PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 62–68 (1988)

Central Sympathoplegic and Norepinephrine-Depleting Effects of Antioxidants (42638)

ANNE E. CHESTER[1] AND FREDERICK H. MEYERS

Department of Pharmacology, University of California, San Francisco, California 94143

Absract. Carbon disulfide (CS_2), tetraethyl lead (TEL), tetraethyl tin (TeET), dithiothreitol (DTT), and gossypol acetic acid (GAA) significantly decreased brain norepinephrine (NE) in rats. The central dopamine (DA) increased after ip administration of CS_2, TEL, and DTT, but decreased after TeET and GAA. The brain serotonin decreased only after TeET. Two doses of DTT decreased the NE longer than one dose (24 vs 2 hr) but did not increase DA. L-DOPA, given SC with DTT, delayed the decrease in NE by 24 hr. The similar behavioral and autonomic effects of each of these compounds suggest a central sympatholytic effect and an antipsychotic type of sedation and rigidity. A possible mechanism is reversible inhibition of dopamine β-hydroxylase through the reduction of the copper ion of the enzyme. Each of these reducing agents, together with the boranes previously studied, has similar behavioral and autonomic effects and a common effect on NE concentration, suggesting that the agents act through a physicochemical property rather than by combination with a cellular component. These data have applications to the toxicity of the single agents. They also provide an index of activity, previously lacking, of systemic antoxidant effect. © 1988 Society for Experimental Biology and Medicine.

Weir and Meyers (1) found that the potent reducing agents deca- and pentaborane decreased the brain levels of norepinephrine (NE) and serotonin (5 HT) in rats and caused changes in autonomic and behavioral function comparable to those of reserpine. The present study examines the pharmacologic effects of five additional reducing agents. The goal is to show that all reducing agents have similar effects on central amine levels and have the same manifest effects and that their potency correlates with their ability as reducing agents rather than with their chemical reactivity or steric properties. A relationship of these similar effects to dopamine (DA) β-hydroxylase is suggested by the reported properties of that enzyme.

From those substances known to be reducing agents, carbon disulfide (CS_2), dithiothreitol (DTT), tetraethyl tin (TeET), tetraethyl lead (TEL), and gossypol acetic acid (GAA) were studied for their effects on the concentrations of catechols in the brain and on autonomic and behavioral functions. These reducing agents were selected because of their toxicologic interest, because some pharmacologic data were already available, or because a therapeutic application is suggested.

Some plausible agents such as vitamin E, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were rejected when an examination of the literature and our own preliminary studies failed to establish evidence of a systemic effect in the experimental animal after oral administration.

CS_2 is an industrial solvent. Not only are the effects of exposure on the cardiovascular, gastrointestinal, endocrine, and behavioral functions described in the human, but also it is the one compound studied here that had previously been reported to alter the levels of central amines (2).

TEL has caused serious illness and death, by effects different than those of inorganic lead. The mechanism of its action has not been established nor has successful treatment been developed (3).

TeET attracted interest because its described toxicologic effects appeared similar to those of carbon disulfide and the boranes (4).

DTT is a common reducing reagent in the chemistry lab and has been used as a protector of sulfhydryl groups in *in vitro* studies of receptors, enzymes, and synaptosomes (5).

GAA is being studied as a potential male antifertility agent due to its relatively reversible induction of oligospermia. Studies have concentrated on the presumed direct effects of gossypol on the testes, but many of the side effects seen in clinical studies cannot be

[1] Present address: Syntex Research, R2-201, 3401 Hillview, Palo Alto, CA 94303.

0037-9727/88 $1.50
Copyright © 1988 by the Society for Experimental Biology and Medicine.
All rights reserved.

explained by this hypothesis. These side effects include gastrointestinal discomfort, electrolyte imbalance, lethargy, loss of libido, and endocrine dysfunction (6).

Materials and Methods. Male Sprague–Dawley rats weighing between 0.230 and 0.300 kg from Bantin–Kingman were kept in an environmentally controlled facility with free access to rat chow and water and with a controlled light–dark cycle. The animals were randomized for treatment received. The rats were decapitated $\frac{1}{2}$ to 48 hr postinjection on a schedule based on observations of the varied onset and duration of action of the agents used (see Table I for details of procedure). Each assay was performed in replicates composed of the pooled brain tissue collected at each postinjection time from one to two control animals and five treated animals. The brain was removed immediately after decapitation and dissected to isolate an area containing the midbrain, thalamus, subthalamus, hypothalamus, and hippocampus following the general outline of Glowinski *et al.* (7). The cerebellum was removed with forceps. To remove the rhombencephalon, a transverse section was made between the pons and the midbrain (all sections were made using a hand-held razor blade). A second transverse section was made at the optic chiasm, separating much of the cerebral cortex from the area of interest. More cortex was removed by dissecting it away, using the third ventricles and the corpus callosum as limits. The brain was then halved longitudinally to decrease the weight of the tissue processed and increase amine extraction. Each half was frozen and weighed. Each half weighed between 0.35 and 0.50 g. If the assay was performed the following day the brains were placed on and covered by polystyrene weigh boats and kept on dry ice overnight.

