PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **180**, 497–504 (1985)

Effect of a Diabetic State on Myometrial Ultrastructure and Isolated Uterine Contractions in the Rat (42208)

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Abstract. Alterations in rat myometrial ultrastructure and *in vitro* uterine contractile responses to oxytocin were examined in estradiol-treated (40 μg/kg) euglycemic and streptozotocin-induced (85 mg/kg) diabetic rats. Myometrial morphology was examined 18, 24, and 36 hr after estradiol administration. At the time points examined, nuclei of myometrial cells from euglycemic and diabetic rats were pleomorphic and contained large areas of heterochromatin dispersed throughout the nuclei. Mitochondria were round to oval in shape and contained a dense matrix with cristae that extended across the mitochondria. Myofilaments were found in both euglycemic and diabetic cells but the relative number of myofilaments in diabetic cells appeared to be less than the number found in myometrial cells removed from euglycemic animals. The number of free cytoplasmic ribosomes in diabetic cells also appeared to be less than those found in euglycemic cells. In order to determine if apparent differences in the number of myofilament found in diabetic myometrial cells could be correlated with changes in uterine contractile responses to hormones, oxytocin dose–response curves (10^{-8} to 10^{-5} *M*) were examined in isolated uteri removed from saline-injected and estradiol-injected (24-hr pretreatments) euglycemic and diabetic rats. The maximal contractile responses (milligrams tension developed per milligrams tissue) in saline-injected euglycemic and diabetic rats were 49 ± 5 and 36 ± 4, respectively, while maximal contractile responses in estradiol-injected euglycemic and diabetic rats were 68 ± 7 and 45 ± 5, respectively. Maximal contractile responses induced by oxytocin in estradiol-treated diabetic uteri were significantly smaller than the contractile responses measured in euglycemic estradiol-treated uteri. This study demonstrates that estradiol-induced changes in both myometrial cell morphology and *in vitro* uterine contractile responses to oxytocin are altered in diabetic rats.

Estradiol plays an essential role in regulating uterine growth and differentiated functions. Uterine growth responses have been characterized as a biphasic process consisting of a series of "early" responses and a series of "late" responses (1). The early responses include increases in water uptake and an activation of RNA polymerase (2) while late responses include increases in DNA, RNA, and protein content (3). The biochemical changes induced by estradiol are followed by cell division and organ growth (4). Growth responses to estradiol have also been characterized in studies of uterine ultrastructure (5–7). The formation of ovoid nuclei containing well-defined nucleoli, the predominance of euchromatin in the nuclei, the formation of an extensive rough endoplasmic reticulum, the development of electron-lucent mitochondria, and eventually the appearance of mitotic figures are typical ultrastructural changes that occur in endometrial cells following estrogen treatment.

A number of hormones have been shown to induce uterine contractions through high affinity membrane-bound receptors (8). Hormone–receptor interactions in uterine tissue lead to calcium influx (9), guanosine 3′,5′ cyclic monophosphate production (10), and prostaglandin biosynthesis (11). These events may be important in initiating uterine contractions. Estradiol regulates the contractile activity of uterine tissue by regulating the concentration of membrane-bound hormone receptors. Estradiol has been shown to stimulate the syn-

0037-9727/85 $1.50

thesis of oxytocin receptors and the changes in the hormone receptor population have been correlated with enhanced responsiveness of the myometrium to oxytocin (12).

The uterine growth response can be profoundly altered by changes in the endocrine state of animals (13, 14). Studies have reported (15, 16) that in diabetic animals estradiol-stimulated endometrial cell growth is significantly reduced. This effect was reversed when insulin was administered to diabetic animals. Estradiol-induced changes in the ultrastructure of the nucleus, nucleolus, mitochondria, and microvilli of endometrial cells from diabetic rats have also been reported to be significantly altered in diabetic animals (17). Since a diabetic state alters endometrial growth responses, these studies were initiated to examine possible changes in myometrial ultrastructure and functions that may also be associated with a diabetic state.

Materials and Methods. *Animals.* Immature female (21 days old) Sprague–Dawley rats weighing 40 to 50 g were used in these studies. Animals were ovariectomized and given 3 days to recover from surgery. In studies with diabetic rats, animals were injected ip with 85 mg/kg of streptozotocin (SZ). Four days after injections, rats with serum glucose levels above 300 mg/dl were considered diabetic. Animals injected ip with 0.9% saline were designated as euglycemic controls. Serum glucose levels in control animals were 96 ± 8 mg/dl. In experiments designed to examine the effect of estradiol treatment on uterine contractile activity, animals were injected with 40 μg/kg of estradiol ip 24 hr prior to sacrifice. Control animals were injected with 0.9% saline. Uteri from animals sacrificed by decapitation were quickly removed and cleaned of adhering fat.

