EFFECTS OF URETHANE DOSE AND TIME PATTERNS ON TUMOR FORMATION

M. WHITE, A. GRENDON, and H. B. JONES
DONNER LABORATORY, LAWRENCE RADIATION LABORATORY,
UNIVERSITY OF CALIFORNIA, BERKELEY

1. Introduction

The carcinogenic action of urethane on the lungs of mice was first noted by Nettleship, Henshaw, and Meyer [1] in 1943. The effect of this chemical is to produce multiple tumors of apparently discrete origin, visible as pearly white nodules on the surface of the lung. Although the quantitative relation between dose and number of tumors (herein termed the dose-effect relation) has been studied by a number of investigators, the available data were not sufficient to test the mathematical model of a two stage mechanism of tumor formation developed by Neyman and Scott [2]. It was apparent from the model that, if the hypothesis is correct, the needed constants might be determined from suitable studies of the changes in the dose-effect relation produced by variation in the time interval between doses.

Henshaw and Meyer [3] and Rogers [4] administered urethane with various time intervals between doses, but their results were not conclusive with respect to the effect of fractionating the dose. Shimkin, Wieder, Marzi, Gubareff, and Suntzeff [5] are presenting a paper in this session concerning their efforts to test Neyman's model. The work reported here was undertaken to supplement the previous data and, by covering a broader pattern of urethane administration, with respect to both quantity and time interval, hopefully to include the particular patterns that would critically test the hypothesis. Additionally, since tumors take time to develop to recognizable size, the effect of time interval from initial injection to sacrifice was introduced as a factor to be studied.

2. Material and methods

The animals used were female, strain A/Jackson mice which were 8½ to 10½ weeks old at the beginning of the experiment. It is to be noted that this is the same strain as used by Shimkin and Gubareff, but that only females were used

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in our experiment, and their age was greater. The choice of sex was based on the greater tractability of the females. This age was chosen because it was known that very young animals are relatively much more susceptible to the carcinogenic effect of urethane when given doses proportionate to body weight [4]. It was our intention to avoid not only the period of high susceptibility but also the transition period when susceptibility decreases rapidly to the adult level, since the uncertainty in this respect would tend to confuse the picture.

Sixteen hundred of these animals were injected according to the time and dose schedule in table I. There are 32 categories defined by dose pattern, that is, the

TABLE I

DOSE PATTERN AND CUMULATIVE DOSE (mg/g BODY WEIGHT)

Asterisks indicate that the same dose was given to two trial groups, one on the date of the first injection and one on the date of the last injection of the groups given 16 injections, in order to check the effect of aging during injections on the susceptibility.

Urethane Dose per Injection	Injection at Intervals of Two Days Number of Injections 1	2	4	8	16	Injection at Intervals of Seven Days Number of Injections 2	4
0.000 (controls)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.0625	0.0625	0.125	0.250	0.500	1.000		
0.125	0.125	0.250	0.500	1.000	2.000	0.250	0.500
0.250	0.250	0.500	1.000	2.000	4.000	0.500	1.000
0.500	0.500*					1.000	2.000
1.000	1.000*						

dose per injection, number of injections, and spacing of injections. Each of these categories included 50 animals. These 50 animals were divided into five sacrifice groups, that is, groups of 10 animals from each dose pattern category were sacrificed at 8, 12, 16, 20, and 24 weeks after the start of injections. There were thus 160 classes distinguishable by dose pattern and time of sacrifice.

The injections were given intraperitoneally. The animals were weighed just prior to injection, and the dose was based on this weight. Five different dilutions of urethane in sterile distilled water were used, so that an approximately constant volume of solution could be injected into each animal to obtain the desired doses. Each animal was given 0.01 ml of solution of the appropriate concentration per gram of body weight. The most concentrated solution corresponded to the usual anesthesia dose of 1.000 mg/g of body weight. The other solutions were ½, ¼, ⅛, and 1/16 as concentrated. The actual concentrations at the time of administration were checked by chemical analysis of each solution according to Kjeldahl's method. This step was taken to insure against the possibility of deterioration of the chemical or error in preparation. Each concentration was given as a single injection of 0.01 ml/g to one group of animals and (except the anesthesia dose) as multiple injections to other groups. The use of multiple anesthesia doses was deemed impracticable because of the risk of death of the animals and unnecessary because of the high rates of tumor induction at lower doses.

Some groups received the multiple injections every other day and some every seventh day. In this way a total dose of 1.000 mg/g body weight was given in one of six ways: as a single dose, in 2 or 4 equal fractions every seventh day, or as 4, 8, or 16 equal fractions every second day. A total dose of 0.500 mg/g of body weight was also given in one of six ways: as a single dose, in 2 or 4 equal fractions at intervals of seven days, or as 2, 4, or 8 equal fractions at intervals of two days. The other doses were similarly administered, as indicated in table I.

There was some concern about whether the aging of the animals between the 1st and the 16th injections would make a significant difference in the results, even though the initial age had been deliberately selected to make such an effect unlikely. To test this point, single doses of 1.000 mg/g and 0.500 mg/g of body weight were administered to each of two groups on the 30th day of the experiment (that is, the day on which the last injection of the series of 16 injections was given) in order to compare the results with those that received similar single doses on "day zero."

The dispersion in age of the mice as received from the supplier was 21 days, so it seemed desirable: (1) to fit animals into the injection schedule in such a way as to reduce the effect of the age variable; and (2) to stratify the samples with respect to age in each of the 160 experimental classes. Animals were serially numbered, with the lowest numbers assigned to the batches of earliest birth date and successively younger batches receiving blocks of numbers in numerical order. Since practical considerations regarding the carrying out of experimental procedures made it necessary to divide the mice into two lots for injection, the older half was injected first and the younger half eight days later; the interval of eight days was a compromise, with respect to mean age difference, that fitted into the feasible operational schedule. (The "eight days" refers to the great majority subject to single doses or dose patterns with intervals of two days. For those on dose patterns with intervals of seven days, it was practically necessary to make this "seven days.") The serially numbered animals were listed in sequence in groups of 160 each, thus establishing five age groups in each half of the total lot with a narrow range of age within each group. The 160 experimental classes were given identifying numbers, and these numbers were randomly assigned to the 160 mice in each age group, thus forming 160 age stratified batches of 5 animals each in the older half and 160 similar batches in the younger half. Thus, each of the 160 experimental classes consisted of 10 animals with age ranges, at time of first injection, of the order of 13 days and containing one representative of each of ten age strata.

To insure random conditions of housing and care of the animals with respect to experimental treatment, the mice were placed in cages of 10 each, in ordinal sequence of their identification numbers. The animals were housed in these cages and removed only on the days when they were injected, at which time they were sorted and temporarily caged according to injection groups. Thus, no dwelling cage had more than one animal from any one experimental class. The cages were rotated from rack to rack and from shelf to shelf on the racks once a week in order to eliminate any room effects. The animals were fed D & G mouse biscuits and water ad libitum, and their cages were changed once a week.

At the scheduled time of injection of each of the 32 dose-pattern categories, the 50 animals of a given category were removed from their dwelling cages, transferred to other cages for treatment, and subsequently returned to their respective dwelling cages. A group of 50 control animals was similarly handled and injected with 0.01 ml of distilled water per gram of body weight, to match the volumes of solution injected into the experimental animals.

At the scheduled time of sacrifice of each group of 10 animals, the individuals were removed from their cages and assembled in one cage for sacrifice by cervical dislocation. The lungs were removed and preserved in Tellyesniczky's fluid, which other experimenters had found well suited for the preparation of lung tissue for ease of recognition of tumors. The number of tumors per lung was determined by examining the surface with the aid of a dissecting microscope. The reliability of the tumor counts depends in large measure on the experimenter's experience in recognizing very small tumors. For consistency, therefore, all counts were made by one person (Miss White), who tested the reliability of her counting technique by twice recounting the first 72 lung specimens examined.

On comparing the sets of three observations per animal for the first 24 animals, the range of variability about the mean tumor count was approximately ± 16 per cent; for the second 24 animals, it was reduced to about ± 10 per cent; and for the third group, to ± 0.8 per cent. All counts after the first 72 specimens were made without repetition. It is assumed that the counts reported, which represent the results of the improvement of Miss White's technique with experience, are as reliably consistent as the statistics on the last group suggest. The counts were made without knowledge of the experimental group to which each specimen belonged.

3. Results

The full data on number of tumors per lung for each of the 1600 mice, together with ages and weights of the animals, are tabulated in the appendix. The statistical summaries are given in tables IIa to IIg, IIIa to IIId, and IVa to IVe, for the experimental groups, and table V for the control groups. Selected portions are presented graphically in figures 1, 2, and 3, discussed later.

