BIOCHEMICAL PHARMACOLOGY OF HARMALINE IN THE RAT

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By

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TABLE OF CONTENTS

I. INTRODUCTION

A. REVIEW OF HARMALINE AND RELATED COMPOUNDS

Harmala alkaloids have attracted the interest of many investigators on account of their effect on the extrapyramidal system, their hallucinogenic properties, their inhibitory activity on monoamine oxidase and the hypothetical role of related compounds in mental illness.

1. Chemistry:

The unsubstituted ring system of these alkaloids is known as β- (or 3-) carboline (Fig. 1). They all bear a methyl group in the 2 position of the carboline nucleus. The degree of saturation of the pyridine ring and the substituents at 3 and 8 positions differentiate them (Fig. 2).

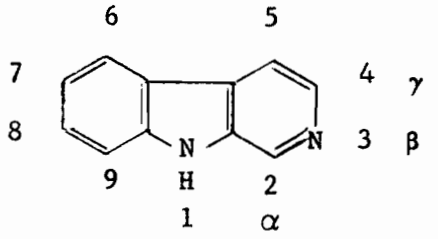
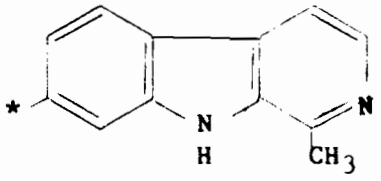


FIG. 1 (From ref. 1)

A different nomenclature is used by Chemical Abstracts. The unsaturated system is called 9H-pyrido (3,4-b) indole (Fig. 3). The pyridine ring-reduced derivatives of it are named as 3H- or 1H-pyrido (3,4-b) indoles. For instance, according to this system, harmaline is 4,9-dihydro-7-methoxy-1-methyl-3H-pyrido (3,4-b) indole and harmine is 7-methoxy-1-methyl-9H-pyrido (3,4-b) indole. Throughout the thesis the β-carboline nomenclature will be used.

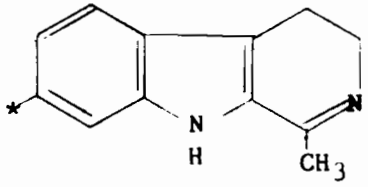
Harmala alkaloids and related compounds:



* = CH_3O : harmine

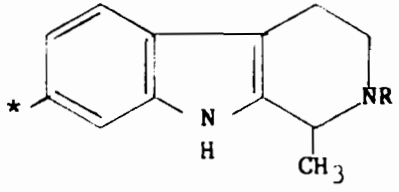
* = HO : harmol

* = H : harman



* = CH_3O : harmaline

* = HO : harmalol



* = CH_3O, R = H : tetrahydroharmine

* = HO , R = H : " " harmol

* = H , R = H : " " harman

FIG. 2

Adapted from Zirkle and Kaiser (1)

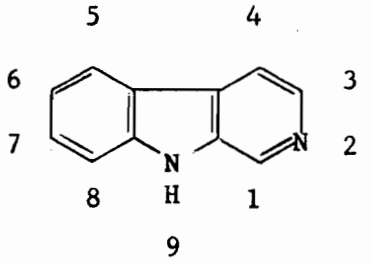


FIG. 3 (From ref. 1)

The harmala alkaloids can be divided in two groups: the methoxyharmans and the hydroxyharmans. The former have a methoxy group at position 8 whereas the latter have a phenolic group there.

Zirkle and Kaiser (1) have recently reviewed in detail the synthesis of the β-carbolines. Groeger and Simon consider tryptophan to be the precursor in the biosynthesis of harmala alkaloids (2).

2. Historical:

Harmaline and harmine were the first alkaloids of this family discovered. They were isolated from the seeds of Peganum harmala, a plant belonging to the order of Rutaceae: harmaline by Goebel just prior to 1841 (3) and harmine in 1847 by Fritzche (4).

In South America, Indians had for a long time used extracts of lianas variously known as "Ayahuasca", "Yagé", "Caapi" in order to induce states of hallucination and intoxication. The corresponding beverages bore the same name (5). In 1927, Raymond - Hamet and Perrot showed that these different plants belonged in fact to the same botanical species named by Spruce, Banisteria caapi (6).

Yagé was thought to bestow the power of telepathy and Bayon, a Colombian scientist, attributed this property to an alkaloid which he named "telepathine". In 1923, Cardenas isolated it and kept the name

telepathine. In 1925, Villalba isolated the same compound naming it "Yageine": another found in much smaller amount was called "Yagenine" (7). Lewin coined the name "banisterine" for the alkaloid produced from Banisteria caapi by Merck (5, 8). Wolfes and Rumpf in 1928, and Elger independently, demonstrated that yageine, banisterine and harmine were the same compound (9, 10).

3. Occurrence:

Peganum harmala recently studied chromatographically by Schipper and Volk contains two major constituents, harmine and harmaline, and two minor ones, harmalol and peganine (vasicine) (11). Re-investigating the same plant, Siddiqui described a new alkaloid, harmidine (12, 13) shown by Robinson to be identical with harmaline (14).

Harmine, harmaline and 1,2,3,4-tetrahydroharmine were extracted from Banisteria caapi (15). Harmine was also isolated from Banisteriopsis inebrians (16), Cabi paraensis (17), Passiflora incarnata (18, 19, 20) and Zygophyllum fabago (21). It has also been found along with harmaline in "parica", a South American drug (22) and in another Banisteriopsis used to prepare "natem" a Peruvian beverage: the latter possibly contains traces of harmaline (23). Other β-carbolines have also been isolated: harman and harmol are present in Passiflora incarnata (18, 19, 20) and in Zygophyllum fabago (20): tetrahydroharman is the main alkaloid of Calligonum minimum (24).

4. Pharmacology:

The pharmacological action of the harmala alkaloids, namely harmine and harmaline, has been extensively studied in protozoa, frogs, mammals and to some extent in humans. Of all their pharmacological

properties, their excitatory action on the central nervous system has retained the attention to this day.

a) Animal studies:

Prior to the investigation of Gunn on harmaline in 1905 (25), Tappeiner and Neuner had studied the general action of harmaline. From their work on frogs and mammals they concluded that harmine and harmaline were convulsive and respiratory poisons. No therapeutic use was foreseen for them (26).

Detailed studies on the pharmacological action of harmaline (25) and harmine (27) have been carried out by Gunn. Alone or with collaborators he also investigated tetrahydronorharman (28), tetrahydroharmine (29), harmalol (30), harmol (31) and its alkyl derivatives (32, 33, 34, 35, 36). In the intact animal and in isolated tissues he found the action of harmaline and harmine qualitatively similar. He observed that the syndrome of effects produced by harmine were not qualitatively altered by reduction to harmaline or tetrahydroharmine. However, substitution of a methoxy group by a phenolic group at position 8, that is, transformation of a methoxyharman to a hydroxyharman removed the convulsant action. Substitution of homologous ethers at position 8 on the harmaline molecule did not greatly alter the general type of action of the drug at least up to the amyl derivative (37). Because harmaline, like quinine, did not have any selective action on tissue and had similar pharmacological properties he suggested that both should be classified as "protoplasmic poisons" (25).

(1) Toxicity:

The minimum lethal dose of harmaline hydrochloride for mammals was established by Gunn to be about 100 mg per kilogram when injected subcutaneously; for harmine hydrochloride it was about 200 mg (25, 27). The weaker activity of harmine had previously been noted by Tappeiner and Neuner. Kreitmair observed later that harmaline administered intravenously to mice was 10-fold more potent than harmine (8). According to Gunn, in mammals death from a lethal dose of harmaline is due to a respiratory arrest by paralysis of the respiratory centre: cardiac arrest is the chief cause of death with harmine (25, 27). Flury who also studied the harmala alkaloids observed that dogs and rabbits could be accustomed to supralethal doses of harmine and harmaline (38).

(2) Effect on the central nervous system:

Both harmaline and harmine exert a stimulant action on the central nervous system. In mammals, according to the dose administered, they provoke clonic convulsions, tremor and other extrapyramidal symptoms. Convulsant action and tremor-inducing property have always impressed the investigators who dealt with these drugs. Harman and harmalan, also β-carbolines, have been said to cause convulsions and paralysis in mice and rabbits (39). Apparent "hallucinations" have been reported in cats with harmaline (25) and in dogs with harmine (6, 40).

The convulsions induced by harmaline do not occur in the animal anesthetized by ether or chloroform (25). Barbiturate anesthesia prevents the appearance of convulsions caused by harmine without affecting other manifestations of the drug (41). General anesthesia with chloralosane does not prevent the appearance of harmine-induced tremor (6).

The tremor provoked either by harmine or harmaline has been frequently described (5, 6, 8, 25). It consists chiefly of a swaying of the head and of generalized trembling of the whole body: the animal, the rat for instance, adopts a typical position with his forelegs stretched forward. The tremor is exaggerated by voluntary movements. Lewin observed in the rabbits that "trembling and shaking reappeared with attempts to move." In the monkey, he noticed that the trembling ceased while the animal was held fast for temperature measurements whereas it was increased by any intended movements (5). The onset of harmine tremor varies slightly with the route of administration (42). It appears within a few minutes after injection and lasts less than one hour (42, 43). In mice, harmine tremor has a cycle recorded at 15-20 per second (42). Similarly other investigators have reported that both harmine and harmaline give a tremor with a cycle of 14-18 per second (44).

Hara and Kawamori (42) found that methamphetamine had a marked synergistic action on harmine tremor. Zetler (43) studied extensively the antagonists of the tremor induced by subcutaneous injection of 7 mg per kilogram of harmine in mice. A variety of drugs was studied: antiparkinsonian agents, ganglioplegics, sedatives, ataraxics, antiepileptics, tryptophan and its derivatives such as serotonin, LSD - 25. He found that the most efficient antagonists were LSD - 25 (lysergic acid diethylamide), serotonin (5-hydroxytryptamine), chlorpromazine, promethazine and apomorphine.

Attempts have been made to localize the site and mode of action of harmine and harmaline. Beer (45, 46) working on cats with intact central nervous system as well as cats without neocortex separated two groups of effects. He attributed the clonic convulsions, the psychic

and sensory changes to the action of harmine on the cortex. He attributed the tremor, delay in movements, the stiffness of extremities and the changes in muscle tone to a direct action on the extrapyramidal system.

Hara (47) used the "extrapyramidal poisons" "harmine and bulbocapnine" in his studies of the extrapyramidal system carried out for the most part in mice. He postulated the existence of a harmine-reactive system and of a bulbocapnine-reactive system. These two systems had opposite actions. The former reacted with an increase in motility and a decrease in muscular tonus. The latter reacted with a decrease in motility and an increase in muscular tonus. Harmine tremor appeared even if the thalamus, the corpora quadragemina and the cerebellum were destroyed. Destruction of the posterior part of the hypothalamus decreased the action of harmine. With removal of both hemispheres, tremor was slight and clonic convulsions were absent. Later, Hara and Kawamori (42) found that removal of the cortex inhibited harmine tremor completely and that destruction or excision of a large enough portion of the striatum also inhibited it clearly. Their conclusion was that harmine, like bulbocapnine, produced its effect by acting on the cortex and the striatum.

Markovic and Giaja (48) have observed that harmine tremor disappears if a rat is exposed to a barometric pressure of 300 mm Hg and the tremor resumes with the return to normal pressure.

Lately, Sourkes and Poirier (49) have investigated the neurochemical bases of tremor and other disorders of movement. Specific unilateral electrolytic lesions in the upper brain stem provoked extrapyramidal disorders. A loss of cells of the substantia nigra on one side was associated with a homolateral depletion of striatal dopamine and

a hypokinesia of the contralateral limbs. A lesion severing the most dorsomedial fibres of the cerebral peduncle and the rubrotegmento-spinal tract was associated with a homolateral depletion of striatal serotonin and choreiform movements of the contralateral limbs. A lesion severing all three tracts resulted in a homolateral loss of serotonin and dopamine and in contralateral hypokinesia with sustained postural tremor. They tested various drugs for their possible effect on the dyskinesias. Only harmine and harmaline had an effect: harmalol, the demethylated derivative of harmaline, was ineffective. Harmaline (5-10 mg I.M.) greatly exaggerated the postural tremor of 5 monkeys and modified the choreiform and ballistic movements of two others to a coarse tremor. Three unoperated animals exhibited only a brief period of shivering (see Table 1). Harmine and harmaline are monoamine oxidase inhibitors (MAOI) and capable of causing an increase in brain serotonin and dopamine. Hence a favorable influence on the dyskinesiashad been expected. Indeed in accord with the postulated role of dopamine depletion in striatal diseases an improvement of the extrapyramidal symptoms should have occurred.

Electroencephalographic studies in dogs have confirmed the stimulatory action of harmine on the central nervous system and the central origin of the motor phenomena (41, 50).

(3) Effect on temperature:

Harmaline and harmine have long been known to cause hypothermia in rats, guinea pigs, rabbits and monkeys (5, 25, 27, 48). No change in temperature is reported in the curarized dog (49) while hyperthermia has occurred in mice (43), cats and dogs (1).

Markovic and Giaja (48) studied the effect of harmine adminis-

Table 1

CORRELATION OF SITE OF BRAIN STEM LESIONS IN MONKEYS AND INDUCED ABNORMALITIES OF MOTOR FUNCTION

Number of monkeys	Side of Lesion	Tracts severed*	Activity of contralateral limbs	
			Spontaneous	After harmaline
1	Left	NS	Hypokinesia	
2	Left	NS, CP	Hypokinesia	No change
1	Left	RTS	Normal	
3**	Left	RTS, NS, CP	Hypokinesia and tremor	Increased amplitude (and slightly reduced frequency of tremor)
2***	Left	RTS, NS, CP	Hypokinesia and tremor	Increased postural tremor
2***	Right	RTS, CP	Choreiform and ballistic movements	Coarse tremor
3	-	unoperated	Normal	No effect except for a brief episode of shivering

* NS = nigrostriatal fibres.

CP = most dorsomedial fibres of the cerebral peduncles.

RTS = rubro-tegmentospinal tract.

** One monkey also showed ataxia and torticollis.

***Same monkeys: lesions extended on both sides of the brain.

Adapted from Sourkes, T.L., and Poirier, L.J. (49)

tered subcutaneously on the body temperature of rats. They found that doses over 10 mg/kg did not increase the hypothermic effect appreciably. No significant hypothermic effect was produced at an ambient temperature of about 30° C. Decreasing the ambient temperature from 21.4° C to 3.5° C did not enhance significantly the hypothermic effect. Hypothermia induced by a dose of about 12 mg/kg at an ambient temperature of 14° reached its lowest point after 45 minutes, remained there for 45 minutes, and returned toward normal 3 hours after the injection. Harmine was thought to produce hypothermia by impeding chemical thermoregulation: there was a complete or almost complete suppression of oxygen consumption in excess of the basal value.

With regard to body temperature, recent investigations have been conducted on the interaction of harmaline with other agents. In rabbits pretreated with harmaline (10 mg/kg S.C.), reserpine (0.5 mg/kg I.V.), administered one hour after, not only failed to cause a fall in temperature but provoked a rise. It was also found that harmaline enhanced the pyretogenic effect of 5-hydroxytryptophan (51).

Lately, Schmidt and Fähse in a study of the effect of various monoamine oxidase inhibitors on the body temperature of rats mentioned again the hypothermic effect of harmaline and harmine. Like other MAO inhibitors, they increased and prolonged the temperature drop following intracerebral noradrenaline injection. As for an intracerebral injection of serotonin a marked hyperthermia followed a short initial drop in temperature (52).

(4) Effect on the cardiovascular system:

Gunn has reported that in mammals small doses of harmaline caused

a rise in blood pressure and bradycardia, while large doses produced hypotension and bradycardia. He attributed this effect to a direct action on the cardiac muscle (25). Decourt and Lemaire also mentioned the hypotensive effect of harmine in dogs and stated it was not of vagal origin (6). In rabbits, Gunn found harmol to be a powerful coronary dilatator (31). In cats, Blum also observed hypotension, bradycardia and often arrhythmias after intravenous injection of harmaline (5 mg/kg) (53). Recent studies confirmed again that harmaline decreases the arterial pressure and slows the cardiac rhythm, but the cardiac output and the pulmonary pressure to not change (54).

(5) Effect on ganglia and myoneural junction:

In anesthetized cats and dogs, large intravenous doses of harmine inhibited transmission through sympathetic ganglia without depressing the function of parasympathetic ganglia (55). Like some other MAO inhibitors tested on dog and rabbit nerve-muscle preparations, harmine caused myoneural blockade (56). Harmaline, as other MAO inhibitors tested on the isolated rat phrenic nerve-diaphragm preparations, produced a reversible block of myoneural transmission and blocked the action of acetylcholine on the denervated diaphragm: this effect is believed to be mediated through a mechanism not involving MAO inhibition (57). The action of harmaline on myoneural activity and its influence on the neuromuscular effects produced by dopamine have recently been studied by Blum (53).

(6) Interaction with other agents:

In mice methoxyharmans (harmine, harmaline, tetrahydroharmine) shortened the reserpine-induced prolongation of ethanol narcosis but the hydroxyharmans did not (51). In rabbits pretreated with harmaline, re-

serpine caused excitation instead of sedation (58), an effect also observed by pretreatment with other MAO inhibitors. In rats pretreated with harmaline the reserpine-induced ptosis does not occur (59) and a conditioned avoidance response is no longer blocked by tetrabenazine (60). Harmaline inhibits the conditioned sound- or light-stimulated flight reaction in the rat (61). Harmine and harmaline can release noradrenaline from peripheral stores after previous injection of amphetamine, a property thought to be unrelated to their MAO inhibitory activity (62).

