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Age and dose-dependent carcinogenic effects of *N*-nitrosomethylurea administered intraperitoneally in a single dose to young and adult female mice

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Abstract. Female Swiss-derived SHR mice aged 3 ("young") and 12 months ("adult") were exposed to a single i.p. administration of *N*-nitrosomethylurea (NMU) at one of four doses: 0, 10, 20, or 50 mg/kg. The mean survival time of the young mice so treated was 440, 325, 398 and 182 days, and of the adult mice 221, 249, 191, and 168 days respectively. The incidence of all kinds of tumours in young mice was 40%, 64%, 77%, and 40%, of malignant tumours 33%, 43%, 57% and 20% of lung adenomas 7%, 14%, 40%, and 20% and of papillomas of the forestomach 0%, 14%, 23%, and 3% respectively. In adult mice these figures were for all kinds of tumours 52%, 52%, 56%, and 50%, for malignant tumours 44%, 52%, 52%, and 40%, for lung adenomas 7%, 0%, 8%, and 20%, for papillomas of the forestomach 0%, 0%, 12%, and 0% respectively. The exposure of adult female mice to various doses of NMU did not significantly increase the incidence of tumours or of malignant tumour incidence in comparison to age-matched controls. At the same time the latency of fatal tumours was shorter in the adult groups than in the young groups. Histoautoradiography of tissues of intact young and adult mice showed that there are no statistically significant age-related differences in the labelling index of forestomach epithelium, endometrium and lung alveolar wall epithelium. Only the labelling index of hepatocytes was decreased in the liver of adult mice in comparison to young ones. Comparison of the present experimental results with the data available on DNA synthesis and on O^6-methylguanine repair in target tissues suggests a requirement for individual monitoring of age-related changes of biomarkers in exposure to carcinogenic agents. The analysis of data on the dose dependence of the carcinogenic effect of NMU against the background of a multistage model suggests an age-related accumulation of stochastically damaged cells for some tissues.

Key words: *N*-nitrosomethylurea – Carcinogenesis – Aging – Mice

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Introduction

It has been assumed that DNA damage is likely to be critical for both cancer and aging (Ames 1989; Cutler 1991), yet at present there is no consensus on the causes of the age-related increase of tumour incidence in either humans or animals. Some researchers believe that this phenomenon results only from accumulation of cell damage induced by carcinogenic agents and/or the increase of the time of exposure to them, and assume that susceptibility to carcinogens remains unchanged with time (Peto et al. 1975, 1985; Stenback et al. 1981). Others consider that it is age-related alterations in the organism itself that promote tumour development (Burnet 1976; Dilman 1985). The results of available epidemiological observations and of numerous experiments, in which animals were exposed to various carcinogens, are rather controversial and do not provide conclusive support for any one view of the cause of any age-related increase in cancer incidence in either humans or animals (Ames 1989; Anisimov 1987, 1989; Zimmerman and Carter 1989; Cutler 1991; Richie and Williams 1991). Analysis of the possible background of these contradictions (Anisimov 1987, 1988) suggests that the problem may be solved by choosing animals of adequate age at the onset of experiments. A major problem, in fact, arises when the tumour latency exceeds the survival time of animals exposed to a carcinogen in old age; this would lead to the mistaken conclusion that older animals are insensitive to the carcinogen. Another problem arises if a carcinogen is given at a dose that induces tumours in the majority of animals: in this case possible age-related differences in susceptibility to the carcinogen may remain obscure. Finally, it is a problem selecting an appropriate statistical treatment of results of experiments where animals are exposed to carcinogens at different ages, when the observed patterns of survival differ from those expected. In a previous study on the dose/response relationship in carcinogenesis induced by a single intravenous administration of *N*-nitrosomethylurea (NMU) to female rats, the effect of age was evaluated and, by designing the experiment with the above factors in mind, such biases in the interpretation of the results were avoided (Anisimov 1988). At the same time it was shown that the sus-

ceptibility of different target tissues to carcinogens, including *N*-nitroso compounds, may depend on the species and, in some cases, on differences in pharmacokinetics and pharmacodynamics according to the route of administration of the carcinogen (IARC 1978; Anisimov 1987; Bartsch 1991). In the present work we have studied the effect of age on susceptibility to carcinogenesis, using: (a) young (3-month-old) and adult (12-month-old) mice, i.e. with a life expectancy sufficient for tumour development; (2) three doses of a directly acting carcinogen (NMU) given intraperitoneally; and (c) statistical tests for a positive trend combined with tests for fatal and incidental tumours, as recommended by IARC for comparison of groups with different survival (Peto's test) (Gart et al. 1986). The results of these experiments, which use a different species and route of administration but the same agent, confirm our conclusions on the correctness in principle of the experimental and statistical approach designed to analyse the problem of age-related susceptibility to carcinogens.

