Br. J. Cancer (1976) 33, 427

THE ROLES OF AGE AT TREATMENT AND DOSE IN CARCINOGENESIS IN C3Hf/Dp MICE WITH A SINGLE ADMINISTRATION OF N-NITROSO-N-METHYLUREA

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Summary.—C3Hf/Dp mice were given a single i.p. injection of 50, 25 or 5 μg/g N-Nitroso-N-Methylurea (NMU) at either 1 or 70 days of age or 50 μg/g at 21 days of age. They were observed until death or until 120 weeks of age.

The two highest doses of NMU produced tumours in a wide spectrum of organs, including the thymus, forestomach, lung, liver (only in males), kidneys, ovaries and orbital glands.

The only two tumour types which appeared to be closely related to the occurrence of death were thymic lymphomata (most of which were found in mice dying before 40 weeks after treatment) and carcinomata of the forestomach. Lifetime analyses are presented concerning the occurrence of these two tumour types as well as the occurrence of any tumour after 40 weeks of age or since treatment.

Incidences of thymic lymphomata were 67·6%, 39·0% and 21·2% in mice receiving 50 μg/g NMU at 1, 21 and 70 days respectively and 17·1% in mice receiving 25 μg/g at 1 day. In the other groups the incidence of thymic lymphomata was zero or negligible. The rate of progression of thymic lymphomata until death was related to both earliness of treatment and dose. On the contrary, incidences and progression of carcinomata of the forestomach were unrelated to age at treatment. Since breakdown of NMU is very rapid and does not require enzymes, these results are considered as evidence that host–tumour interaction differs from organ to organ.

No excess of tumours over the controls was found in mice receiving 5 μg/g either at 1 or 70 days of age.

THE CARCINOGENICITY of N-nitroso-N-methylurea (NMU) in different species of laboratory animals is well established (IARC, 1972). A single administration of this chemical can induce a high yield of tumours at different sites in mice and rats (Druckrey *et al.*, 1964; Graffi and Hoffmann, 1966; Terracini and Stramignoni, 1967; Kelly *et al.*, 1968; Leaver, Swann and Magee, 1969; Frei, 1970; Joshi and Frei, 1970; Terracini and Testa, 1970; Frei, 1971). NMU is also known to undergo rapid decomposition at physiological pH (Druckrey *et al.*, 1967; Swann, 1968). Thus a simple experimental system is provided where some biological aspects of carcinogenesis (incidence, target organs, progression and latent period) can be investigated by changing one parameter of exposure such as age at treatment or dose.

The present study reports a lifetime analysis of the carcinogenicity of a single administration of 5, 25, or 50 μg/g NMU to C3Hf/Dp mice aged 1, 21 or 70 days.

MATERIALS AND METHODS

Inbred C3Hf/Dp mice from this laboratory were used. They were maintained in air-conditioned rooms at the temperature of 20–24°C, in plastic or bakelite cages and given a commercial diet in pellet form

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(Piccioni, Brescia) and tap water *ad libitum*. Recrystallized NMU was obtained through a kind gift from Dr P. F. Swann, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London. NMU was dissolved 0·1% in saline immediately before use and injected once i.p. Eight groups of mice were assembled, including the controls receiving only saline (Group I). Groups II and III were given 25 μg/g NMU at 1 and 70 days of age respectively. Groups IV, V and VI received 50 μg/g NMU at 1, 21 and 70 days of age respectively. Two additional groups were treated with 5 μg/g NMU at 1 day (27♀♀ and 17♂♂) and at 70 days of age (19♀♀ and 20♂♂). Results for these additional groups are not included in the tables and figures as they did not differ from those observed among controls.

Mice treated at 1 or 21 days of age were left with their mothers until weaning, which occurred at 25–28 days of age. At weaning, a few animals from some groups were used for purposes outside the present report. Also 3 mice from the control group were lost during the experiment. All other mice were allowed to die naturally or were killed with ether when moribund or if they survived to 120 weeks of age. Mice were examined daily and weighed at weekly intervals until 10 weeks of age and then at fortnightly intervals.

All mice were submitted to autopsy including opening of the skull but not of the spinal cord. The thymus, liver, kidneys, spleen and orbital glands as well as any organs showing gross abnormalities were fixed in Bouin's fluid and histologically examined in sections stained with haematoxylin and eosin.

Cumulative probabilities of dying with tumours at any site, thymic lymphomata or carcinomata of the forestomach have been calculated according to the formula:

$$P_n = 1 - \frac{(N_1 - t_1)}{N_1} \times \frac{(N_2 - t_2)}{N_2} \times \ldots \times \frac{(N_n - t_n)}{N_n}$$

where P_n represents the probability of having died with that event by the end of week n, N is the number of animals alive at the beginning of each week and t is the number of animals dying with that event during each week (Saffiotti *et al.*, 1972). Since the value of P changes only for weeks when one or more events are recorded, only these weeks are plotted in Fig. 2, 3, 4 and 5.