It is apparent that the data may be grouped for study according to at least three different viewpoints. The data are presented grouped in each of these ways in the tables designated II, III, and IV, respectively. The viewpoints are: in table II, all animals receiving the same total amount of urethane per gram of body weight in the course of the experiment may be considered one group differing only in the fractionation pattern, that is, number of equal fractions into which the total dose is divided and time interval between fractions; in table III,

TABLE II

SUMMARY OF DATA ACCORDING TO FIXED TOTAL DOSE, WITH VARIABLE FRACTIONATION AND TIME INTERVALS BETWEEN DOSES

Doses are reported as mg/g, to be understood as mg/g body weight. Standard errors are shown in parentheses. "Sacrifice time" means the time between start of injections and sacrifice. "Tumor yield" means average number of tumors per animal. The day "30" indicates injected once on day of last injection of animals that received 16 injections, in order to determine whether there was an aging factor between the 1st and 16th injections.

(a) TOTAL DOSE, 4.000 mg/g,
Administered as 16 × 0.250 mg/g at intervals of two days.

Sacrifice Time	Tumor Yield
8 weeks	23.9 (2.95)
12 weeks	42.4 (2.60)
16 weeks	54.7 (4.60)
20 weeks	61.0 (3.07)
24 weeks	64.1 (3.90)

(b) TOTAL DOSE, 2.000 mg/g

Sacrifice Time	Tumor Yield		
	4 × 0.500 mg/g at Intervals of Seven Days	8 × 0.250 mg/g at Intervals of Seven Days	16 × 0.125 mg/g at Intervals of Two Days
8 weeks	13.3 (2.23)	14.1 (1.11)	8.0 (1.88)
12 weeks	30.2 (2.23)	23.6 (2.76)	18.0 (2.15)
16 weeks	39.6 (2.20)	28.3 (1.61)	27.5 (2.19)
20 weeks	43.8 (2.71)	35.8 (2.66)	30.1 (2.30)
24 weeks	47.2 (2.70)	38.0 (1.09)	33.3 (1.62)

TABLE II (Continued)

(c) TOTAL DOSE, 1.000 mg/g
(i) Administered at intervals of seven days.

Sacrifice Time	Tumor Yield				
	1 × 1.000 mg/g				
	On Day "0"	On Day "30"	Avg. Day "0" and Day "30"	2 × 0.500 mg/g	4 × 0.250 mg/g
8 weeks	9.0	0.8	4.9	6.7	3.3
	(1.28)	(0.36)	(1.14)	(1.04)	(0.75)
12 weeks	15.6	9.5	12.5	15.5	9.5
	(1.18)	(1.40)	(1.15)	(2.28)	(1.50)
16 weeks	15.7	15.1	15.4	18.8	11.3
	(1.78)	(1.44)	(1.12)	(2.28)	(0.83)
20 weeks	21.9	16.1	19.0	20.0	17.2
	(1.93)	(1.85)	(1.47)	(3.01)	(1.35)
24 weeks	21.2	26.2	23.7	22.7	16.9
	(2.31)	(1.77)	(1.51)	(2.31)	(1.64)

(ii) Administered at intervals of two days.

Sacrifice Time	Tumor Yield					
	1 × 1.000 mg/g					
	On Day "0"	On Day "30"	Avg. Day "0"' and Day "30"	4 × 0.250 mg/g	8 × 0.125 mg/g	16 × 0.0625 mg/g
8 weeks	9.0	0.8	4.9	5.8	5.1	1.3
	(1.28)	(0.36)	(1.14)	(1.49)	(0.69)	(0.52)
12 weeks	15.6	9.5	12.5	11.4	11.3	6.9
	(1.18)	(1.40)	(1.15)	(2.14)	(1.92)	(1.47)
16 weeks	15.7	15.1	15.4	15.3	12.4	9.8
	(1.78)	(1.44)	(1.12)	(1.60)	(1.04)	(1.32)
20 weeks	21.9	16.1	19.0	15.1	15.2	14.7
	(1.93)	(1.85)	(1.47)	(1.65)	(1.75)	(1.40)
24 weeks	21.2	26.2	23.7	17.7	17.3	13.6
	(2.31)	(1.77)	(1.51)	(2.20)	(1.54)	(1.40)

TABLE II (Continued)

(d) Total Dose, 0.500 mg/g
(i) Administered at intervals of seven days.

Sacrifice Time	Tumor Yield				
	1 × 0.500 mg/g				
	On Day "0"	On Day "30"	Avg. Day "0" and Day "30"	2 × 0.250 mg/g	4 × 0.125 mg/g
8 weeks	3.8 (0.96)	0.3 (0.02)	2.0 (0.62)	3.0 (0.61)	1.6 (0.45)
12 weeks	5.1 (0.53)	2.5 (0.52)	3.8 (0.47)	4.5 (0.62)	4.3 (1.17)
16 weeks	6.0 (1.27)	5.2 (1.05)	5.6 (0.80)	7.0 (1.03)	6.0 (1.01)
20 weeks	9.8 (1.06)	7.2 (1.02)	8.5 (0.77)	9.4 (1.41)	6.7 (0.84)
24 weeks	9.2 (1.20)	9.8 (0.85)	9.5 (0.72)	10.1 (1.29)	7.0 (1.04)

(ii) Administered at intervals of two days.

Sacrifice Time	Tumor Yield					
	1 × 0.500 mg/g					
	On Day "0"	On Day "30"	Avg. Day "0" and Day "30"	2 × 0.250 mg/g	4 × 0.125 mg/g	8 × 0.0625 mg/g
8 weeks	3.8 (0.96)	0.3 (0.02)	2.0 (0.62)	2.7 (0.82)	2.3 (1.02)	1.8 (0.51)
12 weeks	5.1 (0.53)	2.5 (0.52)	3.8 (0.47)	4.3 (0.63)	3.7 (0.61)	4.2 (0.61)
16 weeks	6.0 (1.27)	5.2 (1.05)	5.6 (0.80)	5.2 (0.92)	5.1 (0.46)	3.7 (0.70)
20 weeks	9.8 (1.06)	7.2 (1.02)	8.5 (0.77)	7.4 (0.87)	8.8 (1.17)	5.4 (0.85)
24 weeks	9.2 (1.20)	9.8 (0.85)	9.5 (0.72)	8.7 (0.93)	10.9 (0.98)	8.1 (0.87)

TABLE II (Continued)

(e) TOTAL DOSE, 0.250 mg/g

Sacrifice Time	Tumor Yield			
	On Day "0" 1 × 0.250 mg/g	At Intervals of Seven Days 2 × 0.125 mg/g	At Intervals of Two Days 2 × 0.125 mg/g	At Intervals of Two Days 4 × 0.0625 mg/g
8 weeks	2.1 (0.48)	1.8 (0.36)	0.8 (0.25)	0.7 (0.42)
12 weeks	1.9 (0.50)	2.9 (0.35)	2.3 (0.61)	2.2 (0.25)
16 weeks	2.6 (0.56)	3.1 (0.59)	3.7 (0.54)	3.0 (0.58)
20 weeks	3.5 (0.52)	3.6 (0.34)	2.9 (0.64)	3.6 (0.72)
24 weeks	3.8 (0.87)	3.9 (0.43)	3.9 (0.65)	4.7 (0.68)

(f) TOTAL DOSE, 0.125 mg/g

Sacrifice Time	Tumor Yield	
	On Day "0" 1 × 0.125 mg/g	At Intervals of Two Days 2 × 0.0625 mg/g
8 weeks	0.8 (0.33)	0.0 (0.00)
12 weeks	1.4 (0.71)	0.5 (0.31)
16 weeks	1.5 (0.43)	1.3 (0.37)
20 weeks	2.2 (0.39)	2.2 (0.47)
24 weeks	2.0 (0.45)	1.7 (0.47)

(g) TOTAL DOSE, 0.0625 mg/g
Administered as single dose.

Sacrifice Time	Tumor Yield
8 weeks	1.0 (0.49)
12 weeks	0.7 (0.33)
16 weeks	0.6 (0.22)
20 weeks	0.9 (0.38)
24 weeks	1.2 (0.44)

TABLE III

SUMMARY OF DATA ACCORDING TO FIXED INDIVIDUAL DOSE, WITH VARIABLE NUMBERS OF DOSES AND TIME INTERVALS BETWEEN DOSES

(a) INDIVIDUAL DOSE, 0.0625 mg/g
Administered at intervals of two days.

