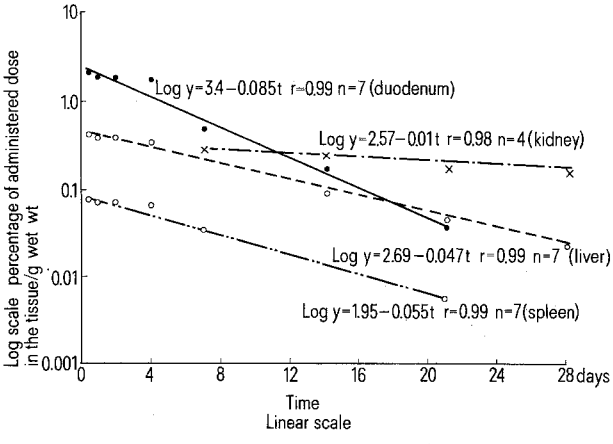
was obtained from Bhabha Atomic Research Center, Trombay, Bombay, India. The rats were divided into 13 groups, each group consisting of 5 animals. A single oral dose of 50 μCi (0.5 ml) of ^{115m}Cd was administered to each rat. The isotope-fed rats were sacrified at various intervals ranging from 30 min to 28 days, and the different tissues, viz the spleen, kidney, liver and a 5-cm long piece of duodenum next to the stomach were then dissected out. The tissues were carefully weighed and digested in 30% KOH at 90 °C for 10 min. The digested samples were monitored for ^{115m}Cd counts employing a solid scintillation counter. ^{115m}Cd counts so obtained were then expressed as the percentage of the administered dose and the latter values were plotted against time (figure). The experimental points were analyzed by the method of least squares to obtain the best-fit curves described by the equation log $Y = mt + C$, Y = percentage of the administered dose per g of wet tissue at a time = t. Half clearance time (HCT) of ^{115m}Cd in the tissues was then calculated from these equations employing the formula $HCT = -\log 2/m$.



Percentage values of administered doses of ^{115m}Cd plotted against time.

Results and discussion. The observations clearly suggest that the peak ^{115m}Cd level, attained following a single oral administration, falls exponentially with time in all the tissues studied, viz the spleen, kidney, liver and duodenum. A highly significant negative correlation is obtained between log (percentage of the administered dose per g of wet tissue) and the time in all the 4 tissues. The striking feature of the present observations is that the duodenum, which attains a peak ^{115m}Cd activity just 8 h post-administration, is also the quickest in clearing cadmium (HCT = 3.5 days). The kidney, though it shows maximum ^{115m}Cd activity 7 days post-administration, retains ^{115m}Cd the longest (HCT = 30 days). The spleen (HCT = 5.5 days) and the liver (HCT = 6.8 days) show intermediate clearance of cadmium, although the liver incorporates much more ^{115m}Cd than the spleen (figure). It is known that the tissues studied in the present report contain metallothionein[4], and this protein plays a significant role in cadmium retention. The biological half-life of cadmium metallothionein has been variously estimated as being between 3 and 5 days[5-7]. The comparatively long HCT of cadmium from both the liver and kidney thus suggests that the degradation of cadmium metallothionein does not lead to the removal of cadmium. Probably the cadmium complexes from the degraded metallothionein are reutilized for the synthesis of new metallothionein molecules[5-7]. There is evidence that liver cadmium is transported to the kidneys by metallothioneins[3] and this may explain the much longer HCT of ^{115m}Cd in the kidneys compared with that of the liver.

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Synaptic development in brains of rats exposed perinatally to ethanol[1]

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Summary. Development of brain synaptosomal uptakes of ^{3}H-norepinephrine and ^{3}H-dopamine in pups whose mothers received ethanol were nearly normal. However, development of synaptosomal uptake of ^{3}H-serotonin was significantly lower than in controls, while uptake of ^{3}H-norepinephrine into synaptic storage vesicles was increased.

The fetal alcohol syndrome is associated with retardation of general growth and decreased mental capacity[2]. In the adrenal medulla of the rat, maternal ethanol administration produces a developmental deficit in adrenal catecholamines which results from an apparent lag in development of adrenal storage vesicles[3]. Alterations also have been reported in brain tyrosine hydroxylase activity (the rate-limiting step in catecholamine biosynthesis) and in synaptosomal uptake of tyramine and subsequent conversion to octopamine in rats born to ethanol-treated mothers[4-6]. It is not certain, however, whether the biochemical changes in the brain represent effects on synaptic dynamics (transmitter synthesis, storage, release) or whether synaptogenesis itself is affected by ethanol exposure. In the present study, synaptosomal uptakes of norepinephrine, dopamine and serotonin have been used as indices of synaptic development, as previous studies have shown that this procedure provides an estimate of the numbers of synaptic terminals for each neurotransmitter[5,7-9]. In addition, storage capabilities for catecholamines were assessed by measurement of norepinephrine uptake into isolated storage vesicles[8,9].