RESULTS

Pre-weaning mortality

Among control mice, 10/72 (13·9%) died during the suckling period. Following treatment with NMU at 1 or 21 days, the proportion of mice dying before weaning ranged from 5/96 (5·2%) in Group V (50 μg/g NMU at 21 days) to 15/83 (18·1%) in Group IV (50 μg/g at 1 day). None of the differences between treated groups and controls was significant at the 0·05 level.

Weight gain

Body growth was depressed in both sexes following 25 or 50 μg/g NMU at 1 or 21 days of age but not at 70 days of age. Growth depression was obvious before weaning and affected all the mice whether they subsequently developed lymphomata or not.

Mortality and evaluation of causes of death

The numbers of survivors at different ages in both sexes of all groups are given in Table I. Over 50% weaned mice were alive at 110 weeks of age in controls. Survival was poorest in Group IV (50 μg/g at 1 day) where all the mice died before 73 weeks of age. In Groups II, V and VI (25 μg/g at 1 day and 50 μg/g at 21 and 70 days respectively) no survivors were left at 100 weeks of age. In comparison with these, mice of Group III (25 μg/g at 70 days) showed a better survival rate, but their lifespan was still shorter than that of the controls.

Distribution of deaths *vs.* time in the whole population of mice under study is represented in Fig. 1, which indicates a bimodal pattern of mortality distribution. All 107 mice dying before 40 weeks of age belonged to Groups II, IV, V or VI and 89 of them had a thymic lymph-

TABLE I.—*Numbers of C3Hf/Dp Mice Surviving at Different Ages Following NMU Injection at* 1, 21 *or* 70 *Days of Age*

Groups	Dose NMU (μg/g)	Age at treatment (days)	No. of mice weaned*	Survivors at weeks of age											
				10	20	30	40	50	60	70	80	90	100	110	120
I	controls	1	♀ 25	25	25	25	25	24	24	24	24	24	21	17	11
			♂ 34	34	34	34	34	34	33	33	31	30	23	21	17
II	25	1	♀ 25	24	20	19	18	18	16	12	2	0			
			♂ 16	15	14	14	13	13	12	7	6	3	0		
III	25	70	♀ 20	20	20	20	20	19	16	14	9	4	0		
			♂ 20	20	20	20	20	19	18	17	16	10	7	4	3
IV	50	1	♀ 44	44	21	12	10	6	3	1	0				
			♂ 24	23	10	4	4	4	3	0					
V	50	21	♀ 38	37	33	22	19	16	9	2	1	0			
			♂ 44	43	38	34	31	27	23	20	8	4	0		
VI	50	70	♀ 41	41	41	40	37	34	27	13	5	0			
			♂ 30	30	30	27	23	23	18	14	11	5	1	0	

* Or treated, at 70 days.

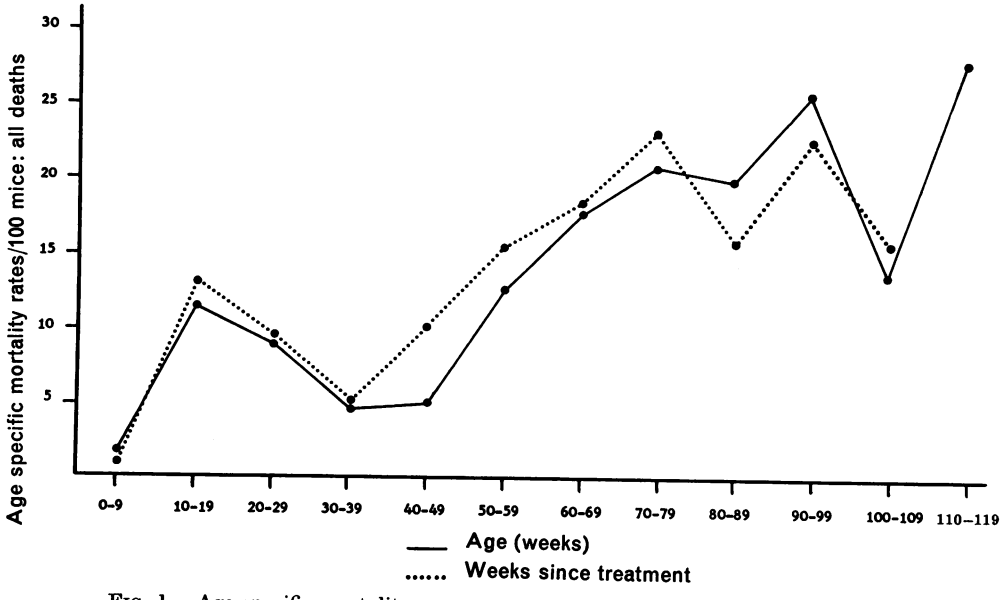

FIG. 1.—Age-specific mortality rates. All groups including controls are pooled.

oma. Five of these mice also had 1–2 small lung adenomata, one had a papilloma of the forestomach and another had a renal adenoma: none of these tumours was likely to be causally related to death. Tumours observed before 40 weeks of age in lymphoma-free mice were 2 lung adenomata, 1 mammary tumour, 1 adenocarcinoma of the intestine and 1 carcinoma of the forestomach.

