Acta anat. *128:* 45–50 (1987)

0001–5180/87/1281-0045 $ 2.75/0

The Effect of Nicotine and Alcohol on the Fertility and Life Span of Rats

A Cytological Analysis

Alphonse Riesenfeld[a], *Heidi Oliva*[b]

[a] Department of Anthropology, Hunter College of the City University of New York, and Institute of Applied Biology, New York; and
[b] Department of Pathology, Mount Sinai Hospital, New York, N.Y., USA

Key Words. Nicotine · Alcohol · Fertility · Life span · Lymphocytes · Polymorphonuclear leukocytes · Inflammation

Abstract. Inbred Fisher and Buffalo rats were exposed to nicotine and alcohol. Fertility was greatly reduced in both strains with nicotine treatments being much more deleterious than alcohol use. Fisher rats tolerated both toxins better than Buffalo rats. Both strains became 'extinct' after one generation of fetal and postnatal exposure to nicotine, but alcohol-ingesting Fisher rats had 3 or more generations of offspring. The total reproductive period was significantly shortened in both strains under the effect of both toxins, as was the total life span. The causes of the teratological effects of both toxins are inflammatory processes as evidenced by the presence of numerous lymphocytes and/or polymorphonuclear leukocytes. Their presence occurs earlier in nicotine than in alcohol use and earlier in Buffalo than in Fisher rats, but the damage done during nicotine treatment is reversible when the procedure is terminated. Inflammation is not transmitted to the newborn offspring of nicotine- or alcohol-treated mothers, but occurs in neonates during the nursing period or later. There is considerable individual variation in the tolerance to both toxins.

Experimental results and clinical observations show a sufficient number of similarities to justify the use of experimental data as a model for further studies on human subjects.

Introduction

Of all the toxic substances consumed by humans, few have a wider use than nicotine and alcohol. In spite of the non-use of alcohol in some areas of the world for religious reasons, nicotine and alcohol are used in literate and non-literate societies and, among some of the people of the Pacific, for instance, the use of tobacco has spread with extraordinary speed [Riesenfeld, 1951].

It is not surprising therefore that a considerable body of information has been accumulated on the many teratological effects of nicotine and alcohol. What is surprising, however, is the almost complete lack of understanding of the mechanism of the effect of these two substances; and Pytkowicz Streissguth et al. [1980] are entirely correct when they say with regard to alcohol: 'the mechanisms through which the effects are produced remain unclear.' Some histological studies have contributed little to the understanding of that mechanism. This study will show that a cytological analysis is capable of clarifying the mechanism of the effect of nicotine and alcohol.

The growth-depressing effects of these two toxins were dealt with in an earlier morphological study [Riesenfeld, 1985].

Material and Methods

In the present study, a total of 574 Fisher and Buffalo inbred rats were used (72 female Fisher, 41 female Buffalo rats in the nicotine experiment; 37 female and 29 male Fisher rats and 39 female and 26 male Buffalo rats in the alcohol experiment, 126 Fisher and Buffalo normal female and 204 normal Fisher and Buffalo male controls).

The reason for this choice of rats is not only the fact that these two strains possess a maximum of homozygosity but also that they differ in

body weight and size [Riesenfeld, 1976], two facts that could have a bearing on possible differences in their tolerance to the toxins involved.

In the nicotine experiment, the sexes were kept together, but only females were injected (0.42 mg/kg). In the alcohol experiment, both sexes were kept together and both were given 20% of alcohol in their drinking water. Both treatments were started at 50 days of age. The reason for beginning both experiments before the onset of puberty was to determine whether the age of the nicotine- or alcohol-treated mother at first parturition differed from that of their normal controls. This obviously, prevented the exclusion of sterile females. However, since the frequency of sterile females is 21% for normal Fisher and Buffalo rats (n = 220, non-littermates), the frequency of infertile experimental rats could be compared with that frequency.

