PROLONGATION OF THE NORMAL LIFESPAN AND INHIBITION OF SPONTANEOUS CANCER BY ANTIOXIDANTS

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Aging may be due in part to the action of endogenously produced free radicals, such as HO• and HO_2• (1). In the first test of this hypothesis (2), mice were given 0.5-2.0% w of various free radical inhibitors in their diet; some of the treated groups were found to live about 20% longer than the controls.

The present experiment was designed to check the positive results of the initial study and to evaluate another antioxidant, hydroxylamine. A pellet diet (to which the chemicals were added before pelleting) was fed, both for convenience and because of the possibility that the period of daily food intake might be spread over a longer time. The impression had been gained in the first experiment that mice on a powdered diet tended to eat most of their daily food within a few hours after it had been placed in the cage. Hence, it was anticipated that on a pellet diet the tissue concentration of inhibitors might be maintained at an elevated level for a longer period of time than on the powdered diet, although on the latter the peak inhibitor concentration might be greater.

MATERIALS AND METHODS

AKR (males), C3H (females), and Swiss (males) were obtained from the Jackson Memorial Laboratory of Bar Harbor, Maine, immediately after weaning. They were fed *ad lib.* a pelleted mouse food to which, before pelleting, had been added (with the exception of the controls) 1 of 4 reducing agents: 1) cysteine hydrochloride, 2) 2-mercaptoethylamine hydrochloride, 3) 2,2'-diaminodiethyl disulfide dihydrochloride, and 4) hydroxylamine hydrochloride; the first 3 compounds were added to the extent of 1% w, while the last was added at 1% w and 2% w. The pelleted foods were prepared in batches at intervals of 1 to 2 months.

The average weight of the mice in each cage was determined monthly. In the case of the C3H and Swiss mice, the number of tumors were also recored; gross tumors were not seen in the AKR mice. After the experiment had been in progress for 6 months, dead AKR mice were autopsied to determine if they had lymphatic leukemia, while dead C3H and Swiss mice were examined (not autopsied) for the presence or absence of gross tumors; unfortunately the data collected on the dead mice through the 15th month were lost. The data obtained after the 15th month on dead C3H and Swiss mice (no AKR's since they had all died) reflected the tumor incidence in the living mice. Thus, most of dead C3H controls had tumors, while virtually none were found in the hydroxylamine groups.

The number of mice in each group at age of 4 months are shown in table 1.

RESULTS AND DISCUSSION

In tables 2, 3, and 4 are presented the average weights of the mice, the number living (corrected for losses due to causes other than unavoidable death by subtracting the number so lost from the number living in each of the months preceding the loss) and of the percentage of the original group—for the purpose of computation this was taken as the number living at 4 months, still living as a function of time in months.* In addition, tables 3 and 4 give the number of animals with tumors in each group as well as the percentage of tumerous mice each month. Table 5 gives the half-survival times (HST) (age at which 50% of the mice are dead) along with corresponding data from the first experiment. In figures 1, 2, 3, the number of living in the controls and 2-mercaptoethylamine, cysteine, and 1% hydroxylamine groups (2% hydroxylamine also in the case of the AKR mice) are plotted as a function of age on a semilog scale, while corresponding data from the first experiment are presented in figures 4 and 5 for comparison purposes.

The half-survival time of AKR mice was prolonged 14.5% by 1% w cysteine hydrochloride ($P < 0.1$),* 8.3% by 1% hydroxylamine hy-

We should like to acknowledge our indebtedness to Dr. Leland Short and Dr. Charles Riggs for their assistance in the care of the mice and to Dr. Hardin B. Jones and Dr. John H. Lawrence for their support of the lengthy experiment conducted at the Donner Laboratory of the University of California, Berkeley, California.

* Chi-square test.

TABLE 1. NUMBER OF MICE IN EACH GROUP AT AGE OF 4 MONTHS

	Controls	2-Mercapto-ethylamine hydrochloride 1%w	2,2'-Diaminodiethyl disulfide dihydrochloride 1%w	Cysteine hydrochloride 1%w	Hydroxylamine hydrochloride 1%w	Hydroxylamine hydrochloride 2%w
AKR	60	42	43	47	38	40
C3H	92	49	48	49	36	24
Swiss	60	48	51	46	51	37

TABLE 2. AKR MICE. EFFECT OF ANTIOXIDANTS ON THEIR LIFESPAN. ADJUSTED MORTALITY DATA AND WEIGHTS.