Twelve mice died with thymic lymphomata after 40 weeks of age, 7 of which also had tumours at other sites (3 lung adenomata, 2 papillomata and one carcinoma of the forestomach, 2 granulosa-cell tumours of the ovary, one renal adenoma and 1 subcutaneous sarcoma). It was assumed that in these 7 mice death had been caused by the thymic lymphoma.

In lymphoma-free mice dying after 50 weeks of age, a frequent observation at autopsy was multiple tumours, none of which could be considered as the main

cause of death with the possible exception of mice with carcinoma of the forestomach. These carcinomata replaced a large part of the stomach wall, invaded the peritoneum and were complicated by purulent inflammation: they could therefore have had a major role in causing death. In 6 animals the only tumour present was carcinoma of the forestomach.

Lifetime analyses were undertaken considering the following end points: 1, death with thymic lymphoma at any age. 2, death with tumours at any site in mice surviving at 39 weeks of age or 39 weeks after treatment (excluding mice with thymic lymphomata). 3, death with carcinoma of the forestomach. Incidences of other tumours have been evaluated considering the proportion of mice developing each tumour type among survivors at 40 weeks of age.

Distribution of tumours

For both sexes of each group Table II reports the number of tumour-bearing animals (TBA), mice dying with more than one tumour and mice developing tumours at specific sites. Only the incidences of extrathymic lymphomata, mammary and subcutaneous tumours were not affected by treatment with NMU. Considering males and females together, in all treated groups the proportion of TBA was over 80% *vs* 55·9% in controls. Mice dying with more than one tumour were 18·6% in controls, 23·5% in mice given 50 μg/g NMU at birth (Group IV, with poor survival rate) and ranged between 46 and 59% in other groups. The average number of tumours per mouse ranged between 0·83 in controls and 2·09 in Group VI.

No sex differences were observed concerning the proportion of TBA and that of mice dying with thymic lymphoma or carcinoma of the forestomach, *i.e.* those parameters for which lifetime analysis has been undertaken on both sexes considered together. In fact, apart from ovarian, mammary, uterine and vaginal tumours, a sex difference in response was detected only for liver-cell tumours. Considering the whole population under study (including the 2 additional groups treated with 5 μg/g NMU) these were found in 15/239 females and 80/205 males ($P < 0{\cdot}001$). The difference between 15/239 females and 6/205 males developing renal tumours is not significant at the 0·05 level.

Induction of thymic lymphomata

Overall incidences and results broken down into 20-week periods after treatment are reported in Table III. Thymic lymphomata were consistently observed in all groups treated with 50 μg/g and in mice receiving 25 μg/g at 1 day of age: cumulative probabilities of dying with thymic lymphoma in these groups are represented in Fig. 2. Incidences in mice receiving 50 μg/g NMU at 1, 21 or 70 days of age (Group IV, V and VI) were 67·6%, 39·0% and 21·2% respectively. Reducing the dose given at 1 day to 25 μg/g NMU (Group II) produced a decrease in the incidence of thymic lymphomata to 17·1%. One thymic lymphoma was found in one mouse out of 40 given 25 μg/g at 70 days of age and none in the controls or in mice receiving 5 μg/g either at 1 or 70 days.

Age at treatment affected both the incidence of thymic lymphomata and the time elapsing between treatment and death caused by a thymic lymphoma.

During the first 20-week period after treatment with 50 μg/g NMU the proportions of mice dying with thymic lymphoma were 35/68, 12/82 and 4/71 respectively in Groups IV, V and VI (the differences between Groups IV and V and Groups IV and VI are significant at the 0·001 level whereas that between Groups V and VI is not significant at the 0·05 level). Between 20 and 39 weeks after treatment, the proportions of mice dying with thymic lymphoma

TABLE II.—*Numbers of Mice Dying with Tumours at Different Sites Following NMU Injections at* 1, 21 *or* 70 *Days of Age*

