Pharmacology Biochemistry & Behavior, Vol. 17, pp. 1077–1082, 1982. Printed in the U.S.A.

Sex-Dependent Biological Changes Following Prenatal Nicotine Exposure in the Rat

DAVID A. V. PETERS AND SHUN TANG

Department of Pharmacology, Faculty of Health Sciences, School of Medicine
University of Ottawa, Ottawa, Ontario K1H 8M5 Canada

Received 10 October 1981

PETERS, D. A. V. AND S. TANG. *Sex-dependent biological changes following prenatal nicotine exposure in the rat.* PHARMAC. BIOCHEM. BEHAV. 17(5) 1077–1082, 1982.—Nicotine was administered to adult female rats in drinking water starting 6 weeks before mating and continuing throughout pregnancy. The litters were cross-fostered to control dams at birth. Prenatal nicotine treatment reduced both the number of male rats born and the male birth weight. Female offspring were not significantly affected. Rearing activity was reduced in male but not female offspring either when tested over a 24 hour period in a home cage environment or during a 10 minute exposure in an open field. Horizontal locomotor activity was reduced only during the first 5 minutes in the open field and again the effect was found only in the males. Baseline plasma corticosterone levels were reduced in both male and female offspring but there was no effect on stress-elevated corticosterone levels.

Prenatal Nicotine exposure Corticosterone Rat Emotionality Motor activity Open field behavior Rearing

OFFSPRING of nicotine-injected rats are reported to show growth and survival deficits [2, 3, 9, 13] as well as behavioral [12,13] and neurochemical [9] abnormalities at maturity. We have previously reported that the procedure of administering nicotine to adult female rats in drinking water before and during pregnancy combined with cross-fostering of the offspring to control dams at birth resulted in significant changes in spontaneous locomotor activity in the adult offspring but neither growth nor survival deficits [14]. These data suggest that at least some of the behavioral changes found in offspring of nicotine-treated rats originate during the prenatal period and are not dependent on the stress of repeated drug injections. It was therefore of interest to study in more detail the effects of oral nicotine administration to pregnant rats on the behavior of offspring.

For the present study nicotine was administered to adult female rats in drinking water as before and the offspring were used to study both locomotor activity and open field behavior. To examine the possibility that the behavioral deficits may have been associated with an altered emotional reactivity of the offspring we also studied the plasma corticosterone response to stress and hypothalamic norepinephrine levels [7].

METHOD

Nicotine was administered to adult female rats as previously described [14]. Briefly, 20 female Sprague-Dawley rats, 150–165 g, received nicotine in drinking water throughout the experiment while 40 matched control rats received water alone. During the first 3 weeks of treatment the nicotine concentration of the drinking fluid was raised every third day until the daily intake was in the range of 6.0±0.2 mg/kg/day of nicotine base and this intake was then maintained for a further 3 weeks before mating. The final intake was achieved with a nicotine concentration of approximately 40 μg/ml. During pregnancy the nicotine concentration was adjusted when necessary to maintain individual drug intakes within the same range. The average daily fluid intake was reduced by 10–20% during the first 3–4 days of nicotine treatment but was not significantly different from control during the remainder of the experiment.

Treatment of Litters

When female rats are exposed to nicotine until weaning the adverse effects on their offspring may originate either before birth or during the suckling period. Postnatal effects may include those resulting from direct exposure of pups to nicotine obtained either from the mother's milk or through access of the older pups to the drinking fluid. Malnutrition of the pups may result from a nicotine-induced blockade of prolactin secretion in the dams [18]. To avoid the adverse effects of postnatal nicotine exposure all nicotine treatment litters were transferred to control dams within 12 hours of birth. A control group was obtained by cross-fostering litters between control dams. All pups were weighed and the litters reduced to 4 male and 4 female pups during the cross-

fostering procedure. Additional litters for which there were no suitable second litters for cross-fostering were discarded. The animals were kept in an independently air-conditioned room with a 12 hour light, 12 hour dark cycle.

Eleven litters in both nicotine and control groups were used for the study, all of which were born within the same 6-day period. This age difference allowed the study to be carried out with animals of identical age even though some sets of readings took up to 6 days to complete. To minimise the influence of interlitter variations within the same group [1,4] not more than 1 male and 1 female rat were used from a single litter for each experiment with the exception of the 24 hour activity study in which pairs of rats were used.