Materials and methods

Animals. Outbred female Swiss-derived SHR mice were purchased from the Rappolovo Animal Farm of the Russian Academy of Medical Sciences at the age of 1.5 months. Animals were kept in polypropylene cages, eight to ten in each, received standard laboratory chow and were allowed to drink tap water ad libitum.

Chemicals. NMU was synthesized at the Laboratory of Organic Chemistry of the N.N. Petrov Research Institute of Oncology. The chemical purity of NMU was 99% as judged by the colour reaction with Griss reagent (Korenman 1970). It was stored at –18° C.

Experiment. NMU was dissolved in standard saline citrate buffer (15 mM sodium citrate, 0.15 *M* NaCl, pH 6.0) and injected intraperitoneally (i.p.) into mice at the age of 3 months ("young" group) or 12 months ("adult" group) at doses of 10, 20 or 50 mg/kg body weight. All animals were injected within 1 h after preparation of the NMU solution. According to available data (IARC 1978) the half-life of NMU in aqueous solutions at pH 6.0 is 24 h. Some of the young or adult animals were exposed to solvent and served as controls (dose=0). Animals were under observation until their natural death or, in some cases, were sacrificed by ether vapour when moribund; both the survival time (from the day of NMU injection) and chronological age at death (from birth) were estimated for every animal.

Pathohistological examination. An autopsy was carried out on all animals that died or were killed. At the autopsy all tumours were evaluated as "fatal" or "incidental" according to IARC recommendations (Gart et al. 1986). The numbers of lung adenomas on the surface of each lobe of the lung were counted as well as the number of papillomas in the forestomach. The liver, kidney, spleen and all macroscopically abnormal organs were fixed in 10% neutral formalin. Routine histological treatment of 5- to 7 µm-thick sections was followed by staining with haematoxylin and eosin with subsequent microscopic examinations. The tumours were classified according to the IARC classification (Turusov 1979), and their numbers per target organ and per tumour-bearing mouse were registered.

Histoautoradiography study. [^{3}H]Thymidine, from v/o Isotop, St. Petersburg, specific activity 10 Ci/m*M*, was injected at 9.00 a.m. i.p., in a single dose of 1 µCi/g body weight, into six female mice aged 3 months and six females aged 12 months. Within 1 h of the injection animals were killed by ether vapour and the forestomach, uterus, liver and lungs were fixed in an alcohol/formalin/glacial acetic acid mixture (6 : 3 : 1) and embedded in paraffin/celloidine. Sections of 5 µm were prepared and covered with a liquid M photoemulsion (Gosniichimphotoproekt, Moscow, Russia), exposed for 4 weeks at 4° C and developed in amidol; the exposure time being the same for every section. Autoradiographs were stained with Meyer's haematoxylin and eosin. Labelled nuclei per 1000 interphase cells were counted only in the upper plane of the autoradiographs. A cell was considered labelled when there were at least three silver grains over its nucleus.

Table 1. Survival of young and adult female SHR mice exposed to single doses of *N*-nitrosomethylurea (NMU)