Group	Dose of NMU (μg/g)	Age at treatment (days)	No. of mice at weaning	Mice dying with tumour (*)	Mice dying with more than one tumour (*)	Mice dying with: Thymic lymphoma	Extrathymic lymphoma	Lung adenoma	Liver-cell tumour	Stomach tumour (†)	Renal tumour	Ovarian tumour	Mammary tumour	Uterine or vaginal tumour	Subcutaneous tumour	No. of other tumours (‡)
I	0		♀ 25	15	7	0	2	6	1	5 (0)	0	3	2	1	5	1 (a)
			♂ 34	18	4	0	1	4	13	0	0	—	—	—	0	5 (b)
II	25	1	♀ 25	20	12	5	1	13	2	10 (5)	0	2	1	1	1	3 (c)
			♂ 16	13	7	2	2	7	9	2 (1)	0	—	—	—	1	3 (d)
III	25	70	♀ 20	18	9	1	0	8	2	7 (2)	0	4	0	6	0	1 (e)
			♂ 20	15	10	0	0	12	12	3 (0)	0	—	—	—	0	1 (f)
IV	50	1	♀ 44	40	11	30	0	11	3	1 (0)	4	0	0	0	0	3 (g)
			♂ 24	21	5	16	0	5	4	2 (2)	0	—	—	—	0	2 (h)
V	50	21	♀ 38	36	16	18	0	15	1	9 (3)	4	9	0	1	0	6 (i)
			♂ 44	41	25	14	1	23	21	19 (12)	1	—	1	—	0	12 (l)
VI	50	70	♀ 41	39	27	6	0	24	2	21 (9)	7	16	4	7	1	5 (m)
			♂ 30	27	15	9	1	18	8	8 (8)	5	—	—	—	0	6 (n)

* Multiple lung adenomata and multiple liver-cell tumours considered as a single tumour.
† In brackets number of mice with carcinoma of the stomach.
‡ See footnotes (in brackets, age at death in weeks).
(a) Ca adrenal (118).
(b) Angioma liver (76), tu orbital gl (113), sarc mediastinum (115), aden pituitary (120), Leydig cell tu testes (120).
(c) Ca skin (74), adenoca pituitary (73), ca skin (79).
(d) Pap skin (81), adenoca subcutaneous (90), Leydig cell tu testes (92).
(e) Tu orbital gl (61).
(f) Meningioma (87).
(g) Angioma mesent lymph node (24), keratoacanthoma skin (45), intracranial neurinoma (52).
(h) Adenoca intestine (25), adenoca pancreas (59).
(i) Tu orbital gl (53), pap tongue (65), ca oesophagus (67), ca skin (67), angioma spleen (68), tu orbital gl (70).
(l) Aden intestine (49), adenoca intestine (51), ca tongue (59), ca anus (62), tu orbital gl (70), ca oesophagus (71), tu orbital gl (73), tu orbital gl (83), tu orbital gl (94), Leydig cell tu testes (94), tu orbital gl (95).
(m) Ca jaw (59), ca tongue (60), tu orbital gl (63), ca skin (63), pap oesophagus (72).
(n) Ca tongue (68), tu orbital gl (78), tu orbital gl (78), tu orbital gl (84), ca skin (84), pap skin (94).

TABLE III.—*Mice Dying with Thymic Lymphoma (TL) Following a Single Injection of NMU Broken Down into Periods Corresponding to Time at Death (Animals of Both Sexes Pooled)*

Group	Dose of NMU (μg/g)	Age at treatment (in days)	Time since treatment (in weeks)								Total			
			0–19			20–39			40–59			60+				
			Alive at start	Dying during period	Mice with TL	Alive at start	Dying during period	Mice with TL	Alive at start	Dying during period	Mice with TL	Alive at start	Mice with TL	Alive at start	Mice with TL		Average time between treatment and death (weeks±s.e.)
															No.	%	
I	controls	—	59	0	0	59	0	0	59	2	0	57	0	59	0	0	—
II	25	1	41	7	4	34	3	1	31	3	1	28	1	41	7	17	29·0±8·4
III	25	70	40	0	0	40	2	1	38	7	0	31	0	40	1	3	—
IV	50	1	68	37	35	31	17	11	14	8	0	6	0	68	46	68	16·5±0·7
V	50	21	82	14	12	68	21	18	47	20	2	27	0	82	32	39	24·5±2·5
VI	50	70	71	4	4	67	10	6	57	30	5	27	0	71	15	21	31·4±4·4

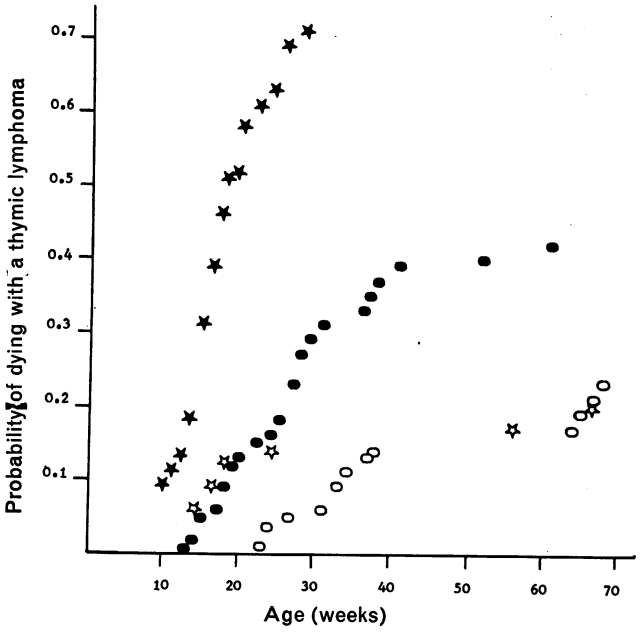

FIG. 2.—Probability of dying with a thymic lymphoma in C3Hf mice given a single injection of NMU.
☆ 25 μg/g at 1 day (Group II)
★ 50 μg/g at 1 day (Group IV)
● 50 μg/g at 21 days (Group V)
○ 50 μg/g at 70 days (Group VI)

