the equilibrium was carried out at 37°C or at 4°C; and b) binding increased with increasing alkalinity over the pH range of 6.7 to 8.1.

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Zn^{65} Uptake by Rat Dorsolateral Prostate as Specific Bioassay for ICSH.* (26131)

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It has been demonstrated(1) that a measurement of the functional capacity of the rat dorsolateral prostate to concentrate Zn^{65} was a more sensitive indicator of ICSH (LH) activity than measurements of either dorsolateral or ventral prostate weight. The results of further studies to ascertain degree of sensitivity and specificity of Zn^{65} uptake as a bioassay for ICSH are reported in this paper.

Methods. Hypophysectomized (hypox) male Wistar rats (16-weeks of age, weighing 300 to 350 g) were purchased from Charles River Breeding Lab., Brookline, Mass. On the 5th day following surgery hormone injections were initiated as follows: 72 hypox rats received daily injections of 5, 20, 40, 100 and 200 μg of ICSH;† 52 hypox rats received daily injections of 200 and 500 μg of FSH,† Growth Hormone,† and Prolactin;† 12 hypox rats received daily injections of 20 and 40 μg of a human male castrate urine extract prepared and purified according to technics outlined by Albert(2). All hormone preparations were injected intramuscularly in 0.2 ml physiological saline, once daily for 6 days. Eighteen hypox rats injected with 0.2 ml physiological saline daily served as controls. On the 6th day of hormone administration all rats received intracardiac injections of Zn^{65} (0.04 μc/g) as outlined previously(3). Twenty-four hours later the rats were sacrificed, the dorsolateral prostates dissected(4), and their radioactive content determined by technics described(3). All rats used in this experiment were inspected grossly for completeness of hypophysectomy. None showed any pituitary remnants. During this investigation the rats were housed under constant

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† Supplies of purified pituitary hormone preparations were generously furnished by the Endocrinology Study Section, Nat. Inst. Health; ICSH (NIH-LH-S-1, ovine), FSH (NIH-FSH-S-1, ovine), growth hormone (NIH-GH-B_2, bovine), and Prolactin (NIH-P-S-3, ovine).

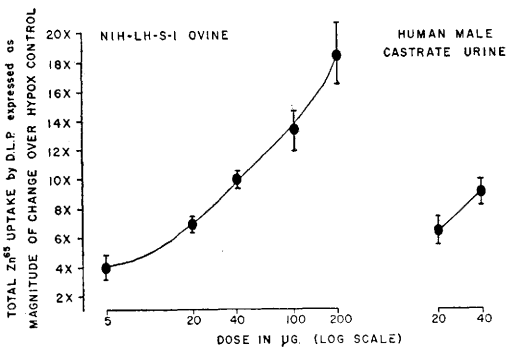

FIG. 1. Effect of various doses of ICSH and of purified male castrate urine extract on Zn^{65} uptake by dorsolateral prostate of hypophysectomized rat (mean ± S.E.M. are shown).

conditions of temperature, humidity and lighting (12-hour light, 12-hour darkness cycle). These experiments were conducted during the months of March, April and May, 1960(3,5,6).

Results. Zn^{65} uptake values, expressed as total counts per minute in dorsolateral prostate (mean ± S.E.M.), were as follows: for 18 untreated hypox controls, 2,500 ± 208; for 5 hypox rats administered 5 µg ICSH, 10,020 ± 2,380; for 18 hypox rats administered 20 µg ICSH, 17,100 ± 1,205; for 13 hypox rats administered 40 µg ICSH, 24,700 ± 1,730; for 10 hypox rats administered 100 µg ICSH, 32,900 ± 4,000; and for 16 hypox rats administered 200 µg ICSH, 46,330 ± 5,610 (Fig. 1). The data indicate that with the 5, 20, 40, 100 and 200 µg of ICSH there was a 4.0-, 6-8-, 9.9-, 13.2-, and 18.4-fold increase, respectively, over the untreated hypox control. Statistical analysis showed that with 5 µg of ICSH the Zn^{65} uptake response differed significantly from that of the untreated hypox control ($P < 0.005$). The response with 20 µg differed significantly from that with 5 µg ($P<0.01$) and from that with 40 µg ($P<0.001$).