Sacrifice Time	Tumor Yield				
	Number of Doses				
	1	2	4	8	16
8 weeks	1.0 (0.49)	0.0 (0.00)	0.7 (0.42)	1.8 (0.51)	1.3 (0.52)
12 weeks	0.7 (0.33)	0.5 (0.31)	2.2 (0.25)	4.2 (0.61)	6.9 (1.47)
16 weeks	0.6 (0.22)	1.3 (0.37)	3.0 (0.58)	3.7 (0.70)	9.8 (1.32)
20 weeks	0.9 (0.38)	2.2 (0.47)	3.6 (0.72)	5.4 (0.85)	14.7 (1.40)
24 weeks	1.2 (0.44)	1.7 (0.47)	4.7 (0.68)	8.1 (0.87)	13.6 (1.40)

(b) INDIVIDUAL DOSE, 0.125 mg/g

Sacrifice Time	Tumor Yield						
	Number of Doses						
		At Intervals of Two Days				At Intervals of Seven Days	
	1	2	4	8	16	2	4
8 weeks	0.8 (0.33)	0.8 (0.25)	2.3 (1.02)	5.1 (0.69)	8.0 (1.88)	1.8 (0.36)	1.6 (0.45)
12 weeks	1.4 (0.71)	2.3 (0.61)	3.7 (0.61)	11.3 (1.92)	18.0 (2.15)	2.9 (0.35)	4.3 (1.17)
16 weeks	1.5 (0.43)	3.7 (0.54)	5.1 (0.46)	12.4 (1.04)	27.5 (2.19)	3.1 (0.59)	6.0 (1.01)
20 weeks	2.2 (0.39)	2.9 (0.64)	8.8 (1.17)	15.2 (1.75)	30.1 (2.30)	3.6 (0.34)	6.7 (0.84)
24 weeks	2.0 (0.45)	3.9 (0.65)	10.9 (0.98)	17.3 (1.54)	33.3 (1.62)	3.9 (0.43)	7.0 (1.04)

TABLE III (Continued)

(c) INDIVIDUAL DOSE, 0.250 mg/g

Sacrifice Time	Tumor Yield						
	Number of Doses						
		At Intervals of Two Days				At Intervals of Seven Days	
	1	2	4	8	16	2	4
8 weeks	2.1	2.7	5.8	14.1	23.9	3.0	3.3
	(0.48)	(0.82)	(1.49)	(1.11)	(2.95)	(0.61)	(0.75)
12 weeks	1.9	4.3	11.4	23.6	42.4	4.5	9.5
	(0.50)	(0.63)	(2.14)	(2.76)	(2.60)	(0.62)	(1.50)
16 weeks	2.6	5.2	15.3	28.3	54.7	7.0	11.3
	(0.56)	(0.92)	(1.60)	(1.61)	(4.60)	(1.03)	(0.83)
20 weeks	3.5	7.4	15.1	35.8	61.0	9.4	17.2
	(0.52)	(0.87)	(1.65)	(2.66)	(3.07)	(1.41)	(1.35)
24 weeks	3.8	8.7	17.7	38.0	64.1	10.1	16.9
	(0.87)	(0.93)	(2.20)	(1.09)	(3.90)	(1.29)	(1.64)

(d) INDIVIDUAL DOSE, 0.500 mg/g
Administered at intervals of seven days.

Sacrifice Time	Tumor Yield				
	Number of Doses				
	1 On Day "0"	1 On Day "30"	1 Avg. Day "0" and Day "30"	2	4
8 weeks	3.8	0.3	2.0	6.7	13.3
	(0.96)	(0.15)	(0.62)	(1.04)	(2.23)
12 weeks	5.1	2.5	3.8	15.5	30.2
	(0.53)	(0.52)	(0.47)	(2.28)	(2.23)
16 weeks	6.0	5.2	5.6	18.8	39.6
	(1.27)	(1.05)	(0.80)	(2.28)	(2.20)
20 weeks	9.8	7.2	8.5	20.0	43.8
	(1.06)	(1.02)	(0.77)	(3.01)	(2.71)
24 weeks	9.2	9.8	9.5	22.7	47.2
	(1.20)	(0.85)	(0.72)	(2.31)	(2.70)

TABLE IV

SUMMARY OF DATA ACCORDING TO FIXED NUMBER OF INJECTIONS WITH VARIABLE INDIVIDUAL DOSES AND TIME INTERVALS BETWEEN DOSES

(a) GIVEN AS 1 INJECTION

Sacrifice Time	Tumor Yield — Total Dose (mg/g) 0.0625	0.125	0.250	0.500 Day "0"	0.500 Day "30"	0.500 Avg.	1.000 Day "0"	1.000 Day "30"	1.000 Avg.
8 weeks	1.0	0.8	2.1	3.8	0.3	2.0	9.0	0.8	4.9
	(0.49)	(0.33)	(0.48)	(0.96)	(0.15)	(0.62)	(1.28)	(0.36)	(1.14)
12 weeks	0.7	1.4	1.9	5.1	2.5	3.8	15.6	9.5	12.5
	(0.33)	(0.71)	(0.50)	(0.53)	(0.52)	(0.47)	(1.18)	(1.40)	(1.15)
16 weeks	0.6	1.5	2.6	6.0	5.2	5.6	15.7	15.1	15.4
	(0.22)	(0.43)	(0.56)	(1.27)	(1.05)	(0.80)	(1.78)	(1.44)	(1.12)
20 weeks	0.9	2.2	3.5	9.8	7.2	8.5	21.9	16.1	19.0
	(0.38)	(0.39)	(0.52)	(1.06)	(1.02)	(0.77)	(1.93)	(1.85)	(1.47)
24 weeks	1.2	2.0	3.8	9.2	9.8	9.5	21.2	26.2	23.7
	(0.44)	(0.45)	(0.87)	(1.20)	(0.85)	(0.72)	(2.31)	(1.77)	(1.51)

(b) GIVEN AS 2 INJECTIONS

Sacrifice Time	Tumor Yield — Total Dose (mg/g) At Intervals of Two Days 0.125	0.250	0.500	At Intervals of Seven Days 0.250	0.500	1.000
8 weeks	0.0	0.8	2.7	1.8	3.0	6.7
	(0.00)	(0.25)	(0.82)	(0.36)	(0.61)	(1.04)
12 weeks	0.5	2.3	4.3	2.9	4.5	15.5
	(0.31)	(0.61)	(0.63)	(0.35)	(0.62)	(2.28)
16 weeks	1.3	3.7	5.2	3.1	7.0	18.8
	(0.37)	(0.54)	(0.92)	(0.59)	(1.03)	(2.28)
20 weeks	2.2	2.9	7.4	3.6	9.4	20.0
	(0.47)	(0.64)	(0.87)	(0.34)	(1.41)	(3.01)
24 weeks	1.7	3.9	8.7	3.9	10.1	22.7
	(0.47)	(0.65)	(0.93)	(0.43)	(1.29)	(2.31)

TABLE IV (Continued)

(c) GIVEN AS 4 INJECTIONS

Sacrifice Time	Tumor Yield					
	Total Dose (mg/g)					
	At Intervals of Two Days			At Intervals of Seven Days		
	0.250	0.500	1.000	0.500	1.000	2.000
8 weeks	0.7	2.3	5.8	1.6	3.3	13.3
	(0.42)	(1.02)	(1.49)	(0.45)	(0.75)	(2.23)
12 weeks	2.2	3.7	11.4	4.3	9.5	30.2
	(0.25)	(0.61)	(2.14)	(1.17)	(1.50)	(2.23)
16 weeks	3.0	5.1	15.3	6.0	11.3	39.6
	(0.58)	(0.46)	(1.60)	(1.01)	(0.83)	(2.20)
20 weeks	3.6	8.8	15.1	6.7	17.2	43.8
	(0.72)	(1.17)	(1.65)	(0.84)	(1.35)	(2.71)
24 weeks	4.7	10.9	17.7	7.0	16.9	47.2
	(0.68)	(0.98)	(2.20)	(4.04)	(1.64)	(2.70)

(d) GIVEN AS 8 INJECTIONS AT INTERVALS OF TWO DAYS

Sacrifice Time	Tumor Yield		
	Total Dose (mg/g)		
	0.500	1.000	2.000
8 weeks	1.8	5.1	14.1
	(0.51)	(0.69)	(1.11)
12 weeks	4.2	11.3	23.6
	(0.61)	(1.92)	(2.76)
16 weeks	3.7	12.4	28.3
	(0.70)	(1.04)	(1.61)
20 weeks	5.4	15.2	35.8
	(0.85)	(1.75)	(2.66)
24 weeks	8.1	17.3	38.0
	(0.87)	(1.54)	(1.09)

(e) GIVEN AS 16 INJECTIONS AT INTERVALS OF TWO DAYS

Sacrifice Time	Tumor Yield		
	Total Dose (mg/g)		
	1.000	2.000	4.000
8 weeks	1.3	8.0	23.9
	(0.52)	(1.88)	(2.95)
12 weeks	6.9	18.0	42.4
	(1.47)	(2.15)	(2.60)
16 weeks	9.8	27.5	54.7
	(1.32)	(2.19)	(4.60)
20 weeks	14.7	30.1	61.0
	(1.40)	(2.30)	(3.07)
24 weeks	13.6	33.3	64.0
	(1.40)	(1.62)	(3.90)

all animals receiving the same dose of urethane per gram of body weight at each injection may be considered one group differing only in the number of times this treatment is repeated and, to a limited extent, in the time interval between treatments; in table IV, all animals receiving the same number of injections may be considered one group differing only in the dose of urethane per gram of body weight given at each injection (and hence in total), and to a limited extent in the time interval between pairs of doses.

