which there is a marked negative sodium balance[12], the lone kidney has an exaggerated rate of renin secretion. Similar adrenalectomy experiments in rats with both kidneys present have shown an increase in PRC that did not markedly differ from that of the present studies[5].

In this study, in which the adrenalectomized animals were not loaded with NaCl or maintained with a substitutional therapy, the degranulation of the juxtaglomerular cells may indicate that their rate of renin synthesis and release predominate over the renin storage. In favor of such an assumption are the elevated values in PRC and the absence of changes in renal renin content of the adrenalectomized uninephrectomized rats as compared with their controls.

In experiments done on rat kidney slices, DE VITO et al.[13] pointed out that the hexose monophosphate shunt is involved in the conversion of hypothetical prerenin to renin. The high macular G6PD activity mostly in adrenalectomized animals may well suggest that, by an unknown mechanism, the macula densa G6PD could be functionally related to the renin secreting cells. It is also possible that the macular G6PD could be functionally independent of the JG cells but could respond to variations in intracellular macula densa cells sodium balance[7,14,15].

Résumé. La surrénalectomie bilatérale chez des rats uninéphrectomisés produit une augmentation très marquée de la concentration de rénine plasmatique, ainsi que de l'activité de la G6PD dans la macula densa. Cependant, malgré une diminution de la granularité des cellules juxtaglomérulaires, on n'observe aucune modification du contenu en rénine du rein. Ces observations indiquent que, dans cette condition expérimentale, le rein qui reste est capable de réagir avec forte augmentation de la sécrétion de rénine.

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Brain Development in Offspring of Rats Treated with Nicotine During Pregnancy

Studies in various mammalian species have attempted to assess the effects of nicotine on the brain, particularly in terms of behavioral responses. In rats and mice, small doses of nicotine seem to facilitate elementary forms of learning and to stimulate spontaneous motor activity[1,2]; larger doses, however, depress spontaneous motor activity[3]. In the sleeping cat, nicotine initially induces EEG activation and behavioral arousal, followed by a period of enhanced slow-wave sleep and activated sleep[4,5], whereas in rabbits, nicotine produces seizure discharges in the hippocampus[6,7]. Most of these studies have been conducted in the adult animal; outside of the finding that nicotine administered to the pregnant female distributes rapidly throughout the body and passes into the blastocyst as well as the implanted fetus[8] and prolongs the duration of gestation[9,10], we know little of the direct or indirect effects of this agent on the developing brain. That fetal development can be affected by adverse conditions imposed on the maternal organism, and result in permanent distortion of maturational patterns at a structural, functional and biochemical level, has been demonstrated[11,12]. Furthermore, significant developmental phenomena as well as manifestations of prenatal influences can be effectively studied in the rat, for the CNS in this species is still relatively immature at birth.

The present study utilizes this species to investigate the effects of nicotine administered to the gestating animal on the development and function of the brain in the offspring. We have chosen to compare the development of seizure activity between offspring of treated and untreated animals, for such electrophysiological data provide useful information on brain maturational patterns under normal and experimental conditions. Although convulsive seizures represent fundamentally pathologic phenomena, when induced experimentally, they offer a measure of the interaction occurring between inhibitory and excitatory systems of the CNS manifested as overt motor activity. Minimal electroshock seizure threshold (EST) and maximal electroshock seizure (MES) patterns were utilized as specific indices of subcortical and whole-brain maturation, respectively[13,14].

Materials and methods. From day 0–21 of gestation, pregnant Long-Evans rats were injected s.c. with 3 mg nicotine per kg body weight, twice daily, and controls were injected with saline on the same schedule. Special care was taken to handle all rats in the same manner to eliminate differences resulting from this variable[15].

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Inasmuch as manifestations of minimal seizure activity cannot be elicited prior to 9 days of age in the rat[16], measurement of EST was initiated in the offspring on the 10th day after birth and continued every other day up to day 24. For EST, threshold stimulus was delivered through corneal electrodes for 0.2 sec with a 60-cycle, alternating-current electroshock apparatus to induce minimal (clonic) seizure activity[17]. The MES patterns were studied after day 22, when the adult tonic-clonic pattern is established in the rat[18]. MES was induced by means of the same apparatus as EST, except that the stimulus applied was approximately 5 times threshold.

Results. As in previous studies[9,10,19], control rats consistently gave birth on day 22 of gestation, whereas in nicotine-treated animals, delivery, on the average, was delayed until day 23, a statistically-significant difference. The extra day in utero has been found to be significant in terms of a 'catch-up' in body weight[9] and this factor may affect other parameters of development as well. In this respect, it is of interest that by 8 days of age, brain weight in offspring of nicotine-treated animals was higher than that of controls – a difference which remained observable at 22 days.