Timed pregnant Sprague-Dawley rats (Zivic-Miller) were housed individually in breeding cages without access to water and were fed a nutritionally complete liquid diet (Sustacal) from the 11th day of gestation. On the 13th day of gestation and thereafter, the experimental group received ethanol (6.8% v/v) in Sustacal while controls received Sustacal made isocaloric and isonutritional to the

Effects of maternal ethanol ingestion on brain development

Age (days)	Body weight (g)		Brain weight (mg)		Synaptosomal uptake (pmole/g brain)					Vesicular uptake (pmole/g brain) Norepinephrine	
					Norepinephrine		Dopamine		Serotonin		
	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Control	Ethanol
2	9.7±0.2	9.0±0.2*	360± 5	337± 3*	11.6±0.9	15.0±0.8*	29.6±0.9	33.7±2.3	89.0± 3.5	3.6±0.4	4.6±0.3
4	12.3±0.1	12.2±0.3	491± 7	467±10	20.3±1.5	16.4±1.8	42.5±3.5	32.3±1.8*	89.5± 4.5	4.9±0.4	6.9±0.9
6	16.7±0.3	14.1±0.5*	663±12	636±18	22.2±6.6	22.4±3.9	50.4±3.4	41.5±2.9	105 ± 8	5.9±0.9	7.6±0.6
9	19.2±0.5	16.3±0.4*	892±10	857± 6*	33.2±6.2	28.9±5.1	62.8±4.3	62.9±4.4	112 ±13	7.0±0.8	8.2±0.2
12	21.9±0.4	20.6±0.7	1090±10	1090±20	40.2±6.4	34.1±4.6	102 ±9	94.5±5.7	174 ±13	7.9±0.7	9.9±0.4*
19	28.7±0.4	22.6±0.2*	1370±20	1240±30*	54.7±6.9	52.5±3.6	–	–	240 ±33	8.8±0.8	11.9±0.5*
Ethanol vs control (t-test paired by age)	$p<0.05$		NS		NS		NS		$p<0.05$	$p<0.002$	

Data represent means and SE of at least 6 determinations at each age. Asterisks denote significant differences ($p<0.05$) by unpaired t-test.

ethanol diet by addition of sucrose. To ensure nutritional equivalence, the control intake was restricted to that consumed by the ethanol group. Ethanol intake averaged 11 g/kg/day. Offspring were killed at intervals of several days postpartum, and the brains were removed, weighed and homogenized in 4 vol. of 0.3 M sucrose containing 25 mM Tris (pH 7.4) and 0.01 mM iproniazid. Subcellular fractions containing synaptosomes or synaptic vesicles were prepared as described previously[9]. For measurements of synaptosomal uptake, incubations contained final concentrations of 0.1 μM ^{3}H-norepinephrine, ^{3}H-dopamine or ^{3}H-serotonin and synaptosomes derived from 10 mg wet weight of tissue in a final volume of 1 ml of Krebs-Henseleit medium containing 1.25 μM iproniazid and 2 μM ascorbic acid. Synaptosomal incubations lasted 5 min at 37 °C with duplicate samples kept on ice to serve as blanks. Labeled synaptosomes were trapped and washed by vacuum filtration[9] and counted by liquid scintillation spectrometry. Synaptic vesicle incubations lasted 4 min at 30 °C (0 °C blanks), with final concentrations of 0.1 μM ^{3}H-norepinephrine and 1 mM ATP-Mg^{2+} and vesicles derived from 133 mg of brain in 1.67 ml of 0.13 M potassium phosphate buffer (pH 7.4). Vesicles were trapped, washed and counted as described earlier[9]. Data are reported as means and SE, and significance determined by the paired and unpaired 2-tailed t-tests. In the paired test, group means of controls and experimentals were paired by age over the course of development; degrees of freedom were calculated as the number of paired means minus 1. The mode of pairing enables comparisons over an extended time period, as opposed to comparison only of individual age points in the unpaired t-test. Pups of either sex were used throughout the studies, but in each case control and ethanol-exposed groups were sex-matched at each time point.

l-Norepinephrine-7-^{3}H (2.2 Ci/mmole), dopamine-2-^{3}H (14.6 Ci/mmole) and serotonin-1,2-^{3}H (25.6 Ci/mmole) were obtained from New England Nuclear Corporation.

Rats whose mothers received ethanol displayed no gross behavioral abnormalities and appeared to nurse normally, but showed a general slowing of growth throughout early postnatal development ($p < 0.05$ vs control body weight by paired t-test); although brain weights tended to be low at each age in the ethanol group, the differences were not statistically significant (table). Development of synaptosomal uptake mechanisms for norepinephrine and dopamine were only occasionally statistically different from controls by the unpaired t-test, and there was no overall effect on the developmental pattern, as differences were not significant by the paired t-test. These data suggest that ethanol exposure during this perinatal period does not produce general retardation of synaptogenesis of catecholaminergic systems. However, uptake of serotonin displayed a small but consistent deficit in ethanol exposed pups ($p < 0.05$ by paired t-test), indicating a possible retardation in serotonergic synaptogenesis. While it is also possible that this difference could result from changes in the general fractionation characteristics of the tissue (e.g. increased fragility), such an explanation seems unlikely, as comparable alterations should then be seen for norepinephrine or dopamine. There was also a consistent elevation of norepinephrine uptake into synaptic storage vesicles ($p < 0.002$ by paired t-test); because norepinephrine uptake into isolated vesicle preparations involves both noradrenergic and dopaminergic vesicles[9], it is not possible from this information alone to determine whether the alteration in storage involves just one or both of these catecholamines. However, a previous study[5] with ^{3}H-tyramine has already implicated alterations in the noradrenergic system during perinatal ethanol. In any case, these results are in marked contrast to

