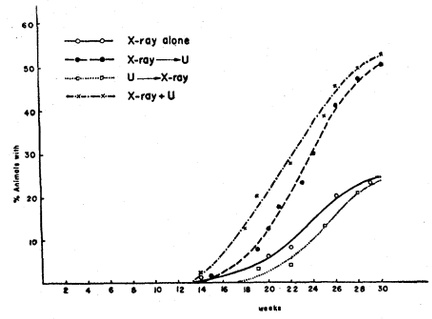

Fig. 1. Incidence of leukemia induced by x-ray alone and in various combinations with urethan.

of extending the interval between the treatments, and other variations that might throw light on the mechanism, are also being investigated.

The possibility of chemical agents acting as promoters of radiation leukemogenesis has not only theoretical interest, but also practical implications for man, with respect to the possible dangers of low doses of radiation, and the debatable question of whether there is, in fact, a threshold dose for leukemogenesis.

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14 March 1960

Influence of Anabolic Steroids on Uptake of Alpha-Aminoisobutyric Acid by Levator Ani Muscle

Abstract. Two representative anabolic steroids caused an increase in the uptake of α-aminoisobutyric acid-1-C^{14} in the levator ani muscle of rats. The distribution ratio between that muscle and the plasma was increased fourfold by the administration of a synthetic anabolic steroid and twofold by administration of testosterone propionate. The determination of this increase may serve as an indicator for the myotrophic effect of anabolic steroids.

The observation of Noall and his coworkers (*1*) that estradiol causes a threefold increase in the concentrative transfer of C^{14}-labeled α-aminoisobutyric acid (AIB) into the uterus of the rat led us to study the effect of anabolic steroids on the uptake of AIB by the levator ani muscle. Clinical studies in this laboratory (*2*) have indicated that a synthetic testosterone analog (*3*), 17-α-ethyl-4-estrene-3-β-17-β-diol-3-propionate, has strong anabolic properties; nitrogen sparing occurred within 24 hours and lasted 8 to 10 days after a single dose (2 mg/kg). It was therefore chosen as one of the test steroids, testosterone propionate being the other.

Sixty-day-old male rats of the Holtzman strain were used. Half of the group were castrated under light anesthesia. Half of the castrated and half of the noncastrated animals were then given 50 mg of the synthetic steroid per kilogram, intramuscularly, and 30 hours later all of the rats were given, subcutaneously, 1 μc of AIB, of the same specific activity (0.8 mc/mmole), per kilogram. Nine hours later the animals were killed by exsanguination. The levator ani muscles were excised, weighed, and homogenized with saline acidified to a *p*H of 5.0 with acetic acid. After centrifugation the supernatants were plated and counted in a thin-window gas-flow counter. The plasma was plated directly and counted. The results are indicated in Table 1.

Table 1. Distribution of AIB in the levator ani muscle on administration of a synthetic anabolic steroid in two different batches of rats (24 each) of the same strain. Each rat weighed approximately 250 gm.

Distribution ratio of AIB*		Mean (± S.E.)	Weight of levator ani†	
Ser. 1	Ser. 2		Ser. 1	Ser. 2
		Castrated—no steroid		
2.1	2.7		48	58
		2.4 ± 0.14		
2.5	2.2		45	52
		Normal—no steroid		
4.8	6.3		53	60
		5.4 ± 0.63		
4.8	6.7		49	60
		Normal—steroid		
7.0	7.2		42	61
		7.8 ± 0.50		
9.2	7.7		45	60
		Castrated—steroid		
9.9	8.2		44	61
		9.4 ± 0.36		
9.9	9.4		47	60

* Each value is from the pooled tissue of three rats. The ratio is counts per minute per gram of tissue: counts per minute per millimeter of plasma. † Each value is the mean for the corresponding three rats in milligrams per 100-gm rat.

