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Effects of Various Drugs on Longevity in Female C57BL/6J Mice

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Abstract. Chlorpromazine, chlorpheniramine, chloroquine, dimethylaminoethyl *p*-chlorophenoxyacetate, aspirin and acetaminophen were individually administered at one or more dosage levels, to groups of female C57BL/6J mice from an average age of 10 months to the end of life. Each of the drugs are known or suspected stabilizers of cellular membranes. Except for two cases of apparently toxic effects, differences in life span between drug-treated animals and control animals were not significant at the $p<0.05$ level. Dimethylaminoethyl *p*-chlorophenoxyacetate, which had previously extended the life span of male Swiss Webster albino mice by 27.3% of the period of administration ($p = 0.039$), produced a modest and not quite significant 5.9% extension ($p = 0.077$). Weight changes were negligible up to 19 months of age, but several of the drug-treated groups averaged somewhat lighter in weight than control mice by 25 months of age.

Key Words
Longevity
Mice
Drug effects

The dimethylaminoethyl (or the closely related diethylaminoethyl) moiety appears in a number of drugs that are known to stabilize lysosomal or erythrocyte membranes, compounds such as those shown in figure 1. The common fragment (in the oval) may account for the common ability of these compounds to stabilize membranes. Phosphatidyl choline, one of the most abundant phospholipid constituents of biological membranes, also contains the dimethylaminoethyl moiety (methylated to choline).

Disruption of cell and organelle (especially lysosomal) membranes due to lipid peroxidation and other causes has been postulated to account for much of the intra- and intercellular damage associated with the aging

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process [2]. Several of the drugs in figure 1 were found to extend fruit fly life span [3].

In the light of these considerations, it was of interest to study the effects of chloroquine, chlorpromazine, chlorpheniramine and dimethylaminoethyl p-chlorophenoxyacetate (meclofenoxate) on mouse life span. Aspirin and acetaminophen were also included in the present study. Aspirin may owe its anti-inflammatory properties to its ability to stabilize lysosomal membranes [1, 5]. Acetaminophen is, like aspirin, a commonly used analgesic and antipyretic drug (though devoid of aspirin's anti-inflammatory and antirheumatic activity).

Materials and Methods

A population of 743 female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) aged 7.9–12.1 months at the start of the trial, was divided into eleven groups, a control group of 152 mice and ten drug-treated groups of 58–60 animals each. The eleven groups were placed 7 or 8 mice per cage and maintained at a temperature of 26 ± 3 °C. The room was lighted with fluorescent lamps from 8.00 a.m. to 5.00 p.m. and had curtained windows which provided daylight and some night light.

Cages were transparent polystyrene with stainless steel or zinc-plated steel cage covers, and measured 8×12×5 inch. The bedding, pine shavings, was changed 3 times weekly. Water bottles were refilled twice weekly. Some food and water was maintained in the cages at all times. Dead mice were recorded at 2- to 3-day intervals. With rare exceptions, cages were not consolidated as the mice died, and the mice lived out their life spans with their original cage mates. All mice received the same standard commercial pellet diet consisting of Lab Blox (Wayne Feed Co., Allied Mills, Chicago, Ill.) and water, both given freely.

Continuously from the start of the trial until all of the mice had died, the drugs under study were added to the drinking water of the ten drug-treated groups in the concentrations shown in tables I–VI. Nothing was added to the water received by the control group.

Results

Animal weights. The weight of all of the animals was measured at intervals of several months in order to determine whether drug treatment caused weight gain or loss. Results of these weight determinations for each of the drug-treated groups and the control group are shown in table I. Drug concentrations are in μg/ml of drinking water.