were similar in Groups IV and V, *i.e.* 11/31 *vs.* 18/68 ($P > 0{\cdot}05$) while it was only 6/67 in mice treated at 70 days ($P < 0{\cdot}05$). Finally, from 40 weeks after treatment, thymic lymphomata were found in 0/14, 2/47 and 5/57 mice treated at 1, 21 and 70 days respectively. The difference regarding the risk of dying with a thymic lymphoma related to the earliness of treatment was therefore much more obvious in the early than in the late phases of the study.

In both Groups II and VI it appears that the relatively low incidence of thymic lymphomata was paralleled by a delayed time of death.

The distribution of thymic lymphomata among the 13 litters given 50 μg/g NMU at birth and the 13 litters receiving the same dose at 21 days was analysed by comparing for each litter the actual number of mice developing lymphomata with the number expected assuming a uniform distribution throughout all litters. No significant differences were found. The sums of χ^2 were 3·07 in Group IV and 8·07 in Group V: with 12 d.f. they are devoid of significance.

Histologically the thymic tumours were poorly differentiated lymphosarcomata.

Tumours other than thymic lymphomata among survivors at 39 weeks

Figure 3 compares the cumulative probability of dying with a tumour at any site (excluding mice with thymic lymphomata) among animals surviving at least 39 weeks after treatment in groups receiving 50 μg/g NMU at 1, 21 or 70 days of age (Groups IV, V and VI). From 50 weeks after treatment the cumulative probability of dying with tumours was somethat higher in Group IV (treated at 1 day of age). On the other hand, no differences appear to exist between mice treated at 21 and 70 days. The average

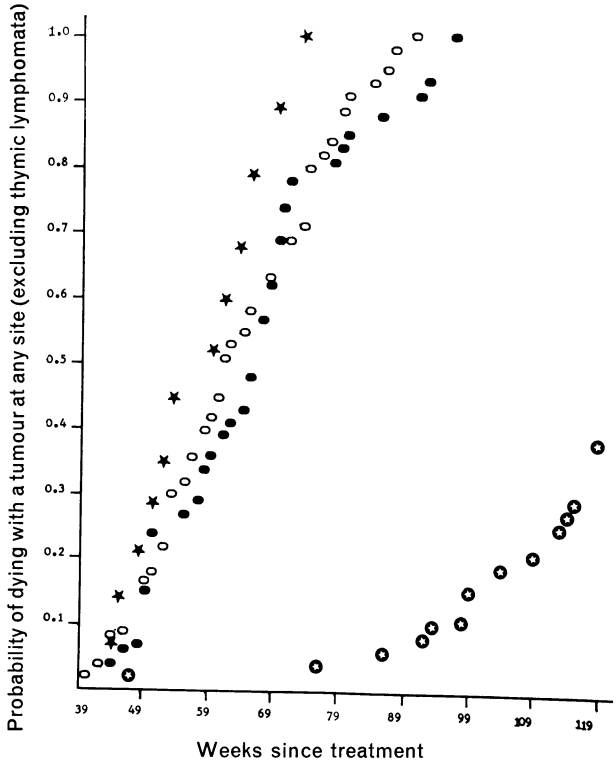

FIG. 3.—Probability of dying with a tumour at any site (excluding thymic lymphomata) from the 40th week after a single injection of 50 μg/g NMU.
✪ Controls (Group I)
✶ Mice treated at 1 day (Group IV)
● Mice treated at 21 days (Group V)
○ Mice treated at 70 days (Group VI)

number of tumours at sites other than the thymus in mice dying between 40 and 73 weeks after treatment (time of death of the last mouse of group IV) was 1·9 per mouse in Group IV, 2·5 in Group V and 2·4 in Group VI. It does not seem that the higher probability of dying with tumours shown by mice of Group IV reflects a broader spectrum of tumour induction when the treatment was given early in life.