(7) Other properties:

High doses of harmaline (60 mg/kg) have been reported to protect against tonic seizures induced by supramaximal electroshock (63). Harmaline was found to increase the cerebral metabolic rate while leaving unchanged the respiratory quotient (64). The level of adenine nucleotides in the rat brain is not modified by harmine (65).

In the isolated perfused heart, harmaline decreased the noradrenaline output without influencing the response to sympathetic stimulation (66). At a concentration of 10^{-5}M harmine was reported to inhibit the noradrenaline uptake (67). Vanov observed that at 5 mg/kg harmaline hydrochloride did not alter the pressor effects of adrenaline or noradrenaline (68).

Some investigators have pointed out a feeling of euphoria or mood improvement associated with administration of harmine (5, 6, 69). Most authors however judge the effect on the mood negligible or nonexistent.

Many other pharmacological properties of the harmala alkaloids have been discovered. They are of less immediate interest and will not

be mentioned in this review.

b) Clinical studies:

Harmala alkaloids have been tried in humans as antiparkinsonian agents; their hallucinogenic property has also been investigated. As MAO inhibitors they have not found any clinical use in psychiatry.

(1) As antiparkinsonian agents:

Interested by Lewin's accounts of Banisteria caapi (5, 70) and by his clinical trial of harmine (banisterine), other physicians utilized that drug in the treatment of the Parkinsonian syndrome. Between 1928 and 1933 numerous publications appeared on this matter.

In 1928, Lewin (5) and Beringer (71,72) reported on their clinical experience with harmine injected subcutaneously at doses varying from 20-75 mg. A dose of 200 mg given mistakenly to a colleague had triggered a severe reaction consisting of a state of collapse, bradycardia, extreme paleness, nausea, excessive vomiting, dizziness, ataxic gait, dullness of consciousness and uncontrollable tremor of the arms and the legs. In patients, Beringer observed that harmine had a beneficial effect on some parkinsonian symptoms, namely rigidity and hypokinesia. It also modified the oculogyric crisis. Tremor was essentially unaffected. The effect appeared 30 minutes after the injection. Its intensity varied from case to case and the optimal dose had to be adjusted experimentally for each patient. The efficacy of the treatment could not be predicted nor the duration of the effect which ranged from a few hours to several days. The drug was also effective in a suppository form.

Many other investigators published their results soon afterwards (73, 74, 75, 76, 77, 78, 6, 79). Eichler observed a favorable effect on

cases of post-encephalitic parkonsonism (75) and so did Decourt and Lemaire (6). Like Beringer, they felt that the main effect was on muscular rigidity and hypokinesia. Eichler found the action of harmaline and harmine practically indistinguishable. Decourt and Lemaire noted that the best response was obtained in young post-encephalitic parkinsonians showing mainly akinesia without tremor. Harmine, they found, lost most of its activity when taken orally: Beringer however was claiming good results with keratinized capsules. Harmalol showed also some efficacy in post-encephalitic parkinsonism (80).

The place of the harmala alkaloids in the antiparkinsonian armamentarium soon receded to the background where it remained for a long period. In 1964, harmine was again found to be an effective antiparkinsonian agent but only in patients whose symptoms were induced by reserpine (69).

(2) As psychotomimetics:

As psychotomimetics the situation of the harmala alkaloids is very complex. It was mentioned earlier that the extract of Banisteria caapi induced hallucinations and that various investigators have reported hallucinations caused by harmaline and harmine in animals.

In 1955, Turner, Merlis and Carl (81) had stated that a careful review of the literature of the past 100 years failed to reveal any record that pure harmine or its related alkaloids had produced visual or auditory hallucinations.

In 1957, Pennes and Hoch (82) gave harmine intravenously (150-200 mg) to non-deteriorated schizophrenics. Half of their subjects experienced visual hallucinations. These visual hallucinations however were present only when the eyes were closed: they disappeared with opening of the eyes. In fact these hallucinations seemed rather to be of the

hypnagogic imagery type. They concluded that at medium or high dosage, harmine fundamentally produced an "acute organic reaction type". This reaction consisted of a semi-delirioid or confusional state with intermittent drowsiness or sleep.

Naranjo in 1959 claimed that harmine produced a paranoid syndrome (83).

Recently, Turner (84) compared on himself the effect of yagé with the effect of the pure alkaloids extracted from it. He experienced vivid imagery only when his eyes were closed. The effect was less intense with the pure alkaloids; with yagé he had in addition nausea and vomiting. No intoxicating effect occurred with harmine or DL-tetrahydroharmine.

5. Biochemistry:

(1) Harmala alkaloids and mental illness:

The search for a biochemical disorder as a cause of mental illness has led to the implication of β-carbolines in psychoses.

In recent years indeed, the hypothesis that endogenous tryptamines could be converted to β-carbolines of the harmala type has been advanced. Part of a review of the recent advances in the field of indole compounds had dealt with this subject (85) and likewise a recent review on the biochemistry of the psychotomimetic drugs (86).

In 1961, McIsaac (87) proposed that in certain cases of mental illness the normal metabolism of serotonin was blocked. Hence abnormally large amounts of melatonin, a skin lightening hormone, were produced and 10-methoxyharmalan, a compound which bears a structural resemblance to harmaline, would be formed by cyclodehydration of melatonin. This compound, labeled as psychotomimetic, is an even more potent serotonin anta-

gonist than harmaline. Therefore it would block further the metabolism of serotonin by a feedback mechanism. Serotonin would accumulate, overflow toward the melatonin pathway and by doing so increase the synthesis of 10-methoxyharmalan. The disruptive action of this compound on behavior was evidenced by its effect on the avoidance-escape behavior in rats: a linear relationship between doses of 10-methoxyharmalan and the number of mistakes in the escape test was found. The compound caused also a short-lasting tremor (88).

McIsaac has demonstrated the possibility of in vitro and in vivo synthesis of a related compound, 10-methoxytetrahydroharman (Farrell's adrenoglomerulotropin) (89). The formation in vivo is obtained by blocking the deamination of 5-methoxytryptamine with iproniazid and by blocking simultaneously the oxidation of acetaldehyde with disulfiram (FIG. 4).

According to other numbering systems the above compounds are also named 6- or 7-methoxyharmalan, 6- or 7-methoxytetrahydroharman. Their respective location and relationship on the tryptophan - serotonin - melatonin pathway is shown in Fig. 5 (90).

The possibility of in vivo conversion of tryptamines to β-carbolines was again recently suggested from studies with substituted α-tryptamines. Indeed a urinary metabolite of α-ethyltryptamine is thought to have a tetrahydro-β-carboline structure (FIG. 6) (85).

Farrell's adrenoglomerulotropin and the urinary metabolite from α-methyltryptamine are the only β-carbolines isolated thus far from animal material (85).

Formation of 10-methoxytetrahydroharman in vivo:

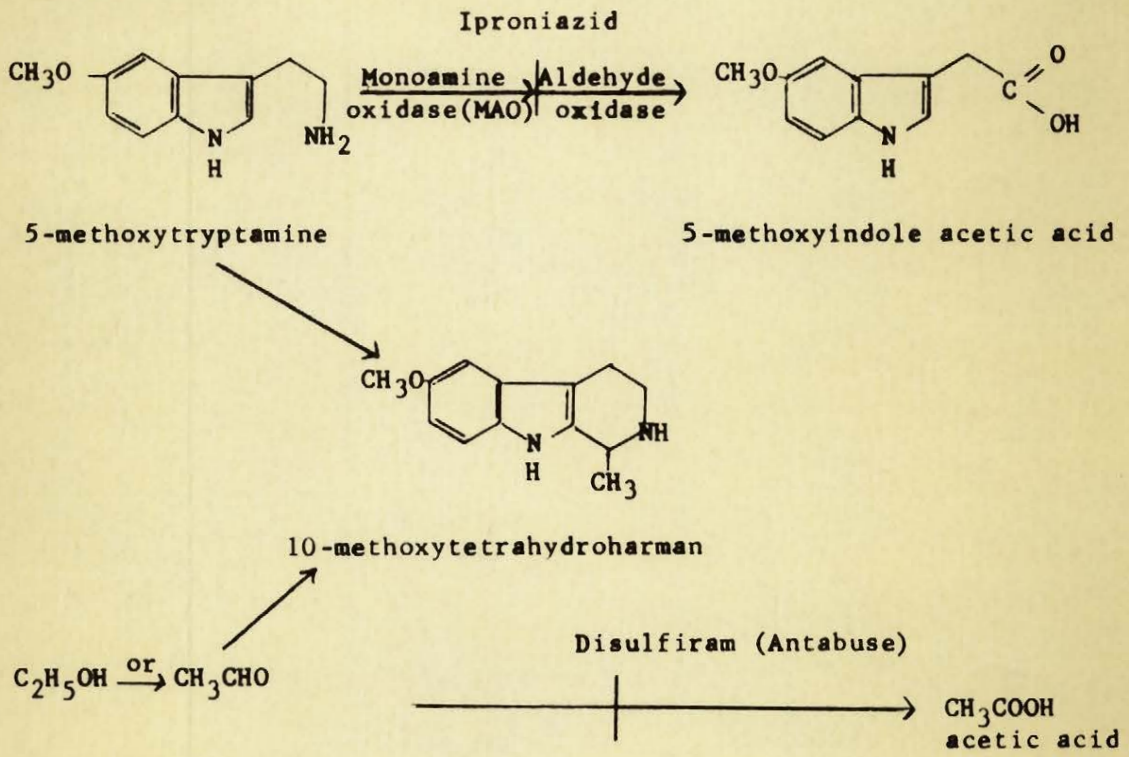
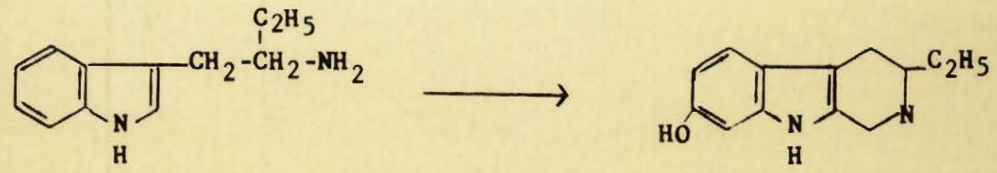


FIG. 4

Adapted from McIsaac (89)

Structure of a urinary metabolite of α-ethyltryptamine:



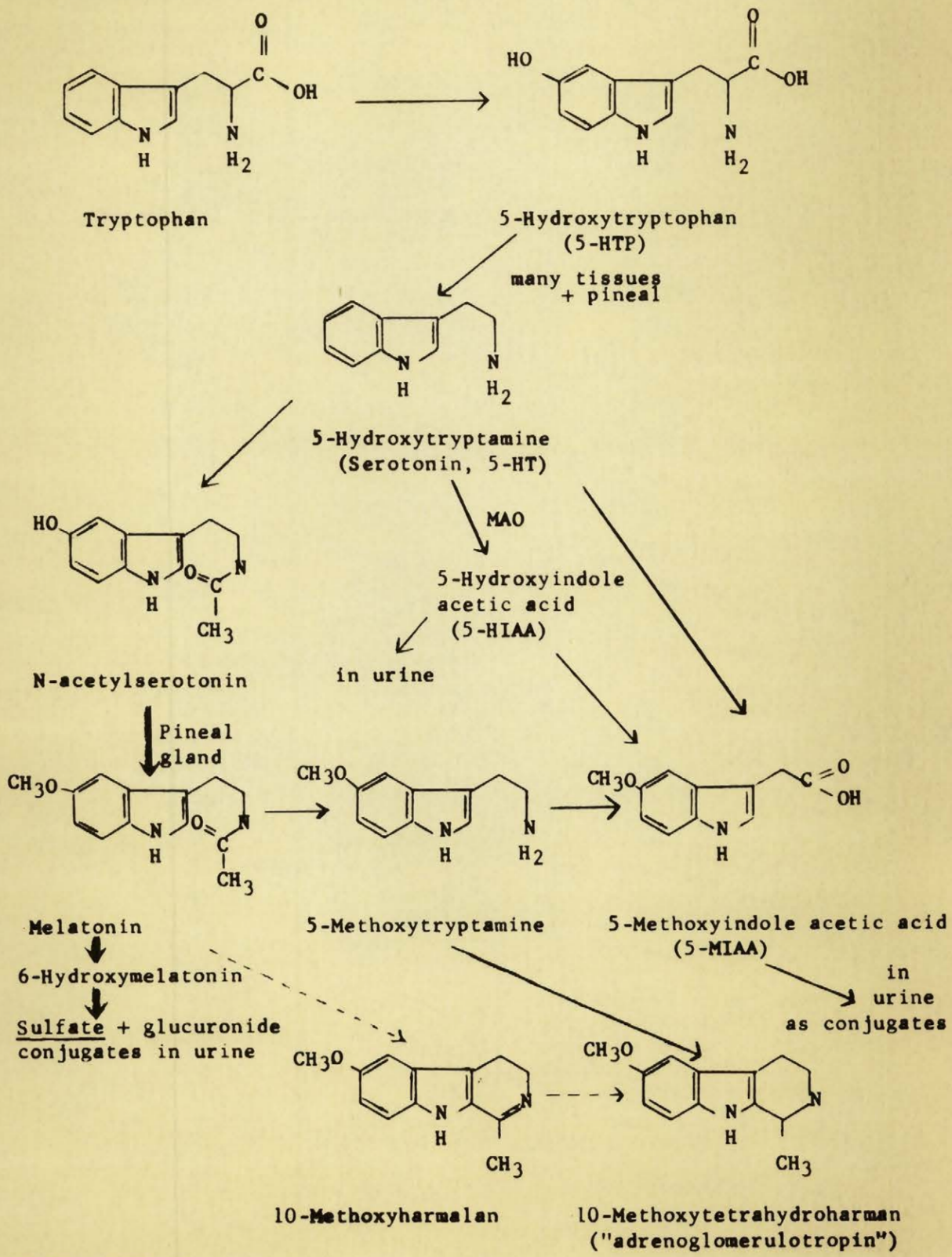
α-ethyltryptamine

metabolite

FIG. 6

From ref. 85.

Formation of 10-methoxyharmalan:



▬ Main Pathway

FIG. 5

Adapted from W.B. Quay (90)

(2) Harmala alkaloids and MAO inhibition:

Monoamine oxidase is the enzyme that effects the oxidative deamination of monoamines in the body. It catalyses the following reaction (91):

$$R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O \longrightarrow R\text{-}CHO + H_2O_2 + NH_3$$

Monoamine oxidase (MAO) occurs in most tissues of mammals, mainly in the mitochondria: the liver contains a high concentration of this enzyme (92) and also certain areas of the brain (93). MAO deaminates many amines of biological interest such as dopamine, tryptamine, serotonin and tyramine. It also acts on adrenaline and noradrenaline although to a lesser extent. This activity of MAO on many substrates explains why the action of MAO inhibitors is not limited to only one amine (94). Since the discovery of MAO by Hare-Bernheim in 1928, many compounds have been found to inhibit the activity of this enzyme.

Monoamine oxidase inhibitors are generally divided in two classes: the hydrazines and the non-hydrazines. A distinction is also made between irreversible and reversible MAO inhibitors. Reversibility refers to the capacity of removing the MAO inhibitors readily from the enzyme's active site by washing the mitochondria or the cells (93, 95).

MAO inhibitory potency:

The harmala alkaloids were the first non-hydrazine compounds discovered to have potent in vivo and in vitro MAO inhibitory activity. This inhibition was short-lasting and reversible (Table 2, 3) (95). Methoxyharmans (harmaline, harmine, 1,2,3,4-tetrahydroharmine) were later found to inhibit significantly liver mitochondria of guinea pigs, but

Table 2

INHIBITION OF MAO IN HOMOGENATES OF RAT LIVER

Compound	Inhibition at 10^{-3} M per cent	Conc.producing 40-60 per cent inhibition
Harmaline	100	10^{-6}
Harmine	100	10^{-6}
1,2,3,4-Tetrahydroharmine	97	10^{-5}
Harman	98	5×10^{-6}
D,L-Tetrahydroharman-3-carboxylic acid	0	-
1-3,4-Dihydroharman-3-carboxylic acid	21	-
α-Carboline	47	-
β-Phenylisopropylhydrazine	100	5×10^{-6}
Iproniazid	90	3×10^{-4}

Procedure: The incubation mixture contained 1.5 ml of rat liver homogenate (equivalent to 500 mg of tissue), 0.5 ml of 0.05 M phosphate buffer (pH 7.4), 1 mg of serotonin, inhibitor and water to yield a total volume of 3.5 ml. Incubation was carried out in air at 37° C for 40 minutes.

From Udenfriend et al. (95)

Table 3

REVERSIBILITY OF HARMALINE INHIBITION

Compounds	Per cent inhibition	
	Before washing	After washing
Harmaline 10^{-5} M	92	38
Iproniazid 10^{-3} M	84	79

Procedure: Rat liver mitochondria (equivalent to 10 gm of liver) were incubated with the inhibitor at 37° C for 30 min. at pH 7.4. The mitochondria were then washed four times with 5 ml of isotonic KCl. Control omitting inhibitor were incubated and washed in the same manner. Following this the washed mitochondria were made up to the original volume. Mitochondria equivalent to 1.5 gm of liver were then incubated with serotonin as described in Table 1.

