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Effects of Nicotine on the Fertility, Cytology and Life Span of Male Rats

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Abstract. Contrary to an earlier opinion that nicotine has no effect on the fertility of male animals or humans, the present experimental study using male inbred Fisher rats demonstrates that the reproductive capacity of the animals is greatly reduced when injected with nicotine, and that the effect is much greater in male than in female Fisher rats similarly injected with nicotine. This is in accord with some earlier histological and morphological studies which have shown that female rodents have a greater tolerance to nicotine than their male counterparts. It is also confirmed by the cytologic observations of the present study. These observations show that, similar to female rats, inflammatory processes, as evidenced by an increased number of lymphocytes and/or polymorphonuclear leukocytes, are responsible for the decrease in fertility. However, the cytological profile is profoundly different in the two sexes: virulent inflammatory conditions begin much earlier in male rats, they are more frequent and, whereas the condition is reversible in the female animal when nicotine treatment is discontinued, it is not in some male rats, and inflammatory conditions persist for the entire life as does infertility. However, the life span of nicotine-treated male rats is greater than in female nicotine-treated rats, although it is shorter in both sexes than in their respective controls; in some male nicotine-treated rats, the life span is greater not only than in their male controls but even than in their female counterparts. Possible explanations for this apparently paradoxical life-prolonging effect of nicotine treatment are reviewed, but the evidence is either conflicting or insufficiently established and requires further study.

In an earlier study [Riesenfeld and Oliva, 1987], we investigated the effect of nicotine on the fertility, cytology and life span of female Fisher rats. Other studies [Lee, 1935; Riesenfeld, 1985], dealing with histological and morphological aspects, have shown that male mice and rats have a lower tolerance to nicotine than females. The vast number of studies, experimental and clinical, on the effects of nicotine has been reviewed by various authors [Larson et al., 1961; Sterling and Kobayashi, 1975; Vogt et al., 1984]. Most researchers, including the present ones, found chronic nicotine or tobacco administration to animals (dogs, rabbits, guinea pigs, rats, mice) associated with various degrees of testicular atrophy or degeneration [Larson et al., 1961] and, in humans, increased incidence of testicular varicocele was found in cigarette smokers [Klaiber et al., 1980]. Spermatological studies have concentrated on the density, motility and morphology of human sperm in smokers and nonsmokers. All authors have noted the many conflicting results of these studies, but some have nevertheless concluded that, on balance, it seems that nicotine has no effect on male fertility. But none of these authors has tried to explain the reasons for the conflicting results, in which some observers found differences between smokers and nonsmokers while others did not. In view of the uncertainty still remaining, the present experimental study seems warranted.

A small number of cytological studies on the effect of nicotine is available [Scheer, 1940; Hopkins and Evans, 1979; Obe and Beck, 1984], and others were reviewed by Larson et al. [1961]. But these studies are equally inconclusive: while some studies found a temporary increase of lymphocytes in nicotine administration followed by a decrease, others found the reverse [Backmann et al., 1925; Takatsuki, 1932; Von Kreuziger et al., 1956], and still others [Garlock, 1935; Küchle et al., 1952] found no changes at all. These studies used only very small numbers of animals (e.g. 1 rabbit, 5 dogs), experimental time was very short (a few minutes or hours), the sex of the animals is frequently not indicated and the only very early chronic experiment by Vas [1894] using rabbits was criticized by Larson et al. [1961] on methodological grounds.

Our investigation differs from these previous studies in three aspects.

(1) The nicotine treatment was carried out on a chronic basis (90 days).

(2) It allows contrast between males and females (from our previous study).

(3) It is based on samples large enough for statistical analysis.