Locomotor Activity

Locomotor activity was measured at 25, 45, 60 and 85 days of age using a microprocessor-controlled equipment as previously described [14]. The objective of the experiment was to monitor movement of the animals in an environment as close as possible to that of the home cage over a continuous 24 hour period. To achieve this objective the cages used in the study were identical to the home cages and the rats were tested in pairs selected from the same litter. The rats were allowed a 2 hour acclimatization period in the equipment before the start of the 24 hour recording.

A standard polypropylene animal cage containing 2 rats of the same sex, age and treatment group was placed between 2 fixed insulated metal plates which served as the capacitance in an oscillator circuit. Movement of the animals within the cage continuously altered the frequency of the associated oscillator so that changes in frequency between successive measurements could be used to detect movement. Eight identical cages were monitored simultaneously and frequency readings were recorded for each cage once every second. Preliminary experiments showed that with a baseline frequency of 1 MHz most movements, including social interactions between the rats and ambulation of one or both rats, produced frequency changes in the range of 20–200 Hz. Only rearing, with or without the support of the side of the cage, produced frequency changes greater than 200 Hz. A single rat rearing with both front paws off the floor of the cage produced a frequency change greater than 1000 Hz. To distinguish rearing movement from other activity the frequency changes greater than 200 Hz were scored separately as "rearing." All other readings greater than 20 Hz were scored as "non-rearing" activity while readings less than 20 were scored as "inactivity." Activity was recorded as the number of 1 second intervals that a change in frequency of the appropriate magnitude occurred.

Automated Open Field

The equipment consisted of a Plexiglas cube with 50 cm sides open at the top and bottom. Three sets of 8 equally spaced infrared beams were used to detect horizontal and rearing movements of a single rat placed in the cube. The three banks were placed horizontally in a removable collar which surrounded the lower half of the cube. Two of the three banks were fixed at right angles to one another 4 cm above the bottom of the cube to monitor movement of the animal in the horizontal plane while the third bank was placed at an adjustable height of 6 to 18 cm above the base to detect rearing. To avoid interference between beams only a single beam was activated at any time. The entire 24 beam system was scanned at a rate of 2 scans each second and the position of the animal within the cube calculated from the pattern of interrupted beams. Depending on the calculated position of the animal an assignment to one of 64 equal squares was made at each scan. From these data were calculated the distance travelled, the number of squares entered, the number of intervals with no detectable activity and the time spent in each of the individual squares. Rearing movements were calculated in terms of both number and duration of rearings.

All animals were tested during the first 2 hours of the light-on period. Each test consisted of a brief electronic check of the system, a 5 second interval during which a rat was placed in the centre of the field and finally, a continuous 10 minute period of data collection. At the end of each test the data were recorded on magnetic tape as 10 successive 1 minute blocks. After removal of the rat the base and sides of the apparatus were cleaned with a mild detergent solution.

TABLE 1

EFFECTS OF MATERNAL NICOTINE ADMINISTRATION ON THE OFFSPRING AT BIRTH

Sex	Group	Pups/litter	Birth weight (g)
Male	Control	6.2 ± 0.3	5.52 ± 0.07
	Nicotine	4.8 ± 0.5*	5.14 ± 0.12*
	%	78	93
Female	Control	6.2 ± 0.4	5.25 ± 0.07
	Nicotine	6.5 ± 0.5	5.21 ± 0.14
	%	105	99

Results are mean±S.E.M. for groups of 28 control litters and 12 nicotine litters.

*Significantly different from control, $p<0.05$ by Student's t-test.

Biochemical Tests

Groups of animals were killed by decapitation at 45 and 85 days of age and a blood sample was collected from each animal for analysis of corticosterone levels. The animals were killed in groups of 2–3 within thirty seconds of removal from a holding room to minimise stress-induced changes in corticosterone levels. A blood sample from each rat was collected immediately in a heparinised tube, frozen in liquid nitrogen and stored at −60° for later assay of corticosterone by a fluorometric procedure [16]. At 45 days of age additional animals were stressed by 10 sec of handling 10 min before killing to measure the corticosterone response to stress. Although this procedure involved a very mild form of stress a similar procedure has been reported to be sufficient to produce substantial change in plasma corticosterone, prolactin and growth hormone [15]. Immediately following the blood collection the brains of the 45 and 85 day-old rats were removed and the hypothalami dissected out on a glass plate cooled in ice. After rapid weighing the samples were frozen in liquid nitrogen and stored at −60° in plastic capsules. Norepinephrine was assayed in these samples by the combined methods of Maickel, Cox, Saillant and Miller [11] and Laverty and Taylor [10].