Dose-related differences in cumulative probabilities of dying with tumours at any age are far more obvious (Fig. 4). For both doses of 50 and 25 μg/g NMU and regardless of the age at treatment, the end point is above 0·9. However, for treatment at either 1 or 70 days there is a consistent difference between the effects of 50 and 25 μg/g, since the two curves are separated by a distance corresponding to 15–25 weeks. A plausible explanation is that the higher dose induced more tumours per animal than the lower one. Between 40 and 73 weeks of age (time of death of the last mouse in Group IV) the average number of tumours per dying animal was 1·1 in Group II and 1·9 in Group IV. Similarly, between 40 and 100 weeks of age (time of death of the last mouse in Group VI) the average number of tumours per dying mouse was 1·3 in Group III and 2.3 in Group VI.

Treatment with 5 μg/g NMU either at 1 day or at 70 days of age did not

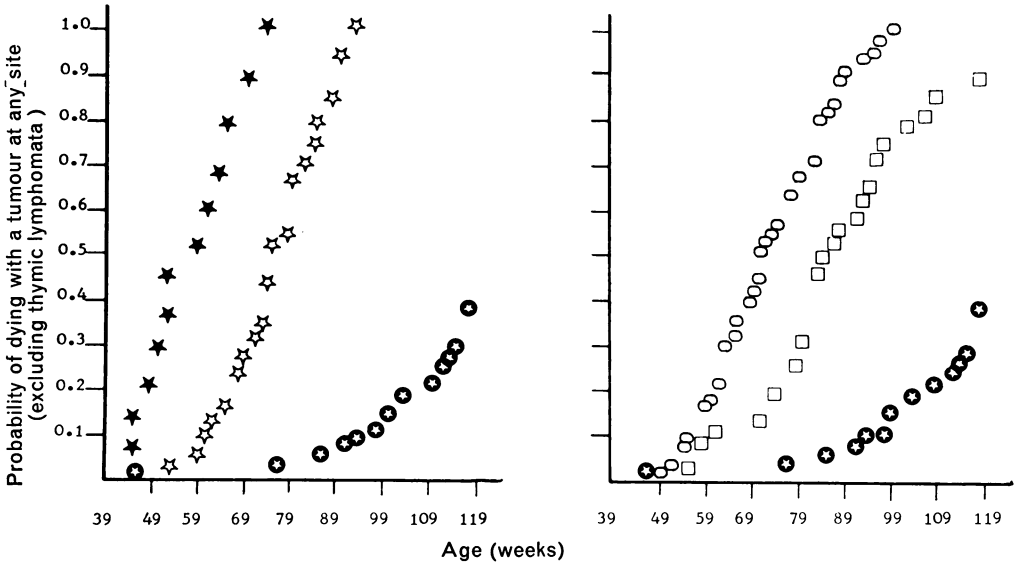

FIG. 4.—Probability of dying with a tumour at any site (excluding thymic lymphomata) beyond the 40th week of age in C3Hf mice given a single injection of NMU.
● Controls (Group I)
☆ 25 μg/g at 1 day (Group II)
□ 25 μg/g at 70 days (Group III)
★ 50 μg/g at 1 day (Group IV)
○ 50 μg/g at 70 days (Group VI)

produce an obvious increase over the controls in the probability of dying with a tumour at any site.

Tumours of the forestomach

Of 87 mice with tumours of the forestomach, 42 had at least one carcinoma of the forestomach and the other 45 had one or more papillomata (Table II). A sizeable incidence of carcinomata of the forestomach was found in Groups V and VI, receiving 50 μg/g NMU at 21 and 70 days of age respectively. Fig. 5 represents the distribution of the cumulative probability of dying with a carcinoma of the forestomach in these two groups: when time since treatment is considered, the two lines are almost overlapping, indicating that the two treatments were equally effective. Average time elapsing between treatment and death with car-

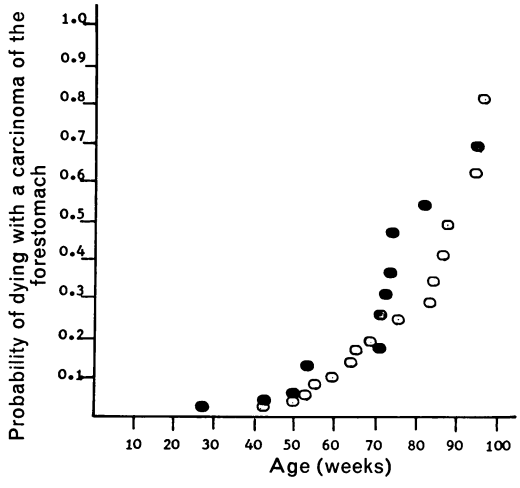

FIG. 5.—Probability of dying with a carcinoma of the forestomach in C3Hf mice given a single injection of 50 μg/g NMU.
● Treated at 21 days (Group V)
○ Treated at 70 days (Group VI)

TABLE IV.—*Incidence of Lung Adenomata, Liver-cell Tumours, Ovarian Tumours, Uterine and Vaginal Tumours and Orbital Gland Tumours in C3Hf/Dp Mice Receiving a Single Injection of NMU Referred to Survivors at 40 Weeks of Age*