Material and Methods

A total of 280 Fisher rats was used: 83 were males, 87 females, and 110 were male controls. Only males were injected with nicotine, 0.42 mg/kg. As in our previous study [Riesenfeld and Oliva, 1987], treatment was started at the age of 50 days for a period of 90 days. The number of daily injections was gradually increased from once daily with 0.21 mg/kg for the first 2 days to twice daily with 0.21 mg/kg during the second 2 days, and then to three times daily during the entire remaining treatment period with 0.42 mg/kg. Injection sites were rotated and injections were intramuscular, as in our previous study. Females were not injected but kept together with injected males. Male fertility was judged by the number of pregnancies and births. Females that died prematurely were autopsied to check for pregnancy. This method of establishing male reproductive capacity seems superior to that of using testicular atrophy or even sperm morphology as criteria, since these methods have given conflicting results in the past. But it should be pointed out that even our method of establishing male fertility can only be considered as an approximation, since a single male could, of course, be responsible for a number of pregnancies. Pregnancies and births were counted during 6 trimesters, the first trimester being the period of puberty and nicotine treatment, the other trimesters representing postnicotine periods.

As in our previous study, cytological analysis was based on postmortem peritoneal washings. The amount of material varied from a few drops to several milliliters. They were received on equal volumes of 50% ethyl alcohol added as a fixative.

When there was no free fluid, the peritoneal cavity was irrigated with 2 ml of 50% alcohol and recovered by needle aspiration.

The fluids were collected for cytological evaluation. First the specimens were centrifuged. Grossly visible sediment was smeared between two slides and fixed with Cytospray. To ensure the greatest cellular yield, scanty specimens were centrifuged in a Shandon cytospin at 1,200 rpm for 10 min and Cytospray-fixed. All specimens were stained with a modified Papanicolaou stain and evaluated using cytological criteria [Koss, 1979].

If cellular degeneration, debris or excessive blood obscuring the cellular detail were found, a diagnosis of 'unsatisfactory' was given. Cell counts were performed only on satisfactory slides. They were classified into the following three categories.

Few: with fewer than 25 leukocytes (lymphocytes and/or polymorphonuclear [PMN] leukocytes, lymph/PMN) per microscopic field, being normal.

Moderate number: with 25–60 leukocytes (lymph/PMN) per microscopic field, indicating mild to moderate inflammation.

Numerous: with more than 60 leukocytes (lymph/PMN) per field, indicating chronic or acute inflammation.

Prima Partum and Fertility

Prima partum delay is well documented for experimental animals treated with nicotine and for women who smoke [Essenberg et al., 1910; Becker et al., 1968; Baird and Wilcox, 1985]. It was observed also by us [Riesenfeld and Oliva, 1987] in one strain of nicotine-treated rats (Buffalo), but not in nicotine-treated Fisher rats in which the mother's average age was 84.30 days (SD 8.27) versus that of untreated controls of 86.70 days (SD 5.21), a difference that is not significant. However, in the present study, in which untreated Fisher females were paired with nicotine-treated males, the mother's average age at prima partum was 100.76 days (SD 15.87). This represents a delay that is significant whether it be compared with normal untreated Fisher females ($p < 0.01$) or nicotine-treated Fisher females ($p < 0.001$).

In our earlier study [Riesenfeld and Oliva, 1987], we have shown that the infertility rate in untreated Fisher control females is 21% and in nicotine-treated Fisher females 50%. In our present sample of 87 normal, untreated Fisher females paired with nicotine-treated males, there occurred 21 or 18.27% pregnancies during the first trimester. This equals a sterility rate of 81.73% and represents an increase in sterility frequency of 60.73% versus that of normal Fisher controls and an increase of 31.73% versus that of nicotine-treated Fisher females.

In the second trimester, there were 31 or 26.97% pregnancies in our sample. Whereas this is a fertility improvement over the first trimester, it still represents an infertility rate of 73.03% or an increase of 52.03% over the infertility rate of normal controls and an infertility increase of 23.03% over the infertility rate of nicotine-treated Fisher females.