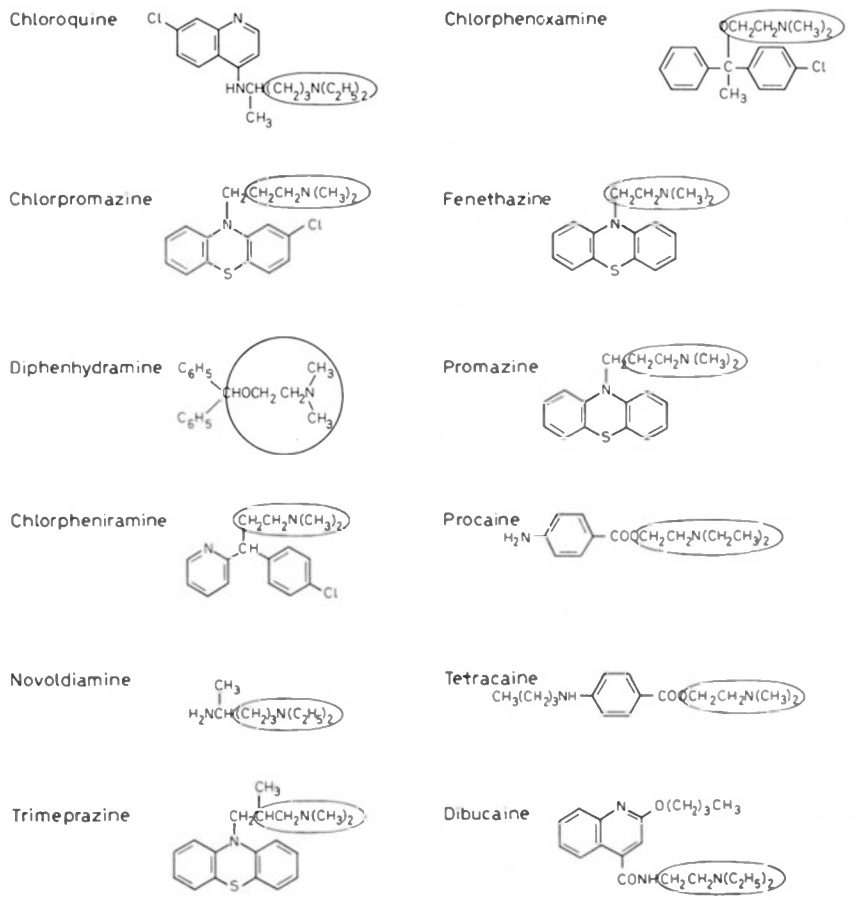

Fig. 1. Membrane stabilizers containing the diethylaminoethyl or dimethylaminoethyl moiety.

Dosage levels. In order to determine the amount of each drug received by the drug-treated groups on a per kg of animal weight basis, the volume of drinking water consumed by each cage was determined at intervals during the trial. Determinations were made over a 4- to 5-day period, averaged to obtain per day consumption, totaled for all mice in a particular drug group and divided by the total weight of all animals in that group (in kg). These results are listed in table II, along with the drug concentration

Table I. Average mouse weight in each group at intervals during trial

Group	Drug concentration μg/ml	Weight (g) at approximate age (months)				
		9	13	19	25	31
Control	–	28.5	30.3	30.2	28.5	28.0
Aspirin (Bufferin)	113	27.8	29.7	30.7	26.8	22.2
Aspirin (Bufferin)	242	28.5	31.2	28.5	29.0	–
Aspirin (Bufferin)	495	29.0	31.4	30.3	24.0	–
Acetaminophen	242	28.5	30.1	30.8	28.1	–
Dimethylaminoethyl *p*-chlorophenoxyacetate	702	29.0	32.0	30.9	24.5	–
Chlorpromazine	16.7	28.8	30.7	31.2	28.5	27.0
Chlorpheniramine	0.762	28.9	31.7	31.8	25.5	–
Chlorpheniramine	2.28	28.0	29.8	30.7	23.6	27.8
Chloroquine	13.2	28.2	29.8	28.6	29.7	23.4
Chloroquine	39.5	29.1	30.9	30.8	28.1	–