From Udenfriend et al. (95)

hydroxyharmans (harmol, harmalol) did not (51). Zirkle and Kaiser have dealt in great detail with the MAO inhibitory activity in vivo and in vitro of harmala alkaloids and related carbolines (1). From compiled in vivo studies they showed that harmaline per os was slightly more effective than iproniazid and so was harmine per os or intraperitoneally: given subcutaneously, harmine was 450 times more potent than iproniazid. They concluded that, despite variations in data collected from various sources, harmine and harmaline, along with tranylcypromine and pheniprazine, were extremely potent MAO inhibitors both in vivo and in vitro. The MAO inhibitory potency in vitro of harmine and harmaline has recently been challenged (96).

Action of amines:

The harmala alkaloids produce their effect rapidly (within one hour) on amine levels and their action lasts only a few hours (95, 97). Doses of harmaline ranging from 5 to 20 mg/kg administered intravenously were found to leave no residue of MAO inhibition in the mouse brain after 24 hours (98). Similar results were obtained with liver MAO (95).

Udenfriend et al. have found an elevation of the serotonin content of brain and liver in rats after injection of harmaline (95). In mice, increase in brain serotonin was also obtained, the response being maximal with 1 mg of harmaline/kg I.V. (99). Pletscher et al. reported that methoxyharmans but not hydroxyharmans caused a marked rise, lasting 6 to 8 hours, of the serotonin and noradrenaline of rat brain (Table 4) (100). An elevation of the brain dopamine in the rat was obtained with harmine at a dose of 30 mg/kg I.P. (101). Sourkes and Poirier have reported an increase in the serotonin content of the striatum in the monkey after

Table 4

INCREASE OF SEROTONIN AND NORADRENALINE IN % ABOVE CONTROLS IN THE RAT BRAIN CAUSED BY VARIOUS HARMALA ALKALOIDS

Compounds	Serotonin	Noradrenaline
Controls	0 ± 5	0 ± 6
Harmine	63 ± 5 S	72 ± 11 S
Harmaline	69 ± 4 S	36 ± 6 S
Tetrahydroharmine	29 ± 3 S	57 ± 3 S
Harmol	6 ± 4 NS	29 ± 10 (S)
Harmalol	3 ± 3 NS	14 ± 8 NS

The harmala alkaloids were administered I.P. in equimolar doses corresponding to 30 mg/kg of harmaline. For the serotonin measurement one injection of harmala alkaloids was given. For the noradrenaline measurement two injections 2 hours apart were given. The amine content was measured 2 hours after the last injection of the alkaloids. S = significant in comparison to controls ($p < 0.01$), NS = non-significant ($p > 0.05$), (S) = $0.01 < p < 0.05$.

Adapted from Pletscher, Gey and Zeller (100)

harmaline (49).

The rate of deamination of serotonin is decreased by harmaline, in mice and man, as evidenced by a decreased amount of 5-hydroxyindole acetic acid in the urine after prior administration of serotonin (95, 102).

Harmala alkaloids are also able to restore the level of serotonin and noradrenaline decreased by reserpine (51).

In the rat heart and brain, harmaline enhanced the 5-hydroxy-tryptophan-induced increase of serotonin (103). It increased in the rat heart the concentration of endogenous noradrenaline and adrenaline (104), but did not elevate the H^3-noradrenaline concentrations 2 hours after injection of the labeled amine (105).

As with some other MAO inhibitors, a "booster" dose effect has been reported with harmaline (99).

Interaction with hydrazines:

In 1959, Pletscher and Besendorf studied the interaction of harmaline with hydrazines. Hydrazines are irreversible long-acting inhibitors. These investigators established the existence of an antagonism between the action of harmaline and that of iproniazid and of L-glutamic acid-α-isopropylhydrazide. They found that in rats and mice, treatment with harmaline one hour prior to the administration of hydrazines antagonized the elevation of brain noradrenaline and serotonin normally produced by the latter. On the other hand harmaline administered 6 to 8 hours after the hydrazines could not counteract the serotonin increase induced by the latter. On this basis they postulated that short-acting reversible inhibitors and long-acting irreversible inhibitors were mutual competitive inhibitors, that is, competing for the same site on the enzyme. They also

suggested the use of such short-acting reversible inhibitors to differentiate the effects due to the MAO inhibitory activity of hydrazines from their pharmacological properties. Indeed if the receptor site on the enzyme was already occupied by the short-acting inhibitor, the effects produced by the long-lasting inhibitor could not be attributed to its MAO inhibitory activity (97). Harmine interacted in the same manner with another hydrazine, β-phenylpropylhydrazine (106). This type of antagonism has recently been confirmed by histochemical studies using the interpeduncular nucleus of the rat brain (93).

As a whole, pretreatment with methoxyharmans diminishes the increase in serotonin and noradrenaline in the brain normally caused by an hydrazine type MAO inhibitor. Hydroxyharmans fail to do so (51).

(3) Metabolism of harmala alkaloids:

According to Zirkle and Kaiser (1) and to our knowledge, no recent studies on the metabolism of harmala alkaloids has been published. In 1911, Flury contended that the metabolites of harmaline were harmine, harmalol, harminic acid and another unidentified compound (38). Harmaline is said to be poorly absorbed from the gut (102) and to be quite toxic (59) in man.

B. NATURE AND SCOPE OF THIS INVESTIGATION

Despite the abundance of data concerning the harmala alkaloids, much basic information about them is lacking.

As mentioned earlier, no recent systematic studies have been done of the metabolic fate of harmaline in the body. Its metabolism was therefore investigated. In view of the close similarity in chemical structure and effects of harmaline and harmine, the metabolism of harmaline was compared to that of harmine.

The hypothermic action of harmaline and harmine has been singled out frequently by investigators. Yet harmaline may behave differently: if administered before reserpine, it had been reported not only to antagonize the fall in temperature induced by the latter, but also to provoke a rise. Harmaline is known to be a potent, short-acting, reversible MAO inhibitor and we wanted to determine if its hypothermic effect is mediated through MAO inhibition and has a specific relation to the level of brain amines. Tremor and hypothermia occurring simultaneously in the species we studied, we attempted to verify if the tremor is based on the same mechanism as for hypothermia and if there is any correlation between these two effects.

II. EXPERIMENTAL

A. HARMALINE METABOLISM

1. General methods:

a) Animals used:

Male rats of the Sprague-Dawley strain were used in all our experiments. Their average weight was about 150 grams. They were purchased from Canadian Breeding Laboratories, St. Constant, Quebec.

b) Chemicals:

Harmaline and harmine were purchased from S.B. Penick and Co., New York. Harmalol hydrobromide used as standard for the thin-layer chromatography was bought from Mann Research Laboratories, New York 6, New York. Their structures have been shown in Figure 2.

Harmaline and harmine were dissolved in water by adding hydrochloric acid to the distilled water and the pH was brought back to 6.0 with sodium hydroxide. These injectable solutions were always prepared in the same manner.

c) Procedure for quantitative determination:

1) Harmaline and harmine assays:

A modification of a method developed by Udenfriend et al. (95) was used.

The tissue homogenate in a glass-stoppered centrifuge tube was made alkaline by addition of 0.5 ml of 20 per cent Na_2CO_3 and 3 ml of 0.5 M borate buffer pH 10. Fifteen ml of $CHCl_3$ were added, the tube was shaken and then centrifuged. Five ml of the $CHCl_3$ fraction were transferred to another tube containing two ml of 0.1 N HCl and the tube was again shaken. To break the emulsion formed in the supernatant and re-

fractory to centrifugation, the tube was frozen and later thawed.

The clear aqueous layer was afterwards transferred to a cuvette. Its fluorescence was measured in the spectrophotofluorometer (Aminco-Bowman) at the appropriate wavelength and compared to standards carried through the entire extraction procedure. Recoveries of harmaline added to lungs, pancreas and heart were quantitative. The recoveries obtained for other organs are also listed in Table 5.

Table 5

PERCENTAGE RECOVERY OF HARMALA ALKALOIDS ADDED TO TISSUES

Drug	Brain	Lungs	Pancreas	Heart	Liver	Kidney
Harmaline	69.6-82.7	100	99.0-105.1	92.0-93.1	86.3-93.0	88.6
Harmine	57.8-70.1	99.8	102.5	82.7-101.7	80.7-94.6	98.4

The samples were examined in the spectrophotofluorometer at activation 380 mμ, fluorescence 480 mμ for harmaline and at activation 332 mμ, fluorescence 413 for harmine.

2) Tissue homogenization:

The organs were homogenized by ultrasound with a Branson Sonifier (Model S-75) with microtip. They were disrupted in five ml of 0.1 N HCl except for the liver whose homogenate was diluted to twenty-five ml with 0.1 N HCl, five ml only being used for the assay. The tissue container was kept in an ice bath during the procedure to avoid heating. The length of time and the power intensity required for sonification varied according to the tissues:

- Brain and pancreas: intensity 5, time: 30-60 seconds.
- Heart and kidneys: intensity 5, time: 60-90 seconds.
- Liver: intensity 6, time: 90-120 seconds.

3) Experiments:

For the determination of the distribution and the concentration of harmaline and harmine in various organs, rats were injected intraperitoneally either with 50 mg/kg of harmaline or 25 mg/kg of harmine. They were killed at intervals after the injection. In each animal, the brain, the lungs, the pancreas, the heart, the liver and the kidneys were removed.

To establish the relative distribution and concentration of harmaline in different parts of the brain, 4 rats were given 50 mg/kg of the drug intraperitoneally. Two were killed 30 minutes after the injection and two after 60 minutes. The brains, sectioned under the posterior colliculi and anteriorly just above the pons, were removed. The cerebral hemispheres were separated from the diencephalon and the mesencephalon. Cerebral hemispheres on one hand and diencephalon plus mesencephalon on the other were assayed for their harmaline content.

The concentration of harmaline and harmine in the brain, heart and liver two hours after intraperitoneal injection of these drugs at various doses was also compared.

Finally, the 24-hour urinary excretion of harmaline and harmine was determined by giving by intraperitoneal route to one rat a 6 mg dose of harmaline and to another 6 mg of harmine. The quantitative determination of these drugs was made by the same method used for tissues.

In each of these experiments control animals were present. The

rats were always killed by decapitation. The material removed from the animal was frozen until the assay took place.

d) Procedure for thin-layer chromatography:

1) Experiment:

Thirty-six male albino rats weighing about 150 gm were divided in 9 groups of four. One group served as control, four groups received an injection of 30 mg/kg of harmaline intraperitoneally and four groups were administered 50 mg/kg of harmine by the same route. The four groups of **harmaline**-injected animals were killed at the following intervals: 30 minutes, 1 hour, 4 hours and 24 hours after the injection. The animals that received harmine were also killed by decapitation according to the same schedule: in the 24-hour group, only two animals had survived by that time. The brains, kidneys and livers were removed, pooled for each group and frozen awaiting analysis. The 24-hour output of urine of the control and the drug-treated animals was also collected.

2) Preparation of the eluates applied on the chromatograms:

The brains and the kidneys were homogenized with the Branson Sonifier in 25 ml of 70% ethanol containing 0.1% concentrated HCl. The homogenates were placed in the freezer overnight. The next day the tubes were shaken for 45 seconds, centrifuged for 20 minutes and the supernatant was collected. Ten ml of the ethanol:HCl solution was added to the residue which was shaken and centrifuged again for the same period of time. This supernatant was also collected and mixed with the first one collected. A five ml aliquot was then transferred to another tube and dried at reduced pressure in a Flash-Evaporator (Buchler Instruments, Fort Lee, N.J.), at a temperature of 32-35° C. Fifty μg of harmaline,

harmalol and harmine were added to aliquots of the extract from the control tissues prior to evaporation: these samples were to serve as standards.

The dried residues were dissolved in 2.5 ml of IN acetic acid and poured in a microcolumn of Dowex 50W, H+. After passage of the solution, the column was washed once with distilled water and eluted with 2 ml of a TEA solution (triethylamine 70 ml, plus 100 ml of acetone brought to a volume of 250 ml with distilled water). The eluate was collected and evaporated to half its volume under an air spray. Finally one drop of the TEA solution was added to each tube before chromatography.

The livers were homogenized in 30 seconds in 100 ml of 70% ethanol containing 0.1% concentrated HCl with a blade homogenizer (VirTis Apparatus). A 40 ml aliquot of the homogenate was taken and kept in the freezer overnight. The next day the tubes were shaken for 45 seconds, centrifuged for 20 minutes and the supernatant collected. A two ml aliquot was transferred to another tube and dried as previously described in a Flash-Evaporator. Twenty-five μg of harmaline, harmalol or harmine were added to aliquots of the control extracts to serve as standards. The same procedure as for brains and kidneys was thereafter followed except that no evaporation of the Dowex eluate was made and no TEA solution was added prior to chromatography.

The 24-hour output of urine collected from the group of control rats and from the harmaline- or harmine-injected animals had been filtered, diluted to 200 ml with distilled water and frozen. Two ml of thawed urine were acidified with 0.2 ml of 8N acetic acid and poured on the Dowex microcolumn. The eluates were employed for chromatography without any other treatment. Ten μg of harmaline, harmalol or harmine were added to 250 μl aliquots of the control group eluate and were used as standards.

3) Preparation of chromatography plates:

The MN-Cellulose Powder 300 (Macherey, Nagel and Co., Düren, Germany) was used in the preparation of chromatography plates. A homogeneous mixture was obtained by mixing 10 gm of cellulose powder with 60 ml of distilled water for 2 minutes with a blade homogenizer. With the Desaga equipment a thin-layer of 0.25 mm of this mixture was applied on the glass plates. The plates were dried and activated in the oven for 60 minutes at 50° C or 30 minutes at 100° C.

4) Preparation of microcolumns:

The resin Dowex 50W X2 (100-200 mesh) was purified by cycling with 5N HCl, then 5N NaOH and finally 5N HCl. Approximately 200 mg of resin was used in the microcolumn (1 ml of suspension of 5 gm in 25 ml of water). The microcolumn consisted of a Pasteur pipette. A glass-bead placed at the lower part of its body allowed the passage of liquid but retained the resin particles.

5) Choice of a solvent system:

Four solvent systems were tested:

- a mixture of 2-butanol, glacial acetic acid and water in relative proportion of 14:1:5
- acetic acid 2%
- a mixture of methylethylketone, propionic acid and water in relative proportion of 75:25:30
- a mixture of 2-butanol, propionic acid and water in relative proportion of 75:25:30 (the abbreviation B : P : W will frequently be employed for this mixture).

This last system was chosen for the plates run in one dimension:

its running time was slow (about 3 1/2 hours) but it gave a good separation of harmaline or harmine and their various metabolites. For the two-dimensional chromatograms, a solution of acetic acid 2% was chosen for the first dimensional separation: its running time was about one and a half hour. The mixture 2-butanol, propionic acid and water (75:25:30) was again adopted for the second dimensional separation. After the first separation of compounds made by acetic acid 2% the plates were dried in the oven for 10 minutes and then exposed in a tank to an atmosphere saturated by vapor of ammonia for 10 minutes. Then they were run in the second solvent system (2-butanol, propionic acid and water). The total time required for a two-dimensional chromatogram was about 4 1/2 hours.

6) Detection of the compounds on the chromatograms by ultraviolet light:

Under ultraviolet light harmaline, harmalol and harmine fluoresce and so do their metabolites on the chromatography plates. Harmaline and harmalol emit a green fluorescence whereas harmine gives a violet blue color. Our chromatograms were therefore alternatively examined under a lamp emitting long ultraviolet light (Blak-Ray, UVL-22) and under another one emitting short ultraviolet light (Mineralight, UVS-11). The former visualized better the green and yellowish fluorescing spots and the latter, as it enhances the luminosity of the violet blue fluorescence permitted the detection of some spots not seen or barely visible under UVL-22. Blak-Ray, UVL-22 and Mineralight, UVS-11 are made by Ultra-Violet Products Inc., San Gabriel, California.

7) Photography of the chromatograms:

The one-dimensional plates were photographed in a darkroom using an Ektachrome Hi-Speed film (Kodak). Wratten filters No. 2 B and

K 2 (Kodak) were superposed on the lens (107). The aperture of the lens was f = 5.6 and the time of exposure was 30 seconds. The UVL-22 was placed at 9.5 inches in front of the plate to be photographed. On the film, the green fluorescent spots were faithfully registered. Unfortunately the violet blue and the yellowish spots also gave a green color. Moreover, the violet blue fluorescence did not imprint on the film unless intense. Finally any spot having too little luminousness was not registered. Therefore explanation will be provided with the photographs whenever necessary.

To obviate some of these problems the two-dimensional chromatograms were photographed with the same type of film but under daylight, after having colored with chalk the areas corresponding to the fluorescent spots. If this procedure has the advantage of showing the colors, the exact location and the approximate size of the spots, it has the disadvantage of giving a less exact idea of the intensity of their fluorescence.

2) Results:

a) Quantitative concentration and distribution:

Harmaline was found in each organ analyzed. The content of harmaline was approximately the same in each tissue except for the pancreas where it was in much higher concentration 30 minutes after the injection. In all tissues, except perhaps the kidneys, the concentration of harmaline reached its peak within 30 minutes. The content of harmaline in the pancreas fell rapidly thereafter until the second hour following the injection. A similar rapid fall in concentration was observed in the heart 30 minutes after the peak concentration was reached in this organ.

In the kidneys, the level of harmaline remained fairly constant from 30 minutes until the fourth hour after the injection. In all other organs, the concentration of harmaline began definitely to decline progressively two hours after the injection of the drug and at the eight hour practically no harmaline was found in any of the tissues analyzed (Table 6, FIG. 7).

Table 6

DISTRIBUTION OF HARMALINE (μg/gm) IN VARIOUS ORGANS

(after injection of 50 mg/kg I.P.)