4. Discussion

This experiment was intended to be a pilot study, with the aim of locating those portions of the dose-effect curves which theoretically would be most sensitive to the effect of dose pattern if the Neyman model is correct. For this reason, the total number of mice that could be practically handled in the experiment was divided to cover a great range of dose patterns and sacrifice times, with the unfortunate consequence that there were undesirably small numbers in each group. This fact must be kept in mind with regard to all conclusions.

In the control groups, as can be seen in table V, there appear to be no signif-

TABLE V

NUMBER OF TUMORS IN CONTROLS,
0.01 ml DISTILLED WATER PER GRAM BODY WEIGHT PER INJECTION

Number of Injections	Tumor Yield							
	16	8	4	2	1	4	2	
Days Between Injections	2	2	2	2	—	7	7	Avg.
Sacrifice Time								
8 weeks	1.0 (0.28)	0.3 (0.22)	0.3 (0.15)	0.4 (0.22)	0.0 (0.00)	0.3 (0.33)	0.2 (0.15)	0.4
12 weeks	0.0 (0.00)	0.5 (0.22)	0.1 (0.10)	0.1 (0.10)	0.2 (0.14)	0.0 (0.0)	0.6 (0.30)	0.2
16 weeks	0.2 (0.13)	0.2 (0.20)	0.5 (0.24)	0.8 (0.49)	0.5 (0.22)	0.6 (0.34)	0.1 (0.10)	0.4
20 weeks	0.8 (0.33)	0.5 (0.31)	0.4 (0.16)	0.4 (0.22)	0.6 (0.30)	0.3 (0.15)	0.1 (0.18)	0.4
24 weeks	0.5 (0.31)	0.1 (0.10)	0.4 (0.22)	0.5 (0.27)	0.5 (0.34)	0.7 (0.26)	0.2 (0.13)	0.4
Average	0.5	0.3	0.3	0.4	0.4	0.4	0.2	0.4

icant differences in the numbers of tumors, regardless of the number or spacing of the injections of distilled water. It was therefore inferred that the solvent (distilled water) had no effect on the number of tumors, that is, that the few tumors which occurred in the controls were spontaneous ones. Since these averaged less than one half tumor per animal, the experimental results were not corrected for the occurrence of spontaneous tumors.

The effect of time in allowing tumors to develop to observable size is illustrated in figure 1. The particular set of doses shown here was selected because the curves were slightly more regular than for other doses; but, in general, the effect seen is an apparent tendency to approach asymptotically some maximum number of tumors that characterizes the effect of a given dose and dose pattern. For the smaller total doses, the curves suggest that the maximum is reached by the time of the last sacrifice period, 24 weeks after initial injection. For the larger doses, the curves continue to rise at the 24 weeks' point, suggesting that a longer interval might have been desirable. For example, the points corresponding to 16 doses in figure 1 are fitted by one branch of a hyperbola asymptotic to a tumor count of 69.9; the count reached in 24 weeks is 64.1. It appears reasonable to focus

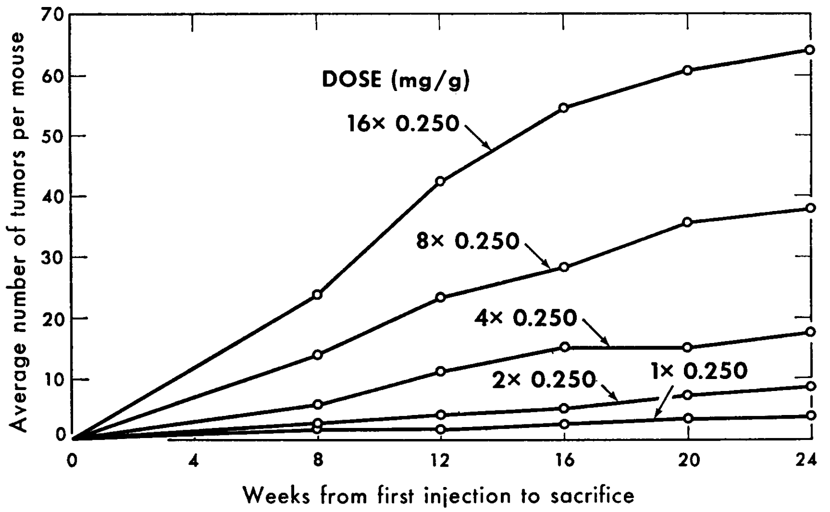

FIGURE 1

Effect of time on tumor development for various multiples of a dose of 0.250 mg/g body weight.

attention, therefore, on the data for the last sacrifice period when attempting to generalize with respect to the ultimate carcinogenic effect of the various modes of administration of urethane. It may be interesting also to speculate on any possible significance in the fact that the hyperbola which fits the 16 dose data intersects the axis of abscissas at about 6 days: May this mean that the first visible tumor occurs at about 1 week after injection?

The effect of varying the time interval between successive injections from two days to seven days is best studied by comparing the four dose patterns that were duplicated with only this time interval as a variable. Extracting these data for the last sacrifice period, we find the average numbers of tumors in table VI.

TABLE VI

EFFECT OF VARYING THE TIME INTERVAL BETWEEN SUCCESSIVE INJECTIONS
Data from last sacrifice period.

Total Dose mg/g	Dose Pattern mg/g	Time Interval	
		Two Days (SE)	Seven Days (SE)
0.25	2 × 0.125	3.9	3.9
0.5	2 × 0.250	8.7 (0.93)	10.1 (1.29)
0.5	4 × 0.125	10.9 (0.98)	7.0 (1.04)
1.0	5 × 0.250	17.7	16.9

In the first and last cases listed, no effect of the time interval is seen. At the 0.5 mg/g dose level, the observed differences are in opposite directions for subdivision into 2 doses and 4. On applying the two sided t test at the 0.01 significance level, neither of these differences appears significant. The four fraction case does appear to show a statistically significant difference at the level of $\alpha = 0.05$; but this is offset by the fact that the equally large experimental groups at each of the four other sacrifice times show differences in both directions (two each way) and of magnitudes that are not significant even when tested at higher values of alpha. Since we know of no plausible explanation for the reversal of direction of the observed differences, we consider this to be a chance variation and are of the opinion that the data for the two day interval and the seven day interval may be appropriately combined. The means of these groups have been used in plotting the dose-effect curves of figures 2 and 3, which are discussed later.

The effect of aging of the animals during the course of the longer series of injections is deemed to be negligible, on the basis of the results obtained from injection of single doses on "Day 0" as compared with "Day 30." Since this interval is approximately 4 weeks, equaling the interval between sacrifice dates, the magnitude of the effect of aging can be noted by inspecting the pairs of columns headed "Day 0" and "Day 30" in table IVa, comparing each line in the "Day 0" column with the line below in the "Day 30" column. In this way, the effect of the time required for tumor development is approximately eliminated. It is apparent that these pairs of values are nearly alike, indicating that the change of age of the animals during even the series of 16 injections probably has no significant effect on the tumor response.

There is a further indication that differences in age were not significant, once the animals had reached the stage of maturity corresponding to the minimum of our sample (8½ weeks). We examined the data for possible correlation between initial age of the animal and number of tumors in each experimental class and between weight of the animal and number of tumors. No correlation was evident with respect to either of these factors.

It may be noted that some of the experimental classes consisted of nine animals rather than the intended ten. In eight instances, randomly selected, only nine mice were assigned to the class because of the fact that eight mice died before the beginning of the experiment. In each of 11 classes, one mouse died during the experiment but before the scheduled sacrifice date. It was agreed in advance that such mice would be eliminated from consideration, even though the death might occur on the day before the assigned date, as happened in one instance.