Table II. Daily water and drug consumption per kg mouse weight

Group	Drug concentration μg/ml	Liquid intake daily, ml/kg mouse weight at months from start			Drug consumption daily, mg/kg mouse weight at months from start		
		9	19	25	9	19	25
Control	–	170	187	146	–	–	–
Aspirin (Bufferin)	113	177	159	204	20.0	18.0	23.1
Aspirin (Bufferin)	242	163	172	–	39.4	41.6	–
Aspirin (Bufferin)	495	156	166	219	77.2	82.2	108.4
Acetaminophen	242	159	142	208	38.5	34.4	50.3
Dimethylaminoethyl *p*-chlorophenoxyacetate	702	140	135	145	98.3	94.8	101.8
Chlorpromazine	16.7	158	176	179	2.64	2.94	2.99
Chlorpheniramine	0.762	173	140	246	0.132	0.107	0.187
Chlorpheniramine	2.28	174	122	204	0.397	0.278	0.465
Chloroquine	13.2	164	189	163	2.16	2.49	2.15
Chloroquine	39.5	153	149	–	6.04	5.88	–

for each group (μg/ml drinking water) and the resulting dosage of drug received daily by the average animal in each group.

Ages at start of drug administration. For various reasons, it was impossible to start all animals on their respective drugs at the same age. An-

Table III. Ages of mice at start of drug administration

Group	Drug concentration μg/ml	Number of mice	Age at start months	Weighed average age at start months
Control	–	20	7.9	
		34	8.1	
		35	8.1	8.5
		39	8.9	
		24	9.3	
Aspirin (Bufferin)	113	60	8.1	8.1
Aspirin (Bufferin)	242	60	8.1	8.1
Aspirin (Bufferin)	495	35	8.1	
		24	9.3	8.6
Acetaminophen	242	60	9.4	9.4
Diethylaminoethyl *p*-chlorophenoxyacetate	702	35	8.1	
		23	9.3	8.6
Chlorpromazine	16.7	18	9.7	
		41	10.7	10.4
Chlorpheniramine	0.762	25	9.4	10.0
		33	10.4	
Chlorpheniramine	2.28	58	9.4	9.4
Chloroquine	13.2	19	9.4	
		40	10.4	10.1
Chloroquine	39.5	35	10.4	11.1
		24	12.1	

imals ranged from 7.9 to 12.1 months of age at the start of drug administration. The actual ages in months of the mice in each group at the start of drug administration is given in table III.

Median life span. Table IV shows the median life span in months for each group, i.e. the age at which 50% of the mice had died, and the difference (in months) between the median life span of each group and the control group. In the last column of the table, this difference is expressed as percent of the average period of drug administration for that particular group. The average period of drug administration is the number of months difference between the weighted average age at start of drug administration and mean life span. It is given in table V.

Table IV. Median life span

Group	Drug concentration μg/ml	Median life span months	Difference from controls months	Difference as percentage of average period of drug administration
Control	–	22.5	–	–
Aspirin (Bufferin)	113	24.1	1.6	10.6
Aspirin (Bufferin)	242	19.7	–2.8	–22.8
Aspirin (Bufferin)	495	22.1	–0.4	–2.9
Acetaminophen	242	23.1	0.6	4.4
Dimethylaminoethyl *p*-chlorophenoxyacetate	702	23.2	0.7	4.7
Chlorpromazine	16.7	22.6	0.1	0.8
Chlorpheniramine	0.762	23.3	0.8	6.3
Chlorpheniramine	2.28	22.7	0.2	1.6
Chloroquine	13.2	20.0	–2.5	–23.6
Chloroquine	39.5	23.7	1.2	10.5

Mean life span. Mean life span (months) and its standard error is shown in table V along with the difference in months between the mean life spans of the various drug-treated groups and the control group. The next column gives the average period of drug administration, the number of months between the weighted average age at the start of drug administration and mean life span. The difference between the mean life span of each group and that of the control mice is expressed as percent of the average period of drug administration for that group. The last column shows the statistical significance, p, of the difference.

Maximum life span. Maximum life span, the total number of months lived by the longest-lived individual in each group, is given in table VI along with the difference in months between each drug-treated group and the control group with respect to this parameter. This difference is expressed as percent of the maximum period of drug administration, defined as the number of months between the weighted average age at the start of drug administration and the maximum life span.

