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EFFECT OF DIMETHYLAMINOETHYL *p*-CHLOROPHENOXYACETATE ON THE LIFE SPAN OF MALE SWISS WEBSTER ALBINO MICE

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INTRODUCTION

THE MOST obvious change which takes place in post-mitotic mammalian cells with age is the gradual accumulation of lipoprotein masses known as lipofuscin or age pigments. These pigments were observed as early as 1842 and therefore constitute one of the oldest, if not the most widely recognized, change which takes place on the cellular level with increasing age.

The phenomenon is very likely associated with the congestive engorgement of lysosomes with undigestible matter, especially organelle membranes. Chemical, fluorescent and electron microscopic studies of lipofuscin pigment have shown that it consists of masses of incompletely hydrolyzed membranes, probably originating from lysosomes, mitochondria and endoplasmic reticulum, whose phospholipid is peroxidized (Tappel, 1968; Brandes, 1966). Tappel and others suggest that lipofuscin pigments may be the debris left over from free radical attacks on these cell components, debris which lysosomes are unable fully to digest. The undigested material accumulates, polymerizes and stays locked inside the cell, a visible record of the damage sustained by the cell. To what extent accumulating lipofuscin interferes with normal cell function is unknown.

Lysosomes may participate in aging processes by at least three mechanisms (Hochschild, 1971*a*). These are:

1. by carrying out injurious lytic activity within cells, either by excessive autophagy or by leakage of lysosomal hydrolases into the cytoplasm through damaged lysosomal membranes whose permeability has been altered,
2. by damage to extracellular structures through exocytic extrusion of enzymes or enzyme leakage following membrane breakdown or cell death, resulting in connective tissue injuries, vascular changes, collagen formation, autoantibody production and other degradative alterations,
3. by inadequately carrying out their lytic activity, as a consequence of becoming congestively engorged with undigestible material (lipofuscin pigment).

Cells and connective tissue damaged in any of these ways may function less efficiently or not at all. Over a period as long as a lifetime, many if not most of the cells in the body may lose some or all of their function in this manner. Loss or malfunction of irreplaceable postmitotic cells would necessarily contribute to age changes. So would cellular damage in tissues comprised of dividing cells, especially if the resultant damage were to limit the mitotic potential of these cells.

Dimethylaminoethyl p-chlorophenoxyacetate

Our interest in the *p*-chlorophenoxyacetic acid ester of 2-dimethylaminoethanol was aroused by reports published by Nandy and Bourne (1966) and Nandy (1968) that 12

weeks of daily injections of the drug substantially reduced age pigment concentration in the neurons of senile guinea pigs. This suggests that the cell may after all be able to clear itself of engorged lysosomes.

Dimethylaminoethyl *p*-chlorophenoxyacetate (also known as centrophenoxine and meclofenoxate) was first synthesized in the French National Scientific Research Center by Thuillier, Rumpf and Thuillier (1959*a*, *b*) by combining the amino alcohol dimethylaminoethanol with *p*-chlorophenoxyacetic acid. The latter is a synthetic relative of one of the several common plant growth hormones known as auxins. In the vegetable cell, *p*-chlorophenoxyacetic acid is a metabolic regulator influencing cellular respiration and glucidic metabolism.

The other half of the molecule, dimethylaminoethanol, is a neurotropic drug used to counteract learning, reading and speech difficulties, shortened attention span, hyperkinetic behavior, impulsive/compulsive behavior, impaired motor coordination, distractability and dissociation. Considering the chemical parentage, it is not surprising that Thuillier *et al.* report that the drug exerts a normalizing effect on certain functions of the central nervous system while at the same time retarding the aging, yellowing and falling of tree leaves if painted on the branches.