Age (Mos.)	Controls			2-Mercaptoethylamine Hydrochloride, 1%			2,2'-Diaminodiethyl Disulfide Dihydrochloride, 1%		
	No. Living	% Surviving	Aver. Wt. (Gm.)	No. Living	% Surviving	Aver. Wt. (Gm.)	No. Living	% Surviving	Aver. Wt. (Gm.)
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	60	100.0	30.2	42	100.0	21.4	43	100.0	25.2
5	59	98.4	30.2	39	93.2	24.7	40	93.2	27.4
6	55	91.7	29.5	31	74.2	24.2	36	83.8	28.0
7	50	83.5	30.5	27	64.5	27.3	35	81.5	27.4
8	42	70.0	28.8	22	52.5	28.4	31	72.2	26.2
9	35	58.5	30.0	20	47.6	26.8	28	65.2	26.4
10	27	45.0	29.4	16	38.2	26.6	20	46.5	24.4
11	22	36.7	24.8	12	28.6	25.4	13	30.2	25.8
12	17	28.4	27.6	11	26.2	26.8	11	25.6	23.2
13	11	18.3	26.8	11	26.2	25.9	2	4.7	20.0
14	9	15.0	27.2	8	19.1	25.6	0	0.0	—
15	8	13.4	29.4	7	16.6	24.3	—	—	—
16	3	5.0	26.6	5	10.9	23.0	—	——	—

Age (Mos.)	Cysteine Hydrochloride, 1%			Hydroxylamine Hydrochloride 1.0%			Hydroxylamine Hydrochloride 2.0%		
	No. Living	% Surviving	Aver. Wt. (Gm.)	No. Living	% Surviving	Aver. Wt. (Gm.)	No. Living	% viving Sur-	Aver. Wt. (Gm.)
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	47	100.0	27.4	38	100.0	29.6	40	100.0	27.8
5	46	98.2	28.8	37	97.5	30.4	40	100.0	29.2
6	46	98.2	28.4	36	95.0	30.2	40	100.0	29.2
7	43	91.6	29.1	32	84.5	30.4	38	95.2	26.3
8	38	81.2	29.9	28	74.0	31.8	34	85.2	27.0
9	35	74.6	29.9	24	63.3	31.2	32	80.2	25.6
10	28	59.7	30.0	21	55.4	28.3	24	60.0	27.6
11	23	49.0	29.2	16	42.2	28.2	21	52.5	28.0
12	21	44.8	29.2	11	29.0	28.6	16	40.0	29.6
13	16	34.1	29.2	6	15.8	30.8	14	35.0	30.6
14	11	23.3	26.8	6	15.8	27.6	14	35.0	29.2
15	7	14.9	25.7	3	7.9	26.6	10	25.0	29.0
16	5	10.6	27.0	2	5.3	25.0	3	7.5	30.0

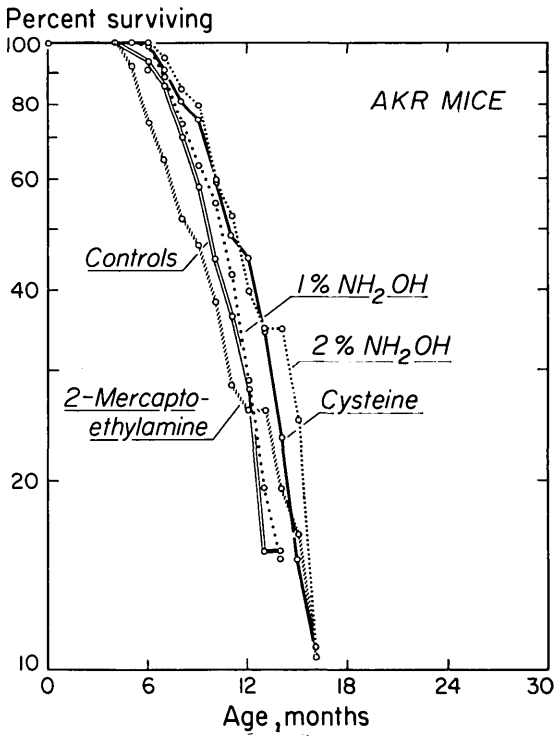

Fig. 1. Effect of antioxidants on the lifespan of AKR (male) mice.