Age when given NMU (months)	NMU dose (mg/kg)	No. mice at start	Survival after NMU±SEM [a] (days)	Survival of mice without fatal tumour	Survival of tumour-bearing mice ±SEM [a] (days)			
					Total tumours	Malignant tumours	Mammary adenocarcinomas	Leukaemias
3	0	30	440±19 (530) [b]	415±30 (505)	493±28 (587)	491±27 (581)	490±29 (580)	493±75 (580)
	10	34	325±29 [*1] (415)	327±40 (417)	401±45 (491)	369±48 [*1] (459)	300±39 [*3] (390)	392±75 (482)
	20	32	398±27 (488)	369±44 (459)	436±23 (529)	435±31 (525)	400±60 (490)	464±87 (554)
	50	41	182±22 [*2] (272)	147±26 [*3] (459)	311±43 [*2] (401)	291±57 [*2] (381)	264±127 (397)	344±67 (434)
12	0	57	221±17 [*6] (586)	220±24 (585)	229±18 (594)	220±19 (585)	195±23 (560)	219±30 (584)
	10	23	249±23 (614)	222±32 (587)	224±27 [*6] (589)	224±27 [*5] (589)	210±46 (584)	159±54 [*5] (524)
	20	25	191±28 [*7] (556)	135±22 [*5] (500)	252±43 [*7] (617)	262±46 [*4] (627)	327±87 (692)	227±41 [*5] (592)
	50	20	168±21 [*1] (533)	155±29 (520)	189±36 [*6] (554)	189±38 (554)	178±44 (543)	238±104 (603)

[a] Mean ± standard error of mean

[b] Parentheses show the chronological age of animals at death (from birth)

[*1–3] The difference from the age-matched control is significant: [*1] $P<0.05$; [*2] $P<0.01$; [*3] $P<0.001$

[*4–7] The difference from the dose-matched young group is significant: [*4] $P<0.05$; [*5] $P<0.02$; [*6] $P<0.01$; [*7] $P<0.001$

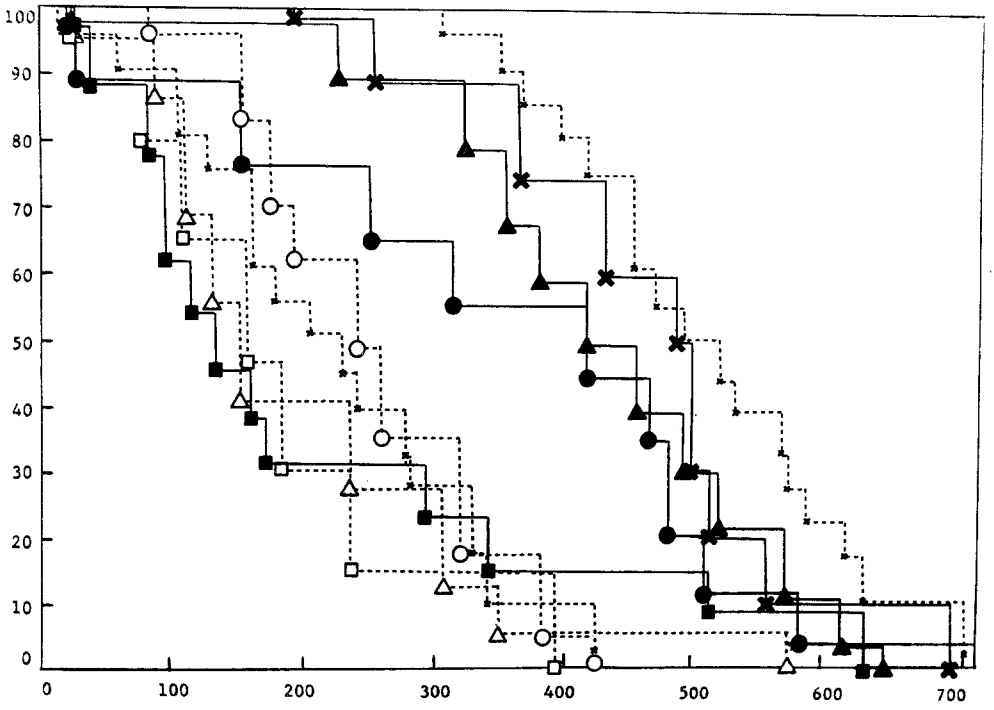

Fig. 1. Survival of young and adult female mice exposed to various dose of *N*-nitrosomethylurea (NMU). *Ordinate:* number of mice (%); *abscissa:* period of observation (days). ×, Young, saline; ●, young, NMU, 10 mg/kg; ▲, young, NMU, 20 mg/kg; ■, young, NMU, 50 mg/kg; ×, adult, saline; *right-hand plot,* from the age of 90 days for comparison with young, saline-treated group; ○, adult, NMU, 10 mg/kg; △, adult, NMU, 20 mg/kg; □, adult, NMU, 50 mg/kg. Symbols represent every third animal (Kaplan-Mayer analysis)