Ultrastructural studies. Uterine horns that were used to study myometrial ultrastructure were fixed in a 2.5% glutaraldehyde solution containing a 0.1 *M* phosphate buffer (pH 7.2) for 2 hr at 20°C. Tissue samples were washed three times in the 0.1 *M* phosphate buffer (pH 7.2) and postfixed in a 1% osmium tetroxide solution containing the phosphate buffer for 1 hr at 20°C. Fixed pieces of tissue were dehydrated at 20°C using gentle agitation in a graded series of ethanol solutions (25, 50, 70, 90, and 100%, 5 min each) followed by immersion in two changes of 100% propylene oxide (10 min each). Dehydrated tissue segments were infiltrated in closed vials using agitation at 20°C for 8 hr with 50% epoxy resin and for 8 hr with 75% epoxy resin (Epon 812: DDSA:MNA:DMP-30,44:37:19:1.5, v:v:v:v, diluted 1:1 and 3:1 with propylene oxide, respectively) and in open vials without agitation at 20°C for 8 hr with 100% epoxy resin. Each uterine segment was then placed in fresh 100% epoxy resin in separate BEEM capsules. The encapsulated tissue/resin preparations were heated 24 hr at 60°C. Embedded tissues were sectioned using glass knives and an MT-1 or MT-5000 Sorvall ultramicrotome (DuPont, Newtown, Conn.). Thin sections, mounted on uncoated 300-mesh copper grids were stained with 2% aqueous uranyl acetate (10 min, 20°C) and Reynold's lead citrate (10 min, 20°C). Stained sections were viewed with a Hitachi HU-11A transmission electron microscope (Hitachi, Mt. View, Calif.) at an accelerating voltage of 50 kV. Ultramicrographs presented in this study are typical observations made from one of the 720 thin section cells per animal and have not been extrapolated for whole cells.

Uterine contractile studies. Uterine horns that were used to study contractile responses were split longitudinally and placed in an isolated organ bath (Phipps and Bird, Richmond, Va.). Uterine horns were bathed in a van Dykes–Hastings buffer (pH 7.4) at 37°C and oxygenated with 95% O_2/5% CO_2. Uterine horns that were placed in the organ bath were exposed to a baseline tension of 0.5 g. A physiograph system (Mark IV Physiograph, Narco Bio-Systems, Houston, Tex.) and myograph transducer (F-60 transducer, Narco Bio-Systems) were used to measure uterine contractions.

In uterine contractile studies, uteri were allowed to equilibrate 15 min in the organ bath before tissues were exposed to hormones. In dose–response studies, uterine horns were exposed to oxytocin and the contractile responses to each concentration of hormone was measured over a period of 3 min. The tissue chambers were washed with hormone-free buffer at least four times between hormone treatments.

The uteri were allowed to regain a baseline tension before the next dose of hormone was used. The magnitude of uterine contractile responses was expressed as milligrams of tension developed per milligrams wet weight of tissue (mg ten./mg tissue). The dose of oxytocin that produced one-half the maximal contractile response was designated as an ED_{50} dose and minimal dose of oxytocin that produced maximal contractile response was designated as an ED_{100} dose.

Chemicals. Chemicals and hormones used in this study were obtained from Sigma Chemical Company (St. Louis, Mo.).

Statistics. Data from these studies were analyzed using the Student t test. Probabilities that were less than $P \leq 0.05$ were considered significantly different.

Results. Most (greater than 95%) cells of the myometrium from estradiol-injected euglycemic (10 animals) and diabetic (10 animals) rats contained pleomorphic nuclei with large patches of heterochromatin (Figs. 1, 2). The heterochromatin was most concentrated along the border of the inner nuclear membrane. Mitochondria in euglycemic and diabetic cells were round to oval in shape, contained a dense matrix, and contained cristae that extended across the mitochondrion. The number of free ribosomes in euglycemic cells appeared to be greater than those found in diabetic cells. The number and cellular orientation of myofilaments in euglycemic and diabetic cells also appeared different. The myofilaments in euglycemic cells in contrast to diabetic cells appeared more abundant and they were orientated along the long axis of the myometrial cells.

