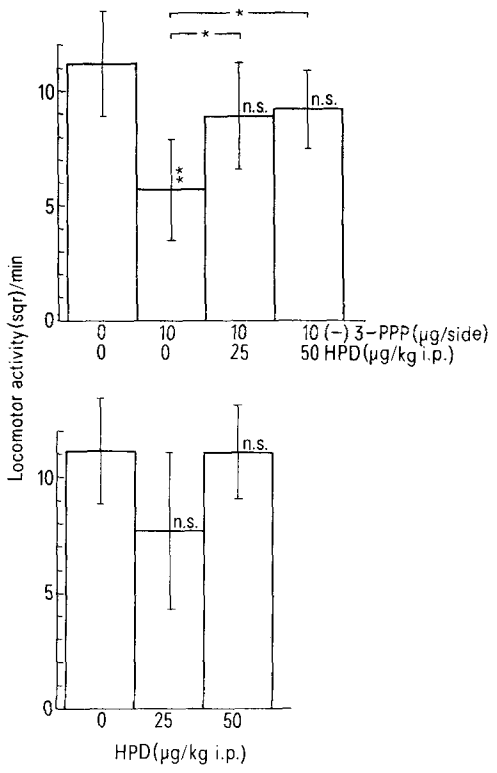

Figure 2. Antagonism by systemically administered HPD of the suppression of exploratory locomotor activity induced by the local application of (-)3-PPP into the nucleus accumbens of the rat. Naive animals were placed in the open field arena 6 min, following completion of bilateral application of (-)3-PPP, 10 µg/side, or saline, 1 µl/side. HPD, 25–50 µg/kg i.p., or 5.5% glucose pretreatment was administered 30 min before locomotor activity recording. The figure shows the exploratory locomotor activity (square root transformation, mean ± SD) of 7 animals/group during 3 min in the open field. The data were subject to an 1-way ANOVA followed by the Newman-Keul's test for individual comparisons[10]. The statistical analysis was performed on the data as grouped in the figure using the same saline controls. Top: $F(3/24) = 7.91$, $p < 0.01$; bottom: $F(2/18) = 4.02$, $p > 0.05$; n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$.

cleus accumbens, in doses below 160 µg/side[4], is not due to a disturbance of motor coordination or other unspecific effects of the drug. Figure 2 (top) shows that, in agreement with our previous observations[4] there was a statistically significant suppression of the exploratory locomotor activity by the local injection of (-)3-PPP, 10 µg/side, into the nucleus acumbens. The effect is probably not due to diffusion to other brain areas since local injection of this or higher doses (20–80 µg/side) into the neostriatum is ineffective[4]. The suppression of the exploratory locomotor activity by (-)3-PPP was statistically significantly antagonized by i.p. injection of HPD, 25–50 µg/kg. HPD by itself had no significant effects on the exploratory locomotor activity (fig. 2, bottom). The present results show (1) that the exploratory locomotor activity suppression induced by (-)3-PPP injected locally into the nucleus accumbens is not due to any disturbance of motor coordination and (2) that the locomotor suppression is most probably due to stimulation of DA autoreceptors, since the effect can be antagonized by the administration of low doses of HPD.

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Little effect of dimethyl sulfoxide on blood-brain barrier to dopamine[1]

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Summary. Rats were treated with dimethyl sulfoxide (DMSO) intraperitoneally or intravenously, and simultaneously with dopamine (DA). The presence of DMSO resulted in small or no increases in brain levels of DA or its metabolites.