Figure 2 shows the dose-effect curves for each of the degrees of fractionation tested, that is, for doses administered in 1, 2, 4, 8, or 16 portions. It will be noted that smooth curves of approximately parabolic or hyperbolic form can be plotted without very significant departures from the observed points. If these curves do indeed represent the phenomena occurring, then it appears that frac-

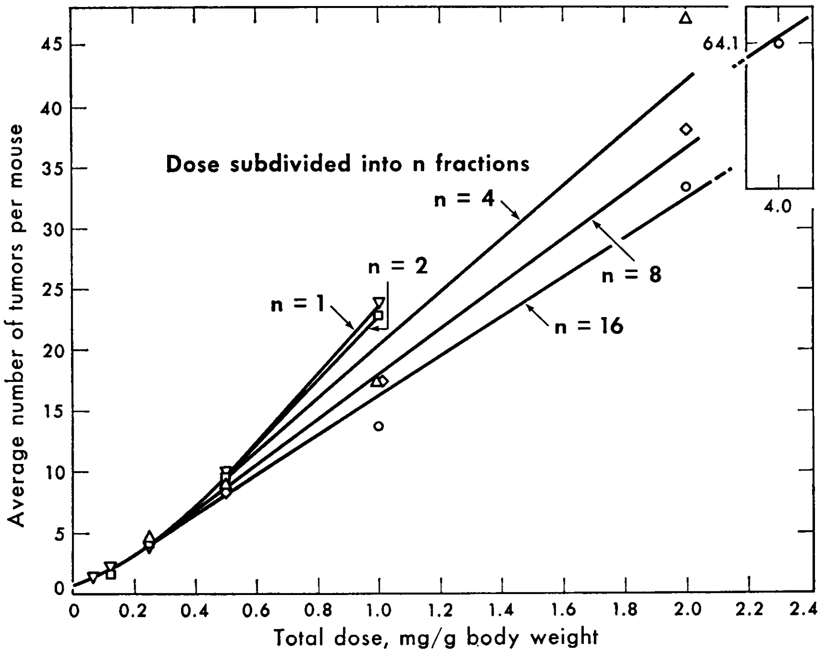

FIGURE 2

Dose-effect curves for tumor production with varying degrees of fractionation of dose.

tionation reduces the effectiveness of doses of 0.5 mg/g and larger but has little effect, if any, on smaller doses. Division into two fractions does little to diminish the effectiveness of doses in the range tested (that is, up to 1 mg/g); but further subdivision does cause fewer tumors to be induced.

Some of this effect is possibly due to the fact that, with the time of sacrifice specified as an interval from the start of injections, the animals that got 8 or 16 doses at two day intervals or 4 doses at seven day intervals were sacrificed at a time significantly closer to their last injection than were the animals that received their total dose within the first few days. It may be conjectured, therefore, that the later doses for the animals with prolonged schedules of injection had not had time to be fully effective before the animals were sacrificed. However, when 1.000 mg/g in 1 dose given at the end of the injection period is compared with 16 doses of 0.0625 mg/g, the single dose was nevertheless more effective than the fractionated dose. There is, therefore, some still unknown factor involved.

This factor may relate to the mechanism by which the mouse rids itself of urethane. If the induction of tumors depends on a second chemically induced carcinogenic event affecting cells altered by a first event, and if the second step may occur very soon after the first, the mechanism of detoxication determines the period in which urethane is present to induce the second event. Skipper, Bennett, Bryan, White, Newton, and Simpson [6] reported that the rate of hydrolysis of urethane decreases with time, whereas Kaye [7] reported finding that the catabolic system, when studied *in vitro*, is saturated at well below the levels normally occurring in mouse plasma following anesthetic doses of urethane and hence detoxifies at a constant rate. Kaye's *in vivo* experiments tend to support the studies *in vitro*. It is possible that liver efficiency is the limiting factor; that is, the liver may not be able to decompose a large dose as fast, proportionately, as it can a small dose. In that event, the tissues would be exposed not only to a higher concentration of urethane but also for a proportionately longer time when a large dose is given.

The results we obtained with 1 large dose and 16 small doses are in the same direction as those obtained by Gubareff and reported here by Shimkin for 1 large dose and 12 small ones, namely, that the single dose is more effective; but the decrease due to fractionation is not as pronounced in our experiment. This might be explained on the basis that their younger animals (approximately 4 weeks of age at the beginning of the experiment) were more susceptible to tumor production at the time when the large dose was given but became appreciably less susceptible by the time the 12th small dose was given. Our animals were at least 8½ weeks old when the experiment was begun, and therefore were past the stage of rapidly changing susceptibility to tumor production. We did not see the pronounced difference in the opposite direction which they obtained when they

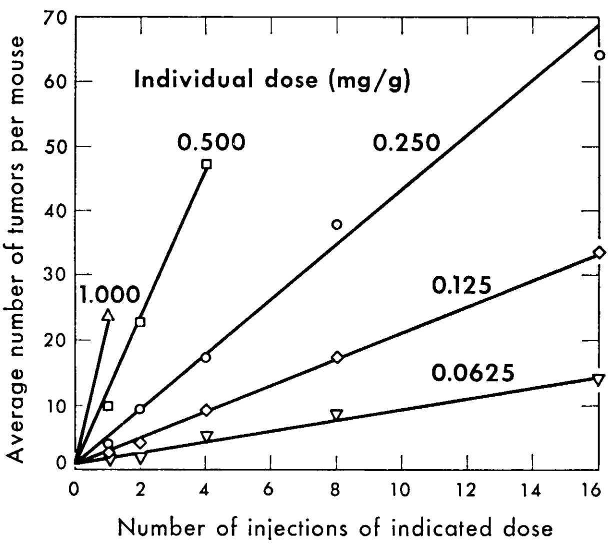

FIGURE 3

Relation of tumor yield
to number of injections of a given size of dose.

gave 0.500 mg/g twice at an interval of 6 days as compared to 1.000 mg/g in 1 dose. Either enhancement or decrease of tumor induction by fractionation is evidence against the one stage mutation theory of carcinogenesis which the more recent two stage model might replace.

There is a slight suggestion in the curves of figure 3 that such enhancement may occur with subdivision of dose. These graphs are straight lines, fitted by eye to the observed points, including the controls. Although no points are very significantly far from their respective lines, the points for a single dose tend to be slightly below the lines while the points for 8 doses tend to be above the lines. The differences are not significant in themselves but suggest a possible pattern of departure. It is proposed to pursue further the study of effects of time pattern on the dose-effect curves by testing other time intervals between doses.

◇ ◇ ◇ ◇ ◇

APPENDIX

Raw data on induction of lung tumors in mice by urethane. In the tables below (tables AI to AIV) giving data on individual mice, line A gives the dose per injection in mg/g body weight; line B, the number of injections given; line C, the total dose in mg/g; line D, the interval between injections in days, column E, the interval between the first injection and sacrifice in weeks; column F, the age in days on the date of the first injection; column G, the weight in grams on the date of the first injection; column H, the number of tumors in the lung. The asterisks indicate that the animal died before the sacrifice date. The — indicates that no animal was assigned.

