quent disaggregation of islet cell insulin–Zn complexes has been suggested as a mechanism for insulin release. (c) Tris is an inhibitor of certain enzymes (1) and may act on glucose metabolism within the islet to allow for the accumulation of a glucose metabolite involved in stimulating insulin secretion. Additionally, cholinesterases are inhibited by Tris (15). Cholinergic mechanisms have been shown to be involved in insulin secretion (16). Investigations of these several alternatives with Tris may prove useful in furthering the understanding of the phenomena involved in insulin release mechanisms.

Summary. Dogs infused with Tris buffer exhibited not only the hypoglycemia reported by others, but also an increase in plasma insulin level as estimated by immunoassay. The induced hyperinsulinemia was much greater in portal vein than in peripheral vein plasma samples. Both hypoglycemia and hyperinsulinemia were much more pronounced when the Tris infused was at pH 10.2 than at pH 7.4. Since Tris molecules are less ionized at higher pH, these findings are in accord with the hypothesis that hypoglycemia induced by Tris is due to this compound penetrating into and inducing release of insulin from the pancreas. The hyperinsulinemia induced by pH 10.2 Tris infusion into the pancreatic artery started later and was more variable than that induced by an equimolar infusion of glucose.

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Effects of Estrogen on Pituitary Prolactin Levels of Female Rats Bearing Median Eminence Implants of Prolactin* (33432)

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Implants of LH (1), FSH (2), ACTH (3) or GH (4) into the median eminence area of the rat hypothalamus have been reported to specifically reduce anterior pituitary (AP) concentration of each of these hormones. Re-

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cently, our laboratory reported that implants of prolactin into the median eminence of rats similarly reduced AP concentration of prolactin (5). Systemic administration of estrogen to rats results in increased AP prolactin concentration (6). It was of interest, therefore, to determine the effects on AP prolactin concentration of injecting estrogen into rats bearing hypothalamic implants of prolactin.

Materials and Methods. Female Sprague-Dawley rats (Spartan Animal Farms, Inc., Haslett, Michigan) weighing an average of 200 g were housed in a temperature (72 ± 2°F) and light-controlled room. The rats were maintained on a diet of Wayne Lab-Blox (Allied Mills, Chicago, Illinois) and fed *ad libitum*.

Implanting procedure. Single stereotaxic implantations of approximately 300 μg of prolactin[3] in cocoa butter (experimental) or cocoa butter alone (controls) were made in the median eminence area of the hypothalamus. The prolactin-cocoa butter mixture or cocoa butter alone was tamped into 23-gauge glass tubing, implanted stereotaxically into the median eminence, and secured tightly to the skull with screws and dental cement.

Twenty-four hr after implantation, the rats were divided into 5 groups and injected (s.c.) once daily for 5 days with different doses (see Table I) of estradiol benzoate (EB) dissolved in corn oil. Twenty-four hr after the last EB injection, the rats were killed by guillotine, the ventral surface of the brain was exposed, and location of the implant was ascertained with the aid of a dissecting microscope. The AP was immediately removed, weighed and frozen (—20°) for prolactin assay.

Prolactin was assayed in 6–8-week-old White King squabs by the intradermal pigeon-crop technique of Lyons (7) as modified by Reece and Turner (8). A direct comparison was made between the control samples and experimental samples within each group by injecting one sample over one side of the crop sac and the other sample over the other side of the crop sac of the same bird. Significance of differences between AP weights, AP prolactin content, and concentration was analyzed by the student *t* test and the *t* test for paired observations, respectively.

Results. The results of this study are shown in Table I. Median eminence implants of prolactin significantly lowered AP prolactin content and concentration in the rats not given EB (Group 1). The 1.0 μg dose of EB (Group 2) showed no ability to increase AP prolactin levels in the presence of the ME prolactin implant, whereas the 5.0 μg dose (Group 3) was only partially effective in increasing AP prolactin levels. In addition, prolactin implants significantly reduced AP weights in rats treated with 1.0 or 5.0 μg of EB. In ME prolactin implanted rats treated with 10.0 μg (Group 4) of EB, AP prolactin levels were increased to the same extent as in control rats given 10 μg of EB and no ME implant of prolactin. None of the prolactin implants and/or EB significantly influenced body weight gains during the short duration of treatment.

Discussion. These results confirm previous observations that median eminence implants of prolactin result in a decrease (5), whereas estrogen administration results in an increase in AP prolactin concentration (6). When the two treatments were combined, the lowest dose of EB (1 μg) was unable to counteract the depressing action of the ME prolactin implants on AP prolactin levels, and the intermediate dose (5 μg) was only partially effective. In contrast, the highest dose of estradiol (10 μg) increased AP prolactin content and concentration to the same degree in rats with an ME prolactin implant as in control rats not given an ME implant of prolactin.

