EFFECTS OF HALOTHANE ON LIFE EXPECTANCY OF MICE

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THE EFFECTS of halothane in animals and humans have been studied in a variety of ways. Practically all investigations have been directed toward quantitation of the acute effects of the drug.

Recently, because of the alleged hepatotoxic properties of halothane, some attempts have been made to investigate the possibility that this drug might produce changes which are either not reversible or very slowly reversible after withdrawal of the drug. For instance, serum transaminase levels have been recorded for as long as two weeks following administration of halothane.[1]

It is worth while to note that if it is true that a drug induces changes which are either not reversible or only slowly reversible, then, by the stochastic theory of mortality,[2, 3] some interesting predictions become possible. In effect it can be forecast that if, following cessation of administration of the drug, functional changes have been induced such that a deviation from a "mean physiologic state"[2] has occurred, then the value for the force of mortality should also change for as long as the after-effects of the drug are present.

It should be obvious that at the present state of knowledge it is not possible to predict a positive or negative effect on mortality rate. Also, in this context, it is probably irrelevant whether the alleged after-effects of the drug are the results of protoplasmic or molecular changes or the by-product of the retention within the cell of traces of the drug not demonstrable by conventional assay methods.

This study was undertaken because the question of halothane toxicity had not been previously approached in this context, and because the development of a methodology capable of demonstrating these phenomena was thought to be needed.

MATERIAL AND METHODS

A batch of 500 white mice, half of them males and half of them females, and all of them born within a 24-hour period, were purchased from a biological supply house. The sexes were separated before puberty and no admixture of the sexes was allowed at any time thereafter.

At the age of ten weeks they were housed in small cages each containing eight animals. When they were twelve weeks old, four groups of 96 mice each were created by selecting for each group six cages of females and six cages of males. At this time each cage was numbered, and the animals within each cage were identified by means of ear punches. A file for each animal in the form of a coded punch card was initiated. The extra animals were destroyed at this time.

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Beginning when they were thirteen weeks old and continuing through the eighteenth week, three groups of mice, as defined above, were anesthetized according to the following routine. All cages belonging to a given group were moved from the cage rack to a flat counter surface. A large clear Plexiglas hood was used to cover all of the cages, and a mixture of halothane in oxygen was delivered by means of an oxygen gauge and a standard Fluotec.* A constant positive pressure (3.0 cm. H_2O) was maintained underneath the hood by means of a water manometer and an escape valve. Anesthesia was continued for three consecutive hours, three times a week on alternate days. One group of mice was subjected to a concentration of halothane of 0.2 per cent, one group to a 0.8 per cent concentration, and one group to a 1.8 per cent concentration.†

The control group was left undisturbed in the cages but for the daily inspection, as indicated below.

Beginning on the day the mice were twelve weeks old and continuing through to the last day of their forty-eighth week, all cages were inspected daily and all dead animals removed. With its death each animal's file card was completed.

At the end of the forty-eighth week, the remaining animals were sacrificed and the experiment terminated. With the exception of the animals sacrificed at this time, no other sacrifices were carried out at any time.

For each animal the following information was entered in the punch card: serial number, experimental group, sex, cage number, identifying ear punches, pathologic features of the liver and lungs, and an appropriate reference as to whether or not the animal had died during anaesthesia.

Results

The duration of life of each animal, expressed in weeks, can be determined from Table I and used to form four life tables illustrated in the subsequent figures.

Because of the fact that dead animals were often promptly eaten up by the survivors in each cage, it is not possible to report the macroscopic or microscopic pathologic findings in more than 50 per cent of the animals.‡

Discussion

Graphic representation of the data contained in the life tables points up two peculiar features.

In Figure 1 the number of animals alive at the end of each week is given by values on the vertical axis, and elapsed time values are given on the horizontal

*The same gauge, the same setting, and the same Fluotec were used for all procedures in this experiment.

†This makes reference to the dial setting of the Fluotec vaporizer. A 10.0 litre/minute flow was employed in all cases. The experiments were carried out in airconditioned quarters where humidity and temperature were kept constant.

‡Most commonly the remains consisted of a badly chewed-up skeleton, although on some occasions only the tail and the skull were to be found. In these cases identification of the dead animal was done by a process of exclusion, after identifying the survivors in the cage.