Figure 3 shows the dose–response relationship between increasing concentrations of oxytocin and isolated uterine contractions. In uteri removed from saline-injected euglycemic rats, the ED_{50} and the ED_{100} doses of oxytocin were 8×10^{-7} M and 9×10^{-6} M, respectively. In isolated uteri removed from estradiol-treated euglycemic rats, the oxytocin dose–response relationship was shifted to the left and the ED_{50} and ED_{100} doses for oxytocin were 3×10^{-8} M and 1×10^{-6} M, respectively. The magnitude of oxytocin-induced uterine contractions was significantly increased in tissues removed from rats pretreated with estradiol. The force of uterine contractions induced by the ED_{50} and ED_{100} doses of oxytocin was 23 and 45 mg ten./mg tissue for saline-treated uteri and 34 and 68 mg ten./mg tissue for estradiol-treated uteri.

Figure 4 shows the oxytocin dose–response curves for isolated rat uteri removed from diabetic rats pretreated with either saline or estradiol. For uteri removed from saline-injected animals, the ED_{50} and ED_{100} doses for oxytocin were 4×10^{-7} M and 3×10^{-6} M, respectively. In uteri removed from estradiol-injected diabetic rats, the ED_{50} and ED_{100} doses for oxytocin were 3×10^{-8} M and 4×10^{-6} M, respectively. The magnitude of oxytocin-induced uterine contractions removed from rats injected with estradiol was not significantly increased over contractile responses induced in uteri removed from saline-injected diabetic rats. The force of uterine contractions induced by the ED_{50} and ED_{100} doses of oxytocin was 20 and 40 mg ten./mg, respectively, for saline-treated uteri and 24 and 48 mg ten./mg, respectively, for estradiol-treated uteri.

Table I summarizes the magnitude of uterine contractile responses induced by ED_{50} and Ed_{100} doses of oxytocin in tissues removed from diabetic and euglycemic rats. The animals were pretreated with estradiol 24 hr prior to the removal of uterine horns. The magnitude of uterine contractions induced by oxytocin was significantly less ($P < 0.05$) in tissues removed from diabetic rats as compared to euglycemic animals.

Discussion. Numerous studies have reported that a variety of hormones and endocrine states alter the ability of estradiol to stimulate normal uterine growth. In hypophysectomized rats, estradiol-stimulated increases in rates of glucose metabolism and DNA synthesis were significantly depressed (13). In a report that followed (14), deficiencies in thyroid hormone production may have been found to be responsible for the altered growth response in hypophysectomized animals. In diabetic animals, uterine growth responses are also altered. Mitotic figure formation and changes in endometrial cell ultrastructure have been reported to be significantly