Stability

The drug is in fact unstable in solution. Within minutes after being dissolved in water, dimethylaminoethyl *p*-chlorophenoxyacetate breaks down into the two components from which it is synthesized. Felder, Pitre and Rescigno (1962) show that the rate of dissociation in aqueous solution is temperature dependent, with a half-reaction time of 5·9 min at 37°C (pH = 7·0) and 33·7 min at 22°C (pH = 7·0). Since less than 1 per cent of the administered substance remains undissociated after 40 min at 37°C, it is highly speculative whether the properties of the drug are unique (as claimed by some authors) or simply those of the hydrolysis products (which they resemble).

Indications

Dimethylaminoethyl *p*-chlorophenoxyacetate has been available in France, England, Germany, Switzerland and more recently in other countries (not including the U.S.) as a normalizer of central nervous system function. Its most frequent uses are in the treatment of depression, mental confusion, anoxia of the newborn, delirium tremens, apathy, disorientation, retardation, memory defects, fatigue, CNS trauma, and such cerebral syndromes in old people as senility, parkinsonism, hemiplegia and chronic febrile illnesses. The drug is also supposed to improve attention, perception and memory. Most of these indications are shared with dimethylaminoethanol in the form of several commercially available acid addition salts.

Effect on age pigment

Bourne (1969) came across the drug through some European connections in 1964 and, because of its interesting clinical properties, decided to study its effects on certain enzymes of the brain. Using the light microscope and stains to assay enzyme activity and distribution

within neurons, Bourne was the first to notice the loss of lipofuscin age pigment from the neurons of guinea pigs. He set to work with Nandy to confirm this interesting observation.

Nandy (1968) reports that about 200 guinea pigs aged 6 months to 6 yr were injected i.m. or i.p. daily for 4 – 12 weeks with dimethylaminoethyl *p*-chlorophenoxyacetate (80 mg/kg body weight). Animals were sacrificed at various ages and after various durations of treatment.

Although scattered lipofuscin pigments were present in neurons as early as 6 months of age, pigmentation was slow to develop until 2 yr of age, after which it increased considerably in most areas of the brain. After the age of 5 yr, pigments occupied a considerable area within the cytoplasm of the neurons.

Animals treated with dimethylaminoethyl *p*-chlorophenoxyacetate showed a notable reduction of lipofuscin pigments in most parts of the CNS. The degree of reduction was largely a function of the duration of treatment, being maximum after 12 weeks. Observations made after varying periods of treatment showed the pigments to occupy a progressively smaller area in the cytoplasm as if gradually shrinking away.

Effect on neuronal enzymes

In attempting to determine the mechanism of action of the drug, Nandy observed that the treatment changed the activity of a number of enzymes within neurons. Succinic and lactic dehydrogenase, cytochrome and monoamine oxidase, acid phosphatase and simple esterase showed reduced activity. In contrast, activity of glucose-6-phosphate dehydrogenase was increased markedly throughout the cytoplasm of the neurons. The degree of this effect was independent of the period of drug treatment.

Succinic dehydrogenase mediates glucose metabolism via the Krebs Cycle. Lactic dehydrogenase participates in the Embden–Meyerhof pathway of glycolysis. Glucose-6-phosphate dehydrogenase is an enzyme of the pentose phosphate pathway for the oxidation of glucose. Because the drug was observed to deactivate the first two enzymes while increasing the activity of the last, Nandy suggests that the drug may divert the oxidation of glucose to the pentose cycle, bypassing the tricarboxylic acid and glycolytic pathways. He also suggests that this diversion may be responsible for the observed elimination of lipofuscin, although the connection is unclear.

Pertinent to the present investigation is the fact that two other enzymes that showed less activity after the drug treatment, namely acid phosphatase and simple esterase, are lysosomal enzymes. Acid phosphatase is the enzyme most commonly monitored as an index of lysosomal enzyme release. We interpret Nandy's results as suggesting that dimethylaminoethyl *p*-chlorophenoxyacetate inhibits the release of lysosomal enzymes *in vivo*.

