(*11*) (Fig. 1*C*). These short fiber bundles apparently constitute the most rostral components of the lateral propriospinal system, adjoining the spinal gray matter on the lateral side. As such, the distribution of these fibers may exemplify the distribution pattern of the entire lateral propriospinal chain, originating in the lateral tegmentum and the zona intermedia and distributing among others to motor and sensory cell groups.

Were this supposition correct, we might predict that the long lateral subcorticospinal system terminating in this very zona intermedia would influence the activity of the secondary sensory cell groups in the posterior horn, in addition to its commonly accepted influence on the motor neurons. This prediction is in striking agreement with the actual findings of a systematic physiological investigation of the medullary cross section (*12*). In that study (*12*) it was found that the points from which the sensory cells in the posterior horn could be influenced dromically were located in the ventrolateral parts of the cross section, an area occupied by the lateral subcorticospinal system. Furthermore, this long, lateral, subcorticospinal system apparently also transmits a subcortical influence on the sensory nuclei cuneatus and gracilis. This is suggested by findings in additional experiments, in the rhesus monkey and the cat, with lesions in the mesencephalon. In these cases, the lateral subcorticospinal system was degenerated and some of its fibers were found to distribute to the basal parts of the nuclei cuneatus and gracilis. On the other hand, some physiological studies (*3*) suggest that the medial bulbar reticular formation influences "all" the sensory nuclei: the spinal posterior horn as well as the nuclei cuneatus and gracilis. In regard to this conclusion some reservations seem necessary (see *5*, *12*). Moreover, in the present material a distinct fiber system from this part of the reticular formation to the region of "all" these sensory nuclei could not be demonstrated. However, this does not allow us to rule out the possibilities of a transmission of reticular activity to the posterior horn through the mediation of the cells in the dorsomedial parts of the anterior horn, which receive long reticulospinal fibers (*13*).

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References and Notes

1. H. G. J. M. Kuypers, *Anat. Record* **124**, 322 (1956); *J. Comp. Neurol.* **110**, 221 (1958); *Science* **128**, 662 (1958); *Anat. Record* **133**, 301 (1959); *Brain* **83**, 161 (1960); W. W. Chambers and C. N. Liu, *Federation Proc.* **17**, 24 (1958).
2. C. G. Bernhard and E. Bohm, *A.M.A. Arch. Neurol Psychiat.* **72**, 473 (1954).
3. K. E. Hagbarth and D. I. B. Kerr, *J. Neurophysiol.* **17**, 295 (1954); R. Hernández Peón, H. Scherrer, M. Velasco, *Acta Neurol. Latinoam.* **2**, 8 (1956).
4. U. F. Lindblom and J. O. Ottoson, *Acta Physiol. Scand.* **29** *Suppl.* **106**, 191 (1952).
5. F. Magni, R. Melsack, G. Moruzzi, C. J. Smith, *Arch. ital. biol.* **97**, 357 (1959).
6. H. G. J. M. Kuypers, *Anat. Record* **124**, 322 (1956); W. W. Chambers and C. N. Liu, *J. Comp. Neurol.* **108**, 23 (1957); F. Walberg, *Brain* **80**, 273 (1957); H. G. J. M. Kuypers, *J. Anat.* **92**, 193 1958).
7. W. J. H. Nauta and P. A. Gygax, *Stain Technol.* **29**, 91 (1954).
8. J. M. Sprague, *J. Comp. Neurol.* **95**, 103 (1951).
9. D. P. C. Lloyd, *J. Neurophysiol.* **4**, 114 (1941).
10. ———, *ibid.* **4**, 525 (1941).
11. R. Lorente de Nó; *A.M.A. Arch. Neurol. Psychiat.* **30**, 245 (1933); M. E. Sheibel, *Anat. Record,* **121**, 362 (1955), and personal communication.
12. U. F. Lindblom and J. O. Ottoson, *Acta Physiol. Scand.* **35**, 203 (1955).
13. These investigations were supported by grant No. B-1131, National Institute of Neurological Diseases and Blindness, U.S. Public Health Service, and grant No. G-6434, National Science Foundation.
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Possible Two-Stage Mechanism in Experimental Leukemogenesis

Abstract. The augmenting influence of urethan on leukemogenesis by x-radiation in mice has been found to operate when the urethan treatment follows the radiation, but not when the sequence is reversed. The result is in keeping with the idea that urethan acts as a promoting factor in leukemogenesis, as defined by the two-stage mechanism hypothesis of carcinogenesis. It may also have a practical bearing on leukemia development in man.

