tically significant. In contradistinction the 66 non-treated ZBC controls all rejected the Ce homologous skin as would be predicted from their genetic differences.

Discussion. Our results agree with the hypothesis which provoked this study. In fact, it was originally postulated that if the state of tolerance requires the presence of living foreign cells in the host it might be possible to transfer this tolerance to another individual of the same strain by injecting at birth the spleen containing the homologous foreign cells necessary to induce this phenomenon. Our observations using the donor-host strain combination Ce⟶ZBC indicate that this has been accomplished, since by treating newborn ZBC animals with living spleen cells taken from ZBC animals tolerant to Ce donor, tolerance to homologous Ce skin was demonstrated.

This observation also agrees with that of Billingham, Brent and Medawar(2) who showed that spleen tissue from tolerant animals contains antigenically active material from the donor strain. Our findings however extend those of the British workers and indicate that in the tolerant host all of the transplantation antigens presumably in the form of many intact cells derived from the original donor are present in the reticuloendothelial tissues of the tolerant host.

Summary. Newborn mice of the ZBC strain injected intravenously with living spleen cells taken from donors of the same strain in which tolerance to Ce tissues was previously induced, became tolerant to this strain and accepted homologous skin grafts from Ce donors. This would indicate that acquired tolerance can be transferred to an isologous individual at birth by the intravenous injection of spleen cells taken from a tolerant donor.

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Hypothalamus and Somatotrophic Hormone Release. (24135)

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Hypothalamic control of somatotrophic hormone (STH) secretion, though not yet clearly demonstrated, may be assumed from data in the literature. Hetherington *et al.*(1), Bogdanove *et al.*(2), and Endröczi *et al.*(3) showed that rats with hypothalamic lesions frequently fail to grow; retardation of body growth has also been reported in dogs in which anterior hypothalamic lesions had been performed early in their life(3). Typical disturbances of carbohydrate metabolism induced by hypophysectomy and by STH deficiency have been found also after hypothalamic lesions involving the region of paraventricular nuclei(4); Spirtos *et al.*(5) observed that lesion-bearing rats often exhibit increased hypoglycemic response to insulin, which can be reduced by STH administration. It is generally agreed that marked retardation of body growth follows transplantation of the pituitary far from the sella turcica (6,7); it is noteworthy that both hypothalamic lesions(2) and hypophyseal transplantation(7) induce disappearance of the STH-producing eosinophil cells of the anterior pituitary. The mechanism by which hypothalamic stimuli may reach the glandular cells of the anterior lobe of the pituitary gland

and regulate release of trophic hormones has been widely discussed(6); results of anatomical and physiological studies support the view that the hypophyseal portal vessels regulate anterior pituitary functions by transmitting an hypothalamic humoral substance(6). Several suggestions have been put forward as to the nature of this hypothalamic neurohumor; attention was called to a possible relationship between antidiuretic hormone (ADH) of the hypothalamic-posthypophyseal system, and release of ACTH(8). This view was mainly based on the demonstration that extracts containing ADH have an ACTH releasing effect in normal animals(9,10), in hypophysectomized animals bearing a functional pituitary graft in the anterior chamber of the eye(7) and in rats with hypothalamic lesions (11). Recently ACTH-releasing activity of posterior pituitary preparations has been confirmed in an *in vitro* system, in this case ascribed to the presence of a contaminant(12, 13). The experiments here reported deal with the possibility of inducing somatotrophic hormone release (STH) by means of the same mediator which is effective in inducing ACTH release.