Time/hr.	Brain	Lungs	Pancreas	Heart	Liver	Kidneys
1/2	*26.0±3.2	33.4	*68.3±15.9	35.0	*26.4±6.3	18.2
1	*21.8±3.1	33.6	39.0	15.5	20.2	22.3
2	*18.5±1.6	26.5	22.4	*13.6±3.8	*18.0±5.3	15.5
4	7.2	18.3	14.8	4.4	9.8	16.0
8	0.18	0.39	0.58	0.15	0.52	1.54

*Mean value of at least 3 animals.

We found that in the brain, harmaline is evenly distributed: there is no difference in its concentration on one hand in the cerebral hemispheres and in the diencephalon and the mesencephalon on the other. A high value obtained once in the latter is most likely due to a technical error (Table 7). It is possible that if analyses of the various nuclei of the brain had been performed, differences in concentration might have been detected.

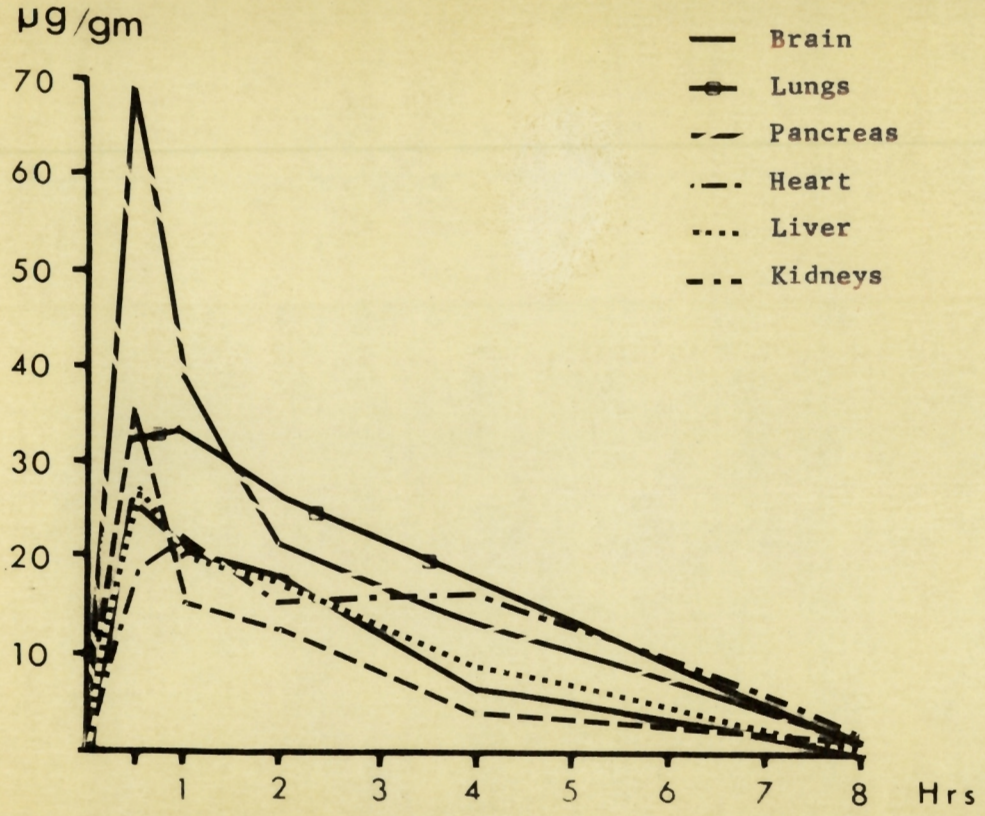


FIG. 7 Distribution of harmaline* (50 mg/kg I.P.)

*values taken from Table 6

Table 7

DISTRIBUTION OF HARMALINE (μg/gm) IN BRAIN*

(after injection of harmaline 50 mg/kg I.P.)

Time (min.) after injection	Cerebral Hemispheres	Diencephalon + Mesencephalon
30	27.9	26.6
	25.4	36.2
60	25.8	21.1
	24.4	24.0

*Four animals.

In each organ analyzed, harmine was found. In all tissues the concentration of the drug reached its peak 30 minutes after the injection and had fallen to very low levels at the end of the first hour. Thereafter it was barely measurable and there was practically nothing left in the tissues at the beginning of the second hour. The lungs, kidneys and pancreas contained similar quantities of harmine. The brain, the hart and the liver had less (Table 8, FIG. 8).

When harmaline and harmine are administered in equal doses a striking difference in their concentration in the organs can be noticed (Table 9). At 10 and 50 mg/kg I.P., the concentration of harmaline in the brain, the heart and the liver is much greater than that of harmine. In the brain and the heart, the concentration of harmaline found seems to be proportional to the dose given but this is not so for the liver.

Table 8

DISTRIBUTION OF HARMINE (μg/gm) IN VARIOUS ORGANS

(after injection of 25 mg/kg I.P.)

Time/hr.	Brain	Lungs	Pancreas	Heart	Liver	Kidneys
1/2	5.99 (4.50)* 3.01	10.81 (8.93) 7.05	11.36 (9.23) 7.10	3.72 (3.32) 2.93	3.10 (2.42) 1.75	9.14 (8.21) 7.29
1	.66 (0.70) .75	.79 (1.34) 1.90	.80 (1.05) 1.31	.39 (0.59) .79	.26 (0.34) .42	1.01 (0.82) .64
1 1/2	.12 (0.17) .23	.19 (0.21) .24	.23 (0.37) .51	.12 (0.16) .21	.08 (0.11) .15	.27 (0.37) .48
2	.02 (0.03) .04	.02 (0.03) .05	.03 (0.06) .09	.02 (0.02) .03	.07 (0.05) .03	.03 (0.05) .07

*Mean value of 2 animals.

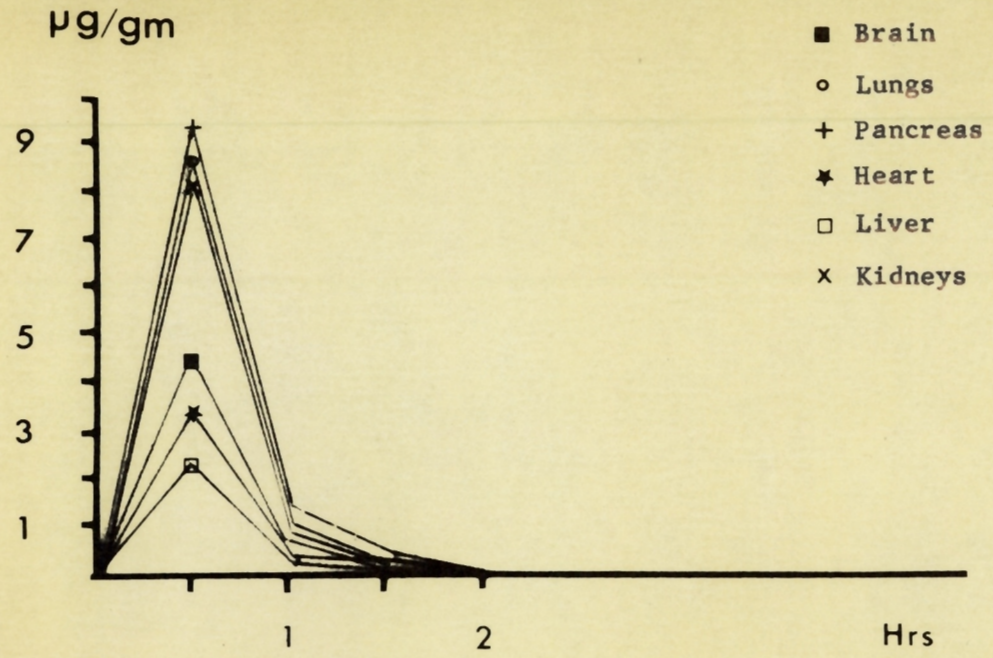


FIG. 8 Distribution of harmine* (25 mg/kg I.P.)

*each point represents the mean value of 2 animals (Table 8)

Table 9

COMPARATIVE CONCENTRATION OF HARMINE AND HARMALINE (μg/gm) IN VARIOUS ORGANS

(after 2 hours)

Dose I.P.	Brain		Heart		Liver	
mg/kg	Harmaline	Harmine	Harmaline	Harmine	Harmaline	Harmine
10	2.88	.11	2.02	.09	5.24	.05
		(.26)		(.23)		(.15)
		.41		.38		.26
50	17.22	1.41	9.18	1.18	12.47	.85
		(1.09)		(1.21)		(.73)
		.78		1.25		.61

A quantitative analysis of the 24-hour urine of the rats injected intraperitoneally with a dose of 6 mg of either harmaline or harmine showed that only 5.4% of harmaline and 0.6% of harmine were excreted unchanged. All these results indicate a marked difference in the metabolism of harmaline as compared to that of harmine.

b) Thin-layer chromatography:

The chromatographic pattern of harmaline was compared to that of harmine in the brain, the kidneys, the liver and the urine. No mention will be made in describing the chromatograms of the compounds also occurring in the control material or of the UVS-11 absorbent substances.

(1) Brain:

Harmaline; one-dimensional chromatogram (FIG. 9):

Ten µl of the brain eluates were applied to the plate at the bottom of each track:

FIG. 9

Tracks:

A = Compounds present after 30 minutes
B = Compounds present after 1 hour
C = Compounds present after 4 hours
D = Compounds present after 24 hours
E = Eluate from control
F = Eluate of harmaline standard
G = Eluate of harmalol standard
H = Eluate of harmine standard
I = Eluate from harmine-injected rats (30 minutes after injection)

In the brain of the rats injected with harmaline, only compound Rf_1 having the same Rf (0.80) and the same green fluorescence as standard harmaline was isolated. The amount present as estimated by the intensity of the fluorescence was approximately the same at 30 and 60 minutes. The spot representing the group of animals killed 4 hours after the injection of harmaline was much less fluorescent. No fluorescence at all was present on the track representing the 24-hour group indicating the complete disappearance of harmaline from the brain after 24 hours.

Harmine; one-dimensional chromatogram (FIG. 10):

Ten µl of the brain eluates were applied to the plate at the bottom of each track:

FIG. 10*

*See footnote of Fig. 9 for description of the tracks. On Fig. 10, track I = Eluate from harmaline-injected rats (30 minutes after injection).

In the brain of the rats which received harmine, two compounds having a violet blue fluorescence like harmine were isolated. The compound present in the greater quantity according to the intensity of its fluorescence had an Rf_1 (0.84) similar to harmine (0.80). The other substance had very little fluorescence and its Rf_2 was 0.39 (it is not visible on the photograph). The fluorescence of these two compounds was more intense on the track representing the group of rats killed 30 minutes after the injection than for the group sacrificed after 60 minutes. No fluorescent spot was visible on the tracks representative of the 4- and 24-hour period, indicating the complete disappearance of harmine from the brain within 4 hours.

Harmaline, harmine; two-dimensional chromatogram (FIG. 11):

Ten µl of the eluate of brains from the group of rats killed 30 minutes after the injection of either drug were applied to the plates:

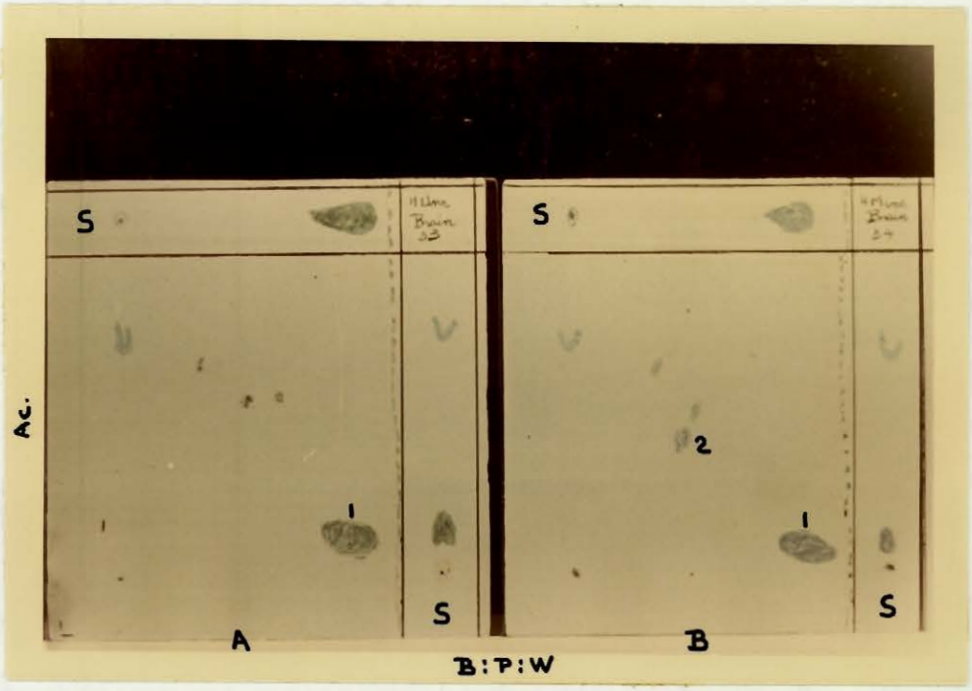


FIG. 11

A = Harmaline chromatogram

B = Harmine chromatogram

S = Track for the eluate of the standard drug: harmaline or harmine

Number = Compound detected on the chromatogram

Non-numbered spots were either UVS-11 absorbent spots or spots also present on the chromatogram of the control tissue

AC = Acetic Acid 2%, first solvent system

B:P:W = 2-Butanol:propionic acid:water (75:25:30), second solvent system.

On the harmaline plate, one green fluorescent spot, No. 1, was found. Its Rf was 0.14 in the first solvent system and 0.86 in the second one. It corresponded to Rf_1, assumed to be harmaline on the one-dimensional chromatogram. There was a questionable faint violet blue fluorescence at the lower edge of the spot: this had been also observed on another chromatogram made under different conditions.

On the harmine plate, two violet blue fluorescent spots were detected. No. 1, the most important had Rfs, 0.08 and 0.85. Compound No. 2, barely visible, had Rfs, 0.40 and 0.41. Compound No. 1 corresponded to Rf_1 (harmine) on the one-dimensional chromatogram and compound No. 2 to Rf_2.

The two-dimensional chromatograms therefore gave results similar to those obtained with one-dimensional chromatograms.

(2) Kidneys:

Harmaline; one-dimensional chromatogram (FIG. 12):

Five µl of the kidneys eluates were applied to the bottom of each track:

FIG. 12

Tracks:

A = Compounds present after 30 minutes
B = Compounds present after 1 hour
C = Compounds present after 4 hours
D = Eluate of harmaline standard
E = Eluate of harmalol standard
F = Eluate of harmine standard*

*Not seen on the photograph but has same Rf value as harmaline standard.

Three compounds were visualized in the kidneys (Table 10):

Table 10

Time (hr.) after injection	Rf_1	Rf_2	Rf_3
1/2	0.77	0.35	0.15
1	0.79	0.32	0.17
4	0.79	-	0.17
Fluorescence	Green	Violet blue under UVS-11	Green

The compound having Rf_1 = 0.77 - 0.79 was assumed to be harmaline. Its Rf was the same as the eluate of standard harmaline. On a previous chromatogram we had found that nothing was left of this compound in the kidneys of the 24-hour group of animals. The compound having Rf_2 = 0.32 - 0.35 had a very faint violet blue fluorescence under UVS-11. It had disappeared within four hours following the injection of harmaline. This compound is not visible on Fig. 12. As estimated by the intensity of the fluorescence, the metabolite with Rf_3 = 0.15 - 0.17 was with harmaline (Rf_1), the substance present in the greater amount. Just as for Rf_1, a previous chromatogram showed no trace of it after 24 hours. Harmaline (Rf_1) and Rf_3 fluoresced as intensely on track A (30 minutes) as on track B (60 minutes). On track C (4 hours) their fluorescence had decreased considerably thereby implying their presence in lesser amount in the kidneys.

Harmine; one-dimensional chromatogram (FIG. 13):

Five µl of the kidneys eluates were applied at the bottom of each track:

FIG. 13*

*See footnote of Fig. 12 for description of the tracks.

In the kidneys of the group of animals killed 30 minutes after the injection, five spots emitting a violet blue fluorescence (green on the photograph) could be visualized (track A), but their number decreased with the lapse of time (Table 11).

Table 11

Time (hr.) after injection	Rf_1	Rf_2	Rf_3	Rf_4	Rf_5
1/2	0.81	0.65	0.33	0.25	0.12
1	0.81	0.65	0.33	0.25	0.12
4	-	-	0.39	-	0.16
Fluorescence	Violet blue fluorescence				

The compound with Rf_1 = 0.81 was assumed to be harmine, its fluoresence and its Rf being identical to that of standard harmine. It was absent on track C (4 hours after injection) indicating its complete metabolism within 4 hours. Two other metabolites, compounds Rf_2 = 0.65 and Rf_4 = 0.25 followed the same pattern. The two other substances, Rf_3 = 0.33 - 0.39 and Rf_5 = 0.12 - 0.16 still present on track C had been eliminated within 24 hours. This had been demonstrated by a previous chromatogram.

As judged by the intensity of their fluorescence, the various compounds in decreasing order of importance were, on track A (30 minutes after injection): Rf_3, Rf_5, Rf_1 (harmine) and Rf_2, and finally Rf_4. The compounds Rf_3, Rf_4 and Rf_5 seemed present in slightly greater amount in the group killed one hour after the injection (track B). Both metabolites Rf_3 and Rf_5 were much less fluorescent in the 4-hour group (track C).

