ited generation capacity for normal cells from human embryos in vitro, that is, about 50 generations, other workers made similar claims of a limited lifetime for hemopoietic (*1*), mammary (*2*), and skin epithelial (*3*) cells in vivo. It has been assumed that the number of stem cells in a tissue remains at a certain level for most of the life-span of an organism, and that a uniformly limited lifetime or renewability of stem cells determines the life-span of the body (*1-3, 6*). There are problems in these interpretations, mainly because of the artifacts, uncertainties, and possible selection mechanisms introduced by serial transplantation of tissues from host to host or by subculture. For the testis system described herein the cells are confined to one organ without transplantation.

Our results with mouse spermatogonial stem cells do not agree with conventional views about the stem cells and aging. They show that the number of spermatogonial stem cells in the mouse decreases exponentially with age, indicating that the lifetime of the mouse spermatogonial stem cells in situ is not uniformly specified but limited in a random manner.

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## 6-Hydroxydopamine and Anticholinergic Drugs

Schallert *et al.* (*1*) reported that depletion of brain catecholamines by the use of intraventricular injections of 6-hydroxydopamine (6-OHDA) resulted in an exaggerated response to anticholinergic drugs such as atropine and scopolamine. They interpreted this finding in terms of an interaction of acetylcholine with dopamine in the central nervous system. Since these authors used a dose and administration regime of 6-OHDA that depletes norepinephrine (NE) (*2*) as well as dopamine, we think it is premature to claim this as evidence for an interaction solely with dopamine. Schallert *et al.* (*1*) used a total dose of 500 μg of 6-OHDA, without first injecting desimipramine to prevent NE neuron destruction. Although they reported that the NE concentration at postmortem was depleted by only 11 percent, this assay was carried out on caudate-putamen, which is generally regarded as a blank region for NE. Certainly, on the basis of previous studies (*2*) in which this amount of 6-OHDA was used, a very severe NE depletion would be expected in regions such as hippocampus, cortex, and hypothalamus which do contain significant NE concentrations. Thus, the authors are not justified in attributing their effects to an acetylcholine-dopamine interaction as opposed to an acetylcholine-NE interaction.

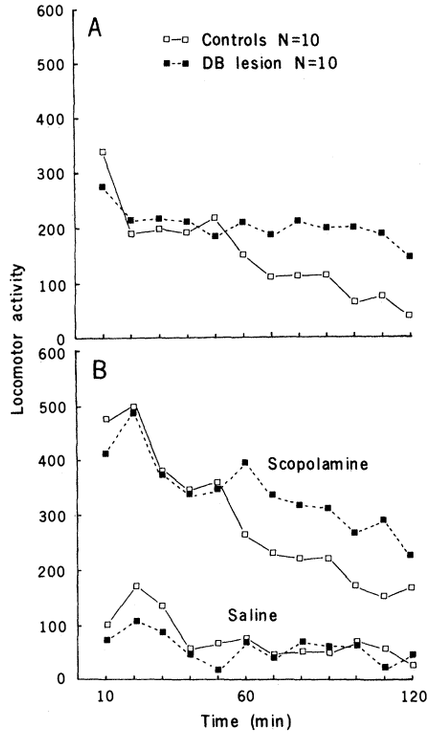


Fig. 1. (A) Locomotor activity of rats with 6-OHDA lesions of the dorsal bundle (*DB*). The activity was measured by means of photocells every 10 minutes for 2 hours. We used ten controls and ten noradrenaline-depleted rats injected intraperitoneally with atropine (10 mg/kg). (B) Locomotor activity in response to intraperitoneal injections of scopolamine (1 mg/kg) and saline.