drochloride ($P > 0.1$), and 17.0% by 2% w hydroxylamine hydrochloride ($P < 0.05$). Unlike in the first experiment, 2-mercaptoethylamine did not prolong the half-survival time. Inspection of figure 1 indicates that 2-mercaptoethylamine hydrochloride may have also had a beneficial effect on the lifespan, although there apparently was an inordinately high loss of animals in this group (every cage was involved) during the first few months, suggesting that their food additive was toxic; no such effect was noted however with the C3H or Swiss mice, although in the case of the latter,

TABLE 3. C3H MICE. EFFECT OF ANTIOXIDANTS ON THEIR LIFESPAN. ADJUSTED MORTALITY DATA. WEIGHTS AND TUMOR DATA.

Age (Months)	Controls					2-Mercaptoethylamine Hydrochloride, 1%				
	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	83	100.0	20.8	0	0.0	44	100.0	21.2	0	0.0
5	83	100.0	21.6	0	0.0	44	100.0	21.8	0	0.0
6	83	100.0	21.6	0	0.0	44	100.0	21.6	0	0.0
7	83	100.0	22.2	0	0.0	44	100.0	21.8	0	0.0
8	81	97.2	23.0	0	0.0	43	97.8	23.2	0	0.0
9	78	93.8	23.2	1	1.3	41	93.2	22.6	0	0.0
10	77	92.5	23.0	5	6.5	41	93.2	23.4	0	0.0
11	76	91.5	23.2	8	10.5	41	93.2	23.8	2	4.9
12	72	86.5	23.6	11	15.3	40	91.0	24.0	1	2.5
13	61	73.4	23.6	11	18.0	38	86.5	23.2	1	2.6
14	43	51.7	24.6	7	16.3	36	82.0	23.6	2	5.6
15	40	48.2	24.0	8	20.0	32	72.8	23.8	3	9.4
16	27	32.4	23.5	4	14.8	30	68.2	23.4	3	10.0
17	22	26.4	24.4	3	13.7	26	59.2	23.2	2	7.7
18	21	25.2	23.6	3	14.3	23	52.3	23.6	4	17.4
19	17	20.4	24.2	3	17.5	20	45.5	23.4	4	20.0
20	14	16.8	27.8	3	21.4	15	34.2	21.4	3	20.0
21	13	15.6	19.0	2	15.4	13	29.5	21.2	2	15.4

C3H MICE. EFFECT OF ANTIOXIDANTS ON THEIR LIFESPAN. ADJUSTED MORTALITY DATA. WEIGHTS AND TUMOR DATA.

Age (Months)	2,2'-Diaminodiethyl Disulfide Dihydrochloride, 1%					Cysteine Hydrochloride, 1%				
	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	48	100.0	20.4	0	0.0	49	100.0	21.2	0	0.0
5	48	100.0	21.6	0	0.0	48	98.0	21.8	0	0.0
6	47	98.0	21.6	0	0.0	47	96.0	21.2	0	0.0
7	46	96.0	21.4	0	0.0	47	96.0	21.4	0	0.0
8	43	90.0	21.2	1	2.3	45	91.8	22.0	0	0.0
9	40	83.5	21.2	1	2.5	42	85.6	22.4	2	4.8
10	36	75.0	22.4	1	2.8	40	81.6	22.2	2	4.8
11	34	71.0	22.8	1	2.9	35	71.5	22.4	1	2.9
12	31	64.6	21.4	1	3.2	31	63.2	22.0	1	3.2
13	23	48.0	20.4	0	0.0	25	51.0	23.2	3	12.0
14	17	35.4	20.0	0	0.0	21	42.8	22.8	4	19.0
15	7	14.6	15.8	0	0.0	18	36.7	22.2	4	22.2
16	0	0.0	—	—	—	15	30.6	24.2	5	33.3
17	—	—	—	—	—	13	26.5	24.6	3	23.0
18	—	—	—	—	—	11	22.4	21.8	1	9.1
19	—	—	—	—	—	6	12.2	21.6	0	0.0
20	—	—	—	—	—	6	12.2	22.4	3	50.0
21	—	—	—	—	—	3	6.1	20.0	0	0.0

TABLE 3. (CONTINUED)

C3H MICE. EFFECT OF ANTIOXIDANTS ON THEIR LIFESPAN. ADJUSTED MORTALITY DATA. WEIGHTS AND TUMOR DATA.