TABLE I

MORTALITY TABLE

Indicating the number of mice dying during each week and the number of mice alive in each group at the end of each week

Weeks of study	Controls		Halothane					
			0.2%		.8%		1.8%	
	Died	Number surviving	Died	Surviving	Died	Surviving	Died	Surviving
12	0	96	0	96	0	96	0	96
13†	0	96	0	96	2	94	19	77
14†	0	96	0	96	1	93	11	66
15†	0	96	0	96	2	91	8	58
16†	0	96	0	96	2	89	5	53
17†	1	95	2	94	0	89	2	51
18†	0	95	0	94	0	89	6	45
19	0	95	0	94	5	84	0	45
20	1	94	1	93	2	82	0	45
21	2	92	1	92	1	81	0	45
22	4	88	1	91	2	79	1	44
23	0	88	1	90	2	77	0	44
24	0	88	1	89	1	76	0	44
25	0	88	3	86	0	76	0	44
26	2	86	1	85	0	76	0	44
27	3	83	5	80	0	76	0	44
28	4	79	13	67	1	75	3	41
29	0	79	0	67	15	60	5	36
30	1	78	4	63	9	51	2	34
31	16	62	0	63	3	48	5	29
32	6	56	0	63	2	46	1	28
33	8	48	10	53	5	41	0	28
34	8	40	13	40	0	41	4	24
35	2	38	1	39	1	40	0	24
36	5	33	1	38	2	38	3	21
37	1	32	3	35	3	35	6	15
38	2	30	1	34	1	34	0	15
39	0	30	0	34	1	33	1	14
40	3	27	0	34	0	33	0	14
41	5	22	0	34	3	30	0	14
42	0	22	0	34	3	27	0	14
43	1	21	0	34	2	25	0	14
44	0	21	0	34	0	25	0	14
45	2	19	0	34	0	25	1	13
46	1	18	1	33	0	25	1	12
47	0	18	3	30	5	20	0	12
48	18*	0	30*	0	20*	0	12*	0

*Sacrificed.
†Anaesthesia administered during this week.

axis. Studying the course of events for those weeks during which halothane was being administered, one sees that there is no noticeable difference between those animals exposed to 0.2 per cent halothane and the controls. However, as the concentration of halothane increases to 0.8 per cent and 1.8 per cent, the mortality rate increases as well. Thus it would appear that the immediate lethality of the drug is directly related to its concentration.

Examining the events occurring during the first few weeks following exposure to halothane, one notices some different patterns. Again, there does not seem to be any difference in the pattern of survival of those animals that inhaled 0.2 per cent halothane and the controls. However, the mortality rate of those animals that inhaled 1.8 per cent halothane is nearly zero and the mortality rate noted in