The distribution ratio of the AIB in the castrated rats injected with steroid was 3.9 times as great as in the castrated animals not injected with steroid. The values for the uncastrated animals injected and not injected with steroid fell between the extremes of these two groups. In the 39 hours of the experiment there was no significant increment in the weight of the levator ani muscle. That the observed effect of the synthetic steroid on the distribution ratio of AIB was not unique to this new compound was indicated by a similar series of experiments in rats of the same strain given 50 mg of testosterone propionate per kilogram. The results for the same experimental groups were 10.2, 12.6, 16.0, and 23.0, respectively. Although the values were higher, the trend was the same, and the distribution ratio in the castrated animal treated with testosterone propionate was 2.2 times as great as in the castrated animals not treated.

The concentrative transfer of AIB into cells is apparently influenced by many endocrine substances, such as insulin (*4*), epinephrine, hydrocortisone, estradiol (*1*), and growth hormone (*1, 5*). Noall and Christensen indicate (*1*) that AIB may be considered a model for the transport of endogenous amino acids into cells and tissues and that the concentrative transfer of these amino acids, under the influence of various endocrine substances, may be the stimulus for protein formation. The anabolic androgens used in these experiments apparently also cause an increase in the transfer of AIB into the levator ani muscle, one of the target tissues of these steroids. It appears, by implication, that the myotrophic effect (and the androgenic effect) results from concentrative transfer of endogenous amino acids into the cells of the target tissues and organs.

Saunders, using the same two steroids (*6*) and the standard myotrophic test of Eisenberg and Gordan (*7*), found only a 10- to 20-percent increase in levator ani weight in 48 hours and a doubling in weight at the end of 7 days. The two- to fourfold increase in the distribution ratio of AIB within 39 hours after administration of the steroids in these experiments would appear to foreshadow the maximum myotrophic effect by at least 5 days. The AIB determination might therefore serve as a monitor for myotrophic activity and give the desired information in a much shorter period of time (*8*).

Note added in proof: Since submission of this report, two other synthetic anabolic steroids, 19-Nor-Δ-4-androstene-17β-ol-3-one-β-phenylpropionate and 17-α-methyl-17β-hydroxyandrosta-1,4-dien-3-one, have been tested in the manner described in this report. The distribution ratios of the AIB were increased 4.6 and 3.4 times, respectively, over the corresponding controls.

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Effects of Ethyl Alcohol on Avoidance Behavior

Abstract. Three albino rats, trained to avoid electric shock, were stomach-loaded with from 1 to 5 ml of 32-percent aqueous solution of ethyl alcohol prior to the experimental session. Small doses produced some increases in response rates and a consistent decline in shocks received. Larger doses produced progressive uncoordination, accompanied by lower response rates and an increase in shocks received.

Avoidance behavior may be maintained by allowing each occurrence of a selected response to postpone a brief shock for several seconds (*1*). In the absence of responses, shocks are delivered at regular intervals. The steady rate of responding and the stable frequency of shocks resulting from this procedure may be altered by pharmacological agents—scopolamine, for example (*2*). In the work described in the present report (*3*) the effect of ethyl alcohol on avoidance behavior in the rat was studied.

Three male albino rats (150 days old) were used for 2 hours daily in a sound-attenuating experimental chamber equipped with a lever. Depression of the lever (a response) activated electric counters and automatic programming equipment. The floor of the chamber consisted of 1/12-in. steel bars. In the absence of lever-presses, electric shocks (3 ma) of 0.5-second duration were delivered to the rat's feet through the floor grids every 20 seconds. The polarity of each grid changed rapidly and irregularly during shock administration. Each lever-press postponed the next shock for 20 seconds. After 90 days of this procedure, each rat was deprived of food (but not water) for at least 15 hours prior to each avoidance session. On alternate days, 3 minutes before the start of the session, a dose of ethyl alcohol was introduced directly into the rat's stomach through a rubber tube inserted orally. The doses were 1, 2, 4, and 5 ml of 32-percent aqueous solution of ethyl alcohol. Each animal (each weighing about 350 gm) received these doses in a different, irregular order. The volume of alcohol per unit of weight represented by the 5 ml dose in a 350-gm rat is equaled in a 175-lb man by a dose of about ¾ quart of 100-proof liquor. Rat WM4 was not given the 5-ml dose. On control days its stomach was loaded with 2 or 5 ml of water or nothing. The number of responses and shocks in each 2-hour session were recorded.