Age (Months)	Hydroxylamine Hydrochloride 1% No. Living	1% % Surviving	1% Aver. Wt. (Gm.)	1% No. With Tumors	1% % With Tumors	2% No. Living	2% % Surviving	2% Aver. Wt. (Gm.)	2% No. With Tumors	2% % With Tumors
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	36	100.0	20.0	0	0.0	24	100.0	19.2	0	0.0
5	35	97.3	21.5	0	0.0	21	87.6	20.4	0	0.0
6	35	97.3	20.8	0	0.0	21	87.6	22.2	0	0.0
7	33	91.6	21.4	0	0.0	21	87.6	21.6	0	0.0
8	33	91.6	21.6	0	0.0	21	87.6	21.4	0	0.0
9	32	89.0	22.8	0	0.0	20	83.5	21.8	0	0.0
10	32	89.0	23.4	0	0.0	16	66.7	22.5	0	0.0
11	30	83.4	23.8	2	6.7	14	58.4	26.0	0	0.0
12	29	80.5	—	1	3.4	14	58.4	25.6	0	0.0
13	28	77.8	23.9	1	3.6	12	50.0	23.8	0	0.0
14	23	64.0	24.8	1	4.3	11	45.8	24.6	0	0.0
15	20	55.5	24.4	1	5.5	10	41.7	22.6	0	0.0
16	16	44.4	24.4	2	12.5	9	37.5	21.6	0	0.0
17	12	33.2	25.8	1	8.4	5	20.8	22.0	0	0.0
18	12	33.2	25.0	1	8.4	3	12.5	—	1	33.3
19	9	25.0	25.0	0	0.0	0	0.0	—	—	—
20	8	22.2	26.2	0	0.0	—	—	—	—	—
21	7	19.4	25.0	1	14.3	—	—	—	—	—

the death rate was somewhat high in the first part of the experiment. 2.2'-diaminodiethyl disulfide is evidently toxic at the 1% w level, since it produced a moderate shortening of the lifespan in the AKR mice as well as in the other 2 strains. The discrepancy between the half-survival time of the controls in the present experiment (9.6 months) and the corresponding figures found in the first (7.5 month, Jackson Memorial Laboratory data; 7.5 months, on powdered diet; and 7.8 months, on a pellet diet) cannot be accounted for. However, even if the data of the first experiment are recalculated, using the higher control half-survival figure obtained in the present work, it is found that 1% w 2-mercaptoethylamine hydrochloride prolonged the half-survival time by 10.4% and 1% w cysteine hydrochloride by 12.5%. The latter figure is essentially the same as that found in the present experiment (14.5%).

2-Mercaptoethylamine hydrochloride (1% w) prolonged the half-survival time of C3H mice from 14.5 to 18.3 months, an increase of 26% ($P < 0.01$); this compound also delayed the onset and incidence of tumors. As can be seen from figure 5, there was some indication in the first experiment that 2-mercaptoethylamine had a life-prolonging effect; the more marked effect noted in the present work may be a result of a more prolonged elevated antioxidant level due to the use of a pelleted rather than a powdered diet or may be in part a reflection of the larger numbers of mice employed. In both experiments the addition of 1% cysteine hydrochloride to the diet had a slight adverse effect on the lifespan; this is in contrast to its beneficial effect on the AKR strain in both studies. It is possible that this difference between the effect of cysteine hydrochloride on the 2 strains is a sex difference. However, this does not seem likely since 2-mercaptoethylamine and hydroxylamine have increased the lifespan of both AKR (male) and C3H (female, 1% w hydroxylamine increased the HST slightly, 7%) mice.

None of the antioxidants studied prolonged the lifespan of the Swiss mice.

The foregoing results probably reflect a balance between the toxic effects and the postulated age-inhibiting effects of the compounds studied on which are superimposed the effects of these substances on tumor initiation and growth.