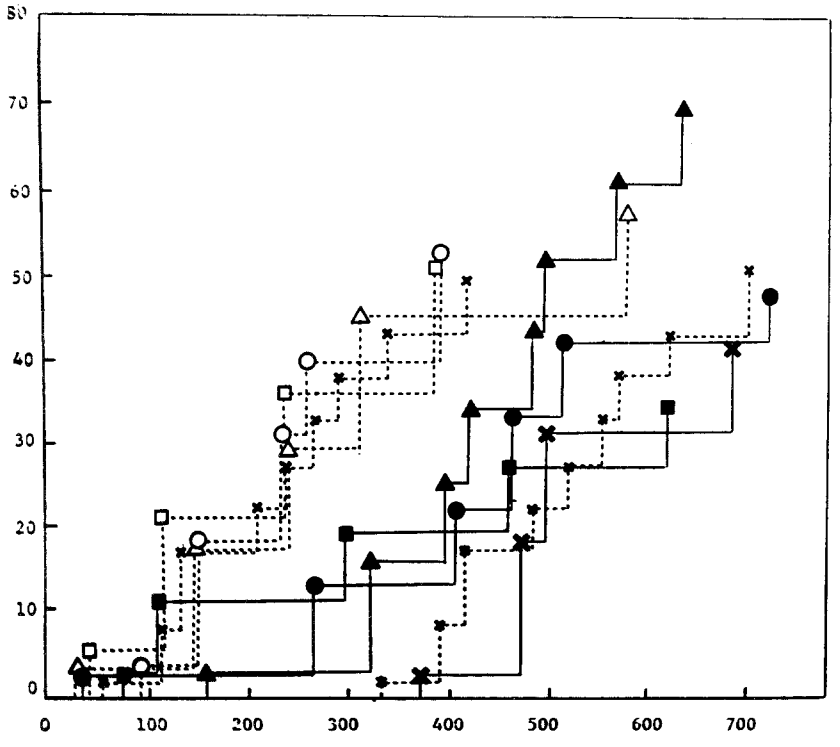

Fig. 2. Total tumour yield in young and adult female mice exposed to various dose of *N*-nitrosomethylurea (NMU). *Ordinate:* number of tumour-bearing mice (%); *abscissa:* period observations (days). Symbols are identified in legend to Fig. 1, and represent every third tumour-bearing animal (Kaplan-Mayer analysis)

Statistics. The results were statistically processed according to IARC recommendations for groups of animals with different survival (Gart et al. 1986). The statistical significance of any apparent increase of the effect with NMU dose was assessed by combining the tests for positive trend for fatal and incidental tumours (Gart et al. 1986). Otherwise Student's *t*-test, the χ^2 and Fischer's exact tests for equality of proportions were used as appropriate (Gubler 1978). The CARTEST program (Gart et al. 1986) and a Hewlett-Packard computer were employed for statistical processing of the data.

Results

Comparison of the life-span parameters for intact mice taken for experimentation at the age of 3 or 12 months showed that survival curves for all animals or for those bearing any or specific tumours as well as the mean age of death of control mice were not significantly different for the two age groups (Table 1; Figs. 1, 2). The mean age at death (chronological

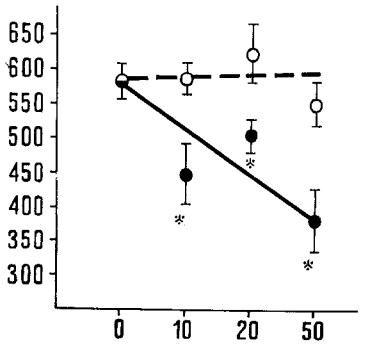

Fig. 3. Relationship between survival of mice of various ages bearing fatal tumours and dose of *N*-nitrosomethylurea (NMU). *Ordinate:* age at death (days); *abscissa:* dose of NMU, mg/kg (logarithmic scale). Data presented as means ± SEM. * The difference from the control group (dose 0) and from the corresponding value for young mice is significant, $P<0.05$

age) of mice that died with any kind of tumour, with malignant tumours with mammary tumours or died without any fatal tumour in control young and adult groups was similar (Table 1).