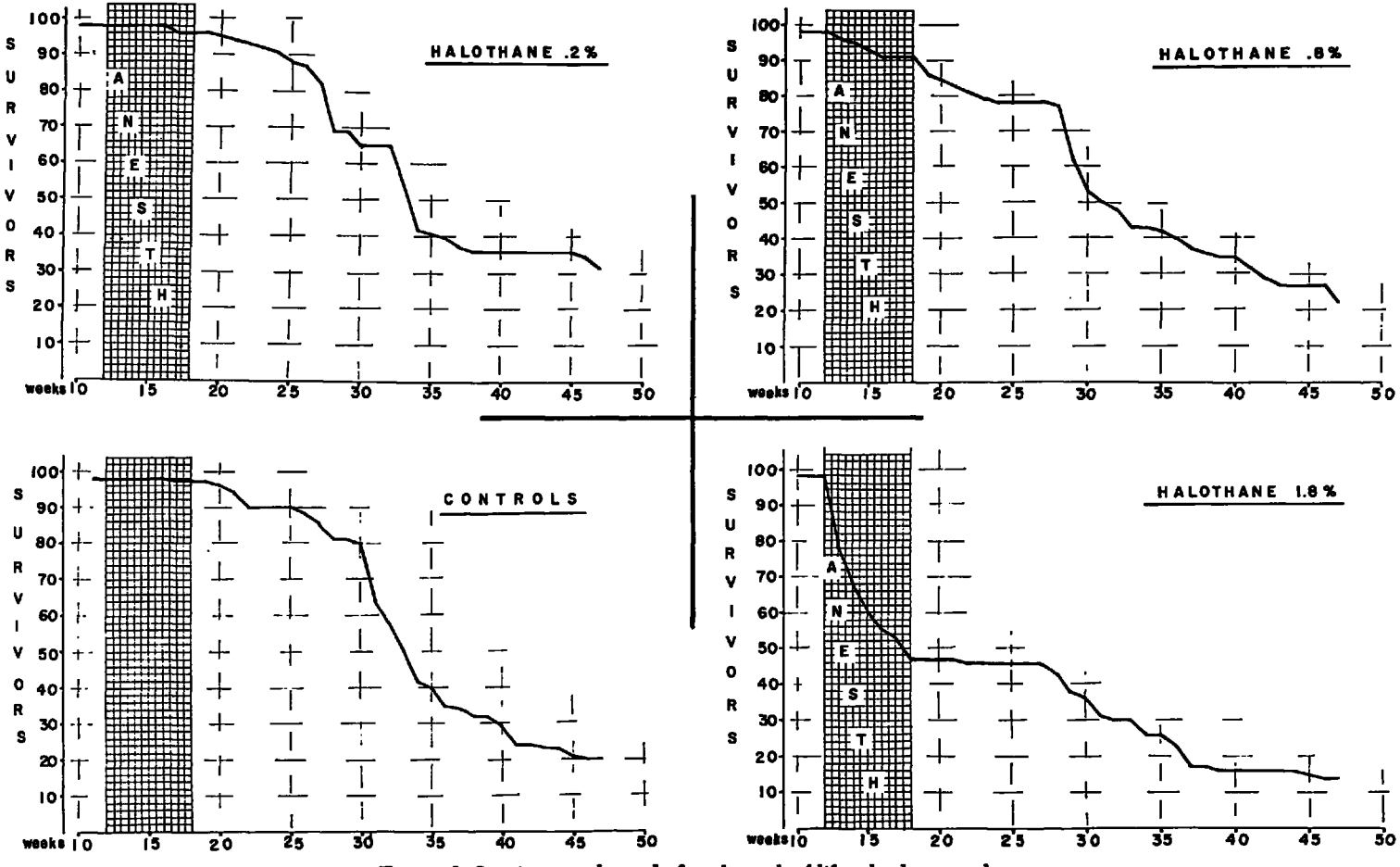

FIGURE 1. Survivors at the end of each week of life, absolute numbers.