This is important because we have obtained data that indicate that a pure NE depletion can enhance the locomotor stimulation in response to anticholinergics. We injected 4 μg of 6-OHDA dissolved in 2 μl of 0.9 percent saline into the fibers of the dorsal noradrenaline bundle in the mesencephalon (*3*). This resulted (Table 1) in a severe and permanent depletion of forebrain noradrenaline in cortex-hippocampus to less than 5 percent of controls, but there was no significant loss of striatal dopamine. Nonetheless, the locomotor response to atropine (Fig. 1A) or scopolamine (Fig. 1B) was significantly potentiated. We thus conclude that the effects reported by Schallert *et al.* (*1*) may not be due to the acetylcholine-dopamine interaction they suggest but to an interaction with NE instead. An acetylcholine-NE interaction is well established in the peripheral nervous system (*4*) and biochemical evidence has suggested that such also occurs in the central nervous system (*5*). Our data indicate that an acetylcholine-noradrenaline interaction must also be considered in the control of locomotor

Table 1. Regional amine assay values obtained at postmortem for control rats and rats injected with 6-OHDA. Values are means with standard error of the mean in nanograms of amine per gram wet weight of tissue.

| Region | Controls | 6-OHDA | Percentage* |
|---|---|---|---|
| Noradrenaline | | | |
| Cortex-hippocampus | 264 ± 6 | 6 ± 1 | 2 |
| Hypothalamus | 2,240 ± 77 | 590 ± 87 | 26 |
| Cerebellum | 219 ± 12 | 271 ± 8 | 124 |
| Spinal cord | 255 ± 6 | 307 ± 12 | 120 |
| Dopamine | | | |
| Striatum | 13,170 ± 570 | 11,570 ± 1,190 | 88 |

*The percentage of control concentrations remaining in lesioned tissues.


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activity and that such an interaction may have been responsible for the observations of Schallert *et al.* (*1*).

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Mason and Fibiger suggest that a decrease in cortical norepinephrine (NE) and not striatal dopamine (DA) may cause the excessive abnormal walking released in otherwise akinetic 6-OHDA-treated rats by anticholinergic drugs. Thus, in contrast to the acetylcholine-DA interaction with which our results (*1*) were consistent (*2*), they assert that an acetylcholine-NE interaction may have been responsible for our observations. We did not preclude this possibility in our original report, but we now regard it as unlikely.

Mason and Fibiger report that in rats with dorsal noradrenergic bundle (DB) damage (which depletes cortical NE relatively selectively), anticholinergic drugs produce what appears to us to be a slightly enhanced amount of some unspecified photocell-detected activity. Locomotion is not a behavioral entity that can be measured by any method one chooses, and its aberrations can take many forms (*1*, *3*). To interpret correctly the behavioral implications of changes in brain neurochemistry, one must adequately analyze the behavioral changes with which they are associated. This is not a simple task, and we do not believe that Mason and Fibiger measured the same form of locomotion that we reported.

We have repeated their procedure of depleting cortical NE by destroying the DB by local application of 6-OHDA. This method produced relatively selective depletion of cortical NE, with lesser effects on striatal DA and NE (see Table 1). However, in animals in which this depletion technique (see DB in Table 2) was used, atropine later did not release excessive locomotion in the running wheel, although in other animals (see NE-DA in Table 2), which received intraventricular 6-OHDA as in (*1*), atropine clearly did so (*4*). Therefore, cortical NE depletion alone, as proposed by Mason and Fibiger, cannot account for our findings. Their photocell-detected increase in activity is not the same as the excessive abnormal walking reported by us. The phenomenon that we report is an enormous behavioral effect—both in quantity and quality. The animals are profoundly and chronically akinetic. Despite this akinesia, within 5 minutes after receiving a single injection of an anticholinergic drug, they begin to walk, as much as 3000 turns in an activity wheel in 6 to 8 hours [some 43,000 steps (*5*), the equivalent of more than 40 km for a human]. They walk with very short steps, a gait suggestive of some forms of Parkinsonism. If they walk into a corner, they are completely trapped, remaining indefinitely without scanning upwards, turning around, or backing out. But if the walls are removed, they once again march inexorably forward.

The excessive walking is not released by atropine immediately after surgery but gradually develops over the next several weeks. One therefore must specify not only the behavioral nature of the phenomenon but also its postsurgical time course.