In the case of the AKR mice, it was shown in the first experiment that the prolongation of life achieved with several antioxidants was not

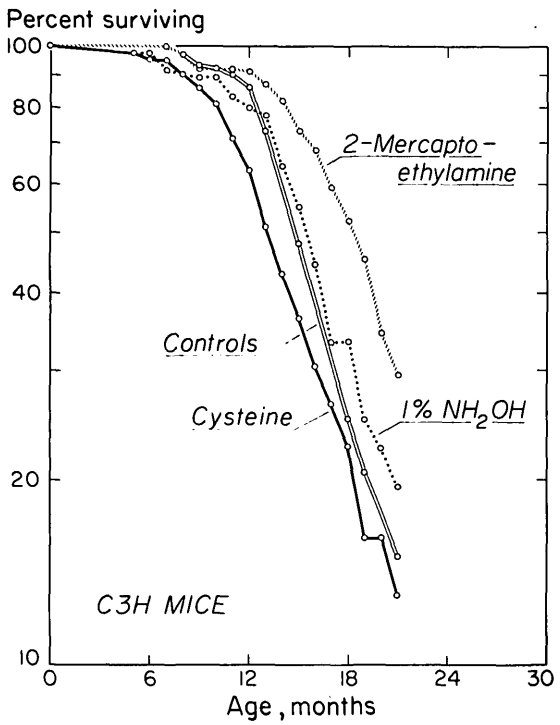

Fig. 2. Effect of antioxidants on the lifespan of C3H (female) mice.

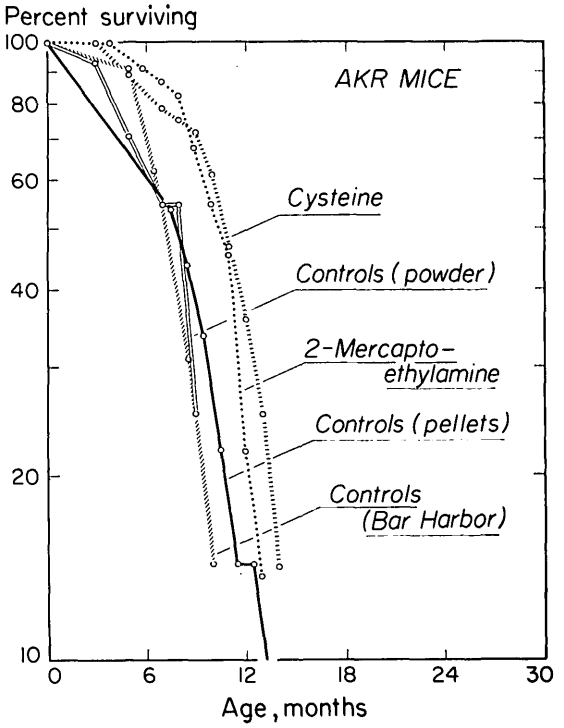

Fig. 4. Effect of antioxidants on the lifespan of AKR (male) mice (data from first experiment).

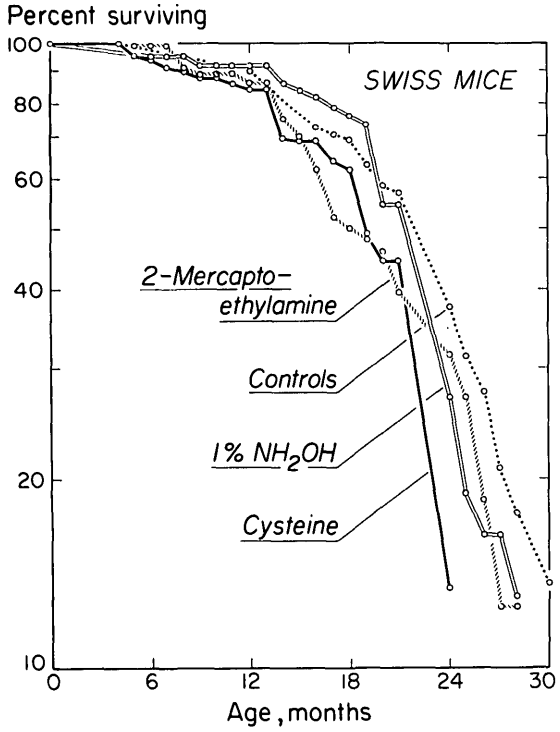

Fig. 3. Effect of antioxidants on the lifespan of Swiss (male) mice.