Methods. Normal and hypophysectomized Sprague-Dawley rats, of both sexes, weighing 60 g were used. They were given basal diet and tap water; hypophysectomized rats received an additional 5% glucose in drinking water. Hypophysectomy was performed by standard parapharyngeal approach. Preparations of whole posterior pituitary lobe extract (PPLE) (Postipofisan, Richter) and purified antidiuretic (Pitressin, Parke-Davis) and oxytocic (Pitocin, Parke-Davis) hormones were used.* These hormones were given by daily intraperitoneal injections (0.5 U/rat dissolved in 0.5 ml of 0.9% NaCl solution) for 10 days; control rats were injected daily with 0.5 ml of 0.9% saline solution. Three groups of normal male rats were given intraperitoneally an STH preparation (Armour, 80 U/mg) in daily doses of 25, 50, and 100 μg/rat, for 4 days. All animals were killed 24 hours after last injection; treatment of hypophysectomized rats started 10 days after operation. The tibias of animals were then prepared for measurement of width of proximal epiphyseal cartilage according to Greenspan's *et al.* standard procedure(14). Only female rats have been used in the previous STH studies; however Geschwind *et al.*(15) established that male rats are sensitive to STH.

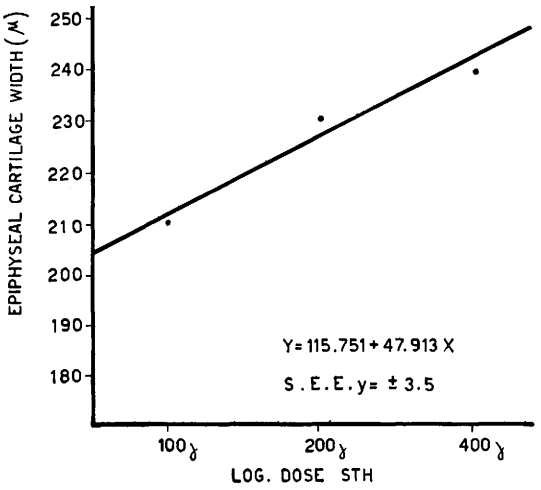

FIG. 1. Effect of exogenous STH on tibia cartilage width of normal male rats.

Results. Fig. 1 shows that an enlargement of the tibia cartilage width may be induced in normal male rats by injecting different doses of STH; the curve, and its regression equation, show that a linear log-dose relationship may be obtained. The results summarized in Table I show that chronic treatment with

TABLE I. Effect of Continuous Injections of Different Posterior Pituitary Preparations on Epiphyseal Cartilage Width of Normal and Hypophysectomized, Male and Female Rats.

Treatment	Sex of rats	No. of rats	Tibia cartilage width (μ) Normal rats	Hypophysectomized rats
.9% NaCl	♂	12	184 ± 8.3	117 ± 11.6
PPLE	♂	13	221 ± 8.1*	113 ± 3.8
.9% NaCl	♂	10	204 ± 3.3	112 ± 15.9
Pitressin	♂	8	236 ± 5.6*	107 ± 17.5
Pitocin	♂	8	199 ± 3.9	
.9% NaCl	♀	8	203 ± 5.8	
PPLE	♀	7	233 ± 6.8†	

* Differs significantly from controls ($P < .001$).
† *Idem* ($.005 > P < .01$).

* We are indebted to Dr. Z. Koreny of Richter Laboratories, and to Dr. D. A. McGinty of Parke-Davis and Co. for generous supplies of posterior pituitary preparations.

PPLE and with Pitressin induces significant enlargement of epiphyseal cartilage width in normal male rats ($P<0.001$); PPLE induces an increase in tibia cartilage width also in normal female rats ($P<0.01$); Pitocin is ineffective in normal male rats. In the hypophysectomized animals PPLE and Pitressin do not produce significant modification of the epiphyseal cartilages.

Discussion. Our experiments clearly demonstrate that different posterior pituitary preparations containing ADH may stimulate proliferation of tibia epiphyseal cartilage in normal rats of both sexes; lack of activity of these same preparations in hypophysectomized animals seem to rule out the possibility of an STH contamination of the extracts used, and to suggest that the stimulus on the cartilage plate is mediated through release of an adenohypophyseal principle; these experiments also suggest that STH may be the hormone involved.