those obtained in the adrenal medulla, where perinatal ethanol causes a definite slowing of catecholaminergic development[3], with a reduction in the number of storage vesicles. It is therefore apparent that the developmental effects of ethanol are different in central vs peripheral neuronal tissues.

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The effects of scorpion venom tityustoxin and ouabain on the release of acetylcholine from incubated slices of rat brain[1]

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Summary. The in vitro release of acetylcholine in slices of rat brain tissue was followed in the presence of tityustoxin and ouabain. At low ouabain concentrations, the release of acetylcholine caused by both ouabain and tityustoxin, was additive. At higher ouabain concentrations the additive effect of tityustoxin and ouabain on the release of ACh was no longer observed.

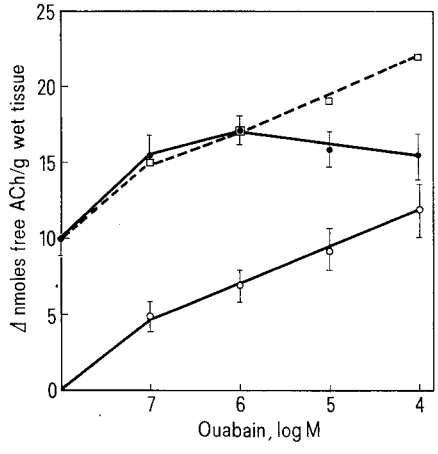
Tityustoxin (TsTX), a toxin purified from the venom of the scorpion *Tityus serrulatus*[2], causes a state of membrane depolarization and an increase in the release of cellular acetylcholine (ACh) in rat brain cortical slices[3]. Previously[4], we have compared the modalities of the release of ACh by TsTX and by ouabain and the effect of these 2 agents on Na^+, K^+, ATPase. Unlike ouabain, TsTX did not inhibit this enzyme activity[4]. Tetrodotoxin failed to reverse the ouabain elicited release of ACh while it successfully antagonized the release of ACh elicited by TsTX[4]. In the presence of Ca^{2+} in the medium, EGTA totally prevented the action of TsTX but not that of ouabain[4]. These results suggest that Na^+, K^+, ATPase was not involved in the release of ACh evoked by TsTX. However, we did not observe[4], as we had expected, an additive effect on the release of ACh by TsTX and ouabain.

The effect of TsTX on ACh release is energy dependent and, at a concentration of 10^{-4} M, ouabain caused almost 40% inhibition in the rates of respiration and ATP hydrolysis in brain cortical slices[5].

Therefore, we decided to compare the release of ACh evoked by TsTX in the presence of different ouabain concentrations that either had or did not have an effect on the rate of respiration and the energy process in brain cortical slices[5].

Material and methods. Tityustoxin (TsTX) was purified by a combination of extraction and chromatographic techniques using Sephadex G-25 and carboxymethylcellulose[2]. Ouabain was obtained from Sigma Chemical Co., Saint Louis, Mo. Albino rats of either sex were decapitated and the brains quickly removed. The preparation of cortical slices, the incubation procedures and the assay were performed as previously described[3].

Results and discussion. The values for the release of ACh were expressed as Δ nmoles of free ACh/g wet tissue (figure). The figures were obtained by subtracting the control value (without TsTX and ouabain) from each of the values obtained in the conditions studied, i.e., in the presence of TsTX or ouabain or combination of the two. Thus, in the absence of ouabain, TsTX 2×10^{-6} M evoked a Δ value of 10.1 ± 0.5 of free ACh/g wet tissue. Ouabain alone, at concentrations of 10^{-7}M and 10^{-6}M, elicited Δ values of 5.0 ± 0.4 and 7.6 ± 0.5 nmoles of free ACh/g wet tissue, respectively. The incubation of TsTX 2×10^{-6}M together with ouabain at the above concentrations, raised the values for the release of ACh to 14.9 ± 0.5 and 17.4 ± 0.6, respectively. The theoretical values (figure, dot-



The effects of tityustoxin (TsTX) and ouabain on the release of acetylcholine from incubated slices of rat brain. ○, Free ACh without TsTX; ●, free ACh with TsTX 2×10^{-6}M; □, theoretical value for TsTX 2×10^{-6} M plus ouabain at the indicated concentrations in abscissa. The mean value ± SEM for 3 experiments are presented. Slices were incubated for 30 min at pH 7.4 in a medium containing (mM): NaCl 136; KCl 2.7; $CaCl_2$ 1.35; NaH_2PO_4 0.36; $NaHCO_3$ 12, glucose 5.5; eserine 0.01. For other details see the text.