RESULTS

Chronic administration of nicotine to female rats

TABLE 2

EFFECTS OF PRENATAL MATERNAL NICOTINE TREATMENT ON LOCOMOTOR ACTIVITY

Age (days)	Non-rearing activity (min/day) Control	Nicotine	%	Rearing activity (min/day) Control	Nicotine	%
			Males			
24	160 ± 3	162 ± 4	101	16.6 ± 2.4	13.0 ± 1.9	78
45	187 ± 4	178 ± 5	95	53.4 ± 2.4	51.2 ± 2.9	96
60	171 ± 3	148 ± 5	87	80.5 ± 4.7	61.3 ± 7.1	76*
85	224 ± 6	212 ± 15	95	66.0 ± 6.7	48.7 ± 5.4	76*
			Females			
24	187 ± 9	201 ± 10	107	21.9 ± 4.7	20.0 ± 2.1	91
45	209 ± 5	204 ± 8	98	60.9 ± 2.8	53.2 ± 3.2	87
60	217 ± 18	207 ± 8	95	62.0 ± 3.6	64.5 ± 4.3	104
85	221 ± 9	249 ± 17	113	72.8 ± 7.6	85.1 ± 11.3	117

Results are calculated as the total duration of activity for a continuous 24 hour period and are expressed as mean±S.E.M. for groups of 8 pairs of rats.
*Significantly different from control, $p<0.05$ by Newman-Keul's test.

produced a small but statistically significant decrease in birth weight of male but not female offspring and slightly reduced the number of male rats born (Table 1). Prenatal nicotine exposure had no effect on postnatal body weight gain and from 7 to 85 days of age body weights were similar to control values.

The effect of prenatal nicotine treatment on locomotor activity is summarised in Table 2. The data are presented as the total duration of activity over a continuous 24 hour period. A 3-way analysis of variance was conducted on treatment, age and sex for each of the two activity measures. The only main effect was a significant sex difference, females showing greater durations of both rearing, $F(1,112)=4.2$, $p<0.05$ and non-rearing, $F(1,112)=22.8$, $p<0.01$ activity. Additional 2-way analyses of variance were then conducted on treatment and age with the sexes separated. Prenatal nicotine exposure was found to significantly decrease rearing acitivty in male, $F(1,56)=7.29$, $p<0.01$, but not female, $F(1,56)=1.03$, subjects while non-rearing activity was not significantly affected in either sex.

A total of 24 rats were tested in the open field for 10 consecutive trials of 1 minute each. Data for two indicators of horizontal movement (squares entered and total distance travelled) and two indicators of vertical movement (the number and duration of rearing movements) as well as the total duration of inactivity and total occupancy time in each of the 64 squares comprising the open field were recorded for each trial. A preliminary $2\times2\times10$ analysis of variance was conducted on treatment, sex and a repeated measure (the 10 consecutive trials) of each of the dependent variables. Both indicators of vertical movement showed a significant sex × treatment interaction which was found to be due to a significant treatment effect in male offspring only. Male offspring in the prenatal treatment group reared less frequently, $F(1,10)=5.99$, $p<0.05$, for a shorter total duration of time, $F(1,10)=6.3$, $p<0.05$, than control males. The two indicators of horizontal movement showed a significant sex effect with females making more square entries, $F(1,20)=9.1$, $p<0.01$, and travelling a greater distance, $F(1,20)=17.9$, $p<0.001$, than males. There was no main treatment effect for horizontal movement. However, males but not females showed a significant treatment × trials interaction which appeared to be due to a decrease in locomotor activity during the first few trials in the open field. The 10 trials were therefore treated as 2 consecutive blocks of 5 trials each and an analysis of variance was conducted for each of the blocks. Table 3 summarizes these data. Only the first block of trials involving male rats showed a significant treatment effect. The male prenatal nicotine group showed significantly fewer square entries, $F(1,10)=5.05$, $p<0.05$, and travelled a significantly shorter distance, $F(1,10)=4.98$, $p<0.05$, than control males. Prenatal nicotine treatment did not appear to affect either the duration or inactivity or the location of the rats within the open field.