Harmaline, harmine; two-dimensional chromatogram (FIG. 14):

Ten µl of the eluate of kidneys from the group of rats killed 30 minutes after the injection of either drug were applied to the plates:

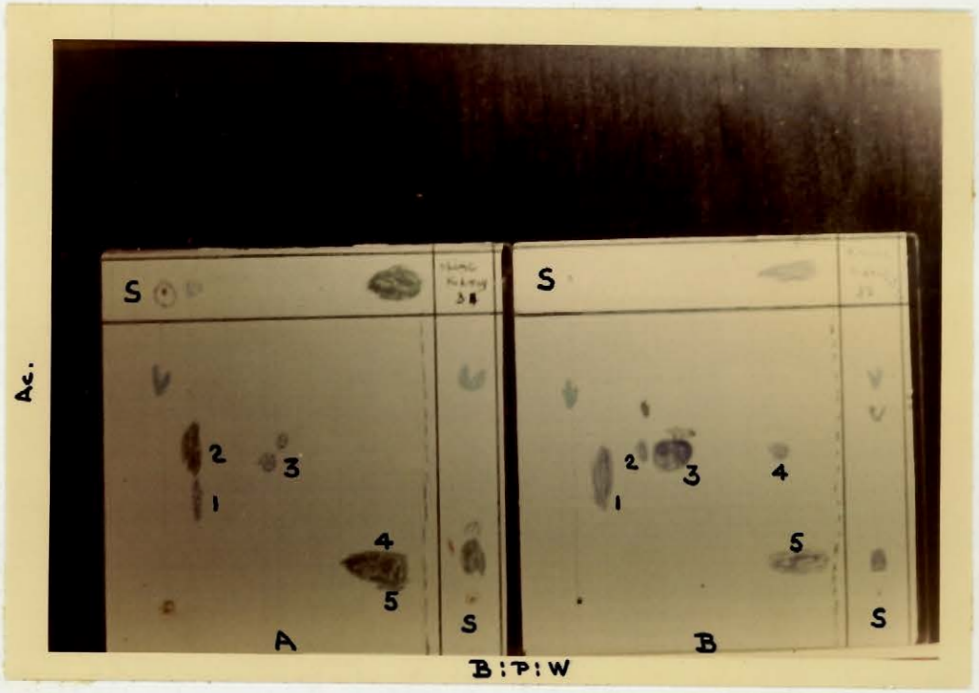


FIG. 14

A = Harmaline chromatogram

B = Harmine chromatogram

S = Track for the eluate of the standard drug: harmaline or harmine

Number = Compound detected on the chromatogram

Non-numbered spots were either UVS-11 absorbent spots or spots also present on the chromatogram of the control tissue

AC = Acetic Acid 2%, first solvent system

B:P:W = 2-Butanol:propionic acid:water (75:25:30), second solvent system.

On the two-dimensional chromatogram of the harmaline eluate (A) five compounds could be detected (Table 12):

Table 12

Compound No.	Rf value in: Acetic Acid 2%	B:P:W	Fluorescence
1	0.37	0.10	Violet blue under UVS-11
2	0.57	0.10	Green
3	0.50	0.38	Violet blue under UVS-11
4	0.14	0.84	Green
5	0.06	0.85	Violet blue under UVS-11

Compounds No. 2 and No. 4 emitted a fairly intense green fluorescence. Compounds No. 1, 3 and 5 were detected under UVS-11 and fluoresced very little. Compounds No. 1 and 2 corresponded to the metabolite Rf_3 in the one-dimensional system, compound No. 3 to Rf_2 and compound No. 4 to Rf_1 which is harmaline in that system. Compound No. 5 had an Rf similar to harmine.

On the two-dimensional chromatogram of the harmine eluate (B), five compounds could also be visualized and they all fluoresced violet blue (Table 13):

Table 13

Compound No.	Rf value in: Acetic Acid 2%	B:P:W
1	0.43	0.10
2	0.52	0.25
3	0.50	0.38
4	0.50	0.78
5	0.11	0.84

Compound No. 1 corresponded to Rf_5 on the one-dimensional chromatogram, compound No. 2 to Rf_4, compound No. 3 to Rf_3 and compound No. 5 to Rf_1. Compound No. 4 had no equivalent on the one-dimensional chromatogram while Rf_4 on the latter was not found here. It may be assumed that Rf_4 was masked by compound No. 5, did not separate from it.

The intensity of the fluorescence was about the same for No. 1 and No. 3 but less for No. 5. Compound No. 2 and 4 were very slightly fluorescent.

(3) Liver:

Harmaline; one-dimensional chromatogram (FIG. 15):

Ten µl of the liver eluates were applied at the bottom of each track:

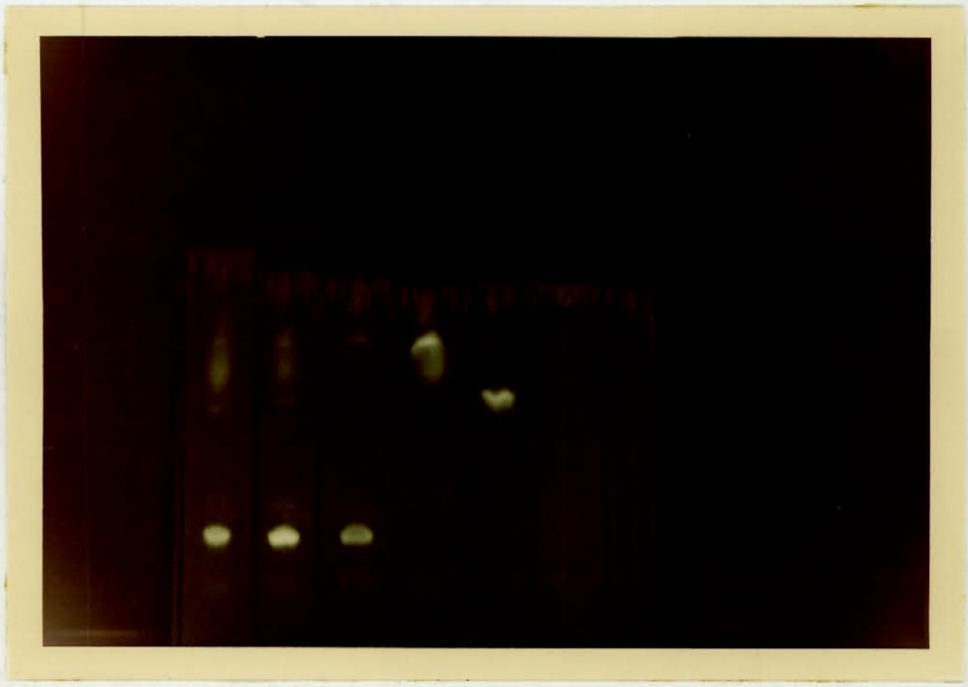


FIG. 15*

*See footnote of Fig. 12 for description of the tracks.

In the liver of the group of animals killed 30 minutes after the injection, five spots were detected. This number remained fairly constant during the 4-hour period (Table 14). A previous chromatogram had shown that nothing was present in the liver 24 hours after injection of the drug.

Table 14

Time (hr.) after injection	Rf_1	Rf_2	Rf_3	Rf_4	Rf_5
1/2	0.71	0.54	0.35	0.24	0.16
1	0.74	0.59	0.37	0.23	0.16
4	0.74	0.59	-	0.23	0.16
Fluorescence	Green	Green	Violet blue under UVS-11	Light Green	Green

The compound with Rf_1 = 0.71 - 0.74 was assumed to be harmaline. The metabolite with Rf_2 = 0.54 - 0.59 was thought to be harmalol as it had the same pale green fluorescence as the standard harmalol and an identical Rf. The compound with Rf_3 = 0.35 - 0.37 emitted a faint violet blue fluorescence under UVS-11: it had disappeared four hours after injection of the drug (track C). The metabolites Rf_4 = 0.23 - 0.24, Rf_5 = 0.16 had a green fluorescence. As evidenced by a previous chromatogram none of these compounds was present in the group of rats killed 24 hours after the injection.

In decreasing order of importance as estimated by their degree of fluorescence, they were: compounds Rf_5, Rf_1 and Rf_2, Rf_4 and finally Rf_3 which is not visible on the photograph.

The fluorescence of compounds Rf_1 (Harmaline), Rf_2 (Harmalol) and Rf_5 was as intense on track A (30 minutes after injection) than on

track B (60 minutes). Except metabolite Rf_5, Rf_1 and Rf_2 had decreased considerably on track C (4 hours).

Harmine; one-dimensional chromatogram:

Ten µl of the liver eluates were applied at the bottom of each track. Better detected under UVS-11, five spots all fluorescing violet blue were seen on the track representing the group of animals killed 30 minutes after the injection. The compounds they represented disappeared rapidly from the liver (Table 15):

Table 15

Time (hr.) after injection	Rf_1	Rf_2	Rf_3	Rf_4	Rf_5
1/2	0.82	0.56	0.31	0.17	0.13
1	-	0.56	0.31	-	0.13
4	-	-	-	-	-

The compound with Rf_1 = 0.82 was assumed to be harmine as it had the same fluorescence and Rf as the standard harmine. Harmine appeared to be present in very little quantity and so were the metabolites Rf_2 and Rf_4. The two major metabolites as estimated by their fluorescence were first, compound Rf_3 and secondly, compound Rf_5: these could easily be seen under UVL-22.

As seen in Table 15, harmine (Rf_1) could not be detected in the liver of the group killed one hour after the injection and all compounds seemed to have been eliminated from this organ within four hours.

Harmaline, harmine; two-dimensional chromatogram (FIG. 16):

Thirty µl of the eluate of livers from the group of rats killed 30 minutes after the injection of either drug were applied to the plates:

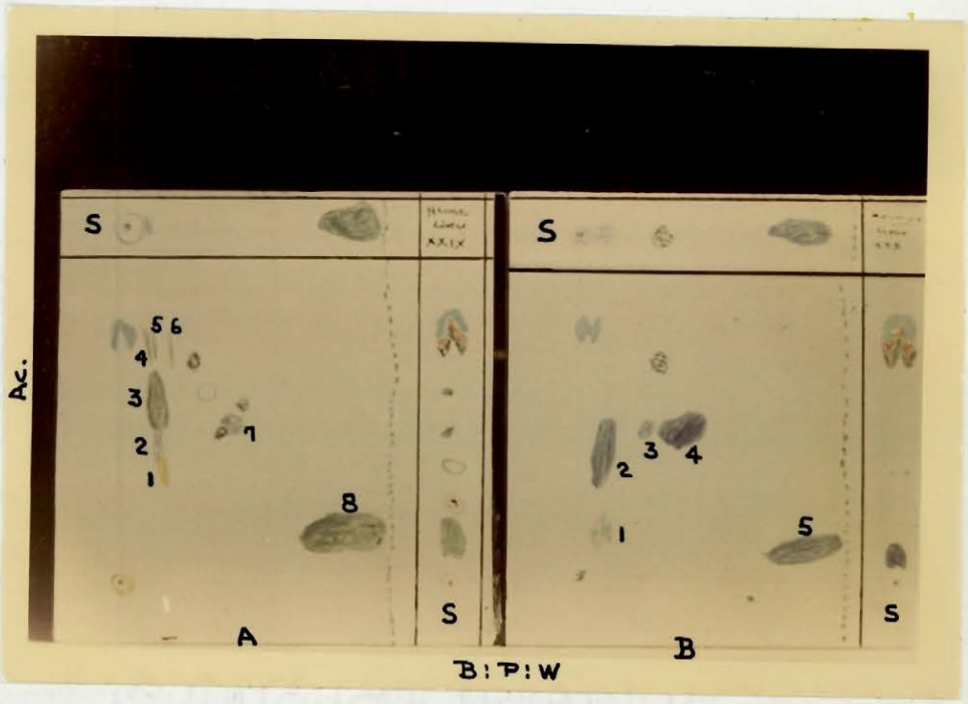


FIG. 16*

*See footnote of Fig. 14

On the two-dimensional chromatogram of the harmine eluate (B), five compounds were visualized (Table 16):

Table 16

Compound No.	Rf value in: Acetic Acid 2%	B:P:W	Fluorescence
1	0.15	0.08	Violet blue under UVS-11
2	0.41	0.08	Violet blue
3	0.48	0.25	Violet blue under UVS-11
4	0.48	0.39	Violet blue
5	0.10	0.87	Violet blue

Compounds No. 2, 4 and 5 were quite fluorescent while No. 1 and 3 had very little fluorescence under UVS-11. Compound No. 1 and 2 could correspond to Rf_5 of the one-dimensional plate, No. 3 to Rf_4, No. 4 to Rf_3, and No. 5 to Rf_1 and Rf_2.

On the two-dimensional chromatogram of the harmaline eluate (A) eight compounds could be detected (Table 17):

Table 17

Compound No.	Rf value in: Acetic Acid 2%	B:P:W	Fluorescence
1	0.35	0.11	Pale yellow
2	0.42	0.09	Violet blue under UVS-11
3	0.57	0.08	Green
4	0.73	0.08	Very faint green
5	0.73	0.11	Very faint green
6	0.73	0.17	Very faint green
7	0.48	0.41	Violet blue under UVS-11
8	0.16	0.86	Green

Compounds No. 3 and 8 emitted an intense green fluorescence while No. 1 had a yellow one. No. 4, 5, 6 were very faintly fluorescing green while No. 2 and 7 gave a violet blue fluorescence under UVL-11 only. It is probable that No. 1, 2, 3, 4 correspond to Rf_5 in the one-dimensional system, No. 5 and 6 to Rf_4, No. 7 to Rf_3 and No. 8 to Rf_1 and Rf_2. In a previous experiment, under slightly different conditions, it had been possible to separate No. 8 into two green spots corresponding to Rf_1 and Rf_2.

(4) Urine:

Harmaline, harmine; one-dimensional chromatogram (FIG. 17, 18):

Fifteen µl of the eluate from the 24-hour urine output of the group of rats injected with harmaline and thirty µl of the eluate of the harmine-injected group were applied to the plate. In the latter group, two animals had died within 4 hours following the injection of the drug:

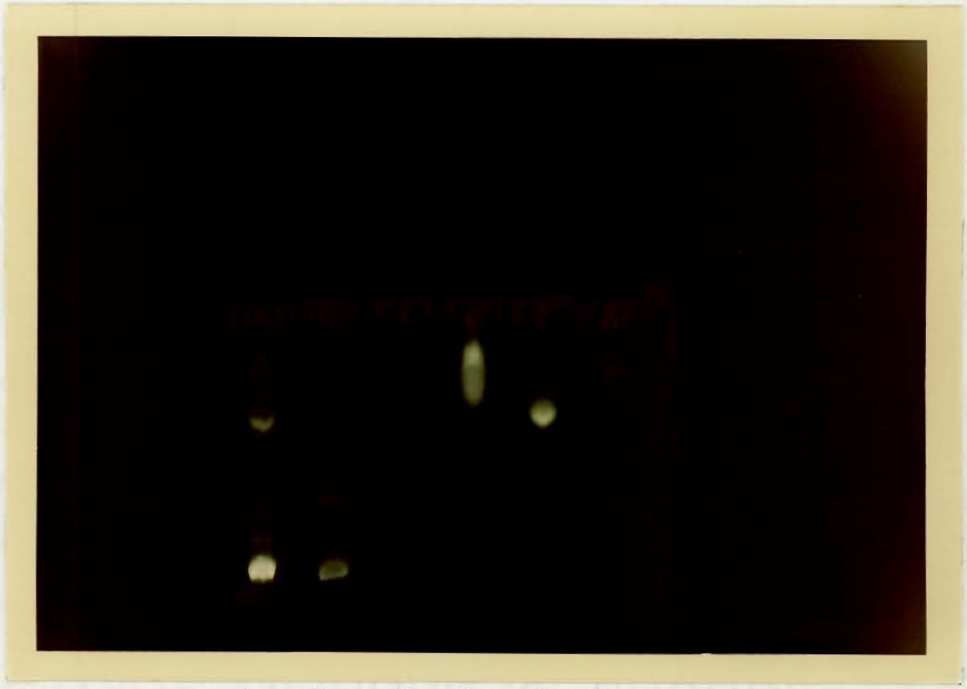


FIG. 17*

Tracks:

A = Eluate from harmaline-injected animals
B = Eluate from harmine-injected animals
C = Eluate from control animals
D = Eluate of harmaline standard
E = Eluate of harmalol standard
F = Eluate of harmine standard (barely visible on photograph)

*FIG. 18 shows the same chromatogram. The fluorescent spots have been colored and the plate photographed under daylight.