Administration of NMU at a dose of 10 mg/kg to female mice aged 3 months was followed by a significant decrease in survival of all animals in the group, as well as by a decrease in the mean life span of mice with malignant tumours or only mammary adenocarcinomas. The same dose of carcinogen administered to mice 12 months old did not influence any parameter investigated. Mice of both age groups exposed to NMU at a dose of 20 mg/kg had the same survival rates as intact controls for any pair of parameters compared. Treatment with the highest dose of NMU at the age of 3 months was followed by a significant decrease in survival of all exposed mice, of mice with any kind of tumour or mice with malignant tumours, as well as of mice that died without any fatal tumour. The mean survival of all mice exposed to the carcinogen at the maximum dose at the age of 1 year was significantly reduced in comparison to the intact age-matched controls while other parameters of survival investigated in this group were not changed significantly (Table 1; Figs. 1, 2).

In general, comparison of survival parameters between mice treated with the carcinogen at different ages showed that for equal doses of NMU a greater reduction of the mean survival time was observed in the young group than that in the adult group. The same phenomenon was registered in relation to the mean survival of any tumour-bearing mice and to some with specific tumours (Table 1) as well as to those bearing fatal tumours (Fig. 3).

It is worth noting that the mean survival time of mice that died without any fatal tumour in the young group was reduced significantly only by the maximal dose of NMU, while the chronological age at death did not change significantly in adult groups exposed to the carcinogen at various doses.

Data on the incidence, localization and morphological type of tumours discovered in both age groups are given in Tables 2 and 3. There are no significant differences in the incidence of all tumours or of only malignant tumours between

Table 2. Tumour incidence in young and adult female SHR mice exposed to single doses of *N*-nitrosomethylurea (NMU)

Age when given NMU (months)	NMU dose (mg/kg)	Effective number of mice	No. tumour-bearing mice		No. tumours		No. tumour-bearing mice					
							Mammary adeno-carcinoma	Leukaemia	Lung		Uterus:	Forestomach:
			Total	With malignant tumours	Total	Malignant			Adenoma	Adeno-carcinoma	adeno-carcinoma	papilloma
3	0	30	12 40%	10 33%	15	10	8 27%	2 7%	2 7%	–	–	–
	10	28	18*1 64%	12 43%	28	14	4 14%	3 11%	4 14%	3*1 11%	1 4%	4*2 14%
	20	30	23*2,4 77%	17*1,3 57%	97	24	7 23%	3 10%	12*4 40%	7*2 23%	4*2 13%	7*4 23%
	50	35	14*2,3,9 40%	7 20%	43	11	3 9%	4*1,10 11%	7 20%	2 6%	–	1 3%
12	0	54	28 52%	24 44%	44	33	12 22%	10 19%	4 7%	1 2%	5 9%	–
	10	23	12*6 52%	12*6 52%	16	15	5*7 22%	3 13%	–	2 9%	2 9%	–
	20	25	14*5 56%	13*5 52%	29	18	5*6 20%	4*4 16%	2*8 8%	2 8%	4 16%	3 12%
	50	20	10*7 50%	8*7 40%	34	11	3 15%	3 15%	4 20%	–	–	–

*1-4 Difference from control of the same age is significant: *1 $P<0.05$; *2 $P<0.01$ (Peto's test); *3 $P<0.05$; *4 $P<0.01$ (Fischer's exact test)
*5-8 Difference from the corresponding index in young mice in the matched test: *5 $P<0.001$; *6 $P<0.01$; *7 $P<0.05$ (Peto's test); *8 $P<0.01$ (Fischer's exact test)
*9,*10 Test on positive trend with dose: *9 $P<0.005$; *10 $P<0.05$ (Peto's test)

Table 3. Localization, type and number of tumours induced by *N*-nitrosomethylurea (NMU) in young and adult female SHR mice

