erythrocytes and actinomycin were injected simultaneously. Closed circles, controls. Actinomycin in the following concentrations: open circles, 10 μg; open triangles, 30 μg; dotted circle, 50 μg; and square, 70 μg.

The 2-day delay in response with actinomycin suggested that the drug could have been metabolized within 2 days, thus allowing the normal response. If this were the case, the injection of a second dose at 2 days should arrest the response further. This possibility was tested. The second dose of 75 μg of drug was lethal to 75 percent of the animals. The serums of those that survived the second dose were assayed, and there was a further slight delay in the response; however, the response was not delayed proportionally.

That the animals could not tolerate the second dose of actinomycin indicates that the first dose of the drug was not metabolized or that the damage from the drug was not repaired. The slight, further delay in response suggested a dose effect. Varying amounts of the antibiotic were injected simultaneously with the antigen to determine the minimum amount required to achieve a delay in antibody production. Animals were killed daily and the antibody titers of their serums were determined. The data for actinomycin concentrations of 10, 30, 50, and 70 μg are presented in Fig. 2. In all cases, there was a delay in the response but no changes in the slope or the maximum titer. After a delay of 6 to 7 hours with 10 μg, the delay was roughly proportional to increasing concentrations of the antibiotic.

A correlative study was made with the enzyme β-galactosidase, which is not as potent an antigen as sheep erythrocytes. The enzyme was purified from *Escherichia coli* ML308 according to the method of Kameyama and Novelli (*9*), and 0.5 ml (54,000 units) was given to male rats, per injection, with Freund's adjuvant in the region of the left popliteal lymph node. Four animals were used for each time interval. The animals were killed and individual serums were collected. Antibody to β-galactosidase was determined by adding a constant amount of enzyme solution to varying concentrations of serum. The tubes were incubated at

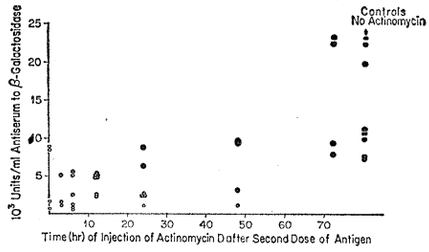

Fig. 3. Effect of actinomycin D on the secondary response of male rates to β-galactosidase.

37°C for 1 hour and at 0° to 4°C for 16 hours. They were centrifuged at 2000 rev/min and the residual enzyme activity in the supernatant fluids was assayed. In rat serum the antibody to β-galactosidase precipitates antigen but does not neutralize the enzyme activity. It forms only as the result of the injection of the enzyme in adjuvant.

An average titer of 0.82×10^3 enzyme precipitating units per milliliter of serum was found at 14 days and a maximum average titer of 6.53×10^3 units at 21 days after a single injection of antigen in adjuvant. Little, if any, antibody was detected at 7 days. No significant differences in titer were observed with actinomycin-treated and untreated animals sacrificed at weekly intervals.

The effect of the antibiotic on the response was evaluated further by administering actinomycin at the time a second dose of antigen in adjuvant was given 10 days after the initial injection. Serum taken on the 9th to 11th day after this second injection had an average maximum titer of 13.5×10^3 enzyme precipitating units per milliliter after 6 days. The drug was given simultaneously with the second injection of antigen or 3, 6, 12, 24, 48, or 72 hours afterward. The data are presented in Fig. 3. The immune response was inhibited only when the actinomycin was administered within the first 48 hours after the antigen. When the drug was administered 72 hours after the second dose of antigen, there was no significant effect on the response. These results and those based on the daily results of the response when sheep erythrocytes were administered are interpreted to indicate a delay in the induction phase and not necessarily an inhibition of antibody production.

It is interesting to note that when 75 μg of actinomycin D was injected into female rats (200 g), there were no toxic manifestations in our experi-

ments. This dose of antibiotic in a 200-g male rat was 40 to 60 percent lethal within 2 days with the syndrome reported by Philips *et al.* (*10*).

If antibody synthesis occurs in a manner similar to induced enzyme synthesis, then a specific messenger RNA is required. In theories based on clonal selection or genetics, the antigen would actuate the transcription of information contained in the genome; in theories not based on genetics the antigen would provide the information for specificity. The effect of actinomycin reported here suggests that a synthesis of a specific messenger RNA may be required prior to the synthesis of antibody. These results do not distinguish between the two theories but they do suggest that a DNA-dependent RNA synthesis occurs during the induction phase and when it is not synthesized, no antibody is produced.

Actinomycin D delays the immune response but does not inhibit the rate of antibody synthesis or the maximum titer of circulating antibody. This finding suggests that the delay is not due to a metabolism of the drug resulting in a dissociation of the antibiotic and DNA. Such a dissociation would affect the rate of antibody production as more and more of the drug is removed from the DNA. Replication of the DNA, however, would allow the DNA-dependent polymerase to function if there were no free actinomycin available to bind to the DNA. This would be consistent with the findings in vitro that concentrations of actinomycin that completely inhibit RNA polymerase inhibit DNA polymerase less than 5 percent (*2*). The dose effect observed implies that more than one round of replication would be necessary with larger amounts of the drug before the DNA could be used for messenger RNA synthesis.