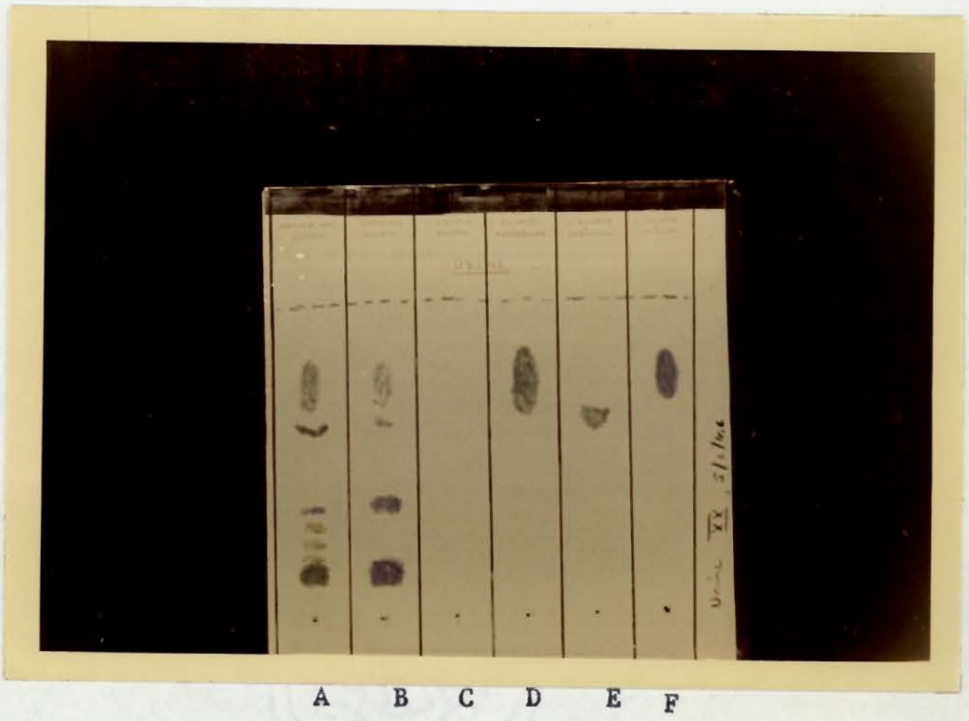


FIG. 18

Harmaline (Track A):

Seven spots of various color and fluorescence intensity were discernible (Table 18):

Table 18

Rf	Rf_1	Rf_2	Rf_3	Rf_4	Rf_5	Rf_6	Rf_7
Compound	0.79	0.61	0.34	0.30	0.24	0.19	0.15
Fluorescence	Green	Green	Blue Violet	Greenish	Yellowish	Yellowish	Green

The compound with Rf_1 = 0.79 was assumed to be harmaline as it had the same fluorescence and Rf as the eluate of standard harmaline. Compound Rf_2 = 0.61 possessed a similar fluorescence and Rf as the eluate of standard harmalol. Compound Rf_2 is believed to be harmalol: indeed if the

dried plate was exposed to fumes of ammonia in a tank for 1 to 2 minutes both compound Rf_2 and the spot of standard harmalol took a yellow color while no other spot on the plate gave any reaction.

Compound Rf_3 = 0.34 was visualized under UVS-11 and emitted a violet blue fluorescence. As listed in Table 18, four other metabolites were also detected. Their amount as estimated by the intensity of fluorescence and size of the spots was in decreasing order: compounds Rf_7, Rf_2, Rf_3, Rf_4 - Rf_5 - Rf_6, and Rf_1. By far the most important metabolite was Rf_7 and ranking second was Rf_2.

Harmine (Track B):

Four spots all emitting a blue fluorescence and better seen under UVS-11 were detected. In order to facilitate the description they will be assigned the same Rf number as the compound found at the same Rf on the harmaline track, (Table 19):

Table 19

Rf	Rf_1	Rf_2	Rf_3	Rf_7
Compound	0.79	0.61	0.34	0.15

As estimated by the intensity of the fluorescence and the size of the spots, the compounds in decreasing order of importance were: compounds Rf_7, Rf_3, Rf_2 and Rf_1. Compound Rf_1 which was barely visible was assumed to be harmine as it had the same fluorescence and Rf as the eluate of standard harmine. Compound Rf_2 had the same Rf as Rf_2 of the eluate of the harmaline-injected animals and also the same Rf as the eluate of harmalol standard: however it fluoresced violet blue whereas the former had

a green fluorescence. Compound Rf_3 appeared to be identical to metabolite Rf_3 in the eluate of the harmaline-injected rats: it was however present in much greater amount here. Compound Rf_7 constituted here the most important metabolite as did compound Rf_7 in the eluate of harmaline-injected rats: they differed in color of fluorescence.

Harmaline, harmine; two-dimensional chromatogram (FIG. 19):

Thirty µl of the eluate from the 24-hour urine output of the group of rats injected with harmaline and fifty µl of eluate from the harmine-injected group were applied to the appropriate plate:

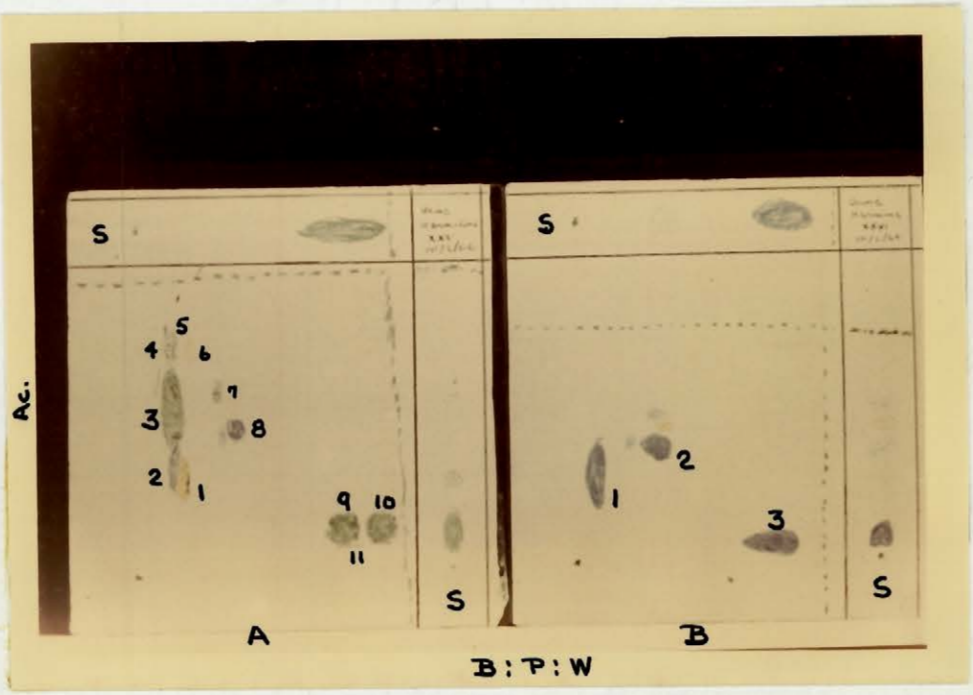


FIG. 19*

*See footnote of Fig. 14.

On the two-dimensional chromatogram of the harmaline eluate (A) eleven compounds could be detected (Table 20):

Table 20

Compound No.	Rf value in: Acetic Acid 2%	B:P:W	Fluorescence
1	0.35	0.18	Yellow
2	0.39	0.14	Violet blue
3	0.60	0.15	Green
4	0.81	0.12	Very pale green
5	0.80	0.15	Very pale green
6	0.79	0.21	Yellowish
7	0.62	0.31	Green
8	0.50	0.38	Violet blue
9	0.15	0.79	Green
10	0.14	0.91	Green
11	0.08	0.80	Violet blue

As estimated by the intensity of the fluorescence and the size of the spots, compounds No. 3, 9 and 10 had about the same importance. Compounds No. 1, 2, 4, 5 and 8 emitted less fluorescence and compounds No. 7, 6, 11 still less: the latter were barely visible.

Seemingly on the two-dimensional chromatogram, the metabolite having the Rf_7 on the one-dimensional chromatogram separated to give compounds No. 2, 3, 4 and 5. Compound No. 1 appeared to correspond to Rf_6 on the one-dimensional chromatogram, compound No. 6 to Rf_5, compound No. 7 to Rf_4, compound No. 8 to Rf_3, compound No. 9 to Rf_2 and finally compound No. 10 to Rf_1. Compounds No. 9 and 10 were believed to be harmaline

and harmalol respectively. The metabolite No. 11, unaccounted for, may have been masked either by Rf_1 or Rf_2 on the one-dimensional chromatogram. A similar compound, located in this way, had also been encountered on the two-dimensional chromatograms of the brain and kidneys of the rats injected with harmaline.

On the two-dimensional chromatogram of the harmine eluate (B), three compounds were visualized. They all had a violet blue fluorescence.

Table 21

Compound No.	Rf value in: Acetic Acid 2%	B:P:W
1	0.37	0.12
2	0.48	0.32
3	0.09	0.81

Both compounds, No. 1 and 2, were quite fluorescent. The size of the spot for compound No. 1 was however larger which indicated its presence in greater amount. Compound No. 3 was much less fluorescent and the spot was elongated.

Compound No. 1 seemed to correspond to Rf_7 on the one-dimensional chromatogram, compound No. 2 to Rf_3 and compound No. 3 to Rf_1 which is believed to be harmine. The metabolite with an Rf = 0.61 on the one-dimensional chromatogram may have been mixed with compound No. 3 as in our two-dimensional system the compounds with a high Rf, that is, near the solvent front, tended to run faster.

3. Discussion:

Sequential thin-layer chromatography of the brain, the kidneys and the liver of the harmaline-injected animals shows conclusively that harmaline is present in these tissues at its highest concentration from 30 to 60 minutes after its injection. After 4 hours, the concentration of the drug has diminished markedly and after 24 hours nothing is found.

Thin-layer chromatography of the same tissues of harmine-injected animals demonstrates that this compound reaches its maximum concentration there 30 minutes after its injection and that little is found after 60 minutes. It is not detectable 4 hours or 24 hours after its injection.

As also shown by thin-layer chromatography, a dose of 50 mg/kg of harmine was metabolized much faster than a dose of 30 mg/kg of harmine.

There is reasonable evidence to suggest that harmaline and harmine have two common metabolites: they have a similar Rf and both emit a violet blue fluorescence. The first common metabolite is visible on the one-dimensional chromatograms of the urines and of all the organs, except the brain of harmaline-injected animals. It has a mean Rf of about 0.35. On the two-dimensional plates, it is located at the center of the chromatogram. Its mean Rfs are about 0.48 and 0.38. The second common metabolite is not discernible on the one-dimensional chromatograms of the harmaline-injected animals. It is indeed located at the bottom of the tracks and is masked by an important spot, fluorescing green. This metabolite is very conspicuous on the one-dimensional chromatograms of harmine-injected animals where it is located also at the bottom of the tracks. On the two-dimensional chromatograms of harmaline-injected animals, the green spot separates into several components amongst which is this violet

blue component. Its mean Rfs are about 0.40 and 0.12 on chromatograms of either harmaline or harmine. It is absent from the brain of the animals that received either drug. These two metabolites are major ones in the metabolism of harmine but are very minor ones in the metabolism of harmaline.

A compound, difficult to visualize and detected at the lower edge of the harmaline spot on the two-dimensional chromatograms of this drug, would have an Rf and a fluorescence similar to harmine. In our solvents, harmaline and harmine have identical Rfs and if present together would practically not separate: harmaline in greater amount on a chromatogram would cover harmine.

On the one-dimensional chromatograms, a compound having the same Rf and fluorescence as harmalol was encountered in the liver (FIG. 15, Rf_2) and was very conspicuous in the urine (FIG. 17, Rf_2) of the harmaline-injected animals. It constitutes there the second metabolite in importance. At the same Rf a violet blue fluorescent compound was found in the animals which received harmine and it could be harmol. It was detected in the kidneys (FIG. 13, Rf_2), in the liver (Table 15, Rf_2) and in the urine (FIG. 18, track B, Rf_2). These compounds that we suppose to be harmalol and harmol do not always separate from the harmaline or the harmine spot in the two-dimensional system.

The metabolites having a low Rf on the one-dimensional chromatograms ($Rf < 0.40$) are probably conjugates and they constitute the most important metabolites in the urine, the kidneys and the liver of the animals injected either with harmaline or harmine. The hydrolysis of the tissue extracts and of the urine followed by chromatography, in order to verify this assumption, has not yet been done.

Our findings with the chromatography of the brain would support the hypothesis that harmaline and harmine themselves and not their metabolites exert an excitatory action on the central nervous system.

From these data, we would speculate that much of harmaline and harmine are demethylated at position 8, most likely in the liver; this would abolish their excitatory action on the central nervous system. Conjugation would occur afterwards. Only a small fraction of either drug is excreted unchanged in the urine.

Harmaline is metabolized more slowly than harmine. This is confirmed both by quantitative determination and thin-layer chromatography.

The metabolism of harmaline is more extensive than that of harmine (FIG. 15, 16, 17, 18, 19) and it appears that some conversion to the harmine series of compounds occurs. The opposite is not true for harmine. Our data would partly substantiate Flury's earlier views on the metabolism of harmaline. He had contended that the metabolites of harmaline were harmalol, harmine, harminic acid and another unidentified compound (38).

The ubiquitous distribution of harmaline justifies Gunn's opinion that it has no selectivity for tissues (25) and this lack of specificity is also true for harmine. The only organ where harmaline was found in greater amount is the pancreas.

B. HARMALINE AND THERMOREGULATION

1. Introduction:

The hypothalamus is the main structure involved in thermoregulation. Its role is to integrate information either of central or peripheral origin and to coordinate the responses of the effector systems.

Its anterior part controls the organism's response to hyperthermia and triggers the heat-loss mechanisms. Its posterior part controls the response to hypothermia and sets in the heat-production mechanisms. This has been conclusively demonstrated by either stimulating or destroying these hypothalamic areas. Thermal balance is achieved through modifications or the respiratory rate, variation in cutaneous blood flow, sweating, piloerection, shivering and complex endocrine and metabolic changes (108, 109, 110).

Many physiological and pharmacological substances exert an action on the body temperature by various mechanisms (110). In recent years, the advent of MAO inhibitors and refinements in biochemical and neuropharmacological techniques have facilitated the study of the physiological role of the biogenic amines. For instance, their ability to alter the body temperature has been noted. Catecholamines provoke a hyperthermia which, according to von Euler, is mostly due to their vasomotor effects, their hyperglycemic action and also their direct action on the central nervous system (111). In different animals, serotonin (5-hydroxytryptamine) has been reported to give either hypothermic or hyperthermic reactions. Its precursor, 5-hydroxytryptophan, produces hyperthermia in rabbits (112).

Recently Feldberg and Fleischhauer have proposed a new concept

of temperature control involving noradrenaline, adrenaline and serotonin (113). These amines were injected in the cerebral ventricles and in the hypothalamus of cats. Adrenaline or noradrenaline when injected in the cerebral ventricles, lowered the rectal temperature for several hours and caused cessation of the shivering accompanying a fever produced by bacterial pyrogens. Intraventricular injection of serotonin induced a long-lasting rise in rectal temperature: this rise was brought down by injection of adrenaline or noradrenaline. Unilateral microinjections of these amines in the anterior hypothalamus produced the same results, but no effect was obtained if they were injected in the ventromedial or posterior hypothalamus. Hence at the level of the anterior hypothalamus, serotonin would be associated with hyperthermia, whereas adrenaline and noradrenaline would be associated with a decrease in body temperature and abolition of shivering.

The level of these biogenic amines in the brain can be modified by many pharmacological agents. MAO inhibitors can increase the brain content of catecholamines and serotonin while a substance like reserpine decreases it.

In an attempt to determine if the hypothermic effect of harmaline is due to its MAO inhibitory property, we thought of utilizing the known antagonism between a reversible MAO inhibitor like harmaline and some irreversible long-acting MAO inhibitors (97). If harmaline acts through MAO inhibition, pretreatment with an irreversible MAO inhibitor would prevent the appearance of hypothermia. By the same token if the harmaline-induced tremor is dependent on MAO inhibition it would not occur either.

In addition we were interested in studying the interaction between harmaline and irreversible MAO inhibitors under various conditions

and finally, if possible, to verify if there is a specific link between harmaline hypothermia and tremor, and brain amines.

This led us to perform experiments designed to investigate:

- the effect of harmaline alone and in presence of an inhibitor of drug metabolism
- the effect of long-acting irreversible MAO inhibitors on body temperature
- the interaction between harmaline and long-acting irreversible MAO inhibitors
- the interaction between harmaline and amine-depleting agents.

2. General method:

The rats were placed in individual plastic cages, 16 to 24 hours before the experiments, in a room where the temperature remained fairly constant (20° - 24° C) (FIG. 20). The room temperature was generally about 22° C. It did not vary more than 1 to 2° C on any given day. The rats received food and water until shortly before the experiment. Then the food was withdrawn.

The rectal temperature was recorded twice at spaced intervals during the period of acclimatisation to the room. It was also recorded twice before the experiment and the mean of these two readings served as baseline. The rectal temperature was taken at least once during the first 30 minutes after the beginning of the experiment, then every 30 minutes for the next 4.5 hours and once every hour thereafter until the eight hour. A reading was taken at the 24th hour in all experiments except one. For the sake of clarity, not all of these temperature measurements will be shown on the graphs presented later. When an experiment extended over 2 days, the rats were fed after the ninth or tenth hour, when the systematic recording of the rectal temperature had been ended. The food was

again withdrawn before the onset of the experiment the next day.

A thermocouple (TRI-R Electronic Thermometer, Model TGL) purchased from TRI-R Instruments, Jamaica, New York, was used. The probe (TR-6) was inserted 1.7 cm in the rectum for the temperature measurements.

Once prepared, the solutions to be injected were kept in the refrigerator until the beginning of the experiment when they were warmed to room temperature. The solutions had always a pH of 6.0 and were injected intraperitoneally. Disposable sterile syringes were used to minimize the risk of bacterial pyrogenic effect.

The weights of the rats varied from 135 to 235 gm. However, during each experiment the weights were always kept within the same range, the biggest difference in weight encountered being 23 gm. The mean variation for all experiments was 12 gm.

At least one non-injected animal and one animal injected with harmaline were used in each experiment.