Dimethylaminoethyl *p*-chlorophenoxyacetate may therefore be active with respect to all three of the postulated mechanisms comprising the lysosomal hypothesis of aging listed above. If so, studies of the effect of the drug on life span in several biological systems could serve as tests of the lysosomal hypothesis. One earlier test of this sort has been reported. The drug extended the mean life span of male fruit flies by 39 per cent ($P < 0{\cdot}0001$), that of female fruit flies by 7 per cent ($P = 0{\cdot}18$) (Hochschild, 1971*b*).

Oral vs i.p. administration

To compensate partly for the rapid dissociation time of the drug, Bourne and Nandy used the injection mode to get the drug rapidly into the system. Hence it was not possible

to determine whether the observed activity was due to the undissociated drug or its breakdown products. We elected to administer the drug orally (dissolved once weekly in the drinking water), to virtually assure that any activity would be due to the separate action of the two hydrolysis products. Besides any effects on longevity, it was of interest to determine whether oral administration would similarly result in a reduction of lipofuscin pigments.

MATERIALS AND METHODS

A population of 63 male Swiss Webster Albino mice aged 8·6 months at the start of the trial, maintained at the University of California, Irvine and obtained originally from Simondson Laboratories, was divided randomly into a control group of 31 mice and a drug-treated group of 32 mice. The two groups were placed 7–8 mice per cage in substantially identical environments and maintained in an animal room at the University at a temperature of 21° ±2°C. The air conditioning system provided a somewhat controlled humidity and 11 air changes per hour. The room was lighted with fluorescent lamps from 7.00 a.m. to 7.00 p.m.

Cages were stainless steel (bottoms and tops) and measured 8 in. × 12 in. × 6 in. They were changed and sterilized two times per week, water bottles being sterilized once weekly. The bedding was pine shavings. Some food and water was maintained in the cages at all times. As the mice died, cages were not consolidated 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, Illinois) and water, both given freely.

From 8·6 to 18·2 months of age and again from 22·2 months of age until all the animals had died, 0·3 g of dimethylaminoethyl *p*-chlorophenoxyacetate per liter was added to the drinking water of the drug-treated group, while nothing was added to the water received by the control group. Survivors were counted bi-weekly except for the period from 15·1 to 20·1 months of age. During this period, the animals received routine care but survivors were recorded only at the beginning and the end of the period. Mice in the drug group drank an average of 9 ml of water per day and had an average weight of 48 g at about 12 months of age, therefore receiving, on the average, about 60 mg dimethylaminoethyl *p*-chlorophenoxyacetate per kg body weight per day. In terms of the hydrolysis products, the mice received about 20 mg of dimethylaminoethanol and about 40 mg of *p*-chlorophenoxyacetic acid per kg body weight per day.

RESULTS

During the 17th month of age (about 9 months after the start of the trial) 2 animals from each group were sacrificed for histochemical determinations of lipofuscin pigment. Brain and myocardial tissue specimens were prepared according to the method of Reichel, Hollander, Clark and Strehler (1968). An u.v. microscope was used to determine pigment density.

No significant difference in the density of lipofuscin pigments was observed between drug-treated and control mice in sagital tissue sections from the Purkinje layer of the cerebellum. Both the drug-treated and the control animals were heavily pigmented, in contrast to the young animals studied for comparison. Similar negative results were obtained for tissue taken from dorsal hippocampus.

On the other hand, significant differences in fluorescent pigment density were found between the drug group and the control group in myocardial tissue. Mice which had been given dimethylaminoethyl *p*-chlorophenoxyacetate showed appreciably less pigmentation.

Brains of the control animals appeared darker in color under visual examination than did those of the drug-treated mice, whose lighter color was similar to that of young animals of the same strain examined on the same occasion. The drug-treated mice were also observed to have less mesentery fat than did the control animals.

Survival curves for the two groups (per cent survivors vertically vs months from the start of the trial horizontally) are reproduced in Fig. 1.