The assays of NE, DA, and 5HT were performed according to the fluorometric method of Jacobowitz *et al.* (8). Amine levels were not done on animals that died before scheduled decapitation.

Voltammetry. An Aminco Bowman CV27 Voltammograph was used with a platinum electrode with reference to a standard calomel electrode. A small amount of gossypol acetic acid (Sigma) was added to 99% acetonitrile with 0.10 *M* tetrabutylammonium perchlorate. A linear increase in voltage was applied at 0.5 V/sec between 0.0 and 2.0 V and the current produced was recorded on a Solec X-Y VP6414S.

Results. *Carbon disulfide.* All rats administered CS_2 showed 50–100% ptosis within 2 hr after injection together with enophthalmia, diarrhea, maximum respiratory effort, and reduced spontaneous movement with little or no response to environmental stimuli. At the dose of 0.75 g/kg (ip), out of 35 rats, 1 died 6 hr after CS_2 injection and 9 died between 8 and 16 hr. Deaths were preceded by convulsions. The mean brain NE concentration was significantly decreased ($P < 0.05$) 1 hr postinjection, reaching a minimum at 8

TABLE I. EXPERIMENTAL PROCEDURE[a]

Experimental compound	Vehicle	Dose (g/kg)[b]	Times experimental rats killed after treatment (hr)	Control animals' time (hr)
CS_2	Corn oil	0.75	1, 2, 3, 8, 16	8
Tetraethyl lead[c] (TEL)	Corn oil	Two doses of 0.15	1, 2, 4, 16, 20	20
Tetraethyl tin (TeET)	Corn oil	0.040	2, 4, 8, 24, 48	24
Gossypol acetic acid	70% DMSO	0.20	2, 4, 8, 12, 16, 20	24
DTT	0.9% saline	0.075	0.5, 1, 2, 3, 24	3
DTT[c]	0.9% saline	Two doses of 0.075	0.5, 1, 2, 3, 24	3
L-DOPA[d]	0.9% saline	0.20	0.5, 1, 2, 3, 24, 26	3

[a] See Tables 2 and 3 for sample size.
[b] All given ip except L-DOPA which was given sc.
[c] Doses were given 4 hr apart.
[d] Experimental rats were given 0.20 g/kg L-DOPA by sc injection and one ip dose of 0.075 g/kg DTT.

hr ($P < 0.001$), and was still significantly decreased after 16 hr ($P < 0.05$). The mean brain DA concentration was increased significantly 2 hr ($P < 0.02$) and 4 hr ($P < 0.001$) after injection. After 8 hr, the mean dopamine concentration was within control values. There was no significant change in the mean brain 5HT concentrations (see Table II).

Tetraethyl lead. Rats administered the two doses of 0.15 g/kg TEL (4-hr interval) developed enophthalmia and ptosis within 1 hr after the first dose. Diarrhea, lethargy, and pink ears were common signs. Red circles, like dried red tears (chromadacryorrhea), were noted on the fur around the eyes of a few rats. Out of 28 rats, 3 died between 16 and 20 hr after the last TEL injection. Deaths were preceded by convulsions. The mean NE concentration in the brain decreased significantly ($P < 0.002$) by 2 hr after the last TEL injection and the maximum decrease was measured at 20 hr ($P < 0.001$). The mean DA concentration increased significantly ($P < 0.05$) 1 hr after injection and remained up until at least 16 hr postinjection. By 20 hr, the DA levels were within control values. There was no significant change in the mean 5HT concentration (see Table II).