In the third and fourth trimesters, there were only four pregnancies, respectively, representing a fertility rate of 3.48% or an infertility rate of 96.52%. In the fifth trimester, there were only three pregnancies, that is a fertility rate of 2.61% or an infertility rate of 97.39%, and there were no pregnancies after that. The advanced age of the females in the fifth trimester (448, 450 and 455 days) cannot be the cause of this infertility, since we observed pregnancies as late as 492 days of age in untreated Fisher controls.

Thus, it follows that our experimental results not only contradict the conclusions of some previous authors that nicotine has no effect on male fertility, but show clearly that the effects on the reproductive capacity of male rats are much more severe than those on the females of the same strain.

However, it should be remembered that the conclusions of some of the previous writers were based not on their own experiments, but merely by weighing the conflicting data in the literature against each other. But our results are entirely in accord with previous experimental studies [Lee, 1935; Riesenfeld, 1985] which have demonstrated that male animals have a lower tolerance to nicotine than females.

Cytology

As in the female Fisher rats of our earlier study [Riesenfeld and Oliva, 1987], reproductive impairment in male rats is caused by inflammatory conditions, as evidenced by an increased number of lymphocytes and/or polymorphonuclear leukocytes. In view of the much more severe reproductive impairment of the male rat treated with nicotine, one would expect this pathological condition to be reflected also in the cytological profile of the male rat. This is exactly the condition we found. When diagnoses were again arranged in chronological order as in our earlier study, it appears (table I) that, out of a total of 37 diagnoses of male nicotine-treated rats, 25 showed numerous lymphocytes and/or polymorphonuclear leukocytes and two were intermediate between a moderate number and numerous leukocytes. This represents 9.99% of the total. In our previous female sample, out of a total of 22 diagnoses, only 3 or 0.66% showed numerous lymphocytes or chronic inflammation, and, whereas all of our postnicotine diagnoses of females were normal, 8 out of a total of 28 postnicotine males showed numerous lymphocytes while 4 were marginal between moderate and numerous (table II). These 12 cases represent 3.36% of the total. In other words, while the damage of nicotine poisoning was reversible in our female rats, it was not in some of our male animals. It might be added that in a test sample of 10 male controls, 300–400 days old, all were normal with only a few or a moderate number of lymphocytes. Moreover, it appears from our previous data that the earliest presence of numerous lymphocytes occurs at 86 days and later of nicotine treatment in female Fisher rats, whereas in our present sample of male nicotine-treated Fisher rats, numerous lymphocytes occur as early as after 2 or 3 days of treatment. If it is kept in mind that at that early time of treat-

Table I. Cytology of nicotine-treated Fisher male rats

Days of treatment	Diagnosis
2	***
3	***
4	***
6	*
7	*
8	*
10	***
13	*
13	***
15	*
15	***
17	***
19	***
20	***
21	***
28	***
32	***
35	***
38	*
40	**/***
41	*
41	**/***
42	***
42	**
46	***
48	***
49	***
50	***
53	**
54	***
63	***
78	*
85	***
85	**
87	***
90	***

* = Few lymphocytes; ** = moderate number of lymphocytes; *** = numerous lymphocytes and/or polymorphonuclear leukocytes.

Table II. Postnicotine male rats

Number of days after termination of treatment	Diagnosis
3	*
10	***
13	***
28	*
33	**/***
34	*
35	*
35	***
37	***
54	***
73	*
82	*
83	**/***
90	**/***
143	*
148	***
227	**/***
245	***
355	*
388	**
408	**
410	*
412	*
413	**
418	*
480	***
500	*
583	**

* = Few lymphocytes; ** = moderate number of lymphocytes; *** = numerous lymphocytes and/or polymorphonuclear leukocytes.

ment only 0.21 mg/kg of nicotine was administered once or twice daily versus 0.42 mg/kg three times daily later (see Methods), it becomes very clear how much less tolerant male rats are to nicotine than their female counterparts.

Life Span

Although there is overwhelming evidence that smoking of tobacco has a life-shortening effect on humans due to, e.g., high blood pressure, emphysema, heart attack, strokes and lung cancer, there are also numerous reports, some anecdotal and others statistical, that show that many groups of aged men in various societies contained large numbers of heavy smokers [reviewed by Larson et al., 1961].