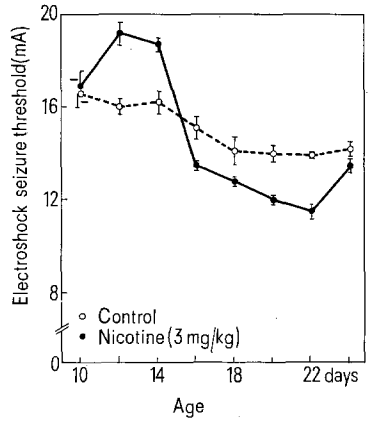

Fig. 1. The electroshock seizure threshold (EST) in offspring of rats injected twice daily throughout pregnancy with 3 mg/kg nicotine or saline (control). With the exception of days 10 and 24, all differences between nicotine and control values were significant: day 18, $P < 0.05$; all other days, $P < 0.001$.

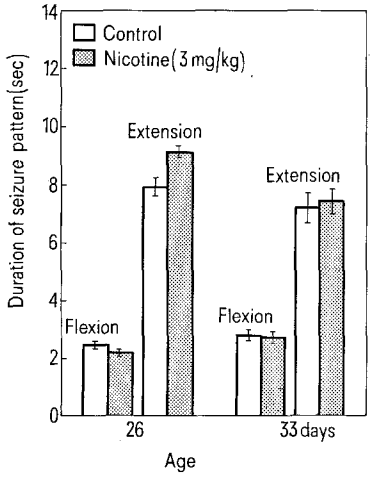

Fig. 2. The maximal electroshock seizure (MES) pattern in offspring of nicotine-injected and saline-injected (control) rats on postnatal days 26 and 33. The duration of extension was significantly longer ($P < 0.001$) on day 26 in offspring of treated rats than in corresponding controls, whereas on day 33, values were comparable between groups.

The developmental pattern of the EST differed markedly between offspring from control and nicotine-treated animals (Figure 1): in controls, the EST decreased slowly from day 10 to 18 and remained at this level until day 24, the last day of testing. In offspring from treated mothers, on the other hand, EST increased from day 10 to 14, dropped below control values on day 16, continued to decrease up to day 22, when, again, it began to rise. MES measurements showed that at day 26, the duration of flexion was shorter and the duration of extension longer in offspring of nicotine-treated rats than in their corresponding controls; these responses returned to control levels by 33 days (Figure 2).

Discussion. The differences in EST between offspring of control and treated mothers from day 10 to day 24 indicate that nicotine induces a transitory effect on the development of seizure activity, most likely involving subcortical inhibitory and excitatory pathways[13]. That cortical development also is altered in offspring of nicotine-treated animals is shown by their MES patterns at 26 days (shorter duration of flexion and longer duration of extension); these responses indicate increased brain excitability[14], which, at this age, may indicate immaturity[20], or other disturbances in CNS maturation. As with EST, MES parameters subsequently revert to control values.

The differences observed in electroconvulsive responses during the first 5 postnatal weeks between offspring of treated and untreated mothers suggest that nicotine administered during gestation prolongs the normal maturational timetable for excitatory and inhibitory systems, either by delaying the development of excitation or accelerating development of inhibition. Although these specific electroconvulsive responses normalize with increasing age (a phenomenon also observed in other parameters of CNS maturation[21]) because of the complexity of events taking place during CNS development, even transient abnormalities occurring during critical maturational periods may have functional repercussions. Indeed, continuing studies of the effects of endogenous and exogenous factors on CNS development consistently reveal that subtle alternations at critical periods of prenatal and postnatal brain maturation, though not always immediately observable, are frequently manifested at the onset of specific functions, or when a specialized demand is placed upon the organism[22].

Résumé. Lorsque le rat a été traité par des injections journalières de nicotine au cours de la gestation, le développement des réponses du jeune rat a l'électrochoc est

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retardé. Ces modifications, qui reflètent des altérations dans la maturation des systèmes inhibiteurs et excitants du système nerveux central au cours de la croissance persistent jusqu'à la 5e semaine post-natale. Ces résultats indiquent que la nicotine est capable de provoquer des modifications dans le développement du cerveau à l'état fœtal.