Baseline plasma corticosterone levels were reduced by maternal nicotine exposure (Table 4). A 3-way analysis of variance conducted on age, sex and treatment showed significant treatment, $F(1,56)=8.51$, $p<0.01$, and sex, $F(1,56)=15.5$, $p<0.001$, effects. At 45 days of age additional animals were handled for 10 sec 10 min before killing as a response to stress test. As expected, handling significantly elevated plasma corticosterone levels, $F(1,56)=67.6$, $p<0.001$, but there were no other significant main effects or interactions.

A 3-way analysis of variance was conducted on age, sex and treatment for the adrenal weight data. The only significant main effect was an age-dependent increase in adrenal weight, $F(1,56)=56.4$, $p<0.001$. Prenatal nicotine treatment resulted in a significant increase in adrenal weight in female offspring, $F(1,28)=5.69$, $p<0.05$, while male offspring showed only a small but statistically significant decrease in adrenal weight at 45 days of age only.

Prenatal nicotine treatment had no effect on hypothalamic norepinephrine levels.

DISCUSSION

Parenteral injections of nicotine (usually 3 mg/kg twice daily) to pregnant female rats are known to lower birth weight, reduce early body weight gain and increase neonatal mortality in the offspring [2, 3, 9, 13]. The adult offspring of rats which received daily nicotine injections throughout ges-

TABLE 3

EFFECT OF PRENATAL MATERNAL NICOTINE TREATMENT ON OPEN FIELD BEHAVIOR OF THE OFFSPRING AT 60 DAYS OF AGE

	Males			Females		
Trial	Control	Nicotine	%	Control	Nicotine	%
			Distance Travelled (cm)			
1–5	284 ± 15	224 ± 24	79*	297 ± 18	290 ± 22	98
6–10	183 ± 9	174 ± 9	95	217 ± 18	230 ± 17	106
			Numbers of Squares Entered			
1–5	20.8 ± 0.9	16.4 ± 2.1	79*	22.6 ± 1.0	23.5 ± 1.6	104
6–10	13.5 ± 1.2	12.3 ± 0.8	91	16.9 ± 0.7	18.4 ± 1.5	109
			Number of Rearings			
1–5	9.4 ± 0.7	6.9 ± 0.9	73*	9.4 ± 0.4	8.2 ± 0.5	87
6–10	6.5 ± 0.7	5.6 ± 0.7	86	6.7 ± 0.8	7.1 ± 0.6	106
			Duration of Rearings (sec)			
1–5	21.3 ± 1.7	16.3 ± 1.6	77*	18.2 ± 1.4	15.5 ± 1.1	85
6–10	16.2 ± 1.4	12.0 ± 1.4	74*	14.9 ± 1.6	15.0 ± 1.6	101

Results are mean±S.E.M. for groups of 6 rats and are calculated as the average values per minute for 2 consecutive blocks of 5 × 1 minute trials each.
*Significantly different from control, $p<0.05$ by Newman-Keul's test.

TABLE 4

EFFECT OF PRENATAL NICOTINE TREATMENT ON ADRENAL WEIGHT, PLASMA CORTICOSTERONE (CS) AND HYPOTHALAMIC NOREPINEPHRINE (NE) AT 45 AND 85 DAYS OF AGE

	Males			Females		
	Control	Nicotine	%	Control	Nicotine	%
Adrenal weight (mg/pr)	22.6 ± 1.1	19.2 ± 1.0	85*	20.1 ± 1.1	24.3 ± 2.1	121
Plasma CS (mg%)—baseline	3.6 ± 0.4	2.0 ± 0.4	56*	5.0 ± 1.2	3.3 ± 1.0	66
—handled†	12.6 ± 2.2	11.5 ± 1.1	91	15.2 ± 1.7	15.3 ± 1.9	101
Hypothalamic NE (ng/g)	911 ± 75	816 ± 66	90	852 ± 49	860 ± 41	101
Adrenal weight (mg/pr)	32.6 ± 1.9	34.7 ± 3.0	107	39.9 ± 1.3	44.1 ± 2.2	111
Plasma CS (mg%)—baseline	2.0 ± 0.5	1.3 ± 0.1	65	6.0 ± 0.9	3.2 ± 0.8	53
Hypothalamic NE (ng/g)	827 ± 71	921 ± 39	111	915 ± 28	878 ± 56	96

Results are mean±S.E.M. for groups of 8 rats.
*Significantly different from control, $p<0.05$ by Student's t-test.
†Handled for 10 sec, 10 min before killing as a response to stress test.

tation and the nursing period performed more poorly than offspring of vehicle treated rats when tested on various appetitive schedules in a double-bar Foringer chamber [12]. However, when nicotine was administered during pregnancy alone both behavioral deficits and growth reductions were less pronounced than when treatment was continued until weaning [12] and neonatal malnutrition may have been a factor in these experiments. Evidence that nicotine injections reduce milk secretion in lactating rats [18] supports this view.