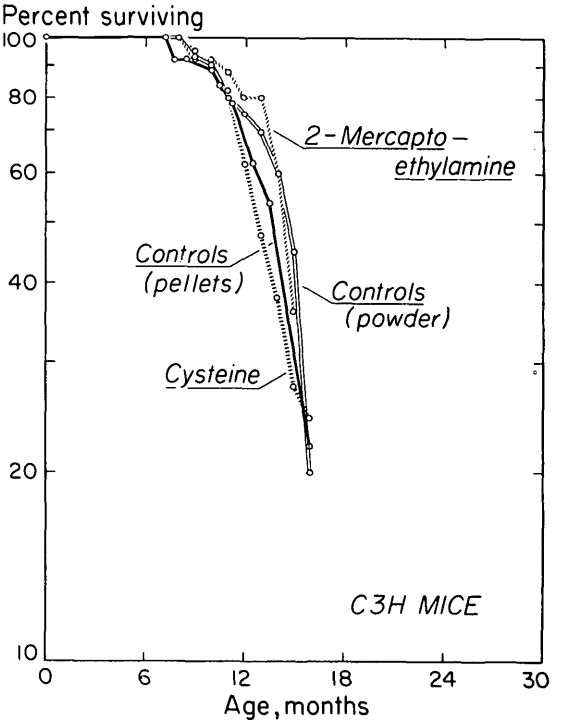

Fig. 5. Effect of antioxidants on the lifespan of C3H (female) mice (data from first experiment).

TABLE 4. SWISS MICE. EFFECT OF ANTIOXIDANTS ON THEIR LIFESPAN. ADJUSTED MORTALITY DATA. WEIGHTS AND TUMOR DATA.

Age (Months)	Controls					2-Mercaptoethylamine Hydrochloride, 1%				
	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	51	100.0	24.6	0	0.0	48	100.0	24.8	0	0.0
5	50	98.0	27.2	0	0.0	47	98.0	26.6	0	0.0
6	48	94.0	29.0	0	0.0	47	98.0	29.4	0	0.0
7	48	94.0	29.6	0	0.0	47	98.0	30.2	0	0.0
8	48	94.0	31.0	0	0.0	44	91.6	31.4	0	0.0
9	47	92.4	32.0	0	0.0	43	89.6	30.6	0	0.0
10	47	92.4	32.8	0	0.0	43	89.6	31.4	0	0.0
11	46	90.2	32.6	0	0.0	43	89.6	32.4	1	2.3
12	46	90.2	32.6	0	0.0	41	85.5	30.8	1	2.4
13	46	90.2	33.0	0	0.0	41	85.5	30.4	1	2.4
14	38	74.5	33.0	1	2.6	36	75.0	30.2	1	2.8
15	37	72.5	32.0	0	0.0	34	70.8	30.4	0	0.0
16	37	72.5	32.0	0	0.0	30	62.5	30.2	0	0.0
17	36	70.6	35.0	0	0.0	25	52.0	30.8	0	0.0
18	35	68.6	35.2	2	5.7	24	50.0	31.0	1	4.1
19	32	62.6	35.0	1	3.1	23	48.0	32.6	1	4.3
20	30	58.8	34.8	1	3.3	22	45.8	32.8	0	0.0
21	29	57.0	33.6	2	6.9	19	39.6	33.4	0	0.0
22	—	—	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—
24	19	37.3	34.2	1	5.3	15	31.2	31.2	0	0.0
25	16	31.4	32.2	0	0.0	13	27.2	30.2	0	0.0
26	14	27.4	33.6	0	0.0	9	18.7	30.6	0	0.0
27	11	21.6	31.4	0	0.0	6	12.5	29.2	0	0.0
28	9	17.6	—	0	0.0	6	12.5	—	0	0.0
29	7	13.7	—	0	0.0	4	8.3	—	0	0.0

SWISS MICE. EFFECT OF ANTIOXIDANTS ON THEIR LIFESPAN. ADJUSTED MORTALITY DATA. WEIGHTS AND TUMOR DATA.