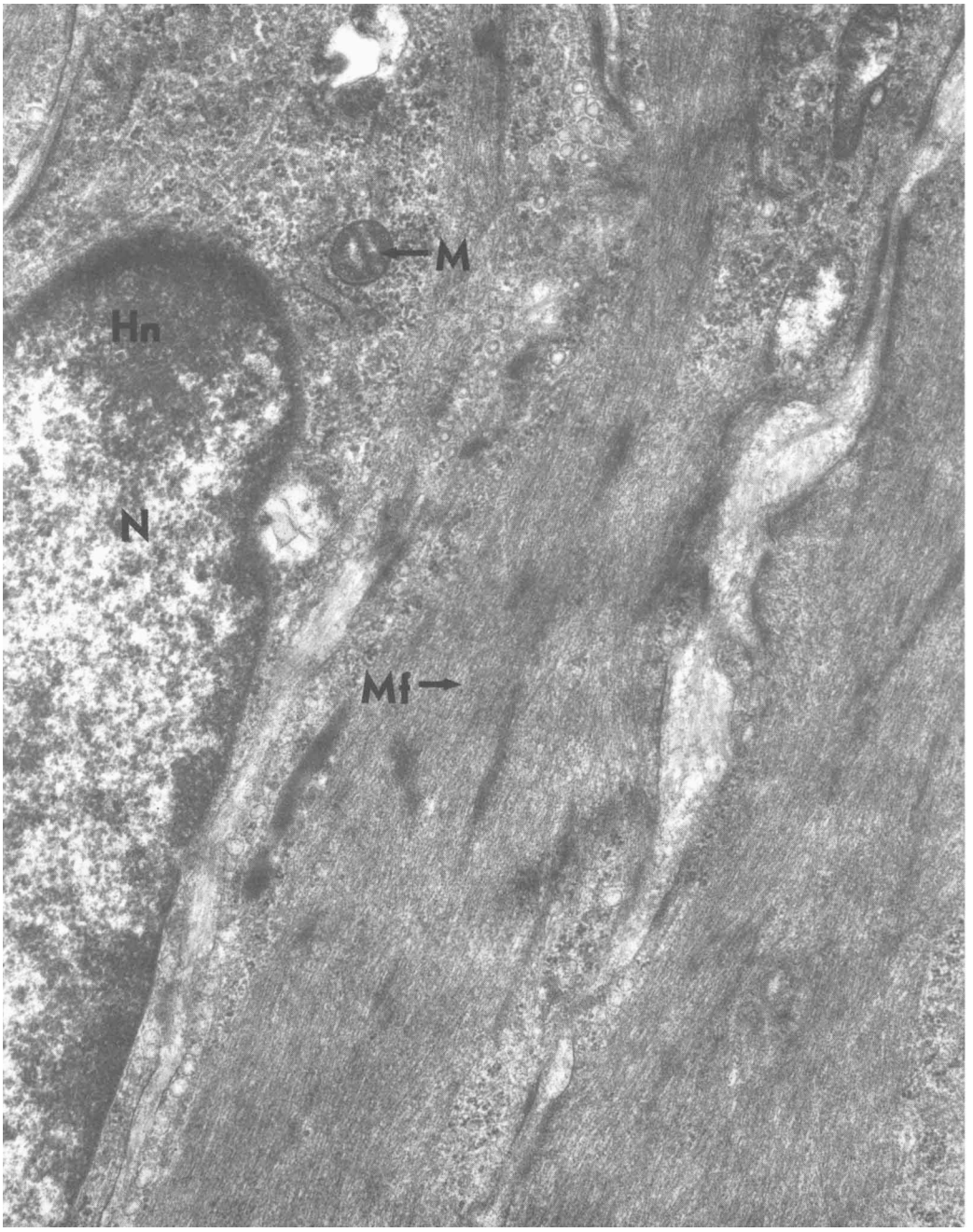

FIG. 1. Electron micrograph of uterine myometrial cells removed from an estradiol-treated euglycemic rat. Labeled structures include nucleus (N), heterochromatin (Hn), mitochondria (M), and myofilaments (Mf). Glutaraldehyde and osmium fixation; stained with aqueous uranyl acetate and Reynold's lead citrate solutions. ×19,500.