It has been suggested that in stressful situations nicotine may affect the stress response by releasing norepinephrine from the hypothalamus resulting in an inhibition of corticosteroid release from the adrenal cortex [8]. When nicotine is administered to pregnant rats by a route which involves stress the effects on the offspring may include those resulting from a nicotine-induced modification of the stress response in the parent. This may explain why offspring of nicotine injected females tended to be more active than offspring of vehicle injected rats but less active than non-injected controls [13].

For our studies we therefore administered nicotine in drinking water to avoid the stress of either multiple drug injections or exposure to a tobacco smoke-filled chamber. We cross-fostered all litters to control dams at birth to prevent the effects of malnutrition due to reduced milk secretion.

In our previous study [14] only half of the litters born to nicotine-treated rats were cross-fostered to control dams at birth. The non-fostered group showed a reduction in nighttime locomotor activity and an increase in daytime activity when tested as adults. Male and female offspring showed similar activity changes. Cross-fostering to control dams reduced the activity changes. Cross-fostering to control dams

reduced the activity changes and it was concluded that part but not all of the effects originated during the early postnatal period. Consistent with our previous report [14] total activity showed only minor drug-induced changes, whereas, when rearing-activity was separated from all other movement it was found that the rearing component showed a significant treatment effect which persisted until at least 85 days of age.

Because the rats were tested in pairs, it is possible that the reduction in duration of rearing activity resulted from a change in the behavioral interactions between the rats. However, rearing activity was also significantly reduced in 60 day-old male offspring when tested singly in the open field.

The open field study also provided evidence that horizontal locomotor activity was reduced in male offspring following prenatal maternal exposure to nicotine. Both the number of squares entered and the total distance travelled were significantly reduced by the prenatal drug treatment although the deficit was seen only during the first few minutes of exposure to the open field. The short duration of this effect may explain why there was no similar sex-dependent effect in the 24 hour study. The reduced locomotor activity on first exposure to the open field did not appear to be due to an increased tendency for the animals in the nicotine treatment group to "freeze" in response to an unfamiliar environment because there was no change in the duration of inactivity.

Changes in locomotor activity in an open field have often been interpreted in terms of an altered emotional reactivity although there is much controversy on the subject. Denenberg [6] suggested that the relationship is dependent on the day of testing. On day 1 of testing, high activity was found to be associated with a high defecation rate and an elevated plasma corticosterone level suggesting a relationship between high activity and a high emotional reactivity. After day 1 low activity was associated with high emotional reactivity. Similarly, a comparison of rats from different sources provided evidence of an association of low exploration and motor activity scores with a high defecation rate, high plasma corticosterone level, high adrenal weight and low hypothalamic norepinephrine [7].

As part of the present study we measured the baseline plasma corticosterone level, the corticosterone response to mild stress and hypothalamic norepinephrine levels. Neither the plasma corticosterone response to stress nor the hypothalamic norepinephrine level was significantly affected by prenatal nicotine treatment. Although there was an apparent association of prenatal nicotine-induced behavioral changes with a reduced baseline corticosterone level in male offspring the female offspring showed a similar corticosterone change but not the behavioral modifications. Even the reduced adrenal weight in 45 day-old male offspring following prenatal nicotine treatment was of doubtful significance since the change was no longer present at 85 days of age. There was, therefore, little evidence that the reduced locomotor activity was associated with an altered emotional reactivity or even to an altered adrenocortical response to stress.

The finding of reduced male numbers at birth is of interest in view of studies which find that pregnant women who smoke tobacco tend to give birth to fewer than usual male offspring (see review in [3]). In our experiments, prenatal nicotine treatment usually affected males more than females. A possible explanation is that the male fetus may accumulate nicotine to a greater extent than the female and therefore be more affected. Evidence that adult male mice showed higher brain nicotine levels than females receiving comparable doses of the drug [17] may support this suggestion. However, the finding of a greater effect on males is not unexpected in view of evidence that male rats are more vulnerable than female rats to disadvantageous conditions during rearing [5].