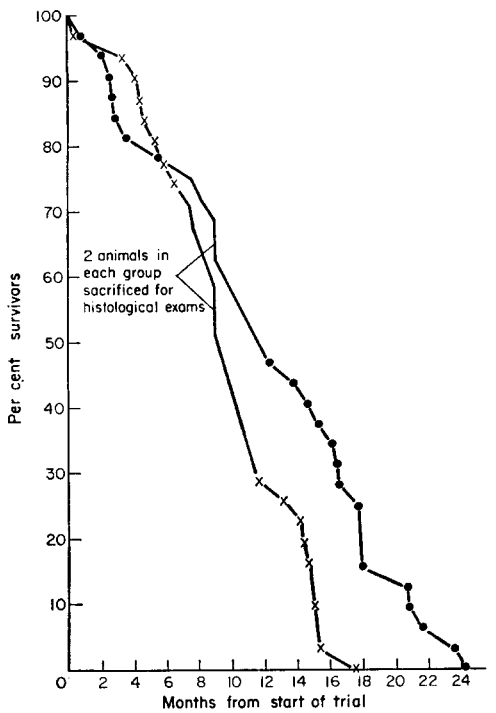

FIG. 1. Swiss Webster Albino mice. Age at start: 8.6 months.

Table 1 below shows the median survival time for each group, i.e. the time at which 50 per cent of the mice had died, measured from the first day of administration of the drug and the percent difference in this factor between the two groups. These data exclude the four sacrificed animals.

TABLE 1.

	Median survival time from start of trial	% over controls
Control group	9·5 months	—
Drug group	12·3 months	29·5%

Mean survival time from the start of the trial and its standard error, again excluding the 4 sacrificed animals, is shown in Table 2 below, along with the per cent difference between the mean survival time of the two groups and the statistical significance, *P*, of this difference.

TABLE 2.

	Mean survival time from start of trial	Standard error	% over controls	Significance against controls P
Control group	9·73 months	±0·81	—	—
Drug group	12·39 months	±1·25	27·3%	0·039

A value of $P = 0{\cdot}039$ is equivalent to a statistical confidence level of 96·1 per cent that life span was extended because of the difference in treatment rather than being the spurious result of chance.

Maximum survival time from the start of the trial, the number of months from the start of the trial lived by the longest-lived individual in each group, is given in Table 3 below. This table also indicates the maximum life span, the age at death of the longest lived survivor in each group, and the per cent difference between the two groups with respect to both factors.

TABLE 3.

	Maximum survival time from start of trial	% over controls	Maximum life span	% over controls
Control group	17·4 months	—	26·0 months	—
Drug group	24·3 months	39·7%	32·9 months	26·5%

Table 4 compares the average weight of surviving animals in the two groups at various times during the experiment. 4·7 months from the start of the experiment, the average drug-treated animal was slightly heavier than the average control animal. Later, 13·8 months from the start of the trial, at the conclusion of the 4·2 month suspension of drug administration, the drug-treated group was significantly lighter than the controls, as it was also at 17·3 months from the start.

TABLE 4.

Age	Months from start of trial	Average weight control group	Average weight drug group
13·3	4·7	45·1	47·8
22·4	13·8	45·5	40·6
	(at conclusion of 4·2 month suspension of drug administration)		
25·9	17·3	—	36·9

It is therefore possible that the observed differences in mortality may be due in whole or in part to appetite spoilage and associated dietary restriction. However, in a subsequent trial of the acetamidobenzoate salt of dimethylaminoethanol, sizable life span extensions were observed in senile male A/J mice without associated relative weight loss (Hochschild, 1973).

It is interesting to note that the drug treatment resulted in extensions not only in mean and median survival time but also in maximum survival time.