Levodopa (L-DOPA), which is the principal therapeutic agent for Parkinson's disease, crosses the blood-brain barrier and is converted by DOPA decarboxylase to dopamine. L-DOPA is used because dopamine, the physiologically active neurotransmitter, does not readily cross the blood-brain barrier[3]. With long-term L-DOPA therapy many patients experience a number of fluctuations in their response such as the 'wearing-off' phenomenon, whereby the effect of L-DOPA fades after a short period of time following each dose, and the 'on-off' phenomenon, whereby the fluctuations are sudden and unpredictable[4]. Some patients with advanced Parkinson's disease become more resistant to L-DOPA and derive less benefit over time. One possible mechanism for the fluctuations and the loss of efficacy could be reduced decarboxylation of L-DOPA to dopamine in brain. DOPA decarboxylase is low in both striatum and substantia nigra in patients with Parkinson's disease as opposed to controls[5]. Hence it would be of some interest to see if it is possible to get peripheral dopamine into the brain and then utilize dopamine directly as the therapeutic agent. This paper reports an attempt to use dimethyl sulfoxide (DMSO) to facilitate the transport of dopamine across the blood brain barrier.

DMSO is an aprotic solvent. At high concentrations it has been utilized in the solubilization of biological membranes. Its unique capability to penetrate the skin has led to its application in the treatment of arthritis. DMSO has been reported to carry other therapeutic agents through the skin as well[6]. It has been a subject of controversy as regards the blood brain-barrier. It has been claimed that DMSO facilitates the passage of pemoline, epinephrine, norepinephrine, horseradish peroxidase, urea, 5-hydroxytryptophan (5-HTP) and L-DOPA into the brain[7-11]. Some of these compounds like 5-HTP and L-DOPA penetrate well even without DMSO, and some virtually do not penetrate at all without DMSO. No results for dopamine have been reported. DMSO has been shown not to enhance the transport of the following compounds into the brain: barbiturates, para-aminohippuric acid, sucrose, glucose, iron, methotrexate, albumin, hexosaminidase A, methionine, cholesterol, and tryptophan[11-15].

Materials and methods. Sprague-Dawley rats (180–200 g males) were used in all experiments. Dopamine (DA) hydrochloride, dihydroxyphenylacetic acid (DOPAC), and epinine were obtained from Sigma (St. Louis, MO). Homovanillic acid (HVA) was obtained from Regis (Chicago, IL). ISO-HVA was synthesized by Dr Sara Ginsburg (Columbia University). DMSO (70%) was obtained from RO-Chem (Atlanta, GA). Alumina was purchased from Bio-Rad (Richmond, CA).

Experiment 1. Rats were injected i.p. with either saline, DMSO (700 mg/kg), DA (75 mg/kg), or a combination of DMSO and DA. The animals were decapitated 1 h later, and the corpora striati were quickly dissected out on an ice cold plate and homogenized 1:15 in ice-cold 0.1 M perchloric acid containing 1 mM $NaHSO_3$ and 1 mM Na_2EDTA. The homogenate was centrifuged (15,000 rpm × 5 min) and the pellet discarded. One aliquot of the supernatant was analyzed for DA and the other aliquot was analyzed for the DA metabolities, HVA and DOPAC, as previously described[16]. Both procedures utilized reverse phase (C18, ODS column, DuPont) high performance liquid chromatography (HPLC) with electrochemical detection, using ISO-HVA as the internal standard for HVA and DOPAC measurements and epinine as the internal standard for DA measurement[16].

Experiment 2. Animals were pretreated with pargyline (75 mg/kg i.p.) 16 h prior to tail vein injection of DMSO (1400 mg/kg) and DA (6 mg/kg), given alone and in combination. Animals were sacrificed 10 min after the i.v. injection. Whole brain was dissected out, homogenized, processed as above, and subjected to HPLC for DA measurement[16]. Since the animals were pretreated with the monoamine oxidase inhibitor, pargyline, the DA metabolites, HVA and DOPAC, were not detectable.

Experiment 3. Animals were pretreated with pargyline (75 mg/kg i.p.) 16 h prior to DMSO and DA injections. DMSO (1400 mg/kg) was injected i.p. and another 1400 mg/kg was injected into the tail vein. DA (6 mg/kg) was injected into the tail vein alone and in combination with the DMSO treatment. The i.p. and i.v. injections were given simultaneously. Animals were sacrificed 10 min after injection, and the whole brain was processed as described above for DA measurement[16]. Statistical analysis was carred out by 2-tailed Student's t-test.