Age (Months)	2,2'-Diaminodiethyl Disulfide Dihydrochloride, 1%					Cysteine Hydrochloride, 1%				
	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors	No. Living	% Surviving	Aver. Wt. (Gm.)	No. With Tumors	% With Tumors
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	51	100.0	23.6	0	0.0	45	100.0	26.2	0	0.0
5	48	98.0	25.2	0	0.0	43	96.5	28.6	0	0.0
6	48	98.0	25.8	0	0.0	43	96.5	30.2	0	0.0
7	47	92.2	27.2	0	0.0	41	91.0	30.8	0	0.0
8	46	90.4	27.4	0	0.0	41	91.0	31.6	0	0.0
9	45	88.4	28.0	0	0.0	40	89.0	32.2	0	0.0
10	45	88.4	29.2	0	0.0	40	89.0	31.8	0	0.0
11	43	84.5	30.4	0	0.0	39	86.6	33.8	0	0.0
12	43	84.5	27.0	0	0.0	38	84.5	33.2	0	0.0
13	43	84.5	26.6	0	0.0	38	84.5	34.0	0	0.0
14	37	72.5	25.0	0	0.0	31	69.0	30.2	0	0.0
15	33	64.8	24.0	0	0.0	31	69.0	32.6	0	0.0
16	17	33.4	23.8	0	0.0	31	69.0	32.4	0	0.0
17	11	21.6	21.8	0	0.0	29	64.5	33.2	0	0.0
18	9	17.7	24.2	0	0.0	28	62.2	32.6	1	3.6
19	9	17.7	23.8	1	11.1	22	49.0	31.6	1	4.5
20	7	13.7	23.6	1	14.3	20	44.4	32.6	0	0.0
21	4	7.8	22.5	0	0.0	20	44.4	32.6	0	0.0
22	—	—	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—
24	0	0.0	—	—	—	6	13.4	32.2	1	16.7
25	—	—	—	—	—	4	8.9	26.0	0	0.0
26	—	—	—	—	—	2	4.4	27.0	0	0.0
27	—	—	—	—	—	2	4.4	27.0	0	0.0
28	—	—	—	—	—	2	4.4	—	0	0.0
29	—	—	—	—	—	1	2.2	—	1	100.0

TABLE 4. (CONTINUED)
SWISS MICE. EFFECT OF ANTIOXIDANTS ON THEIR LIFESPAN.
ADJUSTED MORTALITY DATA. WEIGHT AND TUMOR DATA.

Age (Months)	Hydroxylamine Hydrochloride 1% No. Living	1% % Surviving	1% Aver. Wt. (Gm.)	1% No. With Tumors	1% % With Tumors	2% No. Living	2% % Surviving	2% Aver. Wt. (Gm.)	2% No. With Tumors	2% % With Tumors
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	37	100.0	24.8	0	0.0	37	100.0	23.2	0	0.0
5	37	100.0	28.0	0	0.0	36	97.4	25.6	0	0.0
6	35	94.5	29.0	0	0.0	36	97.4	29.2	0	0.0
7	35	94.5	29.4	0	0.0	35	94.5	27.2	0	0.0
8	35	94.5	31.4	0	0.0	35	94.5	27.6	0	0.0
9	34	92.0	31.2	0	0.0	35	94.5	27.8	0	0.0
10	34	92.0	32.0	0	0.0	35	94.5	31.2	0	0.0
11	34	92.0	33.2	0	0.0	34	92.0	31.8	0	0.0
12	34	92.0	33.0	0	0.0	30	81.0	30.4	0	0.0
13	34	92.0	32.6	0	0.0	29	78.5	31.8	0	0.0
14	32	86.5	33.6	0	0.0	27	73.0	31.8	0	0.0
15	31	84.0	33.6	0	0.0	27	73.0	33.4	0	0.0
16	30	81.2	33.6	0	0.0	25	67.5	30.4	0	0.0
17	29	78.4	34.8	0	0.0	23	62.2	34.0	0	0.0
18	27	75.6	34.8	1	3.7	22	59.5	33.8	0	0.0
19	26	72.8	34.0	1	3.8	22	59.5	32.8	0	0.0
20	19	54.0	33.8	1	5.3	19	51.5	33.4	0	0.0
21	19	54.0	33.8	1	5.3	17	45.8	33.2	0	0.0
22	—	—	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—
24	9	27.0	33.8	2	22.2	8	21.6	35.0	1	12.5
25	6	19.0	31.6	1	16.7	7	18.9	32.2	1	18.9
26	5	16.2	32.0	1	20.0	4	10.8	32.4	0	0.0
27	5	16.2	30.0	0	0.0	4	10.8	32.4	0	0.0
28	4	13.5	—	0	0.0	3	8.1	—	0	0.0
29	4	13.5	—	0	0.0	2	5.4	—	0	0.0