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REFERENCES

BOURNE, G. H. (1969) Private communication.
BRANDES, D. (1966) In: *Topics in the Biology of Aging* (Edited by P. L. Krohn), pp. 149–158. Interscience, New York.
FELDER, E., PITRE, D. and RESCIGNO, A. (1962) *Arzneim. Forsch.* **12**, 931.
HOCHSCHILD, R. (1971*a*) *Exp. Geront.* **6**, 153–166.
HOCHSCHILD, R. (1971*b*) *Exp. Geront.* **6**, 133–151.
HOCHSCHILD, R. (1973) *Exp. Geront.* **8**, 185–191.
NANDY, K. (1968) *J. Gerontol.* **23**, 82–92.
NANDY, K. and BOURNE, G. H. (1966) *Nature, Lond.* **210**, 313–314.
REICHEL, W., HOLLANDER, J., CLARK, J. H. and STREHLER, B. L. (1968) *J. Gerontol.* **23**, 71–78.
TAPPEL, A. L. (1968) *Geriatrics* **23**, 97–105.
THUILLIER, G., RUMPF, P. and THUILLIER, J. (1959*a*) *C. R. Acad. Sci.* **249**, 2081–2083.
THUILLIER, G., RUMPF, P. and THUILLIER, J. (1959*b*) *C. R. Soc. Biol., Paris* **1953**, 1914.

Summary—Dimethylaminoethyl *p*-chlorophenoxyacetate increased the median, mean and maximum survival time from the start of drug administration of male Swiss Webster Albino mice by 29·5 per cent, 27·3 per cent ($P = 0{\cdot}039$) and 39·7 per cent respectively. The drug treatment was associated with some loss of weight.

The drug was previously reported to reduce lipofuscin pigments in the neurons of senile guinea pigs, while concomitantly reducing the activity of the lysosomal enzymes, acid phosphatase and simple esterase. This suggests that the drug is active with respect to three postulated mechanisms involving lysosomes in the aging process.

Within minutes after dissolving in water, dimethylaminoethyl *p*-chlorophenoxyacetate breaks down into dimethylaminoethanol and *p*-chlorophenoxyacetic acid. The observed effects therefore may be those of the hydrolysis products.

Résumé—Dès le début de son administration, le diméthylaminoéthyl *p*-chlorophénoxyacétate augmenta le temps médian, moyen et maximum de survivance des souris mâles Swiss Webster Albino, de 29,5 pour cent, 27,3 pour cent ($P = 0{,}0039$) et 39,7 pour cent respectivement. Le traitement avec le médicament entraîna quelque perte de poids.

Il a préalablement été rapporté que le médicament réduisait les pigments de lipofuscine dans les neurones des cobayes séniles, alors qu'il réduisait en même temps l'activité des enzymes lysosomiques, de la phosphatase acide et de l'estérase simple. Ceci suggère que le médicament serait actif par rapport à 3 mécanismes postulés, impliquant les lysosomes dans le processus de vieillissement.

Dans l'espace de quelques minutes après sa dissolution dans l'eau, le diméthylaminoéthyl *p*-chlorophénoxyacétate se décompose en diméthylaminoéthanol et acide *p*-chlorophénoxyacétique. Les effets observés peuvent donc être ceux des produits d'hydrolyse.

Zusammenfassung—Dimethylaminoäthyl-*p*-chlorphenoxyacetat erhöhte die mediane, mittlere und maximale Überlebenszeit nach dem Beginn der Drogenverabreichung an männliche schweizerische Webster–Albinomäuse um 29.5, 27.3 ($p = 0.039$) bzw. 39.7 Prozent. Die Behandlung mit der Verbindung führte zu einem Gewichtsverlust.

Die Verbindung vermindert nach früheren Berichten die Lipofuszinpigmente in den Neuronen seniler Meerschweinchen, während sie gleichzeitig die Aktivität der lysosomalen Enzyme saure Phosphatase und einfache Esterase senkt. Dies besagt, dax die Substanz hinsichtlich dreier postulierter Mechanismen, welche Lysosomen zum Altersprozex in Beziehung setzen, aktiv ist.

Schon Minuten nach Lösung in Wasser zerfällt Dimethyl-*p*-chlorphenoxyacetat in Dimethylaminoäthanol und *p*-Chlorphenoxyessigsäure. Die beobachteten Effekte können somit auf die Hydrolyseprodukte zurückgehen.