to be the main determinant of fluidity in liposomes[18] and biomembranes, so that membrane enzyme activities may increase or decrease, as dictated by the optimal fluidity level[19]. The present results reveal a high degree of sensitivity of the microsomal membranes to cholesterol, as small changes of the ratio C/PL can produce considerable changes in the microsomal activity.

We recently reported[20] the existence of 2 different cytochromes P_{450} for cholesterol-7a-hydroxylase and for drug oxidation. This was based on the observed differential effect of oestradiol, in vivo, on the activity of these 2 processes. The present results may indicate that oestradiol changes the activity of the endoplasmic reticulum by a mechanism other than by altering its cholesterol to phospholipids ratio.

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Enhancement by pyridoxine of the action of diazepam on spinal presynaptic inhibition[1]

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Summary. In spinal unanesthetized cats, pyridoxine pretreatment significantly enhanced the diazepam-induced increase in dorsal root potentials and dorsal root reflexes.

The predominant action of diazepam on spinal synaptic processes exhibits itself as a pronounced enhancement of the electrophysiological correlates of presynaptic inhibition. Thus there is an increase in the antidromic discharge across the afferent dorsal spinal roots (the dorsal root reflex) as well as in the electrotonic spread of depolarization (the dorsal root potential)[2]. Because of the proposed mediation of primary afferent depolarization by γ-aminobutyric acid (GABA)[2,3], the relationship between the spinal action of diazepam and pharmacological antagonists of GABA, as well as substances which manipulate GABA levels in the CNS, was investigated. Semicarbazide, which reduces GABA levels, and the GABA antagonist bicuculline were found to block the action of diazepam[4,5], which suggested a GABA link in some of the actions of diazepam, particularly those which may underly its skeletal muscle relaxant and anticonvulsant activity.

Since pyridoxal phosphate is a coenzyme known to be required in the biosynthesis of GABA from glutamic acid[6], it appeared likely that a B_6-induced increase in the availability of GABA could enhance the spinal and anticonvulsant actions of diazepam. The possibility that pyridoxine may potentiate the enhancement of presynaptic inhibition by diazepam is explored in this report.

Experiments were performed on adult cats the spinal cord of which was transected at the atlanto-occipital junction under ether. Immediately following the transection, anesthesia was discontinued and the cats were placed under artificial respiration, with end tidal levels of CO_2 maintained between 3 and 3.5%. Both carotid arteries were ligated and the brain made ischaemic by temporary manual pressure applied to the vertebral arteries.

The lumbosacral spinal cord was exposed by laminectomy, the dura sectioned, and a pool of paraffin oil was prepared to cover nerve tissue and maintained at 37 °C by thermostatic control. A heating pad was placed under the cat to

The increase in DRP and DRR in spinal cats 5 min after diazepam, with and without pretreatment with pyridoxine

	B_6/diazepam	Saline/diazepam	t-test (non-paired)
DRP	33.8± 4 (8)*	15.7± 2.9 (9)	$p<0.01$
DRR	109.1±25 (8)	40.5± 12.6 (7)	$p<0.05$

* Mean percent increase in surface area, followed by the SE and the number of experiments in parentheses.

maintain body temperature (monitored by an anal probe) close to 37 °C. All roots from L_5 to S_2 were sectioned extradurally. Bipolar platinum hook electrodes were placed on the dorsal S_1 root for stimulation and on the dorsal L_7 root for recording the dorsal root reflex (DRR). To record dorsal root potentials (DRP), a ball-tipped platinum electrode was placed close to the L_7 dorsal root entry into the spinal cord and the distal hook electrode used as reference. The time constant of the preamplifier was set at 1 sec to record the DRP. Supramaximal square wave stimuli were applied to dorsal S_1 at a rate of 0.25 Hz. Carotid blood pressure was continuously monitored and all drugs were administered through a cannulated antebrachial vein. The areas of the DRR and DRP were integrated by planimetry. After a period of 30 min when control spinal synaptic activity was recorded to ensure stability, the cats were given either pyridoxine hydrochloride (0.17 mg/kg in 2 ml saline) or an equivalent volume of saline, followed in 15 min by 0.2 mg/kg of diazepam (Valium® 1 mg/ml solution). Pyridoxine or saline had no significant effect on the recorded potentials. Diazepam consistently increased the surface area of both DRPs and DRRs. However, this increase was significantly more pronounced in the cats pretreated with pyridoxine. In pyridoxine-pretreated cats, the increase in DRP and DRR, 5 min after diazepam, averaged $33.8 \pm 4\%$ and $109.1 \pm 25\%$ respectively, whereas in saline pretreated cats, the corresponding increase was $15.7 \pm 2.9\%$ and $40.5 \pm 12.6\%$ (table).