Figure 1*a* shows for each rat the total number of lever-presses in a 2-hour session on control days (0) and after doses of 1, 2, 4, and 5 ml of 32-percent ethyl alcohol solution. The responding of each rat declines sharply between doses of 4 and 5 ml. The effect on responding after administration of lower doses is variable: for example, one rat shows a decrease and two rats an increase between doses of 1 and 2 ml.

The number of shocks in a 2-hour session is a more orderly dependent variable (Fig. 1*b*). Each animal receives fewer shocks after a dose of 2 ml of the alcohol solution than after a dose of water. The shock frequency increases above that for water after 4 ml and increases sharply after 5 ml. Low doses of alcohol increase the effectiveness of avoidance responses, although the frequency of responses may not increase (Fig. 1*a*). Higher doses of alcohol decrease the frequency of responding and increase the frequency of shocks.

The relative decrease in response rates following doses of 1 and 2 ml for rat WM5 may be brought about by the decrease in shocks received. This animal's relatively higher rate of responding under control conditions is largely accounted for by bursts of three to four responses per second during and after shocks. A decrease in shock frequency leads to a decrease in the number of such bursts.

An examination of cumulative records of responses during control sessions (water or no dose) reveals a steady rate of responding throughout both hours of the session after a gradual acceleration at the start of the session. A dose of 1 ml produces slight irregularities during the first hour, but the record during the second hour is indistinguishable from the control record. After doses of 2 and 4 ml, responding accelerates markedly and ceases for about 1 minute early in the first hour. The onset of the acceleration is earlier after a dose of 4 ml than after a dose of 2 ml. After 5 ml, responding begins at a rate higher than the control rate.

Extended periods of no responding begin near the end of the second hour after 4 ml and near the end of the first hour after 5 ml. Only one response was made by rat A3 after the end of the first hour with a 5-ml dose, and the experiment was terminated after 27 consecutive shocks had been received. In computing the number of responses emitted and the shocks received (Fig. 1) for rat A3, it was assumed that no further responses would have been emitted and that the shock frequency would have been maximum for the remainder of the second hour of the session. This animal died 12 hours later without recovering from the effects of 5 ml of alcohol.

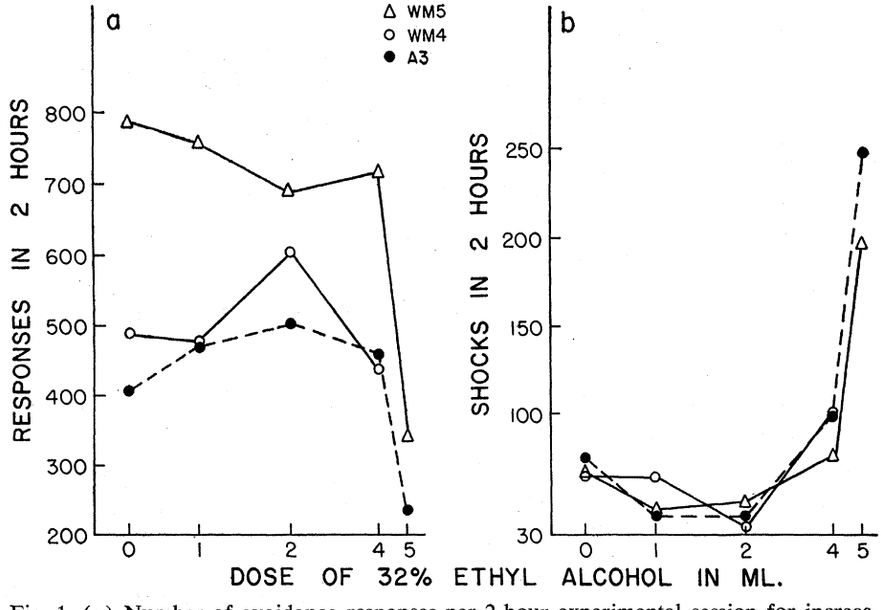

Fig. 1. (*a*) Number of avoidance responses per 2-hour experimental session for increasing doses of alcohol. (*b*) Number of shocks received per 2-hour session. The data for control sessions are shown at 0 on the abscissa. Each point in Fig. 1 is based on from 1 to 5 experimental sessions.