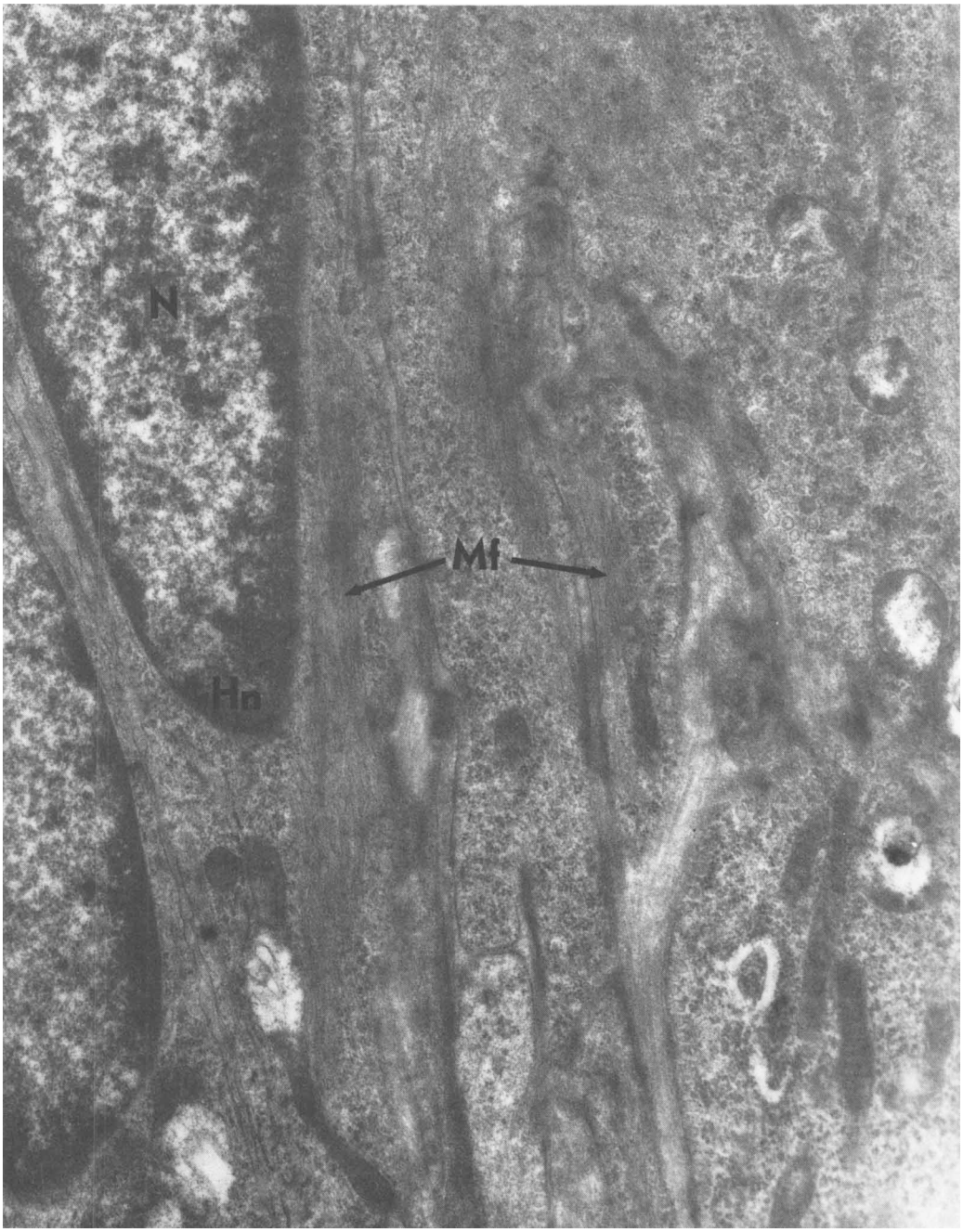

FIG. 2. Electron micrograph of uterine myometrial cells removed from an estradiol-treated diabetic rat. Labeled structures include nucleus (N), heterochromatin (Hn), and myofilaments (Mf). Glutaraldehyde and osmium fixation; stained with aqueous uranyl acetate and Reynold's lead citrate solutions. ×19,500.

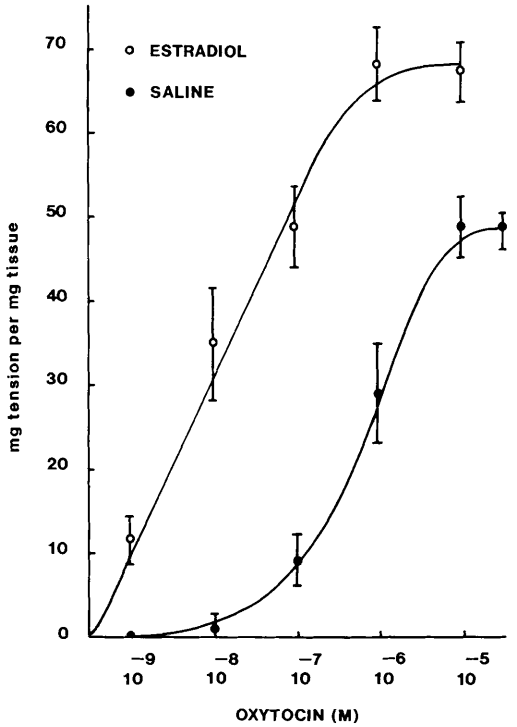

FIG. 3. Isolated uterine contractile responses to oxytocin in tissues removed from euglycemic rats. Animals were pretreated with saline or estradiol 24 hr prior to sacrifice. $N = 8$; data are expressed as means ± SEM.