Pretreatment with pyridoxine also prolonged the duration of action of diazepam. Thus, 30 min after diazepam in B_6 pretreated cats, the DRP and DRR were $22.7 \pm 4.6\%$ and $81.9 \pm 32.4\%$ above control, whereas in saline pretreated cats, the corresponding values were $9.5 \pm 3.4\%$ and $12 \pm 13.7\%$ for the DRP and DRR, respectively. Both values were significantly higher than saline controls ($p < 0.05$).

Both L-glutamic acid decarboxylase (GAD) and GABA-α-ketoglutaric aminotransferase (GABA-T) are B_6-dependent enzymes. Steady state concentrations of GABA in the CNS are normally governed by the GAD activity and not by the GABA-T[6]. The activity of GAD is almost doubled by the addition of its coenzyme to the incubation medium, whereas GABA-T is not activated by pyridoxal phosphate added in vitro[7]. The activity of GAD is an almost linear function of the concentration of pyridoxal phosphate in the brain[8]. The decrease in presynaptic inhibition after depletion of GABA by semicarbazide has been found to be effectively antagonized by pyridoxine[9].

Obviously, many other neurohumoral candidates are B_6-dependent, but the implication of GABA in primary afferent depolarization and the obvious enhancement of presynaptic inhibition by diazepam point to the facilitation of GABA-ergic transmission by pyridoxine as a likely mechanism underlying its potentiation of diazepam's spinal actions. The potentiation of diazepam's anticonvulsant action by pyridoxine is being investigated now.

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Uterine morphology and glycogen deposition of pregnant rats after clomiphene citrate treatment during preimplantation stages[1]

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Summary. Clomiphene citrate did not induce glycogen deposition in the uterine luminal epithelium of pregnant rats as it did in ovariectomized rats. However, the drug did alter the epithelial morphology which may be a factor in its postcoital contraceptive action.

Clomiphene citrate is a highly effective postcoital contraceptive agent in rats and acts either on the blastocyst or the uterus[3,4]. We have reported that clomiphene caused abundant glycogen deposition in the luminal epithelium of ovariectomized rats, an effect not caused by estradiol, and have postulated that the drug's epithelial effect may be a factor in its ability to block implantation[5]. The present experiment tests that postulate.

Materials and methods. Virgin Holtzman rats (220–230 g) were housed with males of proven fertility. Clomiphene citrate (0.5 mg/kg by gavage) was administered at 08.00 h of days (D) 2–4 of pregnancy (D 0 = sperm +). Control and treated rats were killed at 08.00 h of D 5 and 8. 4 rats were bilaterally ovariectomized under ether anesthesia, rested 10 days, treated with clomiphene (0.5 mg/kg) on 3 consecutive mornings, and sacrificed 24 h later. The uteri were fixed in an 80% alcoholic solution of cold picric-acid formalin, and embedded in paraffin. Serial sections were made of all uteri. 3 successive sections were: 1. stained with H and E, 2. treated by the PAS procedure and 3. treated with 1% malt diastase (30 min at room temperature) prior to the PAS technique. Uteri from the rats killed on D 8 were stained with H and E only. The slides were read without knowledge of treatment, and glycogen grades recorded as previously described[5].

Results. None of the 4 clomiphene-treated rats killed on D 8 any gross or microscopic evidence of implantation. The 5 control rats killed on D 8 had normal implantation sites. All controls had microscopic evidence of decidualization on D 5, but decidual cells were not observed in the uteri of the clomiphene-treated rats on D 5.