Although the mechanism and significance is presently not clear, it is evident that prenatal exposure to nicotine results in persistent biochemical and behavioural changes in the rat which suggests that nicotine may be responsible for at least some of the adverse effects on the fetus produced by tobacco smoking by humans during pregnancy.

ACKNOWLEDGEMENTS

This study was supported by grants from the Medical Research Council of Canada and the Ontario Mental Health Foundation.

REFERENCES

1. Abbey, H. and E. Howard. Statistical procedure in developmental studies on species with multiple offspring. *Devl Psychobiol.* **6:** 329–355, 1973.
2. Becker, R. F., C. R. D. Little and J. E. King. Experimental studies on nicotine absorption in rats during pregnancy. III Effects of subcutaneous injection of small chronic doses upon mother, fetus and neonate. *Am. J. Obstet. Gynec.* **7:** 957–968, 1968.
3. Becker, R. F. and J. C. Martin. Vital effects of chronic nicotine absorption and chronic hypoxic stress during pregnancy during pregnancy and the nursing period. *Am. J. Obstet. Gynec.* **110:** 522–533, 1971.
4. Chapman, R. H. and J. M. Stern. Failure of severe maternal stress or ACTH during pregnancy to affect emotionality of male rat offspring: implications of litter effects for prenatal studies. *Devl Psychobiol.* **12:** 255–267, 1979.
5. Crutchfield, F. L. and M. B. Dratman. Growth and development of the neonatal rat: particular vulnerability of males to disadvantageous conditions during rearing. *Biol. Neonate* **38:** 203–209, 1980.
6. Denenberg, V. H. Open-field behavior in rat: what does it mean? *Ann. N. Y. Acad. Sci.* **159:** 852–859, 1969.
7. File, S. E. and S. V. Vellucci. Behavioral and biochemical measures of stress in hooded rats from different sources. *Physiol. Behav.* **22:** 31–35, 1979.
8. Hall, G. H. and C. F. Morrison. New evidence for a relationship between tobacco smoking, nicotine dependence and stress. *Nature* **243:** 199–201, 1973.
9. Hudson, D. B., B. J. Merrill and L. A. Sands. Effects of prenatal and postnatal nicotine administration on biochemical aspects of brain development. In: *Drugs and the Developing Brain,* edited by A. Vernadakis and N. Weiner. New York: Plenum Press, 1974, pp. 243–256.
10. Laverty, R. and K. M. Taylor. The fluorometric assay of catecholamines and related compounds: Improvements and extensions to the hydroxyindole technique. *Analyt. Biochem.* **22:** 269–276, 1968.
11. Maickel, R. P., R. H. Cox, F. P. Saillant and F. P. Miller. A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. *Int. J. Neuropharmac.* **7:** 275–281, 1968.
12. Martin, J. C. and R. F. Becker. The effects of maternal nicotine absorption or hypoxic episodes upon appetitive behavior of rat offspring. *Devl Psychobiol.* **4:** 133–147, 1971.

13. Martin, J. C., D. C. Martin, B. Radow and G. Sigman. Growth, development and activity in rat offspring following maternal drug exposure. *Expl Aging Res.* **2:** 235–251, 1976.
14. Peters, D. A. V., H. Taub and S. Tang. Postnatal effects of maternal nicotine exposure. *Neurobehav. Toxicol.* **1:** 221–225, 1979.
15. Seggie, J. A. and G. M. Brown. Stress response patterns of plasma corticosterone, prolactin and growth hormone in the rat, following handling or exposure to novel environment. *Can. J. Physiol. Pharmac.* **53:** 629–637, 1975.
16. Silber, R. H., R. D. Busch and R. Oslapas. Practical procedure for estimation of corticosterone or hydrocortisone. *Clin. Chem.* **4:** 278–285, 1958.
17. Tepper, J. M., J. R. Wilson and K. Schlesinger. Relations between nicotine-induced convulsive behavior and blood and brain levels of nicotine as a function of sex and age in two inbred strains of mice. *Pharmac. Biochem. Behav.* **10:** 349–353, 1979.
18. Terkel, J., C. A. Blade, V. Hoover and C. H. Sawyer. Pup survival and prolactin levels in nicotine-treated lactating rats. *Proc. Soc. exp. Biol. Med.* **143:** 1131–1135, 1973.