TABLE AI

Data on Individual Mice—I

A	0.0625			0.0625			0.0625			0.0625			0.0625			0.125			0.125			0.125		
B	16			8			4			2			1			16			8			4		
C	1.000			0.500			0.250			0.125			0.0625			2.000			1.000			0.500		
D	2			2			2			2			—			2			2			2		
E	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H
8	71	21	0	72	21	0	72	19	0	70	26	0	71	21	1	73	20	6	73	23	2	72	19	0
	70	22	0	67	23	1	67	18	1	70	20	0	67	24	0	67	21	7	70	22	5	67	21	1
	67	20	1	67	21	3	67	20	4	67	21	0	67	22	0	67	24	18	66	22	6	66	20	0
	66	22	2	66	21	4	66	22	0	66	24	0	66	23	0	68	19	1	66	20	3	66	24	0
	65	23	0	65	18	3	65	20	0	65	21	0	65	26	2	65	20	5	65	22	5	65	18	5
	67	21	0	67	20	1	67	16	0	67	19	0	67	21	0	73	22	13	73	24	10	73	18	10
	67	17	4	67	21	0	67	18	0	67	20	0	67	19	0	67	21	16	67	20	6	67	20	0
	66	22	4	66	21	4	66	20	0	67	16	0	66	19	1	66	23	9	67	21	4	67	18	2
	66	18	0	61	18	2	66	21	0	66	19	0	66	23	1	66	19	1	66	22	6	66	21	4
	60	22	2	60	19	0	60	19	2	—	—	—	60	20	5	61	18	4	61	18	4	60	19	1
12	71	20	8	73	19	1	72	22	3	72	20	0	72	20	0	73	23	11	70	18	7	71	23	5
	70	21	3	70	21	2	70	22	3	67	18	0	70	19	0	70	23	11	70	24	9	67	24	2
	66	22	5	67	19	6	67	22	2	67	21	0	66	22	3	67	22	14	67	22	6	66	19	2
	66	24	5	66	20	3	66	21	3	66	21	3	66	22	0	66	23	10	66	22	3	66	21	6
	65	24	19	65	21	3	65	19	1	65	24	0	65	22	1	65	22	16	65	22	21	65	19	2
	67	19	3	73	19	5	67	18	2	73	23	0	67	21	0	67	22	22	73	23	14	73	25	5
	67	21	8	67	18	6	67	17	1	67	23	1	67	23	0	67	24	22	67	21	15	67	20	7
	67	19	5	67	23	5	67	22	3	66	20	1	66	20	1	66	21	27	67	22	11	66	22	2
	66	20	5	66	23	4	66	20	2	66	18	0	66	20	0	66	21	29	66	18	7	66	19	2
	60	23	8	60	21	7	60	18	2	60	19	0	60	19	2	60	20	18	60	20	20	60	17	4
16	72	24	11	71	22	2	72	21	2	71	21	3	70	19	0	72	25	22	72	16	8	71	22	4
	67	21	8	70	21	6	70	20	4	70	23	1	70	20	2	70	23	33	67	20	10	70	23	7
	66	27	9	66	24	7	67	20	2	66	22	2	67	19	1	67	19	28	66	19	11	66	20	5
	66	23	8	66	21	3	66	20	3	66	22	0	66	21	0	66	23	29	66	22	14	66	19	6
	65	23	6	65	21	4	65	19	0	65	22	3	65	21	1	65	21	14	65	20	8	65	21	6
	73	23	2	73	19	5	67	21	1	73	25	1	73	21	1	73	24	36	67	19	17	73	21	6
	67	20	12	67	22	0	67	24	4	67	22	1	67	18	0	67	20	30	67	16	13	67	22	5
	66	18	11	66	20	5	67	17	6	66	20	2	67	20	0	67	20	36	66	22	11	66	20	4
	66	20	16	66	20	1	66	21	3	66	21	0	66	21	0	66	19	22	61	19	16	66	21	6
	60	19	15	61	15	4	60	20	5	60	19	0	60	21	1	60	22	25	60	21	16	60	21	2
20	73	23	10	73	23	5	73	18	3	71	27	1	71	23	0	70	20	20	73	20	11	70	18	4
	70	21	12	67	19	1	70	24	2	70	19	1	70	19	0	67	22	21	70	23	11	70	22	5
	67	19	13	67	22	6	66	21	3	66	20	2	66	20	0	66	20	25	67	24	12	67	21	5
	66	20	18	66	22	8	66	19	5	66	21	3	66	24	2	66	19	36	66	22	14	66	19	6
	65	22	11	65	20	4	65	18	3	65	23	1	65	22	0	65	22	22	65	22	12	65	24	13
	73	23	17	73	20	10	73	24	9	67	19	1	67	22	0	73	20	36	67	19	8	67	19	8
	67	21	18	67	20	5	67	22	3	67	25	1	67	22	2	67	22	35	67	18	19	67	22	10
	66	24	19	67	19	2	67	24	5	67	20	3	66	22	2	67	25	39	66	21	22	66	17	12
	61	22	21	61	19	7	66	20	2	66	22	4	66	22	0	66	21	32	66	19	18	66	19	14
	60	19	26	60	20	6	61	20	1	60	23	5	60	20	3	60	18	35	60	20	25	60	20	11
24	72	23	6	72	22	7	72	24	7	71	24	1	72	24	4	72	22	27	71	20	9	72	20	9
	70	23	16	67	23	7	70	24	7	66	24	2	70	21	1	66	20	30	70	23	8	70	22	12
	66	24	13	67	22	6	66	21	3	66	21	2	67	22	0	66	21	35	67	23	20	67	22	10
	66	21	12	66	21	8	66	22	3	65	23	1	66	21	0	65	22	29	66	19	21	66	19	4
	65	21	12	65	23	12	65	20	3	67	21	1	65	23	2	67	19	32	65	19	16	65	22	10
	73	21	10	67	21	9	67	20	3	67	23	2	67	22	3	67	23	35	67	21	19	73	21	13
	67	21	20	67	20	12	67	19	6	66	21	0	67	23	0	66	16	39	67	21	21	67	24	10
	66	24	20	66	20	8	66	22	2	66	22	5	66	22	1	66	20	31	66	20	19	67	21	12
	66	20	11	61	21	4	66	19	5	61	19	1	66	23	0	60	18	42	66	19	21	66	22	15
	61	21	16	—	—	—	60	18	8	*	*	*	60	23	1	*	*	*	60	20	19	60	21	14

TABLE AII

DATA ON INDIVIDUAL MICE—II

A	0.125			0.125			0.250			0.250			0.250			0.250			0.250			Controls		
B	2			1			16			8			4			2			1			16		
C	0.250			0.125			4.000			2.000			1.000			0.500			0.250			0.000		
D	2			—			2			2			2			2			—			2		
E	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H
	73	25	0	71	20	0	73	22	23	71	16	20	73	23	11	71	23	3	72	21	4	72	21	1
	70	21	1	67	20	1	67	23	22	70	20	13	67	18	3	70	23	2	67	19	1	70	19	9
	66	19	2	67	22	0	66	22	39	66	16	11	66	23	16	67	21	3	67	23	4	67	21	0
	66	21	0	66	21	1	66	23	8	66	23	10	66	22	0	66	21	6	66	22	4	66	20	0
8	65	21	1	65	23	0	65	21	21	65	19	13	65	24	4	65	19	0	65	17	1	65	24	0
	73	16	1	73	19	2	67	18	22	67	16	20	73	23	2	67	23	3	67	22	0	73	21	0
	67	20	1	67	21	1	67	18	14	67	20	12	67	19	5	67	24	0	67	20	3	67	18	0
	66	19	2	66	24	0	66	21	37	67	20	14	67	21	4	66	22	8	67	22	1	66	22	0
	66	23	0	61	19	3	66	19	26	61	18	16	66	18	5	66	20	1	66	23	1	66	20	0
	60	21	0	60	21	0	61	19	27	60	18	12	60	22	8	60	20	1	60	21	2	60	20	0
	73	23	1	73	20	0	71	21	48	71	20	18	71	18	3	70	22	3	72	15	1	72	21	0
	67	19	1	67	23	1	67	16	35	70	21	26	66	11	9	67	20	0	70	21	2	70	22	0
	67	20	2	66	22	1	66	22	46	66	20	10	66	21	4	66	16	3	66	23	5	67	20	0
	66	21	7	66	23	7	66	21	32	66	20	23	65	20	9	66	23	5	66	22	2	66	23	0
12	65	20	1	65	21	1	65	23	48	65	18	36	67	23	24	65	22	6	65	23	0	65	23	0
	67	23	0	73	18	1	67	21	55	73	24	30	67	19	12	67	19	7	73	21	1	67	20	0
	67	23	3	67	20	1	67	22	48	67	19	10	66	23	17	67	23	4	67	20	4	67	24	0
	66	22	3	66	20	1	66	20	41	67	21	31	66	18	13	67	18	5	66	20	2	66	18	0
	66	22	3	66	22	0	66	21	42	66	19	23	61	20	12	66	24	6	66	16	0	61	20	0
	60	19	2	*	*	*	61	21	29	60	20	29	*	*	*	60	20	4	61	20	2	60	18	0
	72	21	6	72	21	0	71	19	47	76	22	28	73	23	21	72	20	1	71	24	4	73	19	0
	70	24	6	70	24	2	70	20	62	70	22	23	70	22	11	67	21	7	67	20	0	67	19	1
	67	21	4	66	17	1	67	21	54	66	23	28	66	23	14	67	20	4	66	23	4	67	22	1
	66	21	3	66	22	0	66	20	57	66	20	20	66	18	8	66	19	4	66	19	6	66	22	0
16	65	17	1	65	21	2	65	22	51	65	22	36	65	18	9	65	23	7	67	20	1	65	21	0
	67	20	4	73	20	0	73	20	84	67	20	28	67	20	20	67	24	5	67	17	3	73	23	0
	67	24	5	67	19	1	67	18	42	67	22	29	67	19	15	67	21	4	67	19	2	67	20	0
	67	22	4	67	18	2	66	20	36	66	20	32	66	20	23	66	22	7	66	20	3	66	19	0
	66	21	2	61	19	4	66	24	59	66	21	24	66	20	17	66	20	2	66	21	1	66	21	0
	61	19	2	61	16	3	—	—	—	61	18	35	60	20	15	60	15	11	60	21	2	60	20	0
	72	21	5	71	24	1	71	22	57	73	18	21	72	21	12	70	19	5	71	20	2	72	20	1
	70	19	2	67	19	3	70	18	61	70	23	30	67	23	10	70	19	8	70	20	1	70	20	0
	66	21	2	67	21	3	67	17	57	66	21	37	67	25	12	67	20	4	66	22	3	66	21	3
	66	20	3	66	21	1	66	20	49	65	18	37	66	19	11	66	20	10	66	16	5	66	22	1
20	65	23	1	65	21	4	65	22	61	73	21	41	65	23	19	65	20	6	65	25	5	65	23	0
	67	16	0	67	21	4	73	22	60	67	23	48	73	20	15	67	22	10	67	21	6	73	23	2
	67	18	1	67	20	2	67	23	78	67	18	31	67	19	12	67	22	3	67	19	3	67	21	1
	66	20	6	67	15	1	66	20	53	66	19	43	67	21	13	67	20	11	66	18	5	67	18	0
	61	20	4	66	18	1	66	17	78	60	20	34	66	18	21	66	16	8	66	20	2	66	20	0
	61	19	5	60	17	2	60	22	56	—	—	—	60	18	26	60	20	9	61	20	3	60	19	0
	72	17	5	73	22	3	73	20	72	72	20	32	73	18	11	73	19	7	73	24	5	71	21	0
	67	23	5	70	22	1	67	20	44	67	20	38	70	15	15	70	20	6	67	21	1	67	19	3
	66	23	3	67	22	2	67	21	69	67	21	41	66	20	14	66	19	12	67	19	3	67	22	0
	66	20	3	66	23	0	66	18	53	66	19	41	65	22	16	66	24	3	66	20	3	66	21	1
24	65	21	1	65	16	3	73	22	78	65	24	35	67	24	28	65	20	8	65	19	2	65	22	1
	67	19	4	67	23	5	67	17	55	67	21	37	67	17	14	67	20	10	67	18	11	73	22	0
	67	20	8	67	22	2	67	19	60	67	20	36	66	20	16	67	21	9	67	20	3	67	20	0
	66	20	3	66	19	2	66	22	69	66	21	41	66	18	15	67	18	8	67	20	4	66	23	0
	66	17	3	66	19	1	61	18	77	60	17	41	60	20	30	66	22	12	66	22	3	66	21	0
	—	—	—	61	20	1	*	*	*	*	*	*	*	*	*	60	20	12	60	19	3	61	19	0