Site	Type	Number of tumours							
		Young mice				Adult mice			
		0[a]	10[a]	20[a]	50[a]	0[a]	10[a]	20[a]	50[a]
Mammary gland	Adenocarcinoma	8	6	8	3	13	5	6	6
Haematopoetic tissue	Myeloleukaemia	1	–	2	3	6	2	1	1
	Lympholeukaemia	1	3	1	1	4	1	3	2
	Reticulosarcoma	–	–	–	–	–	1	–	–
Ovary	Granulosatheca-cell tumour	–	–	2	1	–	–	–	–
	Haemangioma	–	1	1	1	1	1	–	–
Uterus	Polyp	–	–	–	–	–	–	1	–
	Adenocarcinoma	–	1	4	–	5	2	4	–
Lung	Adenoma	2	5	58	27	10	–	3	22
	Adenocarcinoma	–	3	9	3	2	4	3	–
Adrenal gland	Pheochromocytoma	–	–	–	1	–	–	–	–
Skin	Squamous-cell carcinoma	–	1	–	–	1	–	–	2
Liver	Angioma	–	1	–	–	–	–	–	–
Forestomach	Papilloma	–	6	9	1	–	–	5	–
	Carcinoma	–	–	–	–	–	–	1	–
Kidney	Adenoma	–	–	3	–	–	–	–	–
Bladder	Adenocarcinoma	–	–	–	1	–	–	–	–
Vessels	Angioma	3	1	–	1	–	–	2	1
	Angiosarcoma	–	–	–	–	2	–	–	–
Subtotal	Benign	5	14	73	32	11	1	11	23
	Malignant	10	14	24	11	33	15	18	11
Total		15	28	97	43	44	16	29	34

[a] NMU dose (mg/kg)

control groups taken for injection the age of 3 or 12 months. However, in the adult group more spontaneous leukaemias and uterine adenocarcinomas were observed than in the young group.

A single i.p. administration of NMU at the age of 3 months was followed by development of lung adenomas and adenocarcinomas, ovarian tumours, adenocarcinomas of the uterus and forestomach papillomas. In mice treated with the carcinogen at the age of 1 year, only lung adenomas and papillomas of the forestomach could be said to be induced. Thus, exposure to NMU at a dose of 10 mg/kg was followed by a significant increase in the incidence of total tumours, lung adenomas and papillomas of the forestomach in young mice in comparison with the age-matched controls, but no carcinogenic effect of the same dose of carcinogen was detected in the adult group. As compared to the control group, the administration of 20 mg/kg NMU to young mice was followed by a significant increase in incidence of all tumours, of malignant tumours, of lung adenomas and adenocarcinomas, and of tumours of the uterus and forestomach. In adult mice exposed to the same dose of NMU only tumours of the forestomach were attributable to NMU induction. In young mice exposed to 50 mg/kg NMU the increase in incidence of all tumours, of lung adenomas and adenocarcinomas, and of leukaemias was estimated as well (Table 2).

Statistical treatment failed to reveal significant differences between tumour incidences in mice exposed to any dose of NMU at 1 year old and in the related control. At the same time, the number of lung adenomas per lung-adenoma-bearing mouse treated with the maximum dose of the carcinogen in the adult group was twice that in the controls (5.5 and 2.5 respectively). The number of lung adenomas per lung-adenoma-bearing mouse in mice exposed at 3 months to 0, 10, 20 or 50 mg/kg NMU was 1.00, 1.25, 4.83 and 3.86 respectively, and in mice exposed to the carcinogen at the age of 12 months it was 2.50, 0, 1.50 and 5.50 respectively. There was no dose-related increase in multiplicity of forestomach papillomas in either age group. Thus, the number of these tumours per tumour-bearing mouse in the young group treated with 0, 10, 20 or 50 mg/kg NMU was 0, 1.50, 1.29, and 1.00, and in the adult group 0, 0, 1.67, and 0 respectively.

It is worth noting that the application of Peto's method, recommended by the IARC for comparison of animal groups with different patterns of survival (Gart et al. 1986), helps to show the significance of differences in carcinogenic effect of NMU between the groups matched for dose level. Thus, older mice exposed to NMU at a dose of 10 mg/kg developed more tumours of all kinds, malignant tumours or mammary adenocarcinomas than did the young mice. The incidence of any kinds of tumours, of malignant tumours, of leukaemias

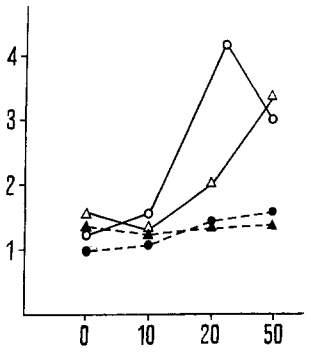

Fig. 4. Relationship between dose of *N*-nitrosomethylurea (NMU) and multiplicity of tumours in young and adult mice. *Ordinate:* mean number of tumours per tumour-bearing mouse; *abscissa:* dose of NMU, mg/kg (logarithmic scale). ○, Young, all tumours; ●, young, malignant tumours; △, adult, all tumours; ▲, adult, malignant tumours