CARL J. WUST
CLARA L. GALL*
G. DAVID NOVELLI
Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tennessee

References and Notes

1. C. J. Wust and L. Astrachan, *Federation Proc.* **21**, 380 (1962).
2. J. Hurwitz and J. T. August, in *Progress in Nucleic Acid Research*, J. N. Davidson and W. E. Cohn, Eds. (Academic Press, New York, 1963), vol. 1, p. 59.
3. G. Giudice and G. D. Novelli, *Biochem. Biophys. Res. Commun.* **12**, 383 (1963).
4. J. Sterzl, *Nature* **189**, 1022 (1961).
5. H. C. Nathan, S. Bieber, G. B. Elion, G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.* **107**, 796 (1961).
6. S. R. Schwartz and J. Andre, in *Mechanism of Cell Tissue Damage Produced by Immune Reactions*, Second Intern. Symp. Immunopathology, P. Grabar and P. Miescher, Eds. (Czechoslovakia Acad. of Sciences, Prague, 1961).
7. Obtained fresh from the University of Tennessee Agricultural Farm and washed extensively in 0.1*M* *p*H 7.0 phosphate-buffered saline prior to use.
8. We thank Merck Sharp and Dohme, West Point, Pa., for the actinomycin D.
9. T. Kameyama and G. D. Novelli, *Arch. Biochem. Biophys.* **97**, 529 (1963).
10. F. S. Philips, H. S. Schwartz, S. S. Sternberg, C. T. C. Tan, *Ann. N.Y. Acad. Sci.* **89**, 348 (1960).

* Summer trainee of the Oak Ridge Institute of Nuclear Studies. Present address: Biology Department, Rosary Hill College, Buffalo, N.Y. † Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

5 December 1963

Gynogenesis in Salamanders Related to Ambystoma jeffersonianum

Abstract. *The oocytes of naturally occurring triploid females of the* Ambystoma jeffersonianum *complex each contain 84 lampbrush chromosomes. This constitutes hexaploidy* (n = *14). The chromosomes are joined into pairs by chiasmata and form 42 bivalents. It is suggested that meiosis in triploid females is preceded by an endomitosis and the resulting sister chromosomes synapse to form pseudo-bivalents. Sperm from diploid males stimulate development of the triploid eggs but do not contribute chromosomes to the triploid nucleus. Bivalents in the oocytes of triploids have twice as many chiasmata as the corresponding bivalents in diploid animals. Such chiasmata cannot result in genetic recombination.*

Natural and continuing populations of triploid females belonging to the *Ambystoma jeffersonianum* complex occur in northeastern North America, where they intermingle with certain populations of the diploid species *A. jeffersonianum* and *A. laterale* (*1*). The triploid females are characterized by having erythrocytes which are considerably larger than those of the diploid animals (*2*). They are accordingly described as large-celled females. The diploid chromosome number for both *A. jeffersonianum* and *A. laterale* is 28 (*1, 3*). Preparations of mitotic chromosomes from the larvae of large-celled females show 42 chromosomes per somatic cell (*1*): We have studied the lampbrush chromosomes in growing oocytes of large-celled females in an effort to determine the mechanism of meiosis in these animals. Chromosomes of the lampbrush type are a feature of amphibian oocytes. They are at a stage corresponding to early diplotene of first meiosis (*4*).

The animals used in this study were collected in Dodge County, Wisconsin; in Washtenaw County, Michigan; and in Sussex County, New Jersey. The blood cells of animals which were thought to be triploid were measured and compared with those of diploid females of *A. jeffersonianum* and *A. laterale*.

Erythrocytes were obtained by cutting the web of one hind foot. The cells were suspended in amphibian Ringer solution and mounted under a coverslip supported with petrolatum. The erythrocytes were drawn with the aid of a camera lucida at a magnification of about 900 times. The drawings represented optical sections through the two longest axes of the ellipsoidal cells. The area of a drawing was circumscribed twice with a planimeter and the total area was divided by 2 to obtain a mean value. At least ten cells from each animal were measured. Cell size proved to be an effective indicator of ploidy. The mean area of erythrocytes from the four triploid females collected in Washtenaw County was 1130 μ^2. The corresponding value for the two females of *A. laterale* collected in Dodge County was 730 μ^2.

The ploidy of each animal was further checked by measuring the amount of DNA per erythrocyte nucleus. For DNA measurements animals were killed and about 0.25 ml of blood was removed from the heart and mixed with 5 ml of 0.01*M* citric acid. The concentration of red blood cell nuclei in this suspension was determined from three separate hemocytometer counts and was adjusted to about 2×10^6 nuclei per milliliter. The diphenylamine reaction (*5*) was used to determine the total DNA in a 1-ml sample of the final suspension. Sperm DNA (*6*) at a concentration of 200 μg/ml in 0.01*N* NaOH was used as a standard. The amount of DNA per nucleus was calculated from