Table V. Mean life span

Group	Drug concentration μg/ml	Mean life span months	SE	Difference from controls months	Average period of drug administration	Difference as percentage of average period of drug admin.	Significance p
Control	–	22.50	±0.36	–	–	–	–
Aspirin (Bufferin)	113	23.25	±0.46	0.75	15.1	5.0	0.119
Aspirin (Bufferin)	242	20.35	±0.41	−2.15	12.3	−17.5	0.0003
Aspirin (Bufferin)	495	22.52	±0.46	0.02	13.9	0.1	0.488
Acetaminophen	242	23.00	±0.44	0.50	13.6	3.7	0.213
Dimethylaminoethyl *p*-chlorophenoxyacetate	702	23.37	±0.39	0.87	14.8	5.9	0.077
Chlorpromazine	16.7	22.48	±0.57	−0.02	12.1	− 0.2	0.488
Chlorpheniramine	0.762	22.68	±0.44	0.18	12.7	1.4	0.388
Chlorpheniramine	2.28	22.08	±0.70	−0.42	12.7	− 3.3	0.279
Chloroquine	13.2	20.72	±0.52	−1.78	10.6	−16.8	0.003
Chloroquine	39.5	22.53	±0.51	0.03	11.4	0.3	0.482

Survival curves. Survival curves for three of the drug-treated groups (percent survivors vertically versus months from the start of the trial horizontally) are reproduced in figures 2–4. The survival curve of the control group (heavy curve) is superimposed on each page. Each drug-treated group is represented by a light curve.

Discussion

With the exception of two cases of mildly toxic effects (242 μg/ml aspirin and 13.2 μg/ml chloroquine), differences in life span between drug-treated animals and control animals were not significant at the $p<0.05$ level. However, dimethylaminoethyl *p*-chlorophenoxyacetate came close with $p<0.08$, corresponding to a confidence of 92% that the life span extension produced in this group was the result of the drug treatment rather than the laws of chance. A moderate extension of life span was produced

Table VI. Maximum life span

Group	Drug concentration μg/ml	Maximum life span months	Difference from controls months	Difference as percentage of maximum period of drug administration
Control	–	32.6	–	–
Aspirin (Bufferin)	113	31.2	–1.4	– 6.1
Aspirin (Bufferin)	242	30.2	–2.4	–10.9
Aspirin (Bufferin)	495	31.5	–1.1	– 4.8
Acetaminophen	242	30.3	–2.3	–11.0
Dimethylaminoethyl *p*-chlorophenoxyacetate	702	30.5	–2.1	– 9.6
Chlorpromazine	16.7	33.9	1.3	5.5
Chlorpheniramine	0.762	29.7	–2.9	–14.7
Chlorpheniramine	2.28	34.4	1.8	7.2
Chloroquine	13.2	30.4	–2.2	–10.8
Chloroquine	39.5	29.8	–2.8	–15.0

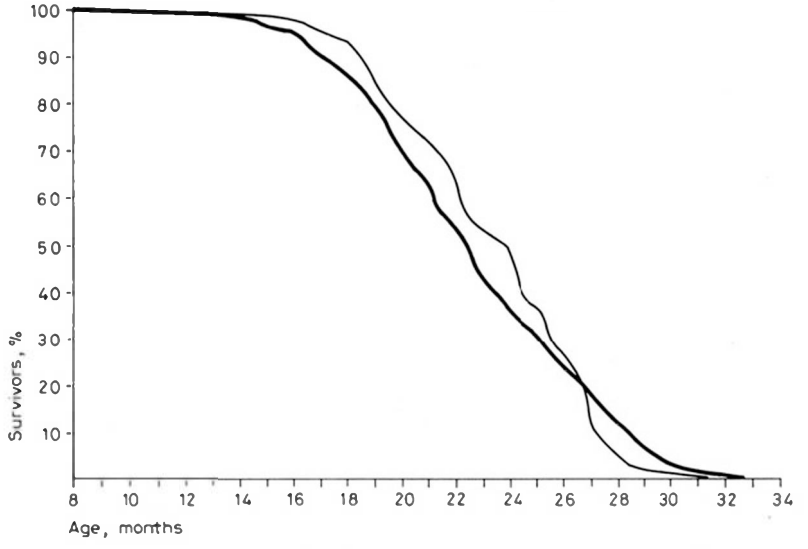

Fig. 2. Survival curve for female C57BL/6J mice receiving Bufferin. Heavy curve: control mice; light curve: mice receiving water containing 113 μg/ml aspirin (Bufferin).

