cluded in the cone-filter combination. Further, both radiation-sparing and radiation-limiting methods were employed to an extent that might be considered impractical in purely diagnostic radiography. Nevertheless, it is clear that a well-planned series of investigative radiographs (with careful shielding) can drastically reduce gonadal exposure from levels currently reported (*5*) to a fraction of the irreducible daily background radiation at sea level.

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Electroencephalographic Desynchronization in Irradiated Rats with Transected Spinal Cords

Abstract. *Rats with transected spinal cords showed electroencephalographic desynchronization and exhibited behavioral arousal in response to x-irradiation of the whole body or the head only, at dose rates between 0.5 and 1.5 roentgens per second. Neither arousal nor desynchronization occurred when only the body of the animal was exposed. Results indicate that neither the circulatory system nor the vagi are essential to the arousal reaction to x-irradiation.*

Hunt and Kimeldorf (*1*) recently demonstrated that behavioral arousal can be produced in rats by exposure of either the head or the body to small doses of x-irradiation for periods as short as 1 second. Garcia *et al.* (*2*) found that electroencephalographic desynchronization occurred in rats within 1 second of the onset of whole-body irradiation, and Hunt and Kimeldorf (*1*) reported comparable latencies for the arousal response to ex-lambda and bregma. Two weeks were

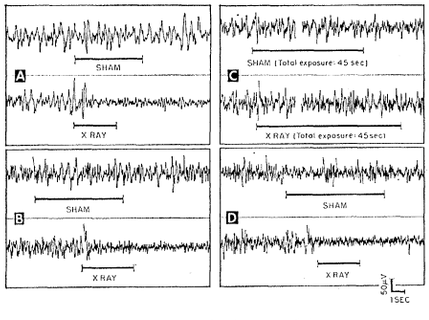

Fig. 1. Electroencephalographic responses of rats to x-irridiation. *A*, Rat with transected spinal cord; irradiation of the whole body. *B*, Spinal transection; irradiation of the head only. *C*, Spinal transection; irradiation of the body only. *D*, Rat that had received sham operation; irradiation of the body only.

posure of only the head or only the body. These latencies are short enough to suggest that exposure to x-rays produces arousal by a direct action on sensory receptors or on the nervous system or by a direct action on both. However, this conclusion was not definitely established by the experiments cited and it is conceivable that primary effects on, or agents transmitted by, the circulatory system secondarily produce neural activation and behavioral arousal. Here we report the results of a study of the electroencephalograms of rats which were exposed to x-irradiation over the whole body, the head only, and the body only after their spinal cords had been transected. The primary objective was to study the mechanism through which the arousal response to x-irradiation is mediated.

Chronic implants of bipolar silver or copper electrodes were made in 60 male Sprague-Dawley rats that were 90 to 180 days old at time of operation. All electrodes were placed epidurally and were located 2 to 3 mm to the left or right of the sagittal suture and about half way between allowed for postoperative recovery. Subsequently, all rats were habituated for 4 hours per day for at least 4 days in cylindrical, Lucite radiation-exposure chambers (7.5 cm in diameter and 26 cm long) before being used in an experiment. Prior to a session of exposures to x-rays, the rats were anesthetized with ether, an incision made in the back of the neck, and the spinal cord surgically sectioned at a level between C5 and T2. After transection of the spinal cord the wound was packed with cotton thoroughly soaked with 1 percent procaine hydrochloride and the skin incision was closed. The sham operations performed on control animals were similar but the spinal cord was not severed. All the rats were put in the Lucite chambers which were then carefully placed in an electrically shielded cage located in the field of the x-ray machine. Cortical electrodes were connected by electrically shielded leads to an electroencephalograph located in a room adjacent to that containing the x-ray unit. A General Electric maxitron x-ray unit operated at 250 kv (peak) and 25 ma (filtration half-value layer, 2.3 mm of copper) was used. During an experiment the x-ray unit remained on at all times and a silent, hydraulically operated shutter was used to control the exposure interval. A lead plate, 0.6 cm thick, was used to shield that part of the animal which was not to be irradiated. Prior to each experiment a Philips type 37471 dosimeter was used to determine dose rates and to check the adequacy of the lead shield. Dose rates used were 0.5, 1.0, and 1.5

Table 1. Number of spinal-transected rats and sham-operated controls showing electroencephalographic desynchronization* on the first two trials.

Dose rate (r/sec)	Trial		Rats tested (No.)
	No. 1	No. 2	
Cord transected; whole body exposed			
0.5	4	5	7
1.0	4	4	5
Cord transected; head only exposed			
0.5	14	17	22
1.0	9	7	11
1.5	6	6	7
Cord transected; body only exposed †			
0.5	0	0	22
1.0	0	0	11
1.5	0	0	7
Sham operated; body only exposed			
1.0	5	4	8

* A change in the pattern of the electroencephalogram from one of high voltage and low frequency to one of low voltage and high frequency.
† The same groups as used above for exposure of head only.

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.

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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