Group	Survivors at 40 weeks	Mice dying with lung adenomata		Mice dying with liver cell tumours		Mice dying with ovarian tumours		Mice dying with uterine or vaginal tumours		Mice dying with tumours of the orbital glands	
		No.	%	No.	%	No.	%	No.	%	No.	%
I	♀ 25	6	24	1	4	3	12	1	4	0	—
	♂ 34	4	12	13	38	—		—		1	3
II	♀ 18	13	72++	2	11	2	11	1	6	0	—
	♂ 13	7	54++	9	69*	—		—		0	—
III	♀ 20	8	40	2	10	4	20	6	30+	1	5
	♂ 20	12	60++	12	60	—		—		0	—
IV	♀ 10	8	80++	3	30	0	0	0	0	0	—
	♂ 4	4	100++	4	100*	—		—		0	—
V	♀ 19	14	74++	1	5	9	47+†	1	5	2	11
	♂ 31	21	68++	21	68+	—		—		6	19
VI	♀ 37	23	62++	2	5	16	43+†	7	19	1	3
	♂ 23	18	78++	8	35	—		—		3	13

+ Difference *vs* controls $P < 0{\cdot}05$.
++ Difference *vs* controls $P < 0{\cdot}01$.
* If ♂♂ of Groups II and IV are considered together $P < 0{\cdot}05$.
† If ♀♀ of Groups V and VI are considered together $P < 0{\cdot}01$.

cinoma of the forestomach was 66·0 ± 6·1 weeks among the 6 mice of Group II, 60·7 ± 4·5 weeks in the 15 mice of Group V and 65·6 ± 4·4 weeks in the 17 mice of Group VI.

In Group III, of 40 mice receiving 25 μg/g NMU at 70 days of age, 2 died with a carcinoma (respectively 46 and 64 weeks after treatment), and 8 had one or multiple papillomata of the forestomach. The overall incidence of 10/40 mice developing stomach tumours was higher than that of 5/59 found in the controls ($P < 0{\cdot}05$). Among mice receiving 5 μg/g NMU at 1 or 70 days of age the incidence of stomach papillomata was, if anything, lower than in the controls.

Other tumours

Target organs of the carcinogen NMU included lungs, liver, kidney, intestine, ovary, uterus and vagina (Table II). The incidences of some of these tumours among survivors at 40 weeks of age are reported in Table IV.

A significant increase ($P < 0{\cdot}01$) of the proportion of mice dying with lung adenomata was found in both sexes in groups given 50 μg/g NMU at any age or 25 μg/g at 1 day as well as in males receiving 25 μg/g at 70 days.

Liver cell tumours were increased over the controls ($P < 0{\cdot}05$) in males receiving 50 μg/g at 21 days as well as in those treated with either 50 or 25 μg/g at 1 day if considered together. No evidence of hepatocarcinogenicity was found in any of the other treated animals.

Renal nodules ranging between 0·2 and 1·0 cm in diameter were observed in 21 mice of either sex receiving 50 μg/g NMU either at 1, 21 or 70 days and in none of the other groups. Histologically, they were benign solid or papillary adenomata, with the exception of one sarcoma and one very atypical epithelial tumour. Mice exposed at 70 days appeared more susceptible than those treated at 21 days, but the difference is of borderline significance.

A total of 37 females died with an ovarian tumour: a few of these were complicated by peritoneal haemorrhage which might have caused death. Histologically, they were of the granulosa-cell type with the exception of one luteoma and one tumour too decomposed for a diagnosis. A significant increase

($P < 0{\cdot}05$) over the controls was observed among mice given 50 μg/g NMU at 21 or 70 days: incidences were 9/19 and 16/37 respectively *vs.* 3/25 in the controls. No ovarian tumours were found in the 10 females treated with the same dose at 1 day and surviving 40 or more weeks.

Twenty-one females at death had a fibroma or fibrosarcoma in the uterus or in the vagina. Only in mice treated with 25 or 50 μg/g at 70 days was there an increase of marginal significance in the incidence of these tumours.

Orbital gland tumours were increased over the controls in males receiving 50 μg/g NMU at 21 or 70 days but not in other groups.

DISCUSSION

In terms of target organs and effective carcinogenic doses of a single administration of NMU to mice, the present investigation is confirmatory of previous studies on other strains of mice (Graffi and Hoffmann, 1966; Terracini and Stramignoni, 1967; Kelly *et al.*, 1968; Frei, 1970; Joshi and Frei, 1970; Terracini and Testa, 1970; Frei, 1971). The sporadic occurrence of neurogenic tumours in the present series as well as in previous studies differs from findings following a single administration of the related compound N-nitroso-N-ethylurea (Searle and Jones, 1972; Vesselinovitch *et al.*, 1974).