Table 4. Labelling index in some tissues of 3- and 12-month-old female SHR mice

Tissue	Labelling index (%)	
	3 months old	12 months old
Forestomach epithelium	11.4±0.44	10.4±0.83
Endometrium	5.4±2.65	8.6±0.48
Liver hepatocytes	13.4±1.97	5.2±1.17 [a]
Lung alveolar wall epithelium	5.5±1.56	3.2±0.59

[a] The difference compared to 3-month-old mice is significant, $P<0.05$

and of mammary adenocarcinomas was significantly increased in the adult group exposed to 20 mg/kg NMU compared to that in the young group. Finally, at a dose of 50 mg/kg, older mice responded to NMU with increasing incidence of all kinds of tumours or of malignant tumours in comparison to dose-matched young mice.

A significant positive relationship between dose and tumour incidence for all tumours, leukaemias and lung adenocarcinomas was found for the young group, but we failed to find a similarly significant relationship in the adult group (Table 2). The number of tumours of all kinds or of malignant tumours per tumour-bearing mouse was increased in proportion to NMU dose in young mice, while this increase was seen only in relation to the total number of tumours in adult mice (Fig. 4).

The histoautoradiographical study of the tissues of mice not exposed to the carcinogen at the aged 3 and 12 months failed to show a significant difference in the labelling index of the forestomach epithelium, endometrium and lung alveolar wall epithelium. However, a clear tendency to an age-related decrease in labelling index of lung epithelium was observed. The labelling index of hepatocytes was decreased in adult mice in comparison to young ones (Table 4).

Discussion

The carcinogenic potential of *N*-nitroso compounds is well known (IARC 1978; Bartsch 1991). A single i.p. administration of NMU to female 6- to 10-week-old mice of various strains induces a wide spectrum of tumours, mainly lymphomas, lung adenomas, and tumours of the kidney, uterus and forestomach in proportion to dose (Frei 1970; Joshi and Frei 1970; Terracini et al. 1976). Our data on the carcinogenicity of various doses of NMU in 3-month-old mice are in accordance with these observations. At the same time, it was shown that exposure of mice to various doses of NMU at the age of 12 months did not significantly change the incidence of all tumours, of malignant tumours or of tumours of any particular localization, in comparison with age-matched controls. Does this mean that NMU administered to 1-year-old female mice failed to induce any tumours? We believe that the following data may be attributed to a carcinogenic effect of NMU in adult mice: (a) the development of forestomach papillomas in adult mice exposed to 20 mg/kg NMU; (b) the increase in number of lung adenomas per lung-adenoma-bearing mouse in adult mice exposed to 50 mg/kg NMU (Tables 2 and 3); (c) the dose-related increase in total tumour number per tumour-bearing adult mouse in comparison to controls (Fig. 4).

Zimmerman et al. (1982) failed to find any differences in the incidence of tumours induced in 3- and 12-month-old male C57BL/6J mice after exposure to a single i.p. administration of NMU at a dose of 37.5 mg/kg. However, these animals were sacrificed 5 months after carcinogen injection, while in our study mice were under observations until their natural death. It is worth noting that the total tumour incidence in our young and adult SHR mice that died before the 6th month after i.p. administration of various doses of the carcinogen was also similar. According to Lijinsky et al. (1991) there were not great differences in tumorigenic response between mice exposed to NMU by skin painting at 8 weeks of age and those starting treatment a year later. However, in each case, whatever small difference there was lay in the direction of a decreased susceptibility of the older mice compared with the young mice to skin carcinogenesis induced by NMU.

In the mechanism of carcinogenesis by nitroso compounds, particularly NMU, their capacity to alkylate DNA guanine in the O^6 position and thymine in the O^4 position has considerable significance (Saffhill et al. 1983; Lijinsky 1991). The persistence of these "promutagenic" bases in the target-tissue DNA at the moment of its replication may bring about mutation and activation of the transforming genes (Saffhill et al. 1983; Sukumar et al. 1983). Numerous studies have shown that the organotropicity of the carcinogenic effect of nitroso compounds depends on (a) the level of alkylation, (b) the efficacy of DNA repair and (c) the proliferative activity of the target tissue (Likhachev et al. 1983; Saffhill et al. 1983; Lijinsky 1991). NMU is a directly acting carcinogen and does not need metabolic activation. The decrease in susceptibility to an initiating effect of NMU in our 1-year-old mice may depend on at least two of the above-mentioned factors.

