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The Effects of Diamox® on the Uterine Response of Estrogen Treated Rats.* (23589)

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(Introduced by Frederick L. Hisaw)

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The earliest detectable response of the rat uterus to estrogen administration is a rapid imbibition of water, followed by a period of cellular proliferation and growth. The tissues of the uterus become edematous and the entire organ enlarges as a result of luminal fluid accumulation.

The drug, Diamox® (2-acetylarnide-1,3,4-thiadiazole-5-sulfonamide) is a powerful inhibitor of the enzyme carbonic anhydrase (1,2). Such inhibition in animals results in the excretion of alkaline urine, diuresis and lowered plasma bicarbonate(3). Perlmutter and Olewine(4) reported that acetazolamide increased the excretion of water about one and one-half times in normal rats. This greatly increased water excretion suggested that Diamox® may also affect the water imbibition of the uterus following administration of estrogen.

Materials and methods. The effects of Diamox® on uterine response to estrogen were studied in both sexually immature and adult rats. The effects on the immature rat uterus were determined by means of the bioassay method of Astwood(5). Animals 21-23 days old and weighing 40 to 50 g were given a single subcutaneous injection of 0.1 µg of estradiol-17β and graded doses of the sodium salt of Diamox®. The uteri were removed at various time intervals,

stripped of fat and weighed immediately. Adult female rats 100 days old were used to determine changes in wet weight, dry weight and volumes of luminal fluid induced by Diamox®. Forty-eight animals were castrated and allowed to remain untreated for 7 days, at which time they were divided into 5 groups and were treated as follows: the first group received no treatment; the second was given 5 µg estrone daily for 3 days; the remaining 3 groups received 5 µg of estrone per day for 3 days and a daily injection of 40, 60 and 80 mg of Diamox®, respectively. The amount of luminal fluid was measured by withdrawing the fluid into a glass syringe fitted with a No. 20 needle. The exact amount of fluid could not be measured by this method, but a fair indication of the relative amount could be ascertained. *Water content* was determined by oven-drying uteri for 24 hours at 90-100°C. Nitrogen content of the dried uteri was measured by Nesslerization after digestion in a selenium-sulfuric acid mixture. The *sodium salt* of Diamox® (Lederle lot No. 7-6015)[§] was dissolved in physiological saline at a concentration of 200 mg per ml. pH of this solution was 9.1. Estradiol-17β and estrone were dissolved in sesame oil. Rats were maintained on a normal diet and given water *ad libitum*. All injections were made subcutaneously either in the scruff of the neck or the skin of the back. The volume never exceeded 0.5 ml.

Results. The experiments in which the

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TABLE I. Effect of Graded Doses of Diamox® on Uteri of Estradiol-Treated Immature Rats.

Treatment	% water*		Dry wt* (mg)		Nitrogen (mg/uterus)		No. animals
	6 hr	30 hr	6 hr	30 hr	6 hr	30 hr	
None	81.2 ± 1.3		4.0 ± .8		.52		25
0.1 µg estradiol	87.2 ± .8	84.2 ± 1.6	4.8 ± .6	5.4 ± 1.0	.56	.63	23
<i>Idem</i> + 20 mg Diamox	83.7 ± 1.6	83.3 ± .7	4.1 ± 1.0	6.2 ± 1.1	.47	.74	12
" + 30 " "	85.4 ± 1.3	83.9 ± 1.4	3.6 ± .7	5.3 ± .7	.50	.65	12
" + 40 " "	82.2 ± 1.9	83.4 ± 1.1	4.4 ± .6	5.2 ± .6	.53	.60	12
" + 50 " "	80.8 ± 2.4	82.7 ± 1.4	4.2 ± .5	5.2 ± 1.0	.49	.62	12

* Mean ± stand. dev.

immature animals were used (Table I) show that acetazoleamide is effective only in blocking uterine imbibition of water at the sixth hour, at which time it normally attains a maximum following injection of 0.1 µg estradiol. The growth phase, as measured by dry weight and nitrogen content at the thirtieth hour, is not disturbed by Diamox®. It is further interesting to note that in the 30 hour experiments the percentage of water is in all cases the same, the difference between any two groups not exceeding the standard deviation of the smaller. Values reported in Table I indicate that the percentage of water at the sixth hour does not vary inversely with dosage of Diamox®. The uteri of control animals injected with 20 mg of Diamox® per day for 3 days

show no deviation from the normal at either the sixth or thirtieth hour.

Although Diamox® inhibited fluid imbibition which normally reached a maximum 6 hours after the injection of 0.1 µg of estradiol, further investigation showed that actually the response was only delayed and occurred at the twelfth hour (Fig. 1). A second injection of Diamox® on the sixth hour did not bring about a further postponement of the reaction. However, in all instances the response was somewhat less at 12 hours when Diamox® was given than at 6 hours when only estradiol was injected. In these experiments, as in the others reported (Table I), Diamox® did not modify uterine growth as shown by insignificant differences in dry weight at 30 hours.