Kawamoto, Ida, Kirschbaum, and Taylor (*1*) reported a striking augmentation of leukemogenesis in mice, whether induced by x-radiation, estrogen, or methylcholanthrene, when the animals were at the same time subjected to urethan (ethyl carbamate) administration. Since urethan alone was entirely free from leukemogenic action, its augmenting influence was described by them as "co-leukemogenic."

In line with earlier investigations on co-carcinogenesis in mouse skin (see *2*), we attempted to analyze the above-mentioned co-leukemogenic action of urethan in terms of the two-stage mechanism hypothesis of carcinogenesis. If the effect were due to a summation of two similar types of action (of which one was too weak to be demonstrated by itself), one would expect the result to be the same whether the urethan preceded or followed the radiation. If, however, the urethan acts as an initiator only, or as a promoter only, then the augmentation should operate in one application sequence or the other, but not in both.

Five groups of C57b1/6 mice, each comprising 75 young adults of mixed sexes, were treated as follows: one group received x-radiation alone; one received urethan alone; one received the two concurrently (to confirm the augmentation reported by Kawamoto *et al.*); one received x-radiation followed by urethan; and one received urethan followed by radiation.

The radiation was provided by a Mühler 250-kv machine (physical factors: 200 kv, 15 ma, 0.5 mm Cu and 1.0 mm Al added filter, 50 cm target mouse distance; output 49.5 r/min). The urethan was injected intraperitoneally as a 10-percent solution in distilled water. The radiation treatment (total body) was administered in 5 doses of 90 r each, at intervals of 5 days, a total of 450 r. The urethan was also administered in 5 doses at intervals of 5 days, the mice receiving 0.2 ml per dose, a total of 100 mg. In the case where the two forms of treatment were given concurrently, the urethan was given immediately before each radiation. Where the two forms of treatment were given during separate periods, the interval between the completion of the one and the commencement of the other was 2 weeks.

The results, after 30 weeks, measured from the time of the first radiation, were as follows: x-radiation alone, leukemia in 17 of 75 survivors (23 percent); x-radiation together with urethan, 26 of 50 (52 percent); x-radiation followed by urethan, 35 of 70 (50 percent); urethan followed by x-radiation, 17 of 74 (23 percent). Urethan alone, after 30 weeks, yielded no leukemia among the 61 survivors. The results, expressed in the form of leukemia incidence curves, are shown in Fig. 1.

The fact that augmentation of leukemogenesis by urethan is obtained when urethan is made to follow the radiation, but not when the sequence is reversed, is in keeping with the idea that urethan acts as a promoting agent in leukemogenesis, as defined by the two-stage mechanism hypothesis of carcinogenesis. This is all the more surprising, since, in the case of skin carcinogenesis, urethan acts as a pure initiator (*3*), while for the lungs it is a complete carcinogen (*4*).

The present experiment is being continued until all the animals die. Meanwhile, further experiments are in progress, involving lower doses of radiation, in the hope that the effect might be observed under more critical conditions—that is, with no leukemia arising in the x-ray control series, or in the group in which the radiation follows the urethan treatment. The effect

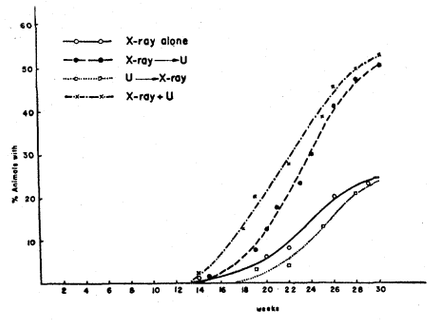

Fig. 1. Incidence of leukemia induced by x-ray alone and in various combinations with urethan.

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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References

1. S. Kawamoto, N. Ida, A. Kirschbaum, G. Taylor, *Cancer Research* **18**, 725 (1958).
2. I. Berenblum, *Ann. Roy. Coll. Surg. Engl.* **21**, 339 (1957).
3. M. H. Salaman and F. J. C. Roe, *Brit. J. Cancer* **7**, 472 (1953).
4. A. Nettleship and P. S. Henshaw, *J. Nat. Cancer Inst.* **4**, 309 (1943).

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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References and Notes

1. M. W. Noall, T. R. Riggs, L. M. Walker, H. N. Christensen, *Science* **126**, 1002 (1957).
2. E. L. Dargan and W. Metcalf, *Surg. Forum, Proc. 45th Congr. Am. Coll. Surgeons* **1959**, 331 (1960).
3. The analog was synthesized by F. B. Colton.