TABLE 5. EFFECT OF ANTIOXIDANTS ON THE HALF-SURVIVAL TIME OF AKR, C3H, AND SWISS MICE.

Group	Age (Months) at Which 50% Are Dead: AKR Present Study	AKR First Study	C3H Present Study	C3H First Study	Swiss Present Study
Control	9.6 ± 1.7*	7.5 ± 1.4 (Bar Harbor) 7.5 ± 2.1 (powdered diet) 7.8 ± 1.9 (pellet diet)	14.5 ± 2.3	14.5 ± 4.6	22.0 ± 4.4
2-Mercaptoethylamine Hydrochloride, 1% w	9.3 ± 2.1	10.5 ± 3.1	18.3 ± 3.9	14.5 ± 4.0	18.0 ± 3.7
2,2'-Diaminodiethyl Disulfide Dihydrochloride, 1% w	9.8 ± 2.2	7.8 ± 2.2	12.9 ± 2.6	14.5 ± 3.9	15.5 ± 3.1
Cysteine Hydrochloride, 1% w	11.0 ± 2.3	10.5 ± 2.9	13.0 ± 2.6	13.0 ± 3.4	19.0 ± 4.0
Hydroxylamine Hydrochloride, 1% w	10.4 ± 2.4	———	15.5 ± 3.6	———	21.0 ± 4.9
2% w	11.2 ± 2.4	———	13.0 ± 3.7	———	21.0 ± 4.9

*Estimate of Standard error.

due to an ameliorating effect of these substances on the spontaneous leukemia that this strain develops, and hence life prolongation could be attributed to the postulated aging process.

Considering the C3H mice, the delay in tumor formation in the 2-mercaptoethylamine group would seem to be due mainly to a slowing down of the aging process, because the curve of tumor incidence versus age is displaced to the right as compared to the controls. On the other hand, the results with 1% w hydroxylamine may be related both to an inhibition of the aging process and to the fact that this compound has been found to produce about 20% extension of the survival time of mice inoculated with Ehrlich ascites tumor (3) (a number of other antioxidants, including cysteine hydrochloride and 2-mercaptoethylamine hydrochloride, were not effective). Thus, it is possible that in the present work the constant presence of hydroxylamine (and possibly to a lesser extent with the other antioxidants) produced an environment detrimental to tumor cells, and thus when they began to appear, they either died or were removed by the natural body defences. The marked depression of the tumor incidence by hydroxylamine suggests the possibility of prophylactic cancer chemotherapy by this or other anticancer agents.

These encouraging results again provide some support for the concept that endogenously produced free radicals contribute to the aging process.

SUMMARY

Antioxidants, in a second experiment, have again been found to prolong the normal lifespan of mice.

2-Mercaptoethylamine hydrochloride (1% w, incorporated into a pellet diet) prolonged the half-survival time of C3H female mice from 14.5 to 18.3 months, an increase of 26%, while hydroxylamine hydrochloride (1% w) produced a slight prolongation, 7%.

Cysteine hydrochloride (1% w) and hydroxylamine hydrochloride (2% w) increased the half-survival time of AKR male mice from 9.6 to 11.0 and 11.2 months, respectively, a prolongation of about 15%.

None of the antioxidants studied, 2-mercaptoethylamine hydrochloride (1% w), 2,2' diaminodiethyl disulfide (1% w), and hydroxylamine hydrochloride (1 and 2% w), prolonged the life of Swiss male mice.

Hydroxylamine hydrochloride (1 and 2% w) produced a marked decrease in the tumor incidence of C3H female mice.

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