Intramuscular nicotine injections were given for 90 days, 3 times daily with injection sites being rotated. The nicotine treatment of the mother was interrupted during the nursing period for 30 days, but the alcohol treatment was never interrupted, and offspring of alcohol-consuming mothers were given alcohol from birth to death.

Peritoneal fluid for cytological analysis was taken by aspiration in all experimental animals at autopsy, but in a few cases, analysis was not possible due to autolysis.

Results

A few earlier studies, in which nicotine was injected into rats [Hoffstätter, 1923; Staemmler, 1936, 1937; Lombard, 1939; Thienes et al., 1946], have shown that the fertility of animals, the number of litters and the number of neonates was greatly reduced and the number of abortions or still-born neonates was increased. However, the strain of rats used in these studies was usually not idientified and a cytological analysis for the elucidation of the mechanism of the etiology was not possible, since some of these studies antedate by many years the full establishment of cytology as a science.

In the present study, in which 96 female Fisher rats and 46 female Buffalo rats were injected with nicotine, 48 or 50% of Fisher female and 27 or 49.7% of Buffalo rats were sterile. This represents an increase of about 29% versus the sterility in normal Fisher and Buffalo female controls in which the sterility rate was 21%. The number of neonates born to injected Fisher females was 76, that of injected Buffalo female 21, as against 729.6 in Fisher controls and 388.2 in identical numbers of Buffalo controls. Both, Fisher and Buffalo rats had litters after termination of their 3-month-long nicotine treatment. These results are basically in agreement with those of previous investigators, albeit more severe. This is probably due to the more rigorous experimental conditions in our experiments as compared to those of previous investigators.

When Fisher and Buffalo rats are compared with each other, it appears that Fisher rats tolerate nicotine treatment better than Buffalo rats as far as the effect on their fertility is concerned. Offspring of mothers that were fetally and postnatally treated with nicotine (F1 nicotine, 3 Fisher and 2 Buffalo females), had no offspring at all or their young died shortly after birth. In other words, nicotine-treated Fisher and Buffalo rats of our sample became 'extinct' after one generation.

In our alcohol experiment, in which 32 Fisher and 31 Buffalo females were used, 8 Fisher or 25% were sterile. This is only 4% more than in normal Fisher controls. In the Buffalo sample, 11 or 34.1% were sterile. This is 13.1% more than in normal Buffalo controls. As previously mentioned, sterility was 50% in nicotine-treated Fisher rats and 49.6% in Buffalo rats. Thus, it follows that not only is nicotine poisoning much more deleterious for fertility than alcohol, but also that Fisher rats tolerate alcohol much better than Buffalo rats. This is particularly evident from the fact that our alcohol-consuming Fisher rats had 3 generations of offspring while alcohol-consuming Buffalo rats had only one generation of offspring and that the total number of young for Fisher rats was 439 (290 for F1, 69 for F2 and 80 for F3) while alcohol-consuming Buffalo females had only 1 generation of offspring with a total of 15 young. If it is kept in mind that Fisher rats are smaller and have a lower body weight than Buffalo rats [Riesenfeld, 1976] and that tolerance to toxins and body mass are generally positively correlated, it follows that the greater tolerance to nicotine and alcohol of the smaller Fisher rats must be due to a genetic factor. Such genetic differences have previously been demonstrated for the tolerance to alcohol in different strains of mice [Swanberg et al., 1977] and interspecific differences in nicotine tolerance were reported by Hoffstätter [1923].