Tetraethyl tin. The rats given 0.040 g/kg TeET developed ptosis, enophthalmia, diarrhea, and lethargy, frequently progressing to catatonia. Some animals developed chromodacryorrhea 8 to 24 hr after the injection of TeET. Out of the eight rats allowed to live beyond 24 hr after the injection; three died between 33 and 48 hr. Death was preceded by convulsions. The animals decapitated after 24 hr postinjection had no reflex kicking after decapitation. The mean concentration of brain NE decreased significantly ($P < 0.002$) by 8 hr, reaching a minimun level at 24 hr postinjection, and was still significantly decreased ($P < 0.02$) after 48 hr. The mean brain DA concentration decreased at 24 hr ($P < 0.01$) and within normal range again by 48 hr. The mean 5HT concentration was significantly decreased ($P < 0.01$) after 8 hr but returned to normal by 24 hr (see Table II).

Gossypol acetic acid. In rats given gossypol diarrhea was not as constant a sign as after the other reducing agents, but the rats did develop some ptosis and enophthalmia and several had chromodacryorrhea. At the dose of 0.20 g/kg, 7 of 22 rats allowed to live longer than 8 hr postinjection died between 8 and 16 hr. Death was preceded by convulsions. The mean brain NE concentration was significantly decreased ($P < 0.01$) 8, 12, and 16 hr postinjection and approached control levels again at 20 hr. The mean brain DA concentration was significantly decreased ($P < 0.05$) 8 hr after gossypol acetic acid injection. There was no significant change in the mean brain 5HT concentration (see Table II).

Dithiothreitol. All rats given DTT developed ptosis, enophthalmia, and lethargy. There were no deaths prior to scheduled decapitation. After one dose of 0.075 g/kg DTT, the mean brain NE concentration was significantly decreased ($P < 0.02$) $\frac{1}{2}$ hr after injection, and within control range 3 hr after injection. The mean concentration of DA was increased significantly ($P < 0.05$) in the brain 1 hr after injection. There was no significant change in the mean brain 5HT concentration. After two doses of 0.075 g/kg DTT (4-hr interval) the mean NE concentration was again decreased ($P < 0.02$) $\frac{1}{2}$ hr after the second injection but unlike one injection, was still significantly decreased ($P < 0.01$) 24 hr postinjection. The mean DA and 5HT concentrations were not statistically significantly changed after the second injection. When 0.20 g/kg L-DOPA was given subcutaneously at the same time as one intraperitoneal dose of DTT, the decrease in mean NE concentration was delayed until 24 hr after the injections (see Table III).

Voltammetry. To verify that GAA was a reducing agent, two voltammograms were made. Both tracings reveal that two electrons are released from GAA, thereby confirming its reducing ability. One electron was removed at 1.10 V and another at 1.47 V (see Table IV).

Discussion. Each reducing agent given in doses that caused similar behavioral and autonomic effects significantly decreased central NE levels. Carbon disulfide, TEL, and DTT significantly increased central DA levels. Tetraethyl tin and GAA significantly decreased central DA levels. Only TeET decreased central 5HT levels. These results

TABLE II. MEAN AMINE CONCENTRATION (μg/g BRAIN TISSUE)

	0.75 g/kg CS_2			0.15 g/kg TEL (two doses with a 4-hr interval)			0.40 g/kg TeET			0.20 g/kg GAA		
(hr)	NE	DA	5HT	NE	DA	5HT	NE	DA	5HT	NE	DA	5HT
(Control)	0.91 ± 0.15 (11)	1.29 ± 0.21 (12)	1.03 ± 0.21 (12)	0.61 ± 0.03 (8)	0.65 ± 0.16 (8)	0.87 ± 0.15 (6)	0.61 ± 0.08 (16)	1.16 ± 0.20 (16)	1.25 ± 0.15 (15)	0.69 ± 0.09 (15)	1.24 ± 0.20 (15)	1.40 ± 0.14 (10)
1	0.74 ± 0.18[f] (7)	1.50 ± 0.21 (7)	1.08 ± 0.17 (7)	0.57 ± 0.07 (6)	0.92 ± 0.30[f] (6)	0.88 ± 0.12 (6)						
2	0.71 ± 0.19[f] (7)	1.65 ± 0.34[e] (7)	1.07 ± 0.14 (7)	0.52 ± 0.05[b] (6)	0.99 ± 0.32[f] (6)	0.86 ± 0.10 (6)	0.60 ± 0.08 (6)	1.17 ± 0.09 (6)	1.23 ± 0.12 (6)	0.63 ± 0.09 (6)	1.13 ± 0.17 (6)	1.45 ± 0.28 (6)
4	0.56 ± 0.18[a] (7)	1.77 ± 0.29[a] (7)	1.10 ± 0.20 (7)	0.53 ± 0.09[f] (6)	1.04 ± 0.30[d] (6)	0.89 ± 0.18 (6)	0.55 ± 0.02 (6)	1.02 ± 0.19 (6)	1.18 ± 0.10 (6)	0.64 ± 0.06 (6)	1.26 ± 0.24 (6)	1.43 ± 0.17 (6)
8	0.53 ± 0.14[a] (6)	1.34 ± 0.22 (6)	1.09 ± 0.15 (6)				0.47 ± 0.04[b] (5)	1.03 ± 0.08 (5)	1.04 ± 0.02[d] (5)	0.53 ± 0.03[b] (5)	1.03 ± 0.16[f] (5)	1.35 ± 0.27 (5)
12										0.58 ± 0.07[e] (7)	1.17 ± 0.11 (7)	1.40 ± 0.15 (7)
16	0.76 ± 0.11[f] (7)	1.13 ± 0.19 (7)	1.19 ± 0.09 (7)	0.50 ± 0.12[f] (7)	0.76 ± 0.19[f] (7)	0.93 ± 0.14 (7)				0.54 ± 0.06[d] (4)	1.08 ± 0.17 (4)	1.40 ± 0.11 (4)
20				0.34 ± 0.08[a] (2)	0.54 ± 0.21 (2)	0.75 ± 0.10 (2)				0.68 ± 0.04 (4)	1.26 ± 0.34 (4)	1.47 ± 0.20 (4)
24							0.44 ± 0.08[a] (7)	0.92 ± 0.14[d] (7)	1.16 ± 0.27 (7)			
48							0.51 ± 0.03[e] (5)	1.08 ± 0.24 (5)	1.13 ± 0.10 (5)			