Although Mason and Fibiger's DB procedure (selective depletion of cortical NE by dorsal noradrenergic bundle damage) did not produce our phenomenon, NE might still play a role in it, a possibility we did not exclude (*1*). Therefore, we treated a group of rats with desmethylimipramine (DMI) and pargyline before we injected intraventricular 6-OHDA (*6*). Such treatment protects striatal NE, while severely depleting striatal DA (see Table 1). This group was severely akinetic but showed only a small increase in walking after atropine injection (Table 2). Therefore, striatal dopamine depletion alone is not suf-

Table 1. Catecholamine assay values obtained postmortem from control rats (medians expressed as nanograms per milligram of wet tissue), and percentages of those control concentrations in samples obtained from various 6-OHDA-treated groups. Abbreviations: DB, 6-OHDA in dorsal noradrenergic bundle; NE-DA, intraventricular 6-OHDA, high dose; DMI, desmethylimipramine prior to 6-OHDA; and NE < DA, intraventricular 6-OHDA, low dose.

| Group | Neocortex-hippocampus | | Striatum | |
|---|---|---|---|---|
| | NE | DA | NE | DA |
| Controls | 0.147 | 0.117 | 0.136 | 4.42 |
| | *Percentages of control concentrations* | | | |
| DB | 19.7 | 76.1 | 55.2 | 82.1 |
| NE-DA | 20.4 | 41.9 | 33.8 | 2.7 |
| DMI | 31.3 | 21.4 | 122.0 | 2.4 |
| NE < DA | 18.7 | 32.1 | 28.3 | 39.6 |

Table 2. Effects of atropine sulfate in 6-OHDA-treated rats and control rats on behavior in the activity wheel as a function of days after surgery. Data are medians, with the range in parentheses. Abbreviations as in Table 1.

| Days after surgery | Number of revolutions (in 8 hours) | | | | |
|---|---|---|---|---|---|
| | Controls | DB | NE-DA | DMI | NE < DA |
| | | *After atropine injection (50 mg/kg)* | | | |
| 1 | 148 (17–270) | 88 (9–270) | 28 (1–152) | 13 (0–596) | 39 (19–401) |
| 5 | 37 (22–196) | 86 (4–1297) | 230 (6–1391) | 60 (13–372) | 856 (23–1143) |
| 10 | 52 (31–56) | 130 (1–1331) | 1395 (169–1970) | 169 (123–359) | 1349 (54–3323) |
| 20 | 33 (25–39) | 147 (2–463) | 1646 (357–3082) | 410 (357–671) | 972 (47–3404) |
| 30 | 154 (97–156) | 152 (1–723) | 1078 (452–3131) | 227 (185–452) | 569 (52–2110) |
| | | *Without atropine* | | | |
| 25 and 35* | 408 (262–494) | 347 (2–947) | 4 (0–220)† | 8 (2–14)† | 120 (39–323) |

*Scores obtained on days 25 and 35 were pooled.

†In totally akinetic rats, periodic restless postural shifts eventually can summate to produce some wheel turning as observed by time-lapse cinematography (*10*).

ficient to produce the phenomenon. Indeed, only when both NE and DA were depleted in the striatum and cortex did the excessive abnormal walking appear in response to blockade of acetylcholine.

Neither an acetylcholine-DA interaction nor an acetylcholine-NE interaction alone can account for all the complexities of the phenomenon we have reported. For instance, an additional group (see NE < DA in Tables 1 and 2), though not akinetic (their locomotion seemed relatively normal when no drug was administered), walked excessively in response to atropine, using normal-sized steps and even running in the activity wheel (*7*). Only when striatal dopamine was also severely depleted (NE-DA) did the short-step aspect of the excessive walking appear (*8*). Atropine seems to interact with both NE and DA in ways and at sites that remain to be more fully understood (*9*). Such understanding will proceed only if brain neurochemistry is done hand in hand with adequate behavioral analysis.