Results. Experiment 1. There was no increase of either striatal DA or its metabolites when DA was injected i.p. with or without DMSO (table 1). When compared to the saline-tested group, DOPAC concentration was slightly increased (38%) ($p < 0.05$) in animals given DMSO plus DA. However, there were no statistically significant differences when animals given DMSO plus DA were compared to those given DMSO alone or DA alone.

Experiment 2. The monoamine oxidase inhibitor, pargyline, caused an increase of DA concentration in whole brain (table 2), as is well known. In the presence of pargyline, the i.v. injection of DA caused a slight (22%) increase of DA in whole brain compared to pargyline-control animals ($p < 0.05$). There were no statistically significant differences when animals given DMSO plus DA were compared with animals given either DMSO alone or DA alone.

Experiment 3. In this experiment DMSO was given by both i.p. and i.v. routes concurrently. When i.v. DA was given along with the DMSO treatment, there was a slight (29%) statistically significant entry of DA into the brain when compared with pargyline controls ($p < 0.002$) (table 3). This difference was less (15%), but still statistically significant, when animals given DMSO plus DA were compared with animals treated with DMSO alone ($p < 0.05$). This difference (12%) did not quite reach the limits of statistical significance when animals given DMSO plus DA were compared with animals given DA alone ($p =$ exactly 0.05).

Discussion. The blood-brain barrier of all higher animals has been shown to be similar anatomically and physiologically[17,18]. Thus, while rats were used for these experiments, the results for humans could reasonably be expected to be similar.

At the concentrations of DMSO and DA utilized, very little entry of DA into the brain was detected. Although, we did not test higher concentrations of DMSO or DA, the concentrations and conditions used in these investigations were selected

Table 1. Effect of intraperitoneal DMSO and dopamine on striatal dopamine uptake and turnover

Treatment group	Dopamine	DOPAC	HVA
Saline	5.96 ± 0.374	0.52 ± 0.068	0.69 ± 0.084
DMSO	6.40 ± 0.315	0.62 ± 0.060	0.85 ± 0.087
Dopamine	5.99 ± 0.565	0.64 ± 0.077	0.69 ± 0.110
DMSO and dopamine	5.97 ± 0.572	0.72 ± 0.057	0.86 ± 0.100

Results are expressed as ng/mg (mean ± SEM) with n = 4. Details for the experimental protocol are given in Methods. There were no significant differences between any of the treated groups vs. the saline group except for DOPAC levels after treatment with DMSO and dopamine ($p < 0.05$). There were no significant differences between any of the DMSO plus dopamine results when compared to treatment with dopamine alone.

Table 2. Effect of intravenous DMSO and dopamine on whole brain dopamine uptake

Treatment group	Dopamine
Control (no pargyline)	0.51 ± 0.023
Control (pargyline alone)	0.68 ± 0.015[a]
Pargyline and DMSO	0.73 ± 0.088
Pargyline and dopamine	0.83 ± 0.065[b]
Pargyline, DMSO and dopamine	0.83 ± 0.084[c]

Results are expressed as ng/mg (mean ± SEM) with n = 4. Details of the experimental protocol are given in Methods. [a] $p < 0.0005$ compared to non-pargyline controls; [b] $p < 0.05$ compared to pargyline-treated controls; [c] $p > 0.05$ compared to pargyline-treated controls.

Table 3. Effect of combination intravenous and intraperitoneal DMSO on whole brain dopamine uptake

Treatment group	Dopamine
Control (no pargyline)	0.45 ± 0.025
Control (pargyline alone)	0.64 ± 0.032[a]
Pargyline and DMSO	0.72 ± 0.053
Pargyline and dopamine	0.74 ± 0.045
Pargyline, DMSO and dopamine	0.83 ± 0.014[b,c,d]

Results are expressed as ng/mg (mean ± SEM) with n = 4. Details of the experimental protocol are given in Methods. [a] $p < 0.005$ compared to non-pargyline controls; [b] $p < 0.002$ compared to pargyline-treated controls; [c] $p < 0.05$ compared to DMSO alone; [d] $p = 0.05$ exactly, compared to dopamine alone.

in order to be similar to those of other studies. For example, the time of sacrifice post-injection was 1 h in the i.p. studies (Experiment 1), which is compatible with the period of increased L-DOPA uptake in the presence of DMSO as measured by De La Torre[8]. Similarly, the time of sacrifice postinjection was 10 min in i.v. studies (Experiments 2 and 3), and this time is compatible with the period of increased epinephrine and norepinephrine uptake in the presence of DMSO as measured by Hanig et al.[9].