In tests in which adult animals were given 3 daily injections of estrone or estrone plus Diamox® (Table II) the luminal fluid was withdrawn before the uteri were removed. Consequently, the percentage of water recorded was that of uterine tissue. Each group initially contained 8 animals but some were rejected due to mishaps in operational procedure; however, none died as a result of treatment.

Daily doses of 40 and 60 mg Diamox® given concurrently with 5 µg of estrone did not alter the percentage of water in the uterine tissues from that reported for the estrogen alone. However, 80 mg of Diamox® under the same conditions appeared to depress both uterine wet and dry weights. There also was a reduction in uterine luminal fluid as the dosage of Diamox® was increased. When Diamox® alone was administered to castrated females in doses of 60 mg per day for 3 days the percentage

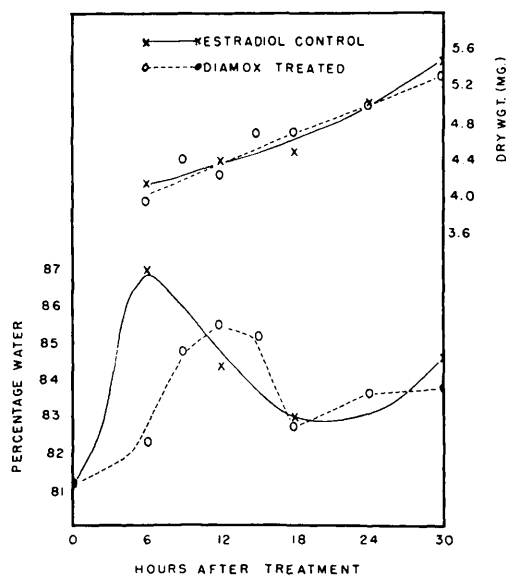


FIG. 1. Relationship of time to effects of Diamox® (40 mg) on uterine response to estradiol (.1 µg) stimulation.

TABLE II. Effect of Graded Doses of Diamox® on Uteri of Estrone-Treated Mature Castrated Rats.

Treatment*	Wet wt† (mg)	Dry wt† (mg)	% water†	Luminal fluid (ml)	No. animals
None	112.9 ± 4.0	20.9 ± 1.0	80.4 ± .7	Unmeasurable	8
5 µg estrone	218.9 ± 10.2	45.5 ± 3.2	80.5 ± .5	.6	7
<i>Idem</i> + 40 mg Diamox	189.8 ± 14.0	37.9 ± 2.2	81.1 ± .2	.4	5
" + 60 " "	218.3 ± 10.7	39.6 ± 1.5	80.5 ± .5	.2	6
" + 80 " "	172.5 ± 10.5	33.6 ± 1.6	80.9 ± .5	.2	7

* Animals inj. daily for 3 days.

† Mean ± stand. dev.

uterine water was 79.6 ± 0.5 , which was not significantly different from that indicated for untreated castrates (Table II).

Analyses of the ionic content of uterine fluid under these conditions showed no differences between estrone treated animals and those given both estrone and Diamox®. The sodium concentration in luminal fluid from animals given estrone was 121 mEq/l and potassium 42 mEq/l, while for those given estrone and Diamox® it was 122 mEq/l and 44 mEq/l, respectively.

Discussion. It is known that Diamox® causes an increase in excretion of water and an elevation of bicarbonate in the urine(3). Associated with this condition when Diamox® and estrogen were injected simultaneously, is a failure of the imbibition of fluid by the uterus during the first 6 hours. However, the effect of estrogen is only postponed as an accumulation of fluid, though somewhat reduced, occurs at about the twelfth hour. This shift in time of fluid imbibition does not influence the growth response of the uterus as seen at 30 hours.

The postponement of fluid imbibition from the sixth to the twelfth hour when Diamox® is given with estrogen is also a point of interest. If Diamox® is eliminated from the body more rapidly than estrogen an effective amount of estrogen may be present for a longer period than an inhibitory amount of Diamox®. However, a second dose of Diamox® administered on the sixth hour does not inhibit the delayed response of the twelfth hour nor does it modify uterine growth. This failure of a second dose of Diamox® to inhibit fluid imbibition is more difficult to explain but it may be that

the animals become so acidotic they are unable to respond to a second injection.

There is also a question as to whether Diamox® produces its effect by a direct action upon the uterus. Mediation through carbonic anhydrase inhibition is doubtful; however, since Lutwak-Mann(6) has reported that the uterus of the rat does not contain a detectable amount of this enzyme. It is more probable that the effects of Diamox® as seen in the uterus are due to the general diuretic response of the animal.

Summary. Diamox® (40 mg) postpones the peak of water imbibition in the uterus of estradiol treated immature rats from the sixth to the twelfth hour. It is, however, incapable of blocking the normal growth response occurring at 30 hours. Daily doses of 40 and 60 mg of Diamox® given concurrently with 5 µg of estrone per day for 3 days to castrated adults did not alter the percentage of water in the uterine tissues from that reported for the estrogen alone. It is suggested that imbibition of water and the proliferative phase observed in the uterus under estrogen stimulation represent independent reactions.

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