Reviews on the role of age at treatment in chemical carcinogenesis lead to the conclusion that neonates are more susceptible than older animals to the induction of lymphomata, liver-cell tumours and lung adenomata, whereas results for other target organs are less definite (Toth, 1968; Della Porta and Terracini, 1969). The particular susceptibility of some organs of newborn mice to chemical carcinogenesis has been attributed to a series of host-related factors such as metabolic maturation, immunological or hormonal conditions, different functional status of the target organs, *etc.* (Toth, 1968; Della Porta and Terracini, 1969).

The progressive decrease of the susceptibility to thymic lymphoma induction from birth to 10 weeks of age confirms previous studies with NMU itself (Terracini and Testa, 1970), urethane (De Benedictis *et al.*, 1964; Vesselinovitch, Mihailovich and Itze, 1970), DMBA (Toth, Rappaport and Shubik, 1961) and N-nitroquinoline-1-oxide (Nishizuka, Nakakuki and Sakakura, 1964). In one of such studies, (C57BL $\times$ C3H)F_1 mice aged 25 weeks were found completely resistant to treatment with urethane at a dose which was highly active in newborn mice (Vesselinovitch *et al.*, 1970).

On the other hand, when a comparison is made between groups of the carcinogenic action on all organs excluding the thymus, age at treatment seemed to play a minor role. Analyses of tumour incidences in individual organs is limited by interference caused by intercurrent deaths. However, hepatocarcinogenicity was confined to male mice receiving 25 or 50 μg/g NMU at 1 or 21 days of age. In a previous study on the same colony of C3Hf/Dp mice, a single administration of 1 mg urethane at birth was equally effective on both sexes (Della Porta *et al.*, 1967).

The susceptibility to carcinogenesis of the ovary and orbital glands seems to be lower in newborns than in animals aged 21 or 70 days, in confirmation of previous studies with urethane and N-nitroso-N-ethylurea (Vesselinovitch and Mihailovich, 1967; Vesselinovitch *et al.*, 1974).

In the present series, the probability of dying with a carcinoma of the forestomach following 50 μg/g was the same in mice treated at 21 days and in those treated at 70 days of age. Among animals given 25 μg/g NMU, the proportion developing benign or malignant tumours of the forestomach was 12/41 in those treated at birth and 10/40 in those treated at 70 days of age. Although 6 of the former had a carcinoma *vs.* 2 of the latter, these differences are hardly significant. In a previous study with

N-nitroso-N-ethylurea, mice treated at birth were slightly less susceptible to stomach carcinogenesis than those treated at 15 or 42 days of age (Vesselinovitch *et al.*, 1974).

Lifetime analysis in the present series indicates that lymphomata following exposure to 50 μg/g NMU at birth not only are more frequent but also progress to death more rapidly than those induced later in life. By and large this is confirmatory of previous investigations with NMU (Terracini and Testa, 1970), DMBA (Toth *et al.*, 1961) and urethane (De Benedictis *et al.*, 1964) as well as with Gross' passage A virus (Axelrad and Van der Gaag, 1962).

The difference in the mechanism of carcinogenesis reflected in this result might take place either at the time of the interaction between carcinogen and cell components or during tumour development. It is unlikely that age at treatment affects the persistence in the target organ of NMU, as this is an unstable compound, rapidly broken down without enzymic activation (Druckrey *et al.*, 1967; Swann, 1968). Morphological differences of the thymus between newborn and adult mice (Axelrad and Van der Gaag, 1962) do not appear to be involved, since thymic lymphomata induced in newborn mice with low doses of NMU progressed relatively slowly. In addition, no morphological differences were found between lymphomata occurring in mice treated at birth and those found in mice treated later in life.

The different rate of progression of thymic lymphomata related to age at treatment more likely reflects differences in tumour–host relationships during the early phases of tumour development. "Natural" age-related differences such as immunosurveillance and the hormonal situation do not seem to be relevant since thymic lymphomata induced by a low dose of NMU at birth progressed as slowly as those induced by a high dose given at 10 weeks of age.

On the other hand, an age-related systemic effect of NMU on the general status of the animals is reflected in the degree of body growth inhibition related to age at treatment. A role of NMU-induced immuno-depression is possible, but the impact of age at treatment on this effect has not been investigated (Parmiani, Colnaghi and Della Porta, 1971). This hypothesis is compatible with the theory that chemical induction of lymphomata in mice is mediated by viral activation (Kaplan, 1967).

Age-related factor(s) affecting tumour-host relationship do not appear to be involved in the mechanism of carcinogenesis for the stomach, where progression was independent of age at treatment. This discrepancy between thymic lymphomata and carcinomata of the forestomach is reminiscent of a previous observation that the immunological status of NMU-treated rats does not affect equally the induction of tumours of the nervous system and that of tumours of the bladder (Denlinger *et al.*, 1973). This again suggests that host–tumour interaction may be quite different according to the organs involved.

The authors are grateful to Dr G. Della Porta for helpful discussions and to Mr R. Rubertelli and Ms G. Pasquini for their technical help.

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