A number of experimental studies have reported that prima partum was delayed in rats treated with nicotine during pregnancy [Essenberg et al., 1910; Becker et al., 1968] and a delay in conception was reported also for smoking women [Olsen et al., 1983], but another study [Buncher, 1969] has shown a relationship between cigarette smoking and a shortening of the gestation period. Our data (table I) show that the first parturition in nicotine-treated Fisher rats occurs 2 days earlier than in their controls, but the difference is not significant. However, in nicotine-treated Buffalo rats, it occurred 21 days later than in their controls and this difference is significant ($p < 0.05$). In our alcohol experiment, the first parturition was delayed for F1 in Fisher rats, but the delay was not significant; however for F2 it was significant ($p < 0.01$) and even more so in F3 ($p < 0.001$). However, it should be kept in mind that in our alcohol experiment the teratological

Table I. Effect of nicotine on first and last parturitions

	Sample size	Mother's age at first and last parturitions, days				
		normal rats	nicotine-treated	alcohol-treated F1	alcohol F2	alcohol F3
Fisher						
First parturition	10	86.7 ± 5.2	84.3 ± 8.3 NS	112.3 ± 41.9 NS	99.9 ± 10.0**	118.0 ± 34.9***
Last parturition	10	386.3 ± 70.0	175.0 ± 81.4***	230.3 ± 23.3***	150.3 ± 41.0***	UK
Buffalo						
First parturition	10	84.5 ± 2.7	105.4 ± 25.6*	102.1 ± 23.3**	–	–
Last parturition	10	464.8 ± 104.1	174.8 ± 33.8***	248.7 ± 91.4***	–	–

Results are means ± SD. For statistical evaluation the Student t test was used. NS = Not significant; UK = unknown (females still fertile at end of experiment); – = no second-generation offspring.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. controls.

effects of alcohol were compounded, since males and females ingested alcohol and the severe histological and atrophic effects on the testes of alcohol-consuming rats have been demonstrated by Mankes et al. [1982]. For alcohol-consuming Buffalo rats, the first-parturition delay was significant in the first generation ($p < 0.01$) and there was no second generation. In other words, alcohol-consuming Buffalo rats became 'extinct' after the first generation and Fisher alcohol-consuming rats after the 3rd or possibly 4th generation. However, our experiment was terminated after the 3rd generation of alcohol-consuming Fisher rats with some of the experimental F3 rats surviving.

In order to fully appreciate the teratological effects of nicotine and alcohol, the life span (in days) of our two experimental groups was determined and compared with their normal controls. It appears from our data (table II) that the lifespan of both strains and sexes is shortened under the effect of nicotine and alcohol and that the results are highly significant in all cases. Moreover, it appears that alcohol is less deleterious than nicotine, results which are in accord with the growth-depressing effects of the two toxins as reported in a previous study [Riesenfeld, 1985].

Pathological studies on the cause of the teratological effects of nicotine and alcohol have so far dealt only with the histology of the ovaries and testes of experimental animals. Nakazawa [1931] found the ovaries of nicotine-injected rats to be atrophic. Unbehaun [1931] found increased follicular atresia, increased connective tissue, but no tumors. Staemmler [1936, 1937] found the ovaries of injected rats normal and the testes atrophic. Sodano [1934] found the ovaries of nicotine-injected rats normal, but the tubes and uteri showing inflammatory changes. However, his sample consisted of 4 rats only. Our own histological analysis (analysis by Dr. J.A. Daino, Dept. of Pathology, Deepdale General Hospital, Little Neck, N.Y.) of the ovaries of nicotine- and alcohol-treated rats of both strains showed no morphological or pathological changes. As most previous studies antedate of the establishment of cytology, no cytological analysis was possible. Cytological diagnoses of this study were arranged in chronological order.

Table II. Life spans (days) of experimental and control rats

Sex	Sample size, n	Rats		
		normal	nicotine-treated	alcohol-treated
Fisher				
Male	110	374.0 ± 220.0		
	29			153.4 ± 79.8***
Female	66	502.3 ± 218.8		
	73		110.4 ± 76.9***	
	37			163.8 ± 89.5***
Buffalo				
Male	94	412.6 ± 255.8		
	26			158.2 ± 79.9***
Female	60	472.0 ± 252.6		
	39		131.0 ± 83.4***	
	41			146.6 ± 107.9***

Results are means ± SD. For statistical evaluation the Student t test was used. *** $p < 0.001$ vs. controls.