FIG. 20 Experimental Setting

3. Chemicals: (FIG. 21a, 21b)

(1) Inhibitor of drug metabolism:

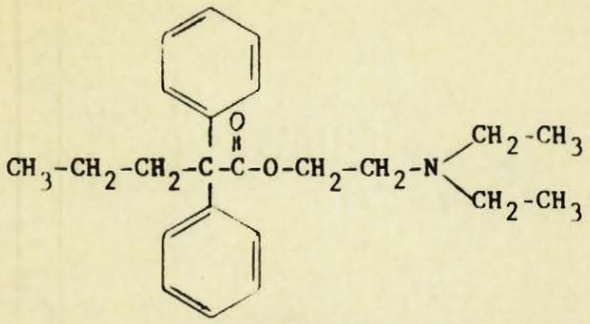
β-Diethylaminoethyl diphenylpropylacetate (SKF-525A) was obtained from Research Laboratories, Smith, Kline and French Labs., Philadelphia.

(2) Irreversible inhibitors of monoamine oxidase:

β-Phenylisopropylhydrazine (Catron, JB-516, pheniprazine) was obtained from Schering Corporation, Bloomfield, N.J. Phenylcyclopropylamine sulfate (Parnate, tranylcypromine, SKF-385A (trans)) was obtained from Research Laboratories, Smith, Kline and French Labs., Philadelphia. Pargyline (Eutonyl) was obtained from Abbott Laboratories, North Chicago, Ill. Iproniazid (Marsilid) was obtained from Hoffmann-LaRoche Limited, Montreal.

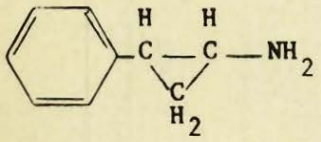
(3) Amine-depleting agents:

Reserpine (Serpasil) was obtained from Ciba Corporation, Montreal. α-Methyldopa hydrochloride (Aldomet) and L-α-Methyl-para-tyrosine were given to us by Merck Sharp and Dohme Research Lab., Rahway, N.J.

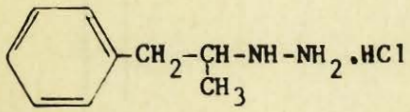


β-DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE

(SKF-525A)



TRANYLCYPROMINE



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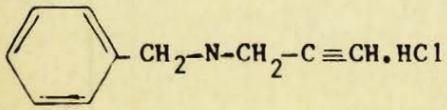
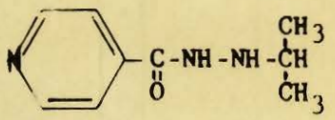
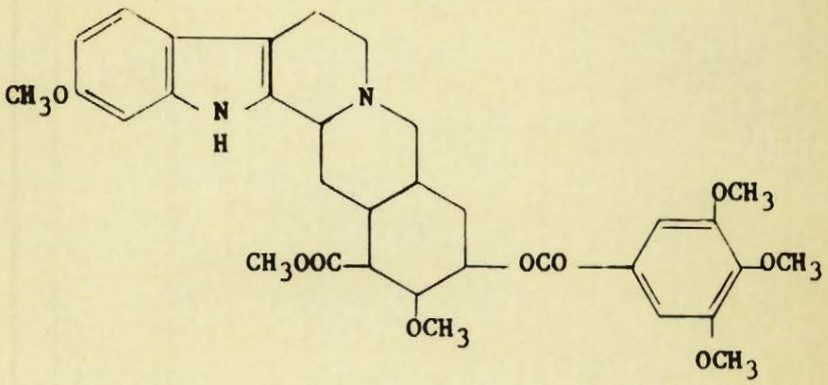


PARGYLINE

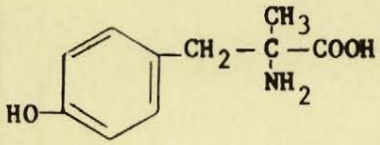


IPRONIAZID

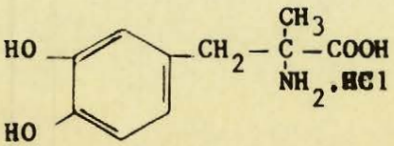
FIG. 21a



RESERPINE



L-α-METHYL-PARA-TYROSINE



L-α-METHYLDOPA HYDROCHLORIDE

FIG. 21b

4. Analysis of data:

The data were analyzed by an "analysis of variance" technique described by Winer (114) for a one-factor experiment ("drugs") with repeated measures ("time"). Where differences due to drugs were significant, a t test was applied comparing the effect of each drug with that of its own control. The number of comparisons made were equal to the number of degrees of freedom corresponding to the "Drug-effect". Any difference with a probability of less than 0.05 was considered significant.

Where the data showed a marked deviation from a normal distribution, the analysis of variance was not used. The sign "+" in the tables indicates that a fall in temperature failed for various reasons to be detected by statistical analysis, that is, failed to be statistically significant.

In all our experiments, the variations in the temperature of the control animal were very small.

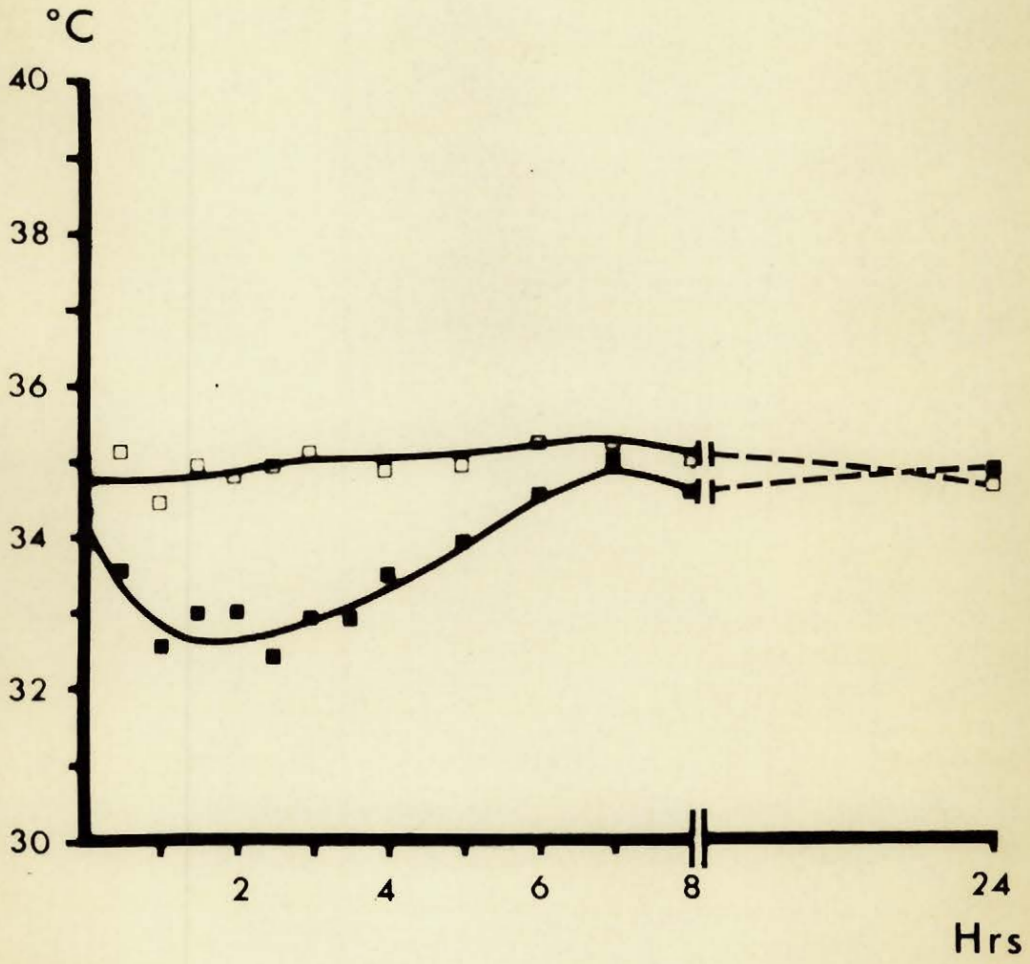
5. Experiments and results:

a) Effect of harmaline alone and in presence of a metabolic inhibitor:

(i) Harmaline alone:

At 30 mg/kg I.P. harmaline always provoked hypothermia and tremor, but the response was by no means uniform. The tremor and the fall in temperature began usually a few minutes after the injection.

In general the temperature fell about 1.6° C within one hour, remained at that level for about two hours and was back to normal approximately five hours after the injection (FIG. 22). Greater decreases in temperature than this often occurred. The tremor, exaggerated by voluntary movements or triggered again by provoked movements, lasted



□ = Control

■ = Harmaline 30 mg/kg I.P.

Each dot represents 5 animals

p = < .0005

FIG. 22 EFFECT OF HARMALINE ON BODY TEMPERATURE

about 2 - 3 hours during which it waned progressively. It practically disappeared while the rats were being held fast for temperature measurements.

When two injections of harmaline were administered 24 hours apart, that is, on two consecutive days, the fall in temperature seemed a little more pronounced on the second day, whereas the tremor appeared less intense.

The fall in temperature induced by harmaline at 30 mg/kg was always significant, most frequently at the .001 level of probability. For a group of 5 rats the significance reached the .0005 level.

At 10 mg/kg, harmaline also induced hypothermia and tremor. Both had a shorter duration and the latter was less intense.

(ii) Harmaline and SKF-525A (β-diethylaminoethyl diphenylacetate):

SKF-525A is a metabolic inhibitor reported to slow down the degradation of various drugs, that is, to prolong their action (115, 116, 117). It is said to inhibit microsomal enzymes that act on drugs.

As we wanted to see if it would exert any effect on the metabolism of harmaline, namely on its hypothermic and tremor-inducing properties, the following experiment was designed:

Experiment:

Each animal received one of the following treatments:

T_1 = SKF-525A, 30 mg/kg.

T_2 = SKF-525A, 30 mg/kg followed one hour after by an injection of harmaline, 10 mg/kg.

T_3 = harmaline, 10 mg/kg.

T_4 = harmaline, 10 mg/kg and a simultaneous injection of SKF-525A.

Results:

All drugs provoked a significant fall in body temperature of the rats (Table 22, FIG. 23):

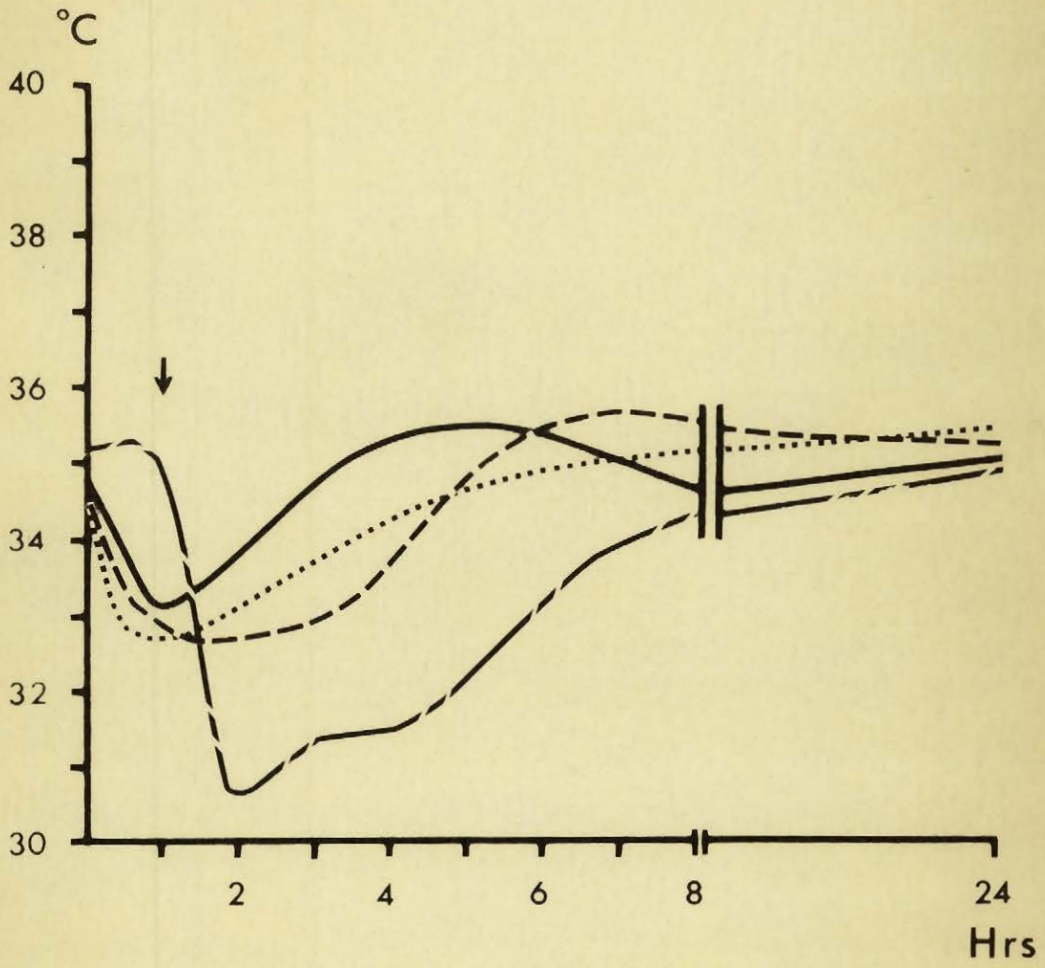
Table 22

Treatment	Maximum fall in temperature in ^{o} C*	P
T_1	- 1.7°	< .001
T_2	- 4.5°	< .001
T_3	- 1.6°	< .01
T_4	- 2.5°	< .001

*Lowest drop recorded deviating from mean initial temperature.

The drop in temperature was more pronounced and lasted longer in the rat which received SKF-525A followed by harmaline than in the animal given harmaline 10 mg/kg alone. The same applies to the tremor whose appearance was delayed. In the rat given harmaline and SKF-525A simultaneously the tremor was also of much longer duration than for the rat receiving harmaline alone but the fall in temperature had about the same duration. In this case the tremor was not delayed. No shivering occurred in the rat which received only SKF-525A despite the hypothermia sustained.

In this experiment a further statistical analysis was made of the effect of each treatment as compared to the others. The degree of fall in temperature was not significantly different for T_1 as compared to T_3 and for T_3 as compared to T_4. The drop in temperature was significantly greater, at the .01 level of probability, for T_2 as compared



SKF-525A (30 mg/kg I.P.) followed after 1 hour (↓) by harmaline (10 mg/kg I.P.)

SKF-525A (30 mg/kg I.P.) administered simultaneously with harmaline (10 mg/kg I.P.)

SKF-525A (30 mg/kg I.P.)

Harmaline (10 mg/kg I.P.)

FIG. 23 INTERACTION OF HARMALINE AND SKF-525A

to T_4 and, at the .001 level, for T_2 as compared to T_3.

From the overall results, we can deduce that SKF-525A itself possesses a hypothermic action, can slow down the metabolism of harmaline and potentiates its activity.

b) Effect of long-acting irreversible MAO inhibitors on body temperature:

Experiment:

Each of four rats was injected intraperitoneally with an irreversible MAO inhibitor as follows:

- tranylcypromine: 10 mg/kg
- pargyline: 50 mg/kg
- iproniazid: 100 mg/kg
- pheniprazine: 25 mg/kg

In another experiment this same procedure was repeated for tranylcypromine and pargyline.

In the case of three rats, one that had been injected with tranylcypromine, one that had been injected with pargyline and the one which received iproniazid, a second injection was given 24 hours after the first one.

Results:

All four drugs lowered the temperature of the injected animal. The combined results of the two experiments are presented in Table 23:

Table 23

Treatment	Dose/kg I.P.	Maximum fall in temperature in ^{o} C *	P
Tranylcypromine	10	- 1.9^{o}	< .001
		- 1.6^{o}	+
Pargyline	50	- 1.9^{o}	< .001
		- 4.3^{o}	< .001
Iproniazid	100	- 2.3^{o}	< .001
Pheniprazine	25	- 3.3^{o}	+

*Lowest drop recorded deviating from mean initial temperature.

+Fall in temperature not detected by statistical analysis.

In one experiment, tranylcypromine caused a fall in temperature of 1.9^{o} C occurring 1.5 hours after the injection. This had been preceded by a rise in temperature. The temperature remained low for about 3 hours and had returned slightly above normal level at the 8th hour where it remained thereafter (FIG. 24). In the other experiment, the temperature began to drop immediately and one hour after the injection had fallen by 1.6^{o} C. It gradually returned to a normal level 4 hours after the injection but continued to rise until the sixth hour. It was back to the initial level at the 24th hour.

In two different experiments, pargyline did not give the same degree of fall in temperature. In one case, after a brief rise, the temperature dropped by 1.9^{o} C one hour after the injection. It rose progressively and went a little above the initial temperature. A second

fall occurred but at 4 hours the temperature rose again and remained slightly above the initial temperature of the animal thereafter (FIG. 25). In the second case, the drop was more pronounced. The temperature had fallen by 4.3° C two hours after the injection. After a return to a normal level around the 5th hour, it continued to rise. Twenty-four hours after the injection it was back to the initial temperature. The falls in temperature in the animals injected with pargyline were always significant at the .001 level of probability.

Pheniprazine provoked an immediate fall in temperature which reached its lowest point 1.5 hours after the injection of the drug. The temperature remained at that level for about 2 hours, then rose progressively but remained lower than the initial level thereafter (FIG. 26).

Iproniazid caused a drop of 2.3° C in 1.5 hours. The temperature returned near normal 2.5 hours after the beginning of the experiment and remained at that level thereafter, except for a brief fall of 1.5° C at 3.5 hours.