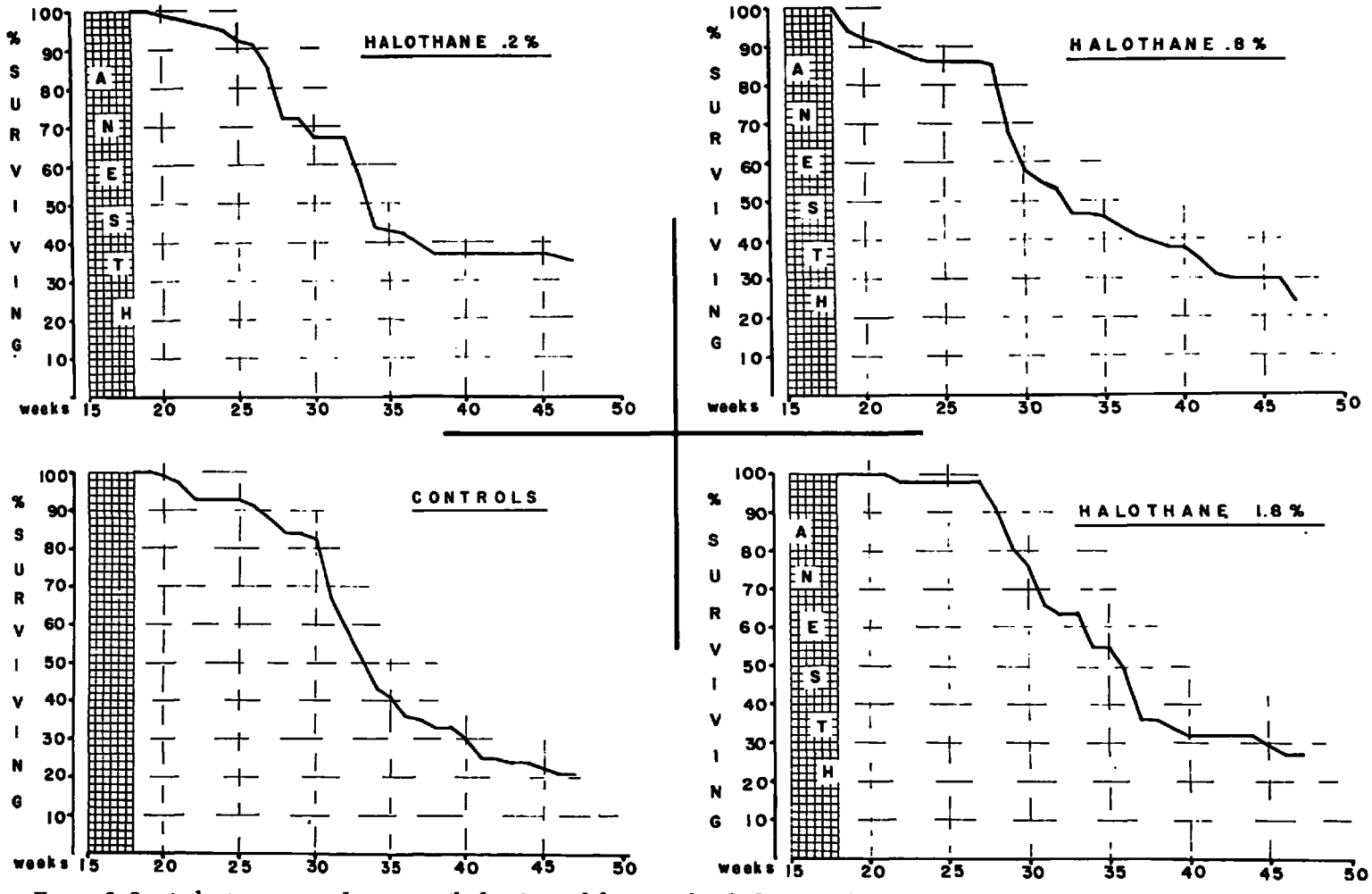

FIGURE 2. Survival rates, expressed as percentile fractions, of the animals which survived the six weeks during which anaesthesia was administered. Note the early sharp drop in survivors among the group which inhaled 0.8 per cent halothane.

those animals exposed to 0.8 per cent halothane is greater than in any other group (Fig. 1). One might conclude that in the former group the high concentration of the drug disposes immediately of all but the hardiest subjects, while in the latter group, even though the concentration is not high enough to dispose immediately of the weakest subjects, it is strong enough to reduce for some time thereafter their resistance to other causes of death.

Another interesting feature which can be noted in Figure 1 is the sharp increase in the mortality rate noted in all groups at about the thirtieth week of life. (This appears to occur somewhat earlier in the group of animals subjected to halothane at the 0.8 per cent concentration level.) If the data are now rearranged so that survival is expressed as percentage of the animals alive at the end of the six-week experimental period (Fig. 2), one notes that the result of this natural process is approximately the same in all groups, i.e., of those animals that survived the experimental period, a fraction (between 45 and 55%) similar in all groups were alive at the thirty-third week of life.

Since this phenomenon does not appear to be related to previous exposure to halothane at any concentration, or even to non-exposure to the drug, one may speculate that this is a natural cut-off point in the life expectancy of mice living under our laboratory conditions. This being granted, albeit not proven, one may then wonder whether or not any halothane effect persists beyond this "natural" cut-off point, which also happens to be very close to the median life expectancy in the control group (223 days or 31.8 weeks).

Accordingly, the data were rearranged in such a manner that the number of mice being alive in each group on the 223rd day was taken to be the total population for that group (100%) and a survival curve constructed for each group from that time on. The results of this procedure are shown in Figure 3, and there does not appear to be any gross difference between the survival patterns in the various groups. Thus it would appear that if an animal survived at least as long as the median survivor in the control group, his chances for further survival were not influenced by previous exposure to halothane.

With reference to the predictions made on the basis of the previously mentioned stochastic theory of mortality, the conclusion appears justified that fluothane affects the internal balance (configuration of Sacher and Trucco[2]) of the organism in such a way that it alters its mean physiologic state, and thereby modifies the force of mortality, even at a time when the drug is not being administered.

As an outgrowth of these observations, one must conclude that occasionally untoward effects of some drugs may not be measurable in terms either of immediate death or of immediate functional changes or "toxicity."

Summary

An investigation of the effects of repeated halothane administration to mice during the early period of their adult life has shown that at high concentrations the drug is immediately lethal to a significant number, and that at very low concentrations it has no significant immediate lethal effects. In the intermediate