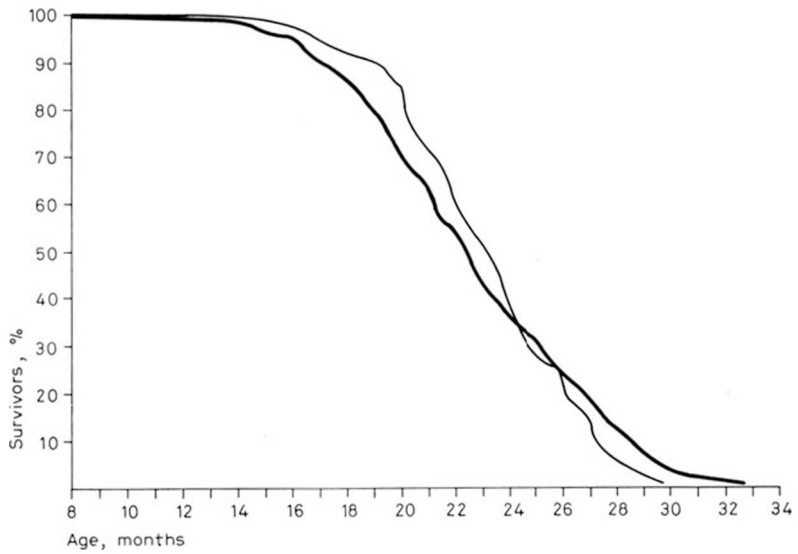

Fig. 3. Survival curve for female C57BL/6J mice receiving acetaminophen. Heavy curve: control mice; light curve: mice receiving water containing 242 μg/ml acetaminophen.

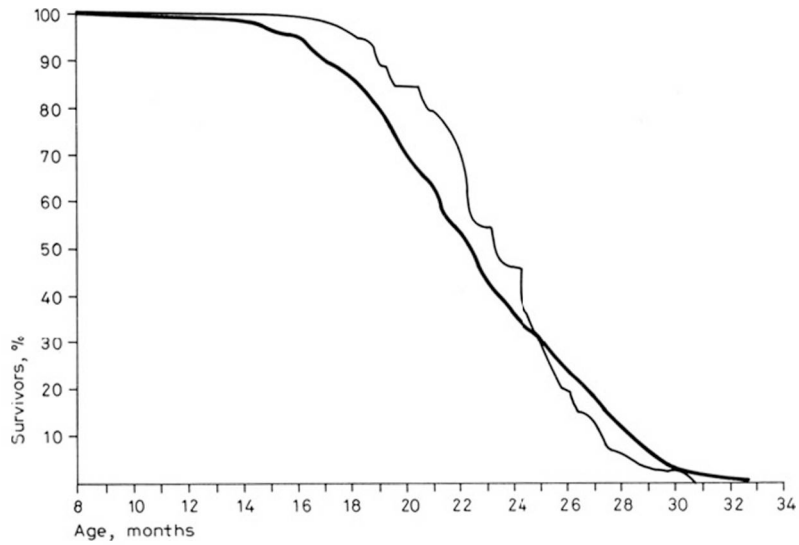

Fig. 4. Survival curve for female C57BL/6J mice receiving dimethylaminoethyl *p*-chlorophenoxyacetate. Heavy curve: control mice; light curve: mice receiving water containing 702 μg/ml dimethylaminoethyl *p*-chlorophenoxyatate.

by this drug among the first 50% of the animals to die, but the net effect of this extension was almost cancelled by accelerated deaths among older animals, that is, among the last 50% to die. The exceptionally high dosage level of dimethylaminoethyl *p*-chlorophenoxyacetate used in this experiment, equivalent to about 7 g/day in a 70-kg man, may have contributed to this result. In another experiment [4], an extension of life span equal to 27.3% of the period of administration ($p = 0.039$) was noted at a lower concentration (300 μg/ml) of this drug in male Swiss Webster albino mice.

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