Note. Values are mean μg/g brain tissue ± standard deviation. L-DOPA given sc; all others ip. For test of significance, L-DOPA at same time period used for control values. Number in parentheses is sample size. Student's two tailed *t* test.

[a] $P < 0.001$.
[b] $P < 0.002$.
[c] $P < 0.005$.
[d] $P < 0.01$.
[e] $P < 0.02$.
[f] $P < 0.05$.

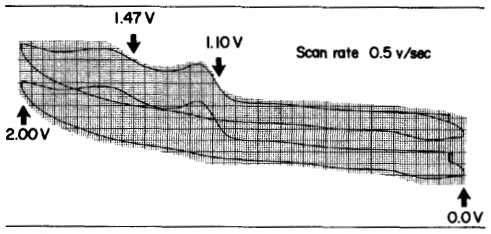
TABLE III. MEAN AMINE CONCENTRATION (μg/g BRAIN TISSUE)

	0.75 g/kg DTT			0.75 g/kg DTT (two doses with a 4-hr interval)			0.20 g/kg L-DOPA + 0.75 g/kg DTT			0.20 g/kg L-DOPA		
(hr)	NE	DA	5HT	NE	DA	5HT	NE	DA	5HT	NE	DA	5HT
(Control)	0.66 ± 0.08 (12)	0.89 ± 0.19 (12)	1.13 ± 0.15 (14)	0.66 ± 0.08 (12)	0.89 ± 0.19 (12)	1.13 ± 0.15 (14)						
0.5	0.55 ± 0.05[e] (5)	1.16 ± 0.30[f] (5)	1.08 ± 0.08 (5)	0.54 ± 0.09[e] (6)	0.95 ± 0.24 (6)	1.24 ± 0.17 (6)	0.61 ± 0.10 (5)	2.81 ± 0.93 (5)	1.24 ± 0.15 (5)	0.64 ± 0.05 (4)	2.52 ± 0.62 (4)	1.34 ± 0.12 (4)
1	0.54 ± 0.06[d] (5)	0.91 ± 0.24 (5)	1.08 ± 0.12 (5)	0.55 ± 0.09[f] (5)	1.03 ± 0.21 (5)	1.11 ± 0.17 (6)	0.67 ± 0.11 (5)	3.18 ± 1.03 (5)	1.17 ± 0.20 (5)	0.67 ± 0.06 (5)	3.91 ± 1.73 (5)	1.34 ± 0.08 (5)
2	0.54 ± 0.04[d] (5)	0.97 ± 0.25 (5)	1.04 ± 0.16 (5)	0.55 ± 0.08[f] (5)	0.94 ± 0.24 (5)	1.17 ± 0.21 (6)	0.69 ± 0.09 (5)	2.49 ± 0.73 (5)	1.18 ± 0.13 (5)	0.66 ± 0.06 (5)	2.87 ± 1.00 (5)	1.28 ± 0.07 (5)
3	0.58 ± 0.04 (5)	0.97 ± 0.32 (5)	0.98 ± 0.12 (5)	0.53 ± 0.06[c] (6)	1.01 ± 0.26 (6)	1.16 ± 0.10 (6)	0.63 ± 0.11 (17)	3.23 ± 1.93 (17)	1.24 ± 0.17 (13)	0.68 ± 0.12 (14)	3.07 ± 1.31 (14)	1.23 ± 0.14 (10)
24	0.63 ± 0.02 (4)	0.90 ± 0.11 (4)	0.98 ± 0.06 (4)	0.54 ± 0.13[d] (5)	0.85 ± 0.21 (5)	1.05 ± 0.04 (5)	0.56 ± 0.05[d] (7)	3.04 ± 1.26 (7)	1.24 ± 0.08 (7)	0.66 ± 0.05 (5)	2.64 ± 0.84 (5)	1.27 ± 0.09 (5)
26							0.67 ± 0.05 (5)	1.73 ± 0.31 (5)	1.42 ± 0.05 (5)	0.73 ± 0.09 (4)	3.11 ± 1.65 (4)	1.34 ± 0.16 (4)