In view of the much more serious impairment of fertility in male than in female rats and the concordant much more serious pathological conditions in the male as reflected in the cytological profile, it was natural to expect a more serious effect on the life span in the male than in the female nicotine-treated rat. However, our results show that this is not the case. Whereas average life span in female nicotine-treated Fisher rats was 110.44 days (SD 76.86) versus 502.32 days (SD 218.8) in 66 female controls [Riesenfeld and Oliva, 1987], it was 180.48 days (SD 66.2) in our male nicotine-treated rats versus an average life span of 373.98 days (SD 220.0) in 110 male controls. This represents a highly significant loss of life span in both sexes of nicotine-treated Fisher rats ($p < 0.001$). However, t values for the differences versus the normal controls (13.70 for females and 6.94 for males) show that the life-shortening effect of nicotine is greater in the female than in the male rat. Similarly, when the life span in nicotine-treated males (180.48 days) is compared with that of nicotine-treated females (110.44 days), the difference is highly significant ($p < 0.001$) in favor of the male animals.

Moreover, our sample of nicotine-treated male rats contains 10 animals (8.3%) with an average life span of 571.4 days (SD 301.5). This is significantly longer not only than in normal male controls ($p < 0.001$) but even in the longer-living female controls ($p < 0.05$). Such an almost paradoxical finding is actually not isolated. Wehner et al. [1976] found a significantly longer life span and decreased weight in male hamsters exposed to cigarette smoke than in their untreated controls ($p < 0.01$).

In the literature of experimental gerontology, only two methods have so far been shown to produce a significant prolongation of life span in rodents, viz. caloric restriction [Ross, 1972; Weindruck and Walford, 1982; Eklund and Bradford, 1977; Borkan et al., 1982; Berg, 1960; Berg and Simms, 1960; Riesenfeld, in preparation] and castration [Hamilton, 1948, 1965; Talbert and Hamilton, 1965]. The first explanation could possibly be ruled out for the following reasons: the opinion has frequently been expressed that people who smoke eat less, and that opinion is backed up by a vast body of clinical and experimental information. But the authors who have reviewed that information [Wack and Rodin, 1982; Gruneberg, 1985] have shown that various other factors are involved in the inverse smoking/body weight relationship and that, furthermore, the evidence is conflicting. Thus, it has been shown that food intake in nicotine-treated rats is not reduced significantly [Schechter and Cook, 1976; Classen and Bättig, 1976]. Moreover, if reduced food intake in nicotine-treated rats were a cause of life span prolongation, it would apply to

both sexes alike, which, according to our data, is, however, not the case. Therefore, it would seem more likely that a castration-like effect might be the cause of life prolongation in some of the male nicotine-treated rats. This cause would be in accord with the frequent testicular atrophy, the male-induced delay of prima partum, the more serious impairment of fertility and the more virulent cytological condition in the male rather than in the female nicotine-treated rat. In this connection, a recent study by Mordes et al. [1984] is of interest. These authors have shown that anorexia occurs in male rats bearing transplanted Leydig cell tumors, but that little anorexic effect occurs in female rats. If it can be shown that similar anorexic effects can be produced by the testicular abnormality caused by nicotine treatment, it might be conceivable that reduced caloric intake could be a factor in the life-span-prolonging effects in some male rats and hamsters. However, the evidence at this time is still either conflicting or insufficiently established and therefore needs further study.

Future cytological studies using the results of the present analysis as a model should extend their scope to other, higher species and concentrate on the following questions: (1) what are the sexual differences in the effects of nicotine in a given species; (2) what are the age differences, and (3) what are the differences of reversibility in the two sexes and age groups after nicotine use is discontinued.

It might be that by clearly dividing groups according to sex and age some of the conflicting results of previous studies will be avoided, and by increasing our knowledge of nicotine-induced testicular abnormalities the mechanism through which these affect life span might be better understood.

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