Zn^{65} uptake values for the human male castrate urine extract were as follows: for 6 hypox rats administered 20 µg extract, 15,960 ± 2,900; for 6 hypox rats administered 40 µg extract, 22,440 ± 2,277. Fig. 1 shows that with 20 and 40 µg of extract there was a 6.4- and 9.0-fold increase in Zn^{65} uptake, respectively, over the untreated hypox control. Statistical analysis showed that with the low dose of 20 µg of extract the Zn^{65} uptake response differed significantly from that of the untreated hypox control ($P< 0.001$). Within the limits of error of the assay method, the human male castrate urine preparation was equipotent with the ICSH standard used, as judged from the parallel line multiple dose-response curves.

Zn^{65} uptake values in hypox rats administered 200 and 500 µg of Growth Hormone and of Prolactin did not differ significantly from that of the untreated hypox control. Zn^{65} uptake values in hypox rats administered 200 and 500 µg of FSH indicated the presence of approximately 1-2% ICSH activity.

Discussion. Previous investigations have shown that the amount of administered Zn^{65} taken up by the rat dorsolateral prostate depended upon androgen level(7,8). The functional capacity of the gland to take up Zn^{65} was diminished by removal of testes or pituitary, but could be maintained near control levels in the castrate by suitable doses of testosterone propionate(7) and in the hypox rat by maintenance doses of chorionic gonadotrophin or testosterone propionate(8). A more recent communication(1) demonstrated that with administration of 200 µg of ICSH to hypox rats total Zn^{65} uptake by the dorsolateral prostate was increased approximately 18-fold over that of the untreated hypox control, suggesting use of this technic as a possible bioassay for ICSH activity. In the experiments reported here it was demonstrated that doses of ICSH as low as 5 µg could be detected by virtue of a 4-fold increase in Zn^{65} uptake values over those noted in untreated hypox controls. That this method is suitable for a bioassay of clinical material was indicated by the fact that an extract of human male castrate urine gave a dose-response curve which was identical in slope with that of the standard ICSH preparation used.

These studies have also shown that the Zn^{65} uptake technic was a specific measure of ICSH activity as has also been claimed for the ventral prostate assay method(9). There was no significant response to either

growth hormone or prolactin with doses as high as 200 and 500 μg. The presence of ICSH activity in the FSH preparation used is borne out by the reports of others.‡ The problem of LH or ICSH activity in preparations of FSH is a topic of much discussion(10). Woods and Simpson(11) have recently stated, "No follicle stimulant of natural origin (pituitaries of any species, menopause or castrate serum or urine) is known which did not at some relatively low multiple of the minimal dose for follicle-development also show uterine stimulation and luteinization or interstitial cell stimulation."

Summary. Experiments have been reported which indicate that a measurement of Zn^{65} uptake by the dorsolateral prostate of the hypophysectomized rat is a sensitive and specific bioassay for ICSH activity.

‡ Report on LH (ICSH) contamination in NIH-FSH-S-1, ovine by the Endocrinology Study Section, Nat. Inst. Health.

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Endocrine Effects of Sulfoxone and of Two Nitriles as Compared with Amphenone.* (26132)

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Amphenone (3, 3-bis-(p-aminophenyl)-butanone-2), has been shown to exert marked endocrine effects on intact mammals through inhibition of formation of adrenal cortical and thyroid hormones by those glands and by a direct effect on the genital tract of the female(1). Because of the report of endocrine changes in patients treated with a sulfone(2) and because of the structural resemblance of the "parent sulfone" diaminodiphenylsulfone (DDS) to amphenone, it was decided to test the disodium formaldehyde sulfoxylate derivative of 4, 4′ diaminodiphenylsulfone (sulfoxone, "Diasone",® Abbott Pharmaceutical Co.) for such actions. Two steroid analogues bearing some structural resemblance to amphenone were also tested: 2, 3-bis-(p-hydroxyphenyl)-valeronitrile (SC-3402 of the G. D. Searle Co.), and 2, 3-bis-(p-hydroxyphenyl)-proprionitrile (Searle SC-4473). The formulae are given in Fig. 1.

Methods. The intact rats were immature females from 23 to 28 days of age obtained from the Holtzman Co., Madison, Wisc. Ovariectomies were performed on a group of these rats at this age one week before beginning drug administration. The hypophysectomized rats were adults of a Sprague-Dawley strain from the Hormone Assay Labs., Chicago, Ill. They had been hypophysectomized about 2 months prior

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