It appears from our data (table III) that the overriding result of all observations in our nicotine and alcohol experiments is the presence of lymphocytes. It is well established that the presence of lymphocytes indicates chronic inflam-

Table III. Cytology of nicotine-treated female rats

Strain	Treatment, days	Lymphocytes	Diagnosis
F	3	few	normal
F	8	few	normal
B	9	numerous	CI
B	10	numerous + PMN	acute I
F	18	few	normal
F	20	moderate	mild I
F	24	moderate	mild I
F	29	moderate	mild I
F	38	moderate	mild I
F	41	few	normal
F	49	few	normal
F	52	few	normal
F	63	few	normal
F	67	few	normal
F	68	few	normal
F	75	few	normal
F	83	few	normal
F	86	numerous	CI
F	88	numerous	CI
F	90	few	normal
F	91	few	normal
F	91	numerous	CI
F	0, neonates, 14 and 38 days old, never injected	very few	normal
F	0, 48 days old, never injected	few	normal
F	29	moderate	mild CI
F	74	moderate	mild CI
Post-nicotine female rats			
F	6, neonates, (20 days old)	few	normal
B	28	few	normal
B	155	few	normal
F	245	few	normal
F	422	few	normal

B = Buffalo strain; CI = chronic inflammation; F = Fisher strain; I = inflammation; PMN = polymorphonuclear leukocytes.

matory processes. While a small number of lymphocytes is normal, a moderate number is indicative of a mild inflammation. Numerous lymphocytes and/or polymorphonuclear leukocytes indicate a chronic inflammation [Koss, 1979]. It appears from our data (table III) that mild inflammation in Fisher nicotine-treated females occurs at 20 days of treatment at the earliest while in Buffalo females numerous lymphocytes (≙ chronic inflammation) occur as early as after 9 and 10 days of treatment. This is in accord with the lesser fertility of Buffalo females as compared with their Fisher counterparts in nicotine poisoning.

The offspring of nicotine-treated mothers, which are not yet themselves treated, are normal (very few lymphocytes), as are the offspring of nicotine-treated mothers born after termination of their mother's treatment. In other words, the inflammatory condition of the pregnant nicotine-treated female is not transmitted to the embryos but appears in the offspring as early as at 29 days (moderate number of lymphocytes) of nursing by a nicotine-treated mother, even though the nicotine treatment of the mother was interruped during the nursing period. Fisher females, in which nicotine treatment was terminated after 90 days, are normal (few lymphocytes) as early as 6 days after termination of the treatment and in Buffalo females even as early as 28 days after termination of the nicotine treatment, and the damage is reversible. This is in accord with the reversibility of testicular damage in the human male after termination of nicotine poisoning as reported by Phillips [1943]. However, there are considerable individual variations as indicated for instance by some Fisher females with 20, 24 and 29 days of nicotine treatment with a moderate number of lymphocytes (mild inflammation) and even PMNs while others are still normal (few lymphocytes) even after 90 days of treatment.

In alcohol ingestion, inflammatory processes indicated by the presence of lymphocytes occur also (table IV), but generally much later than in nicotine treatment, thus, again, testifying to the more serious teratological effect of nicotine over alcohol. Again, neonates (14 days old) of alcohol-ingesting Fisher mothers were normal. The earliest cases of acute inflammation (numerous lymphocytes and PMNs) occurred in a 3rd generation of alcohol-exposed Fisher male at 17 days of age, at 89 days of alcohol ingestion in an adult Buffalo male and at 150 days in a Fisher male, while another Fisher male was still normal at 310 days of alcohol ingestion. This testifies again to the wide range of individual differences in alcohol tolerance.