A slight enlargement of the cartilage width may be produced by thyroxine and testosterone(15,16); it is then conceivable that TSH and ICSH through activation of their target glands may act as stimulators of the epiphyseal cartilage plate. A TSH-releasing activity of posterior pituitary preparations(17,18) may suggest release of TSH as a possible explanation of the results here described; however, Geschwind *et al.*(15) found that very large doses of exogenous thyrotrophic hormone are required to duplicate the effects of small amounts of STH on the tibia test. As to the role of ICSH, very little is known about the action of posterior pituitary principles on release of gonadotrophic hormones. Pitocin could be the stimulus for the pituitary-gonadal system, as judged by output of urinary gonadotrophin and fractionation of 17-ketosteroids(19,20). But according to our results Pitocin is not active on the cartilage plate; moreover liberation of ICSH after giving posterior pituitary preparations could play a role only in male animals(15), whereas their effectiveness on the cartilage width occurs in both sexes. Prolactin might also duplicate the effects of STH(21-23) as Marx *et al.*(16) reported a slight enlargement of the epiphyseal cartilage after giving prolactin but Geschwind *et al.*(15) demonstrated that this effect was sex-dependent, because of its presence only in male rats. In the present experiments the effect of posterior pituitary preparations on the cartilage plate was evident both in female and in male rats; moreover should this effect be due to prolactin, it would also be present after giving Pitocin, which is very active in promoting prolactin release(23,24).

Summary. 1) Chronic treatment with posterior pituitary preparations containing ADH induces significant enlargement of tibia cartilage width in normal male and female rats; oxytocic hormone is ineffective in normal animals. 2) In hypophysectomized animals posterior pituitary hormones do not produce modification of the epiphyseal cartilages.

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Increased Creatine and Creatinine Excretion after 17α-Ethyl-19-Nortestosterone.* (24136)

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Among biological processes, the rate of creatine synthesis in mammals is remarkable for its constancy. Creatine is formed in the liver by methylation of guanidinoacetic acid (1). It then diffuses freely throughout the body: concentration of free creatine in various cells and body fluids is approximately equal. Creatine, particularly in muscle, is phosphorylated to form the labile phosphocreatine, thus concentration of total creatine in cells is higher than that of free creatine(2). There is a renal threshold for creatine(3), and under normal circumstances very little appears in the urine. Creatinine, the end product of creatine metabolism, arises from creatine or phosphocreatine by non-enzymatic dehydration(4). At closely regulated pH and temperature of body, its rate of formation appears to depend only on concentration of creatine. In man, about 1.64% of the creatine body pool is converted to creatinine each day (5). Creatinine does not enter into any metabolic reactions and ultimately is completely excreted in the urine. While fluctuations in excretion in consecutive short term collections are known to occur, creatinine content of successive 24 hour collections is quite uniform (6,7). Since a steady state of formation and excretion is established, even in illness, the measure of combined creatine and creatinine chromogen in urine is also a measure of creatine synthesis. The factors which regulate rate of synthesis of creatine are not well known. Changes in peripheral requirements for creatine do not seem to modify its production(8). However, Wilkins, Fleischmann and Howard(9) found that methyltestosterone, but not testosterone, increased markedly the formation of creatine. This communication reports a similar, perhaps more profound increase in creatine synthesis as judged by excretion of total creatinine chromogen after oral administration of 17α-ethyl-19-nortestosterone.

Materials and methods. Six apparently healthy adults were given 30 mg 17α-ethyl-19-nortestosterone (Nilevar) daily by mouth. A 24 hour urine collection was obtained prior to and after 6 weeks of drug administration for determination of creatine, creatinine, total creatinine chromogen and uric acid. Subjects were placed on a creatine-free diet for 4 days before urine collections were obtained. Creatine was determined by the method of Anderson, *et al.*(10), creatinine was measured by the method of Owen, *et al.*(11), and uric acid was determined by the method of Brown(12).

Results. Increase in excretion of creatine and total creatinine chromogen was observed in all subjects. Mean daily excretion of creatine was 81.4 mg before and 473.3 mg after 6 weeks of drug with a mean increase in creatine excretion of 393.7 mg. Mean daily excretion of total creatinine chromogen was 1.58 g before and 2.35 g after 6 weeks of drug

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