Note. Values are mean μg/g brain tissue ± standard deviation. L-DOPA given sc; all others ip. For test of significance, L-DOPA at same time period used for control values. Number in parentheses is sample size. Student's two-tailed *t* test.

[a] $P < 0.001$.
[b] $P < 0.002$.
[c] $P < 0.005$.
[d] $P < 0.01$.
[e] $P < 0.02$.
[f] $P < 0.05$.

TABLE IV



allow conclusions and inferences relating to mechanism of action, to the toxicity of the single agents, and to the study of antioxidants in general.

By what mechanism can six compounds of greatly differing chemical structures but with one common physical property cause similar biochemical, physiological, and behavioral effects? The correlation cannot be explained in terms of the receptor theory with binding to a limited number of macromolecules on the surface of an effector cell or to an enzyme. The receptor concept places structural requirements onto the compound which must be met if binding is to occur. In the present case, a chemical property, reducing ability, appears to be correlated with a set of similiar pharmacologic effects and, therefore, structural requirements are unnecessary.

Inhibition of DA β-hydroxylase (DBH) has been shown to increase DA, decrease NE, and cause behavioral effects such as seen here by CS_2, DTT, TEL, TeET, and GAA.

DBH is a copper-containing enzyme that undergoes cyclic oxidation reduction as it hydroxylates DA. The enzyme is part of a complex system which is studied primarily *in vitro*. Molecular oxygen is the only oxygen source it uses (9). Ascorbate is the most effective reducing agent, occurs endogenously in high concentrations, and therefore is believed to be the natural electron donor.

DBH is inhibited by various compounds, many of which are metal-chelating agents or phenylethylamine analogs. Some of the more potent inhibitors such as substituted thioreas, have a hydrophobic side chain similiar in size to the aromatic ring of the phenylethylamines. A hydrophobic pocket in DBH has been proposed to explain these observations. Carbon monoxide and peroxide are examples of irreversible DBH inhibitors. Carbon monoxide is assumed to irreversibly bind to the copper I form of the enzyme. Using data on the effect of hydrogen peroxide on bovine erythrocyte superoxide dismutase, an enzyme which requires the oxidation of copper I to copper II for activity, it has been suggested that hydrogen peroxide inhibits DBH by reducing the oxidized form of the enzyme and the resulting hydroxyl radical irreversibly inactivates it by oxidizing an adjacent histidine residue necessary for DBH activity (9). In the single reported case of congenital DA hydroxylase deficiency, a similar pattern of ptosis, lethargy, and hypotension was reported (10).

Further studies will explore the possibility that the reducing agents investigated here are reducing the copper ion in DBH but, unlike hydrogen peroxide, in a reversible manner. The fact that dopa delays the decrease in NE after administration of DTT indicates that DBH may be involved.

That TeET and GAA, the two strongest reducing agents studied (see Table V), have other effects is not surprising. Few agents have just one effect. The decrease in DA by TeET and GAA and the decrease in 5HT by TeET may not be related to their reducing potential. However, it is interesting to note that a decrease in DA may be caused by inhibition of tyrosine hydroxylase, and a decrease in 5HT by inhibition of tryptophan hydroxylase. Both of these enzymes require a reducing agent (tetrahydrobiopterin) for activity. Tetraethyl tin and GAA could be inactivating tyrosine hydroxylase with TeET

TABLE V. REDUCTION POTENTIALS

Compound	Reduction potential E (V)	Reference
Dithiothreitol	−0.33 at pH 7	(5)
Carbon disulfide	−0.68[a]	
Tetraethyl lead	−1.26[b]	(14)
Gossypol acetic acid	−1.47, −1.10[b]	See Results
Tetraethyl tin	−1.76[b]	(14)

[a] Calculated from Gibbs' free energy for CS_2; see Ref. (15).