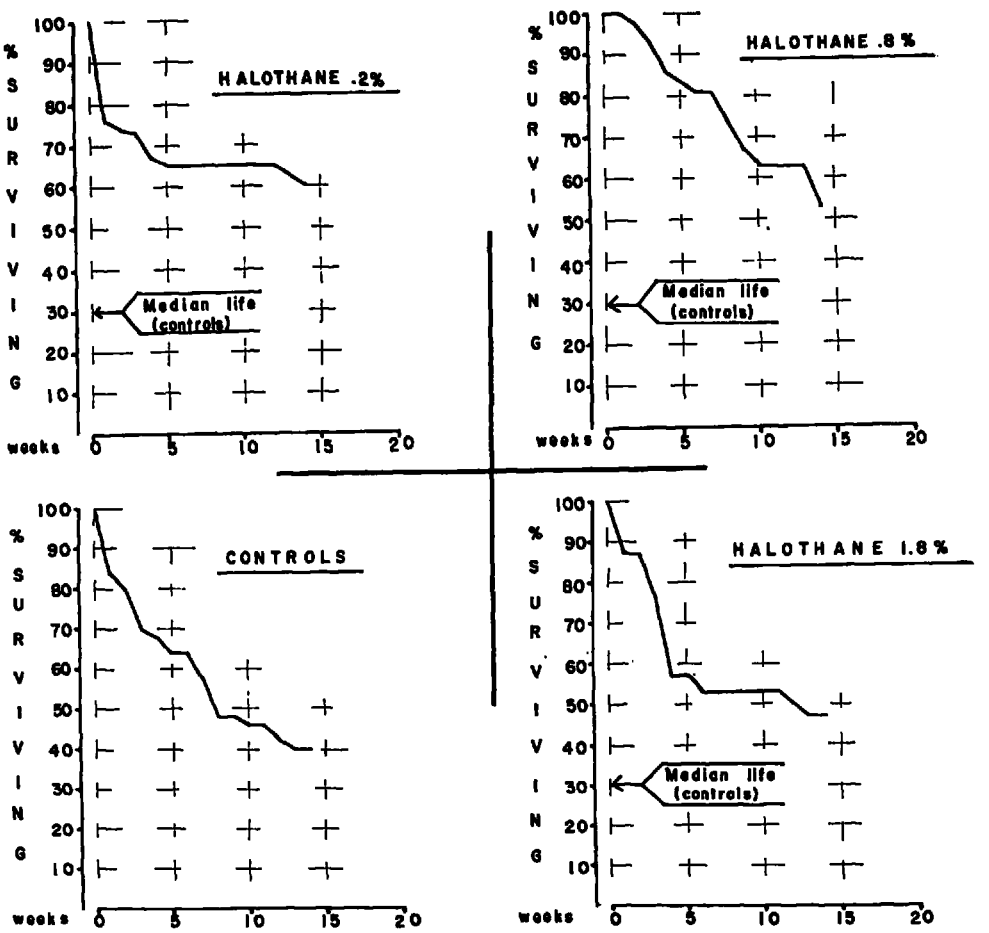

FIGURE 3. Survival rates in the various groups expressed as percentile fractions of the number of animals alive in each group on the day of the death of the median survivor in the control group (223 days).

concentration range the drug has a moderate lethal effect which does not disappear with cessation of administration of the drug but continues to exert its effects for an additional three- to six-week period.

Once an animal has reached the expected median of life, as determined in the control group, its life expectancy is not affected any longer by previous exposure to halothane in any concentration.

Some considerations about the assessment and evaluation of the toxicity of certain drugs, implicit in these results, have been discussed.

RÉSUMÉ

On a administré de l'halothane à trois groupes de 96 souris. On a utilisé comme contrôle un quatrième groupe de 96 souris.

Toutes les souris étaient nées le même jour et on les avait identifiées avec

précision à l'age de douze semaines. De la treizième à la dix-huitième semaine d'âge, on a anesthésié trois fois par semaine, durant trois heures chaque fois, les animaux des groupes servant à l'expérience. Un groupe a reçu l'halothane à 0.2 pour cent, un groupe l'halothane à 0.8 pour cent et un groupe l'halothane à 1.8 pour cent. Le groupe de contrôle n'à reçu aucune anesthésie.

Chaque jour, on a visité les cages et on a enlevé les animaux morts. A la 48e semaine on a terminé l'expérience, et à l'aide des données receuillies, on a préparé les tables de survie.

Ces tables ont révélé qu'il n'y a aucune différence de survie entre les animaux qui n'ont rien reçu et ceux qui ont reçu l'halothane à 0.2 pour cent. Parmi les animaux anesthésiés à l'halothane à 1.8 pour cent, plusieurs sont morts au début de l'expérience. Par la suite, la survie se compare à celle des animaux de contrôle.

Les animaux qui ont inhalé de l'halothane à 0.8 pour cent ont présenté une singulière augmentation du taux de mortalité durant les quatre ou cinq semaines qui ont suivi le temps des expériences.

L'orsqu'un animal survivait aussi longtemps que la moyenne des animaux de contrôle, le nombre des jours qui lui restaient à vivre n'était pas influencé par l'administration antérieure du médicament, quelle qu'en ait été la concentration.

D'après ces résultats, on peut supposer qu'une étude de toxicité aigue ou qu'une épreuve fonctionnelle isolée, durant la période aigue, peuvent être peu significatives quant aux propriétés toxiques d'un produit.

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