The action of the ME implant of prolactin in depressing AP prolactin has been shown to be mediated through an increase in hypothalamic production of prolactin inhibiting factor (PIF) (5). Such implants result in atrophy of the mammary glands (5) and inhibition of lactation (9) in rats, indicating that both synthesis and release of prolactin by the AP are inhibited. Estrogen can increase AP prolactin synthesis and release, both by a direct action on the AP and by

[3] NIH-P-S-8 ovine prolactin (28 IU/mg)

TABLE I. Effects of Estradiol Benzoate (EB) on AP Prolactin Levels of Female Rats Bearing Median Eminence Implants of Prolactin.

Group	Treatment: Type of ME implant	Treatment: Dose of EB (μg)	No. of rats	Final body wt.[e] (g)	AP wt.[e] (mg)	No. of pigeons	Prolactin content[e] (IU/AP)	Prolactin conc (IU/100 mg of AP[e])	p[f]
1	Cocoa butter	0.0	14	214.6 ± 3.2	8.76 ± 0.38	16	0.168 ± 0.08	1.94 ± 0.44	<.0005
	Cocoa butter + prolactin	0.0	13	214.4 ± 4.2	8.39 ± 0.31	16	0.092 ± 0.02	1.09 ± 0.28	
2	Cocoa butter	1.0	12	211.3 ± 4.1	11.37 ± 0.38[a]	19	0.254 ± 0.06	2.24 ± 0.49	<.0005
	Cocoa butter + prolactin	1.0	14	214.0 ± 2.2	10.21 ± 0.31[b]	19	0.103 ± 0.03	1.01 ± 0.25	
3	Cocoa butter	5.0	14	211.3 ± 3.3	13.36 ± 0.47[c]	20	0.376 ± 0.06	2.81 ± 0.45	<.01
	Cocoa butter + prolactin	5.0	15	206.0 ± 3.1	11.08 ± 0.34[d]	20	0.241 ± 0.04	2.17 ± 0.35	
4	Cocoa butter	10.0	13	216.6 ± 2.7	14.40 ± 0.45	17	0.511 ± 0.14	3.55 ± 0.94	NS
	Cocoa butter + prolactin	10.0	14	210.1 ± 3.0	13.20 ± 0.60	17	0.600 ± 0.17	4.54 ± 0.94	

[a/b] $p < 0.05$.
[c/d] $p < 0.01$.
[e] Mean ± SE.
[f] p value refers to prolactin content and concentration.

depressing PIF production by the hypothalamus (10). Apparently when the two lower doses of estradiol were injected, the action of the ME implant on AP prolactin predominated. However, the largest dose of estradiol given, 10μg, was able to completely counteract the effect of the ME prolactin implant on AP prolactin levels. This dose of estradiol may have acted both at the AP and hypothalamic levels. It is of interest that AP weight was equally increased by the 10-μg dose of estradiol in both the control and ME prolactin implanted rats.

Several workers have reported on estrogen uptake by the hypothalamus and AP. Eisenfeld and Axelrod (11) observed that administered estradiol—^{3}H was approximately 5 times as concentrated in hypothalamic tissue and approximately 60 times as concentrated in AP tissue as in plasma. This may indicate a specific affinity by the AP and hypothalamus for estrogen. It would be of interest to determine to what extent uptake of estrogen by these two tissues may be modified by an ME implant of prolactin. The present findings, as well as the observation that high circulating levels of prolactin can depress AP prolactin levels in rats (12), suggest that circulating levels of prolactin may normally alter the actions of estrogen on the AP and hypothalamus.

Summary. A median eminence implant of prolactin significantly lowered anterior pituitary prolactin content and concentration in female rats. Injection of 1.0 μg of estradiol benzoate daily for 5 days was unable to counteract this action of the ME implant of prolactin, whereas 5 μg was only partially effective. However, injection of 10.0 μg of estradiol benzoate daily was as effective in increasing anterior pituitary prolactin levels in the presence as in the absence of an ME implant of prolactin. These results suggest that circulating levels of prolactin, by acting on the hypothalamus, may be able to modify the action of estrogen in promoting prolactin secretion.

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Glomerular Lesions in a Strain of Genetically Diabetic Mice* (33433)

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Glomerular lesions, similar to those found in human diabetes mellitus were observed in nonobese, genetically determined, spontaneously diabetic mice (1). The lesions were not found in pen-bred Swiss albino control animals under the same laboratory conditions.

The hereditary diabetic mice of the KK

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