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4. Note that the cortical NE values for the two techniques were comparable (Table 1). For the DB group, we injected 4 μg of 6-OHDA locally into the dorsal noradrenergic bundle in four rats and 8 μg in four additional rats (the data for the two doses were pooled because the results were similar). For group NE-DA, seven rats were treated with large doses of 6-OHDA intraventricularly (200 μg into each lateral ventricle and 100 μg into the third ventricle). Treatment with pargyline (50 mg/kg) preceded the 6-OHDA treatment in two of these rats.
5. By a step, we mean the average distance between two successive footprints made by the same hindleg on one side of the body.
6. In the DMI group, three rats received intraperitoneal injections of DMI (25 mg/kg) and pargyline (50 mg/kg) 30 minutes prior to receiving intraventricular injections of 6-OHDA (the same dose as group NE-DA).
7. In the NE < DA group, four rats were treated intraventricularly with smaller doses of 6-OHDA (100 μg into both lateral ventricles) with no special prior treatment.
8. Median step sizes were: control, 14.4 cm; DB, 14.6 cm; NE-DA, 9.0 cm; DMI, 5.0 cm; and NE < DA, 14.6 cm.
9. For example, brainstem or spinal mechanisms may also be involved. In our study, the two groups that were the most active after atropine treatment (group NE-DA and group NE < DA) showed little or no convulsive kicking behavior when they were decapitated. All rats in groups DMI and DB, which did not show hyperactivity to atropine, showed the prolonged rapid and powerful decapitation-released kicking behavior that occurs in control animals. Thus, the lack of kicking was not correlated with degree of akinesia (which presumably is a striatum-related effect) but seemed to be correlated with the propensity to show atropine-induced hyperactivity in the wheel. Although the hyperkinesia induced by atropine in group NE < DA was blocked by haloperidol (2 mg/kg, a dopamine receptor blocking agent), it was not blocked by haloperidol in group NE-DA, in which striatal dopamine was even more severely depleted. The role of other transmitters should be explored.
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## Age Affect and Replicative Life-Span of Fibroblasts of Diabetic, Prediabetic, and Normal Donors: Another Look at the Data

The purpose of this technical comment is twofold. The first is to correct some numerical and biostatistical errors in our recent report (*1*). The second is to describe the results of further analysis of the data.

The statistical methods used were not described in (*1*) and are outlined here for clarification. Pearson's correlation was used to calculate the relation between age of donor ($x$) and total mean population doublings ($y$). Linear regression was used to fit a least-squares line to the data. Comparisons among the mean replicative life-spans were made by one-way analysis of variance followed by unpaired $t$-tests between all pairs of means corrected for degrees of freedom. A test for linear trend among group means was also performed. We performed the analyses on an IBM 370/168 computer using the Data-Text program package (*2*).

Tables 1 and 2 summarize the previously reported data with corrections as indicated. With reference to the text of the previous report, two corrections should be noted. In the first paragraph of the first column of page 782 (*1*) the linear trend was not significant ($P = .215$) as indicated, and therefore does not support the hypothesis of progressive decrease in replicative capacity with increasing predisposition to diabetes. Then in the middle of the second column on the same page, the statement should have indicated the lack of a *significant negative* correlation between age and total mean population doublings in the normal group rather than implying lack of a negative value when it was indeed present, but not statistically significant.

The presence of these errors was unfortunate; we thank Senner (*3*) for causing us to review the data analyses again. Other problems were noted as indicated in Tables 1 and 2 and have now been corrected.

Although the mean ages did not differ significantly among the three study groups, the age ranges and age distribu-

Table 1. Summary of previously reported data (*1*).

| Study group | $N$ | $r$ | Regression line ($x$ = age, $y$ = total mean population doublings) | $P$ |
|---|---|---|---|---|
| Normals | 25 | −.279* | $y = 59.47^* - .17x^*$ | .177 |
| Prediabetics | 21 | −.579* | $y = 66.57 - .46x$ | .006* |
| Diabetics | 26 | −.431* | $y = 59.31^* - .29x^*$ | .020* |
| Combined | 72 | −.392* | $y = 60.47^* - .27x^*$ | .001 |

*Values that were in error in (*1*).

Table 2. Summary of previously reported data (*1*) (means ± standard deviation).

| Study group | Age (years) | Age range (years) | Total mean population doublings | Total mean population doublings adjusted for age |
|---|---|---|---|---|
| Normals | 44.3 ± 17.5 | 15 to 76 | 51.76 ± 10.92 | 52.54 ± 11.05 |
| Prediabetics | 40.0 ± 15.0 | 19* to 62 | 48.29* ± 11.88 | 47.84 ± 10.88 |
| Diabetics | 40.2 ± 19.2 | 14 to 76 | 47.54 ± 13.10 | 47.14 ± 11.02 |

*Values that were in error in (*1*).


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