TABLE AIII

DATA ON INDIVIDUAL MICE—III

A	Controls			Controls			Controls			Controls			0.125			0.125			0.250			0.250		
B	8			4			2			1			4			2			4			2		
C	0.000			0.000			0.000			0.000			0.500			0.250			1.000			0.500		
D	2			2			2			—			7			7			7			7		
E	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H
	73	19	2	71	20	1	73	22	0	72	22	0	75	19	2	73	20	1	75	21	3	73	23	6
	70	24	0	70	18	0	70	19	0	70	23	0	72	21	4	72	24	1	69	21	2	69	19	0
	66	24	0	67	23	0	66	25	0	66	21	0	69	20	0	68	21	4	69	22	4	69	20	4
	66	23	0	66	19	0	66	24	2	66	19	0	68	22	0	68	21	1	68	21	4	68	20	2
8	65	20	0	65	23	0	65	22	0	65	20	0	67	19	1	67	23	0	67	24	8	67	21	3
	67	16	0	73	19	1	67	16	0	67	19	0	68	21	2	74	23	2	74	18	0	68	21	3
	67	19	0	67	17	0	67	20	1	67	22	0	68	20	3	68	21	2	68	21	2	68	20	6
	66	18	0	66	21	0	67	19	0	67	21	0	67	22	0	67	21	3	68	19	3	68	20	2
	66	18	1	66	22	1	66	19	0	66	21	0	67	18	3	67	20	2	67	20	1	67	17	1
	60	19	0	61	20	0	60	23	1	60	21	0	61	24	1	61	22	2	61	16	6	62	20	3
	70	21	0	71	23	1	72	21	0	71	23	0	75	21	9	73	20	2	74	21	3	74	22	8
	70	24	0	67	21	0	70	19	1	67	21	0	69	18	9	69	20	2	72	22	14	69	20	5
	66	19	1	67	22	0	66	18	0	66	21	0	69	21	1	69	21	2	68	23	12	68	22	3
	66	23	1	66	20	0	66	23	0	66	21	0	68	17	0	68	21	2	68	20	4	68	22	5
12	65	22	0	65	20	0	65	20	0	65	17	2	67	21	3	67	21	3	67	23	5	67	22	4
	73	22	1	73	22	0	73	25	0	73	26	0	68	19	1	74	24	3	74	21	10	74	20	4
	67	20	0	67	20	0	67	22	0	67	20	0	68	19	3	68	23	4	68	21	13	68	22	7
	66	20	2	66	22	0	66	19	0	66	18	0	67	20	4	67	21	2	68	21	5	67	19	5
	66	20	0	66	20	0	61	19	0	66	20	0	67	19	3	67	23	4	67	19	15	67	21	2
	60	20	0	60	21	0	61	21	0	60	19	0	61	21	10	61	22	5	61	18	14	62	21	2
	73	21	0	72	23	0	72	21	0	71	20	0	74	21	4	73	21	1	73	22	12	73	22	9
	70	23	0	67	16	2	70	22	1	70	22	1	72	20	4	72	20	2	72	22	16	69	25	1
	67	14	0	66	19	0	67	23	1	66	23	0	69	20	3	68	22	7	68	20	8	68	18	8
	66	20	0	66	20	1	66	21	1	66	23	2	68	23	8	68	23	3	68	22	11	68	23	13
16	65	19	0	65	23	1	65	21	0	65	21	0	67	20	3	67	21	4	67	20	12	67	22	6
	67	22	0	73	23	1	73	22	5	73	25	0	68	20	8	74	18	3	68	20	15	74	20	6
	67	20	2	67	20	0	67	22	0	67	23	0	68	20	8	68	18	5	68	19	10	68	20	10
	67	19	0	67	20	0	66	20	0	66	23	1	67	21	5	67	22	2	67	22	10	67	20	5
	66	21	0	66	20	0	66	18	0	66	21	0	67	21	4	67	23	1	62	17	11	62	19	7
	60	19	0	—	—	—	60	18	0	60	20	1	62	22	13	61	19	3	61	15	8	61	17	5
	73	22	1	71	18	0	73	24	0	71	20	0	75	19	4	74	22	3	72	22	17	75	21	4
	67	17	0	67	22	0	67	18	0	67	17	0	72	21	10	69	22	2	72	24	22	69	18	5
	67	20	0	67	22	0	66	21	1	66	22	0	68	22	10	69	21	3	68	19	10	69	19	8
	66	18	0	66	23	0	66	23	1	66	19	2	68	20	3	68	20	4	68	24	25	68	24	10
20	65	23	0	65	21	1	65	22	0	65	20	0	65	22	8	67	20	2	67	23	18	67	21	5
	73	19	0	73	21	0	73	20	0	73	18	0	74	20	5	74	20	4	68	18	17	74	19	9
	67	22	0	67	21	1	67	23	0	67	19	2	68	20	5	68	22	4	68	20	15	68	22	19
	66	21	0	66	20	0	66	22	0	66	20	0	67	21	10	67	21	4	66	19	16	68	21	13
	66	20	1	66	18	1	66	17	0	66	23	0	67	22	7	67	21	5	67	18	13	67	20	11
	60	16	3	60	18	1	60	19	2	60	19	2	61	18	5	61	20	5	62	20	19	61	21	10
	73	22	1	72	22	2	71	17	1	73	19	0	73	20	3	74	22	6	75	23	12	75	23	7
	67	21	0	67	20	1	67	24	0	67	19	0	72	22	8	72	21	4	72	21	16	69	21	7
	66	23	0	67	20	0	66	19	2	67	20	3	69	20	11	68	20	3	69	20	16	68	20	10
	66	22	0	66	19	0	66	20	0	66	19	0	68	22	13	68	23	5	68	17	29	68	20	5
24	65	24	0	65	24	0	65	24	0	65	22	0	67	19	6	67	23	5	68	20	16	67	23	10
	73	19	0	73	17	0	73	23	0	67	20	0	68	20	8	74	21	2	74	22	17	68	19	11
	67	22	0	67	21	0	67	20	2	67	19	0	68	19	2	68	22	4	68	22	17	68	20	14
	66	20	0	66	21	1	66	21	0	67	20	0	68	21	6	68	20	3	67	19	9	67	19	6
	66	19	0	66	19	0	66	18	0	66	20	0	67	22	6	67	23	2	67	20	19	67	16	18
	60	20	0	60	20	0	60	21	0	60	18	2	62	19	7	61	18	5	61	20	18	61	19	13