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Effect of Alkali Cations on the Interaction Between Detergents and Erythrocyte Membranes

The degree of complement (C)-mediated lysis of mammalian erythrocytes (E) with high K^+ and low Na^+ content, including human E, depends on the alkali metal cation present in the reaction mixture[1-3]. Thus, the highest degree of lysis was obtained in 145 m*M* K^+, and the lowest, in Na^+. Arrangement of the alkali metal cations according to their ability to enhance C lysis resulted in the selectivity series K > Rb > Li > Cs > Na or K > Li > Rb > Cs > Na[1,3]. The facilitating effect of K^+, Rb^+ and Cs^+ occurs primarily on the final stage of the reaction of C with E[2]. Therefore, it became important to ascertain whether the effect of alkali cations was specific for C lysis or whether it also occurred in hemolysis of non-immune nature. We explored this problem using the detergents Triton X-100 (Tr) and Na desoxycholate (DOC). We also investigated whether the alkali cations modify the degree of solubilization of purified E membranes (M) caused by Tr and DOC.

Material and methods. Blood from normal humans was collected in ACD solution and used within 2 days. The E were washed 3 times at 4°C with veronal buffer containing 145 m*M* NaCl, 0.5 m*M* $MgCl_2$ and 0.15 m*M* $CaCl_2$, pH 7.3[4] (Na buffer). The E were suspended in cold Na buffer and standardized photocolorimetrically to 6.25×10^8 E per ml. For hemolysis studies 0.4 ml were transferred to tubes and centrifuged. To the sedimented E was added 1 ml ice-cold Na buffer or buffers identical to the Na buffer except that 145 m*M* K, Rb, Li, or Cs was substituted for 145 m*M* Na. Then an aqueous solution of the detergent was added (25 µl of 0.2% [v/v] Tr or 50 µl of 0.9% [w/v] DOC) to yield 0.076 m*M* Tr or 1.03 m*M* DOC. The E were suspended and incubated at 37°C for 1 h, with mixing. The reaction was terminated by addition of 2 ml of an ice-cold veronal buffer identical to the buffer used initially, centrifugation of the unlysed cells, and measurement of the degree of lysis[4]. E suspensions subjected to this procedure but incubated without detergent served as blanks.

M solubilization studies were performed with M obtained from human E that were washed at 4°C wiht isotonic phosphate buffer, pH 7.4, and lysed with 5 m*M* phosphate buffer, pH 7.4[5]. The M were washed twice with this buffer and twice with Na buffer. Then they were suspended in Na buffer and the concentration was adjusted to an O.D. of 0.5 at 550 nm, which corresponded to 4.4×10^9 *M* particles per ml, as determined with a Coulter counter. 2 ml were transferred to tubes which were centrifuged at 30,000 g for 20 min. To the sedimented *M* was added 1 ml ice-cold Na buffer or buffers containing the other alkali metal ions. Then an aqueous solution of the detergent was added (50 µl of 10% [v/v] Tr or 50 µl of 10% [w/v] DOC) to yield 7.4 m*M* Tr or 11.5 m*M* DOC. The procedure then continued as described above for hemolysis of E. The reduction in turbidity at 550 nm was used to measure the degree of M solubilization.

Results and discussion. The results (Table) indicate that the alkali metal cations markedly influence the degree of lysis of human E caused by Tr and DOC, in a manner that is characteristic for each detergent. Thus, the activity series obtained with Tr was K > Rb = Cs > > Na ⩾ Li, and that with DOC was Li > Rb = Cs > K > Na. In contrast, the degree of E M solubilization produced by Tr and DOC was independent of the alkali metal ion present in the reaction system. Identical results

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Effect of alkali metal cations on degree of hemolysis of human erythrocytes and on solubilization of human erythrocyte membranes by Triton X-100 and by Na desoxycholate

Alkali metal ion present during reaction (145 m*M*)	% Erythrocytes hemolyzed (Mean ± S.E., 8 experiments[a])		% Membranes solubilized (Mean and range, 4 experiments[a])	
	Triton	DOC	Triton	DOC
Na	19.6 ± 5.4	22.7 ± 4.1	32.1 (17.2–38.8)	76.1 (60.8–63.0)
K	87.5 ± 1.4	30.9 ± 6.5	36.2 (33.5–38.8)	77.2 (64.8–84.4)
Rb	76.1 ± 5.9	80.7 ± 6.1	32.5 (25.3–38.8)	79.1 (68.6–84.4)
Cs	76.3 ± 6.3	80.4 ± 9.0	34.3 (29.5–41.7)	81.5 (68.6–88.7)
Li	17.7 ± 2.6	93.7 ± 0.7	25.0 (20.2–27.6)	81.5 (68.6–88.7)

[a] Each experiment was carried out with type 0, Rh^+ erythrocytes obtained from different donors.