The earliest appearance of mild chronic inflammation in the Fisher females of our sample occurred at 373 days of alcohol ingestion (moderate number of lymphocytes) and in Buffalo females as early as 91 days, while some Buffalo females were still normal at 475 days of alcohol consumption. But in a Fisher female with 492 days of alcohol ingestion, chronic inflammation (numerous lymphocytes) was found.

A number of similarities in clinical observations and experimental results on the deleterious effects of nicotine and alcohol are known. A few of these resemblances are:

Table IV. Cytology of male and female rats consuming alcohol

Strain	Sex	Treatment, days	Lymphocytes	Diagnosis
F	female	30	few	normal
F	female	51	few	normal
F	female	70	few	normal
B	male	77	few	normal
B	male	89	numerous + PMN	acute I
B	male	90	few	normal
B	female	90	few	normal
B	female	91	few	normal
B	female	91	numerous	CI
B	female	106	few	normal
B	female	111	few	normal
F	female	146	few	normal
B	female	150	few	normal
F	male	150	numerous	normal
B	male	158	few	normal
F	male	168	few	normal
F	female	169	few	normal
F	female	180	few	normal
F	female	230	few	normal
F	male	247	few	normal
F	male	310	few	normal
B	female	347	numerous + PMN	acute I
F	female	373	moderate	mild CI
F	female	423	few	normal
B	female	475	few	normal
F	female	492	numerous	CI
First generation				
B	male	85	few	normal
F	male	239	few	normal
F	male	247	numerous	CI
F	male	274	few	normal
F	female	303	few	normal
Second generation				
F	male	171	few PMN	normal
F	male	176	few	normal
F	female	180	few	normal
F	female	180	few	normal
F	male	207	few	normal
F	male	224	moderate	mild CI
Third generation				
F	male & female neonates		few	normal
F	male	17	numerous	acute I
F	male	25	few	normal
F	male	33	few	normal
F	female	36	few	normal

B = Buffalo strain; CI = chronic inflammation; F = Fisher strain; I = inflammation; PMN = polymorphonuclear leukocytes.

spontaneous abortion in smoking women and experimental animals [Kline et al., 1977], premature birth or delay of conception in nicotine use [Buncher, 1969; Simpson, 1957], changes in the menstrual cycle in women working in tobacco factories [Unbehaun, 1931], lower birth weight [Abel, 1980; Riesenfeld, 1985], testicular atrophy in experimental animals and sterility in cigarette-smoking or alcohol-drinking men and the reversibility of the damages. The many similarities in teratological effects of alcohol in humans and laboratory animals were summarized by Pytkowicz Streissguth et al. [1980].

If there still remained any doubt about the extraordinary accord between clinical and experimental observations, that doubt was removed by a recent clinical study by Baird and Wilcox [1985]. In young women the authors found reduced fertility associated with cigarette smoking, a delay in conception and reversibility of the damage after cigarette smoking was stopped, in other words, the same effects as observed in our experimental animals. However, the authors still must admit that 'the mechanism by which smoking impaired a woman's reproductive capacity had not been determined.'

It seems that the reason why all these studies failed to identify the mechanism of the teratological effects of the toxins involved, is the fact that the investigators confined themselves to merely establishing statistical correlations of cause and effect. However, in view of the many similarities of the experimental and clinical data, it is hoped that the present cytological study will serve as a model for further investigations on human subjects.

References

Abel, E.L.: Smoking during pregnancy: a review of effects on growth and development of offspring. Hum. Biol. *52:* 592–626 (1980).

Baird, D.D.; Wilcox, A.J.: Cigarette smoking associated with delayed conception. J. Am. med. Ass. *253:* 2979–2983 (1985).

Becker, R.F.; King, J.E.; Little, C.R.D.: Experimental studies on nicotine absorption during pregnancy Am. J. Obstet. Gynec. *103:* 942–946 (1968).

Buncher, C.R.: Cigarette smoking and duration of pregnancy. Am. J. Obst. Gynec. *104:* 942–946 (1969).