First, it may depend on the increase of activity in DNA (O^6-methylguanine)-methyltransferase in target tissues in adult mice in comparison to young mice. Thus, it was shown that in C57BL/6 mice the activity of the liver methyltransferase significantly increases from the age of 5 weeks to the age of 22 weeks, persists at the same level up to the age of 91 weeks and subsequently decreases (Nakatsuru et al. 1989). A

similar pattern of age-related changes in methyltransferase activity was observed in ICR mice. The level of the enzyme increases from the age of 8–9 days for 7–8 weeks and then remains more or less the same in liver, lung, brain and ovaries of 65- to 75-week-old female C3Hf and C57BL/E mice (Washington et al. 1989). The activity of DNA (3-methyladenine)-*N*-glycosylase has a broad specificity toward all three *N*-alkylpurines (3-alkyladenine, 7-alkyladenine and 3-alkylguanine), is increased in liver, lung, brain and ovaries of mice of two strains from the age of 8–9 days to 49–56 days and is significantly decreased later on (Washington et al. 1983). NMU is also an effective inducer of unscheduled DNA synthesis in vivo. Bond and Singh (1987) have shown an age-dependent decrease in NMU-induced unscheduled DNA synthesis in bone marrow of mice. Thus, there is no direct correlation between age-related changes in the activity of systems involved in repair of DNA damaged by NMU in various tissues and their susceptibility to the carcinogen.

Secondly, the age-related changes in DNA synthesis rate in some tissues may modify susceptibility to the carcinogen. An age-related slight decrease in DNA snythesis was observed in kidney cells of nephron tubules and mouse oesophagal epithelium basal layer, and an insignificant decrease in lung alveolar wall cells (Thrasher 1971; Simnett and Heppleston 1966; Cameron and Thrasher 1976). We failed to observe any statistically significant age-related decrease in labelling index of forestomach epithelium, lung alveolar wall epithelium and endometrium in one-year-old in comparison to 3-month-old female mice, while DNA synthesis in hepatocytes was significantly decreased (Table 4). Thus, available data on age-related changes in MT and DNA synsthesis activity in target tissues could only in part explain the results of our experiment. There are the wide species, strain and individual variability of these and some another biomarkers of exposure to carcinogens at any age as well as the wide variability in the patterns of their age-related changes (Anisimov 1987; Likhachev et al. 1992; Monakhov et al. 1992). The monitoring of such kinds of the biomarkers in the target tissues of indiviudal animals may be very helpful for interpretation of results of both experimental and epidemiological studies on the age-related susceptibility to carcinogens.

To answer the question of whether NMU-induced carcinogenesis in rats of various ages is a single- or multistage process, we studied the relationship between the logarithm of the number of tumors per animal and the logarithm of the carcinogen dose (Anisimov 1988). The calculations of corresponding regression coefficients showed the age-related decrease of their value. The analysis of these results within the framework of a multistage model of carcinogenesis suggests age-related accumulation, in different tissues, of cells stochastically lesioned and in the "late" stages of becoming malignant (Anisimov 1988). In present experiment the incidence of tumor developed in target tissues of mice exposed to NMU at the age of one year was decreased in comparison to that in mice exposed to the same doses of the carcinogen at the age of 3 months. At the same time the latency of fatal tumors was shorter in older group. This observeration suggests the age-related accumulation of initiated cells in some tissues during natural aging. The results of the experiments on carcinogenesis induced in rodent liver, colon, uterus or skin by chemical carcinogens or tumour promoters (Reuber and Glover 1967; Stenback et al. 1981; Turusov et al. 1981; Ebbesen 1985; Anisimov 1987, 1989; Ward et al. 1988; Kraupp-Grasl et al. 1991), as well as data on the molecular events in senile DNA sufficient for carcinogenesis (Cutler and Semsei 1989; Anisimov 1992) are in line with this conclusion.

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