Also, the concentrations used in Experiment 1 for i.p. injection of DA and DMSO are 75 mg/kg and 700 mg/kg, respectively. These are basically identical to those utilized by De La Torre[8] in testing i.p. injected L-DOPA (75 mg/kg) and DMSO (750 mg/kg). Under these experimental conditions there was a large DMSO-mediated increase of L-DOPA in the brain[8]. In other experiments in our laboratory we have seen that this amount of L-DOPA given i.p. to rats even without DMSO will result in large increases in DOPAC and HVA levels.

In Experiments 2 and 3 the i.v. dosage of DA (6 mg/kg) compares well with the intravenous doses of epinephrine and norepinephrine (5 mg/kg) utilized by Hanig et al.[9].

In Experiment 2, limitations on the maximum volume of 70% DMSO solution injectable by tail vein (approximately 0.4 ml of 1400 mg/kg solution) preclude an exact comparison with the injections of DMSO/epinephrine and DMSO/norepinephrine in jugular vein, in which 2750 mg/kg was injected[9]. However, 1400 mg/kg DMSO (Experiments 2 and 3) is approximately twice the concentration used for facilitating L-DOPA entry into the brain with intraperitoneal DMSO[8].

Experiment 3 was designed to increase the amount of DMSO injected and because of the report that horseradish peroxidase only entered the brain significantly if both i.v. and i.p. routes of administration of DMSO were utilized simultaneously along with an intravenous bolus of the peroxidase[10]. However, in our experiments this combination approach had little effect since statistical significance was not reached between DA alone and DA with DMSO (12% increase) (table 3). This can be compared with the 34% and 38% increase found with epinephrine and norepinephrine, respectively, in the presence of DMSO[9]. Any differences might be attributable to the fact that the results for norepinephrine and epinephrine were obtained in neonate chicks where the blood brain barrier is not fully developed[18].

Our results are consistent with non-passage of large amounts of DA through the blood brain barrier. Considering the large doses of DA and DMSO given and the need to inject DMSO i.v., the small increases we found would not warrant a therapeutic trial of DMSO and DA in Parkinson's disease.

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Preadult lethality in four populations of *Drosophila melanogaster* treated with formaldehyde

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Summary. Samples from 4 populations of *D. melanogaster* were treated with formaldehyde by the larval feeding method, and induced lethality was scored. The results showed relevant differences among the populations analyzed.

Formaldehyde is a contaminant and mutagen originating from many sources, and distributed widely in the human environment[1]. It is used in the textile industry, in agriculture and also in the manufacturing of paper, cosmetics, resins, etc.[2].

Formaldehyde is a strong mutagen for *D. melanogaster*, inducing high increases of sex-linked recessive lethals, visible mutations, gynandromorphs, dominant lethals, deficiencies, duplications, translocations, and inversions[3,4]. Mutagenic properties of formaldehyde have also been described for *Neurospora crassa*[5], *Escherichia coli*[6], and *Saccharomyces cerevisiae*[7].

In spite of the wide distribution of this toxin, no studies have been performed to demonstrate its effects on partial components of the fitness of an organism, such as fertility, developmental rate, etc. This paper shows preliminary results on the effects of formaldehyde on the lethality in 4 populations of *D. melanogaster* from different sources.

Material and methods. The flies used in this work were caught during 1972 and 1975 in Asturias (Spain). Each population was established by the capture of at least 40 pairs, and maintained by mass culture. The Felguera and Oviedo populations were obtained in those towns, which are exposed to several kinds of contamination. The populations Teverga and Naranco were caught in the village of Teverga and on Naranco mountain, places apparently less contaminated. Two types of