Essenberg, J.M.; Schwind, J.V.; Patras, A.R.: The effect of nicotine and cigarette smoke on female albino rats and their offspring. J. Lab. clin. Med. *25:* 708–717 (1910).

Hoffstätter, R.: Experimentelle Studie über die Einwirkung des Nicotins auf die Keimdrüsen und auf die Fortpflanzung. Arch. pathol. Anat. *244:* 183–213 (1923).

Kline, J.; Stein, L.A.; Mervyn Susser, B.C.; Warburton, D.: Smoking: a risk factor for spontaneous abortion. New Engl. J. Med. *297:* 793–795 (1977).

Koss, L.G.: Diagnostic cytology, vol. 1, 2; 3rd ed. J.B. Lippincott, Philadelphia 1979).

Lombard, D.F.: The effect of chronic nicotine poisoning on the growth and reproduction of albino rats. Thesis University of Southern California, Los Angeles (1939).

Mankes, R.F.; LeFevre, K.F.; Benitz, I.; Rosenblum, I.; Bates, H.; Walker, A.I.T.; Abraham, R.: Paternal effects of ethanol in the Long Evans rat. Toxicol. envir. Hlth *10:* 871–878 (1922).

Nakazawa, R.: Der Einfluss der chronischen Nicotinvergiftung auf die Funktion der Geschlechtsorgane der weiblichen Ratten. Jap J. med. Sci. Pharmacol. *7:* 1–7 (1931).

Olsen, J.; Rachootin, P.; Schiodt, A.V.: Alcohol use conception time and birth weight. Epidemiol. Commun. Hlth *37:* 63–65 (1983).

Phillips, L.G.: Cigarette smoking a factor in sterility. Hawaii med. J. *2:* 249 (1943).

Pytkowicz Streissguth, A.; Landsman-Dwyer, S.; Martin, J.C.; Smith, D.W.: Teratogenic effects of alcohol in humans and laboratory animals. Science *209:* 353–361 (1980).

Riesenfeld, A.: Tobacco in New Guinea and the other areas of Melanesia. R. anthrop. Inst. *81:* 69–102 (1951).

Riesenfeld, A.: Constitution and body proportions in different strains of rats: a study of race formation in breeding isolates. Acta anat. *94:* 159–183 (1976).

Riesenfeld, A.: Growth depressing effects of alcohol and nicotine in two strains of rats. Acta anat. *122:* 18–24 (1985).

Simpson, W.J.: A preliminary report on cigarette smoking and the incidence of prematurity. Am. J. Obstet. Gynec. *73:* 808–815 (1957).

Sodano, A.: Ricerche sperimentali sull'influenza della nicotina sulla funzione genitale della donna. Archs Ostet. Ginec. *41:* 559–569 (1934).

Staemmler, M.: Nikotin und Keimdrüsen. Münch. med. Wschr. *83:* 658–659 (1936) and Chem. Abstr. *31:* 8016 (1937).

Swanberg, K.M.; Crumpacker, D.W.: Genetic differences in reproductive fitness and offspring viability in mice exposed to alcohol during gestation. (Abstract 6258). Behav. Biol. *20:* 122–127 (1977).

Thienes, C.H.; Lombard, C.F.; Fielding, F.J.; Lesser, A.J.; Ellenhorn, M.J.: Alterations in reproductive functions in white rats associated with daily exposure to nicotine. J. Pharmac. exp. Ther. *87:* 1–10 (1946).

Unbehaun, G.: Untersuchungen über die Einwirkung des Nicotins auf das Ovarium der weissen Maus. Arch. Gynaek. *147:* 371–383 (1931).

Received: Nov. 29, 1985
Accepted: May 21, 1986

Dr. Alphonse Riesenfeld,
Department of Anthropology,
Hunter College of the City University of New York,
and Institute of Applied Biology,
New York, NY (USA)