[b] Compared to a standard calomel electrode whose potential is 0.24 V.

additionally inactivating tryptophan hydroxylase. Tyrosine hydroxylase also requires ferrous ion for activity, which GAA has been shown to be capable of binding (11).

These results also have important applications to the compounds considered singly as environmental agents. Exposures to CS_2 and TEL still occur, but the chronic effects of GAA are perhaps of the greatest immediate interest.

The presumption that GAA, a putative male contraceptive, acts primarily on the testes needs to be questioned, for our observations suggest a relationship to agents known to alter the release of hypothalmic-releasing factors and gonadotropins. GAA chronically administered to humans not only caused lassitude but also decreased serum testosterone levels, oligospermia, and loss of libido in the male and regression of the endometrium in women (12). These effects are similar to those seen in workers exposed to CS_2 (13) and may be related to the apparent ability of NE to stimulate and of DA to inhibit the release of gonadotropin releasing factors.

Study of these known reducing agents provides quantifiable evidence of antoxidant effect. With the antoxidants now being studied in lipid peroxidation and aging, for example, the tocopherols, BHA and/or BHT, there is great difficulty in establishing in exposed animals whether physiologically active doses are being given or distributed.

We are very grateful to Dr. Gary TerHaar of the Ethyl Corporation for providing the tetraethyl lead and to Ralph Stearns in the laboratory of Dr. Paul Ortiz de Montellano at the University of California, San Francisco, for the voltamographic data.

1. Weir F, Meyers FH. The similar pharmacologic effects of pentaborane, decaborane and reserpine. Ind Med Surg **35**:696–701, 1966.
2. Magos L, Jarvis L. The effects of carbon disulphide exposure on brain calecholamines in rats. Brit J Pharmacol **39**:26–33, 1970.
3. Beattie A, Moore N, Goldberg A. Tetraethyl lead poisoning. Lancet **27**(7766), 12–15, 1972.
4. Barnes JM, Stoner H. The toxicology of tin compounds. Rev Pharmacol **11**:211–231, 1959.
5. Clealand W. Dithiothreitol, a new protective reagent for SH groups. *Biochemistry* **3**(4):480–482, 1964.
6. Liu G. Clinical study of gossypol as a male contraceptive. Reproduction **5**:189–193, 1981.
7. Glowinski J, Inverson L. Rational studies of catecholamines in the rat brain. J. Neurochemistry **13**:655–669, 1966.
8. Jacobowitz D, Richardson J. Method for the rapid determination of norepinephrine, dopamine, and serotonin in the same brain region. Pharmacol Biochem Behav **8**:515–519, 1978.
9. Rosenberg R, Lovenberg W. Dopamine-β-hydroxylase. In: Yaudim M, Ed. Essays in Neurochemistry and Neuropharmacology. New York, Wiley, Vol 4:pp163–209, 1980.
10. Man IN'T, Veld AJ, Boomsma F, Moleman P, Schalekamp MADH. Congenital dopamine beta-hydroxylase deficiency. Lancet **1**:183–187, 1987.
11. Quian S, Wong Z. Gossypol: A potential antifertility agent for males Annu Rev Pharmacol Toxicol **24**: 329–360, 1984.
12. Kuo-Fen C, Wei-Yu W, Min-Yi T, Peng-Ti C. Endometrial changes after administration of gossypol for menorrhagia. Amer J Obstet Gynecol **138**(8):1227–1229, 1980.
13. National Institute for Occupational Safety and Health. Occupational Exposure to Carbon Disulphide. Washington DC, U.S. Department of Health, Education, and Welfare, 1977.
14. Klinger R, Kocke K. Heterogeneous rates of electron transfer. Application of cyclic voltammetric techniques to irreversible electrochemical processes. J Amer Chem Soc **102**:4790–4798, 1980.
15. Johnson A. Bond energy terms. In: Senning A, Ed. Sulfur in Organic and Inorganic Chemistry. New York, Dekker, 1972.

Received May 8, 1987. P. S. E. B. M. 1988, Vol. 187.
Accepted September 21, 1987.