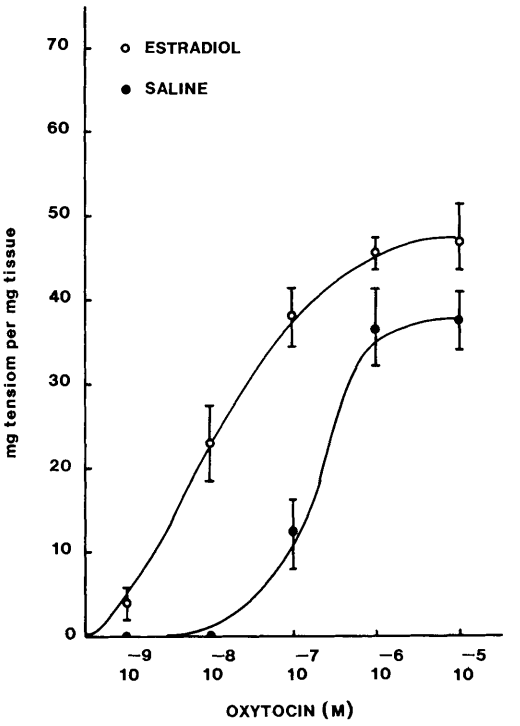

FIG. 4. Isolated uterine contractile responses to oxytocin in tissues removed from diabetic rats. Animals were pretreated with saline or estradiol 24 hr prior to sacrifice. $N = 8$; data are expressed as means ± SEM.

altered in diabetic animals (16, 17). Results from this study further support the observations that uterine myometrial structure and function are altered by a diabetic state. Altered uterine responses to estradiol that are associated with a diabetic state may be a partial explanation of general reproductive failure that is common in diabetic females (18, 19).

Uterine contractile responses induced by oxytocin are profoundly altered by circulating levels of estradiol. Estradiol administration has been shown to cause an up-regulation of oxytocin receptors in myometrial tissue and this effect was correlated with an increase in the responsiveness of uterine tissue to oxytocin. In our study, the magnitude of uterine contractile responses induced by oxytocin in estradiol-treated diabetic rats was significantly less than in euglycemic control animals. This effect could have resulted from the failure of

TABLE I. ISOLATED UTERINE CONTRACTILE RESPONSES TO ED_{50} AND ED_{100} DOSES OF OXYTOCIN IN TISSUES REMOVED FROM EUGLYCEMIC AND DIABETIC RATS

	Magnitude of uterine contractile responses			
	Euglycemic rats		Diabetic rats	
Dose of oxytocin	SAL	E_2	SAL	E_2
ED_{50}	24 ± 4	35 ± 4	20 ± 3	23 ± 3
ED_{100}	49 ± 5	67 ± 7	36 ± 4	45 ± 5

Note. The magnitude of uterine contractile responses is expressed as milligrams tension developed per milligrams tissue. Contractile responses represent means ± SEM of eight rats. Contractile responses were measured in tissues removed from saline-injected (SAL) and estradiol-injected (E_2) animals. Injections were made 24 hr prior to sacrifice. The magnitudes of uterine contractile responses to ED_{50} and ED_{100} doses of oxytocin are significantly different ($P < 0.05$) in estradiol-treated euglycemic rats as compared to estradiol-treated diabetic rats.

estradiol to stimulate an up-regulation of oxytocin receptors in diabetic tissues or alternatively it may have resulted from the lack of myofilament development. Further studies are needed to characterize the effect of a diabetic state on estradiol-induced changes in hormone receptor populations in uterine tissue.

Although the precise role that insulin plays in regulating uterine growth is unknown, three potential mechanisms are discussed. First, in a previous study of diabetic animals, retention times of estradiol in nuclei of uterine tissue was found to be shortened. Alterations in the retention times of estradiol were also correlated with uterine tissue atrophy (20). This effect may have been expected since it is recognized that uterine growth and differentiation is dependent on the period of time that estradiol remains sequestered in the nuclei of target tissues (21). Retention times of estradiol in myometrial cells examined in this study may also have been shortened and this effect could have altered certain aspects of myometrial growth and differentiation. Regulation of adenosine 3′,5′ cyclic monophosphate (cAMP) levels in uterine tissue may provide a second mechanism for controlling uterine growth. Insulin has been reported to enhance cell division in certain cell lines (22) by lowering the levels of cAMP. The absence of insulin in animals used in this study may have altered cAMP levels in myometrial cells and as a consequence altered the normal growth response. Finally, the effect of a diabetic state on uterine growth may be a nonspecific effect that is the result of hyperglycemia, acidosis, or some other metabolic disturbance associated with a diabetic state (23).

These studies have shown that a diabetic state alters estradiol-stimulated changes in myometrial ultrastructure and *in vitro* uterine contractile responses.

This research was supported by the Rochester, New York, Regional Affiliate, Inc. of the American Diabetes Association; NIH Grant HD-08615; and the John P. McGovern Foundation. G.M.S. is the recipient of an NIH Research Center Development Award and J.L.K. is a recipient of an NIH Clinical Investigator Award.

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Received May 29, 1985. P.S.E.B.M. 1985, Vol. 180.
Accepted July 22, 1985.