TABLE AIV

DATA ON INDIVIDUAL MICE—IV

A	0.500			0.500			Controls			Controls			0.500			0.500			1.000			1.000		
B	4			2			4			2			1 (Day 0)			1 (Day 30)			1 (Day 0)			1 (Day 30)		
C	2.000			1.000			0.000			0.000			0.500			0.500			1.000			1.000		
D	7			7			7			7			—			—			—			—		
E	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H	F	G	H
	75	22	8	73	24	4	74	21	0	75	20	0	73	21	3	102	23	0	73	20	8	102	23	0
	72	21	29	69	19	10	72	23	0	69	22	0	67	20	10	101	23	1	67	19	11	98	21	2
	69	21	10	69	22	7	68	25	3	68	20	0	66	22	5	97	23	1	66	19	11	97	26	0
	68	22	14	68	20	5	68	20	0	68	20	0	66	22	0	97	25	0	66	20	6	97	26	0
	67	22	10	67	20	4	67	18	0	67	19	1	65	22	3	96	27	0	65	22	6	96	26	2
8	74	20	14	74	24	8	74	23	0	74	21	0	68	22	3	103	25	1	74	20	5	97	21	0
	68	21	4	68	22	3	68	22	0	68	20	0	68	20	2	97	25	0	68	21	8	97	25	3
	67	22	21	68	18	6	68	18	0	67	21	1	67	17	2	96	19	0	67	20	8	96	24	0
	67	24	12	67	22	6	67	21	0	67	21	0	67	20	2	96	20	0	67	20	8	91	25	0
	62	21	11	61	18	14	61	20	0	—	—	—	61	18	8	90	24	0	61	19	19	90	22	1
	73	24	42	73	18	10	75	22	0	74	21	0	71	21	6	104	23	3	72	25	13	104	23	11
	72	21	41	67	20	11	69	20	0	72	17	1	67	21	3	98	24	4	67	20	13	101	22	9
	69	22	27	68	22	21	68	20	0	69	20	0	67	20	7	97	27	0	66	22	11	97	24	19
	68	20	28	68	20	9	68	22	0	68	20	0	66	22	2	97	26	5	65	21	20	97	24	13
12	67	24	36	67	20	18	67	19	0	67	22	3	65	21	6	96	23	1	74	19	15	96	26	4
	68	16	25	68	19	20	68	22	0	74	22	1	68	18	6	97	27	3	68	22	19	97	19	6
	68	23	30	68	21	29	68	19	0	68	21	0	68	22	5	97	26	1	67	20	12	97	26	11
	67	21	22	68	18	15	68	22	0	67	21	0	67	20	5	96	19	1	67	21	20	96	24	5
	67	20	27	67	19	18	67	18	0	67	21	1	67	17	4	96	28	3	62	24	17	91	25	10
	61	20	24	61	18	4	71	20	0	61	19	0	62	20	7	90	21	4	*	*	*	90	22	7
	74	20	31	74	21	16	75	22	0	75	21	0	71	21	11	102	26	6	72	22	17	102	25	21
	69	21	35	69	24	21	69	20	0	72	20	0	70	21	4	101	25	3	67	22	10	101	25	14
	68	20	36	69	22	22	68	19	0	68	21	0	66	20	4	97	23	6	66	22	11	98	22	11
	67	22	36	68	21	18	68	20	0	68	20	1	66	20	5	97	22	5	66	21	16	97	23	12
16	68	22	51	67	25	24	67	21	0	67	19	0	65	20	3	96	25	13	65	19	11	96	22	9
	68	19	44	68	20	20	68	20	2	74	24	0	74	20	4	97	23	4	74	20	12	97	27	10
	68	20	35	68	20	32	68	21	3	68	20	0	68	21	5	97	23	1	68	21	14	97	25	19
	62	20	47	67	18	6	68	18	0	68	21	0	67	19	4	96	25	5	67	21	16	96	25	18
	61	20	41	67	20	19	62	23	0	67	17	0	67	20	14	96	23	2	62	18	22	96	22	16
	*	*	*	61	18	10	61	19	1	61	18	0	—	—	—	90	23	7	61	18	28	91	20	21
	72	24	49	72	22	24	75	25	0	73	19	0	73	21	12	102	25	12	71	24	24	103	24	18
	72	23	56	72	23	16	69	22	1	69	21	0	70	21	9	101	23	11	70	21	17	101	23	21
	68	19	53	69	22	32	69	23	0	69	20	0	67	20	9	98	27	7	66	20	19	97	20	20
	68	19	46	68	20	20	68	20	0	68	19	0	66	20	7	97	25	8	66	21	20	96	25	7
20	67	21	36	67	18	23	67	19	0	67	20	0	65	21	10	96	25	7	65	23	25	103	26	11
	74	20	37	74	19	13	74	18	0	68	22	1	68	21	10	103	28	6	74	21	36	97	24	22
	68	22	45	68	18	2	68	21	0	68	23	0	68	21	11	97	25	6	68	19	16	96	18	9
	68	23	50	68	18	33	67	20	1	67	19	0	68	22	16	96	25	8	67	21	24	96	25	18
	67	22	30	62	16	12	67	18	1	67	17	0	67	20	3	96	26	0	67	20	23	90	24	19
	61	14	36	62	22	25	61	23	0	61	19	0	61	20	11	90	26	7	61	17	15	*	*	*
	74	23	44	74	21	16	74	23	1	75	19	0	73	22	6	103	22	11	73	22	18	104	21	18
	69	23	46	72	21	24	72	20	0	72	22	1	67	19	14	101	25	9	70	24	16	101	25	25
	68	22	41	69	19	19	69	21	1	68	20	0	67	22	8	98	24	12	66	20	21	97	25	28
	68	22	55	68	20	14	68	23	0	68	17	0	66	21	11	97	24	4	66	23	11	97	19	17
24	67	21	50	68	22	16	67	21	2	67	19	0	65	23	15	96	22	14	65	22	28	96	26	28
	74	20	32	74	19	35	68	18	2	74	23	0	74	22	10	97	23	9	74	19	21	103	26	34
	68	25	47	68	22	30	68	20	0	68	21	0	68	21	11	97	25	11	68	20	25	97	27	33
	68	21	54	67	19	31	67	21	1	67	20	0	67	22	4	97	27	8	67	20	34	96	25	26
	67	22	62	67	18	24	67	19	0	62	19	0	67	20	9	96	24	11	62	19	17	96	23	29
	61	19	41	61	18	18	61	17	0	61	19	1	61	20	4	90	23	9	*	*	*	90	23	24

REFERENCES

[1] A. Nettleship, P. S. Henshaw, and H. L. Meyer, "Induction of pulmonary tumors in mice with ethyl carbamate (urethane)," *J. Natn. Cancer Inst.*, Vol. 4 (1943), pp. 309–319.

[2] J. Neyman and E. L. Scott, "A two-stage mutation theory of carcinogenesis," *Bull. Inst. Internat. Statist.*, Vol. 38 (1961), pp. 193–200.

[3] P. S. Henshaw and H. L. Meyer, "Minimal number of anesthetic treatments with urethane required to induce pulmonary tumors," *J. Natn. Cancer Inst.*, Vol. 4 (1944), pp. 523–525.

[4] S. Rogers, "Age of the host and other factors affecting the production with urethane of pulmonary adenomas in mice," *J. Exp. Med.*, Vol. 93 (1951), pp. 427–449.

[5] M. B. Shimkin, R. Wieder, D. Marzi, N. Gubareff, and V. Suntzeff, "Lung tumors in mice receiving different schedules of urethane," *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability*, Berkeley and Los Angeles, University of California Press, 1967, Vol. 4, pp. 707–719.

[6] H. E. Skipper, L. L. Bennett, Jr., C. E. Bryan, L. White, Jr., M. A. Newton, and L. Simpson, "Carbamates in the chemotherapy of leukemia. VIII. Over-all tracer studies on carbonyl-labeled urethan, methylene-labeled urethan, and methylene-labeled ethyl alcohol," *Cancer Res.*, Vol. 11 (1951), pp. 46–51.

[7] A. M. Kaye, "A study of the relationship between the rate of ethyl carbamate (urethan) catabolism and urethan carcinogenesis," *Cancer*, Vol. 20 (1960), pp. 237–241.