When a second injection of iproniazid, at the same dosage, was given 24 hours after the first one to the iproniazid-treated animal which had a fall of 2.3° C the first day, a drop in temperature of 1.5° C was observed; the temperature curve had a shape similar to that of the first day (FIG. 27). A fall of 1.3° C also occurred in the pargyline-treated animal, which had a drop of 4.3° C the first day, when a second injection of pargyline, at the same dosage, was repeated 24 hours after the first one. However, the tranylcypromine-treated rat that had a fall of 1.6° C the first day did not show any variation in temperature when given a second injection of tranylcypromine. The falls in temperature which occurred after the second injection of either iproniazid or pargyline failed to be detected by statistical analysis.

FIG. 24: Effect of an injection of tranylcypromine (10 mg/kg I.P.)

FIG. 25: Effect of an injection of pargyline (50 mg/kg I.P.)

FIG. 26: Effect of an injection of pheniprazine (25 mg/kg I.P.)

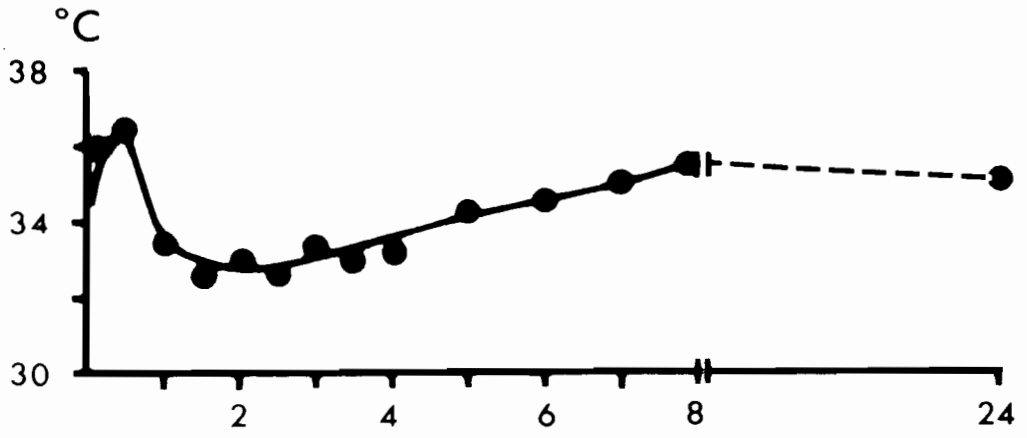


Fig. 24

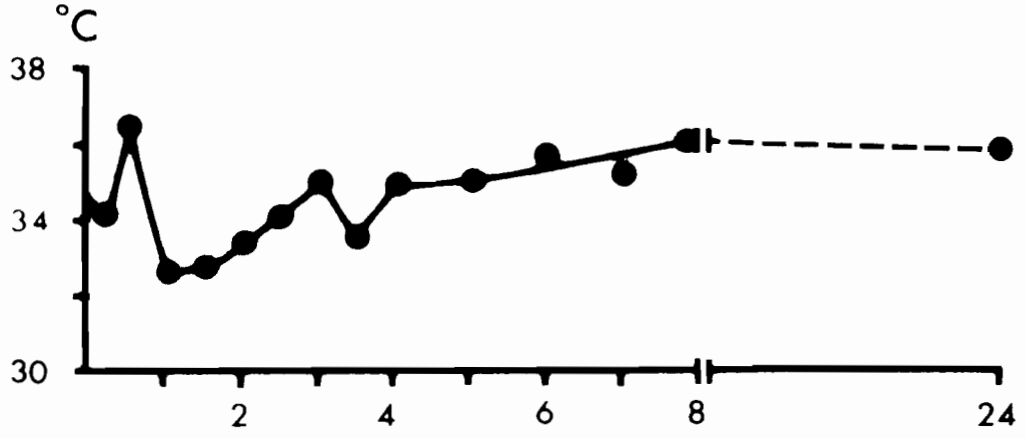


Fig. 25

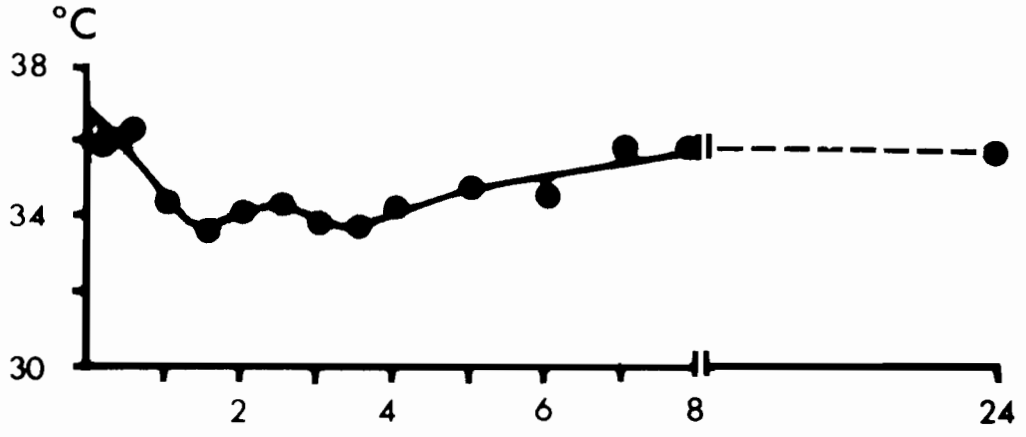
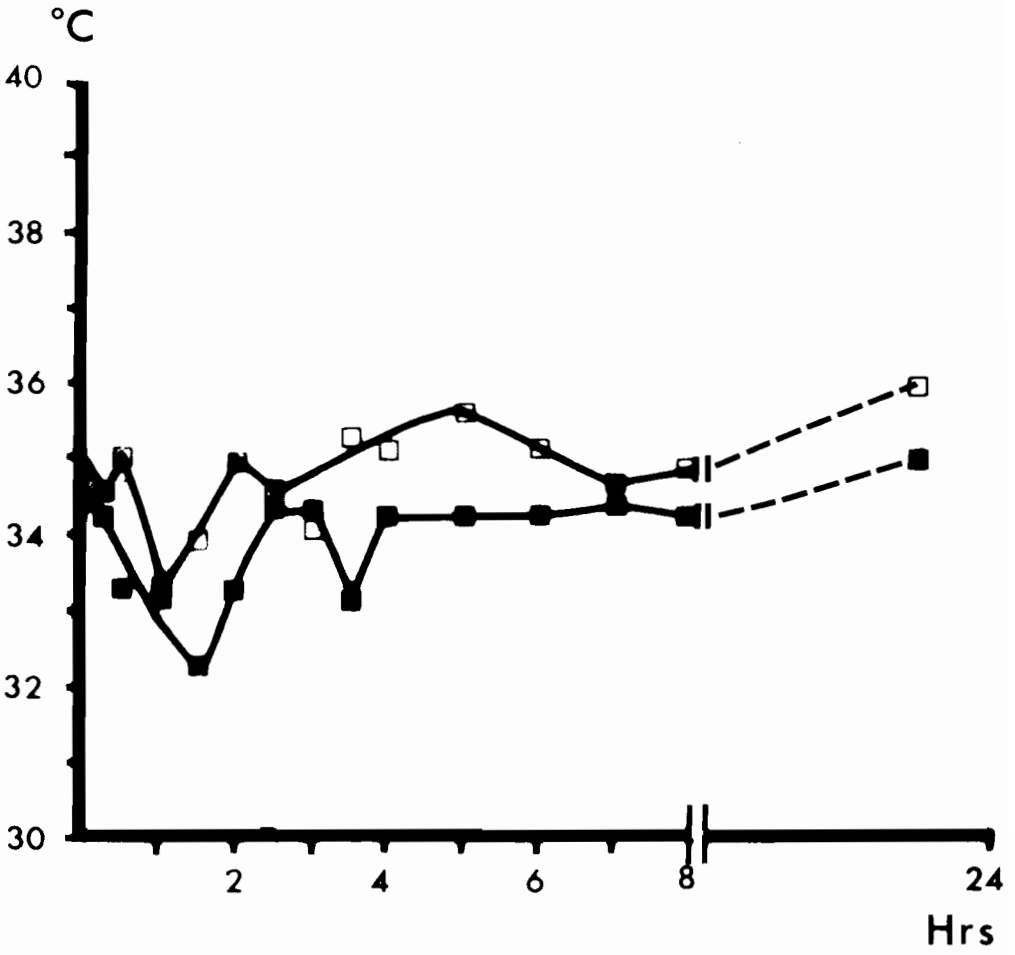


Fig. 26



■ First day

□ Second day

FIG. 27 EFFECT OF IPRONIAZID (100 mg/kg I.P.) ADMINISTERED ON TWO CONSECUTIVE DAYS

c) Interaction between harmaline and long-acting irreversible MAO inhibitors:

Three types of experiments were performed:

(i) a long-acting irreversible MAO inhibitor was administered to a rat; 24 hours later harmaline was given

(ii) harmaline was given to a rat, and one hour after a long-acting irreversible MAO inhibitor was injected

(iii) harmaline and a long-acting irreversible MAO inhibitor were injected at the same time.

(i) Irreversible MAO inhibitor followed by harmaline after 24 hours:

Experiment:

Harmaline 30 mg/kg was injected into rats which had received, 24 hours previously, by intraperitoneal route either tranylcypromine 10 mg/kg, pheniprazine 25 mg/kg or pargyline 50 mg/kg.

Results:

The results obtained are listed in table 24:

Table 24

Harmaline injected into a rat pretreated with:	Maximum fall in temperature in ^{o} C*	P
tranylcypromine	- 4.4^{o}	< .001
pheniprazine	- 2.2^{o}	+
pargyline	- 1.1^{o}	NS

*Lowest drop recorded deviating from mean initial temperature.

+Fall in temperature not detected by statistical analysis.

NS not significant.

The rat which had been pretreated with tranylcypromine showed an intense hypothermia statistically significant at the .001 level. The hypothermia began within half an hour of the injection of harmaline, reached its lowest point at the second hour and slowly returned to normal about 7.5 hours after the injection (FIG. 28).

The animal pretreated with pheniprazine had a marked hyperthermic reaction reaching its peak one hour after the injection of harmaline. This was followed by a sharp decrease in temperature that reached its lowest point 2.5 hours after the animal had received harmaline, and by a gradual return to normal around the eight hour (FIG. 29).

The rat pretreated with pargyline showed a brief initial decline in temperature but then also had a very marked hyperthermic reaction one hour after the harmaline injection. This reaction subsided rapidly and the temperature returned to normal about 2.5 hours after the beginning of the experiment. It remained close to the initial temperature thereafter (FIG. 30).

The appearance of the tremor normally induced by harmaline was not prevented by pretreatment with long-acting irreversible MAO inhibitors. Both the rat pretreated with pheniprazine and the one pretreated with pargyline were more excited during the first hour. During this experiment, they exhibited a tremor which was more intense than the rat receiving only harmaline. The animal pretreated with tranylcypromine showed a tremor similar to that of the rat injected with harmaline alone but of a longer duration.

(ii) ,Harmaline followed by an irreversible MAO inhibitor after one hour:

Experiment:

This type of experiment was performed twice. The procedure used

FIG. 28: Effect of harmaline (30 mg/kg I.P.) in a rat pretreated 24 hours before with tranylcypromine (10 mg/kg I.P.)

FIG. 29: Effect of harmaline (30 mg/kg I.P.) in a rat pretreated 24 hours before with pheniprazine (25 mg/kg I.P.)

FIG. 30: Effect of harmaline (30 mg/kg I.P.) in a rat pretreated 24 hours before with pargyline (50 mg/kg I.P.)

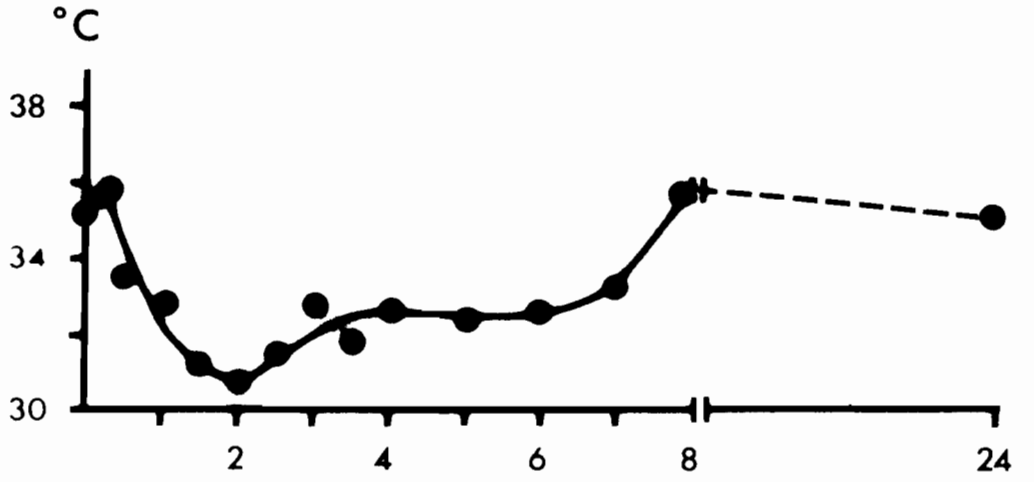


Fig. 28

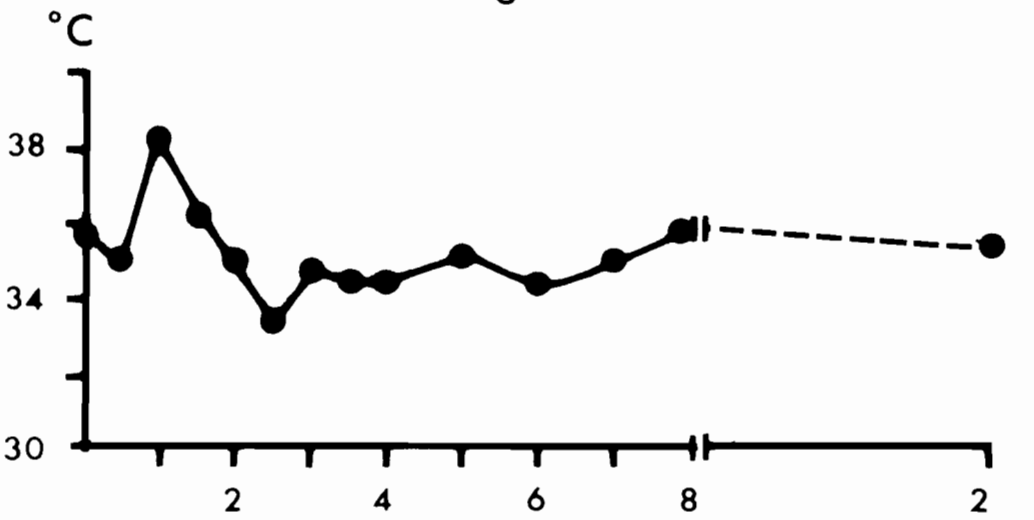


Fig. 29

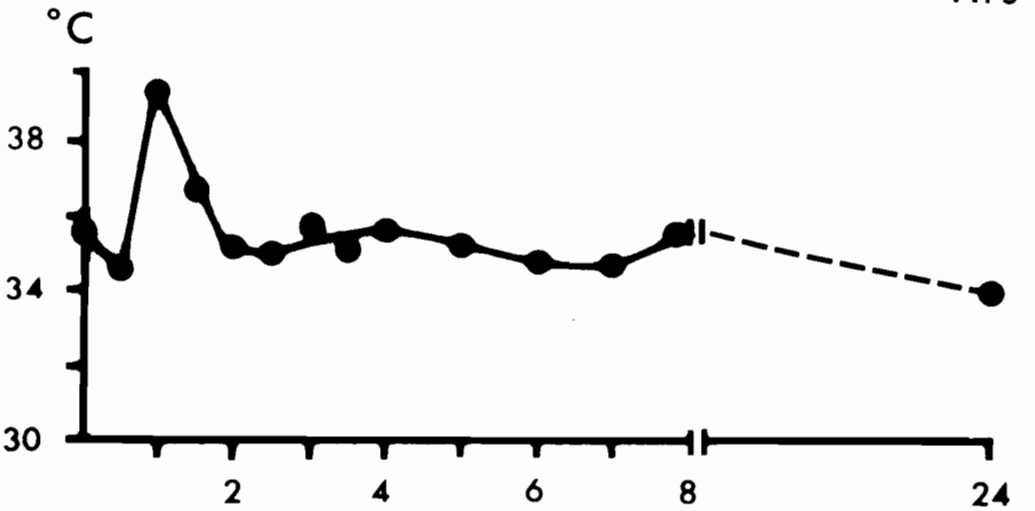


Fig. 30

and the overall results obtained on these two different occasions will be described here. In one experiment the temperature measurements were discontinued 8 hours after its beginning.

A rat received an injection of harmaline 30 mg/kg I.P. and one hour after was administered by intraperitoneal route a long-acting irreversible MAO inhibitor, either pheniprazine 25 mg/kg, tranylcypromine 10 mg/kg, pargyline 50 mg/kg or iproniazid 100 mg/kg.

Results:

Pheniprazine, tranylcypromine and pargyline reversed fairly rapidly the hypothermia induced by harmaline (FIG. 31, 32, 33). Within 60 to 90 minutes, they brought the body temperature back to the initial level or to a slightly higher level. A fairly sharp drop in temperature subsequently ensued reaching its lowest point between the fourth and the sixth hour after the onset of the experiment in most animals. This secondary hypothermic reaction had a duration varying from 2.5 to 4 hours. The body temperature returned to normal by the seventh or eighth hour of the experiment. The general aspect of the curve was biphasic, of a sinusoidal type. The same experiment repeated with tranylcypromine reproduced again the same effect in another rat: pargyline also given to another rat stopped again the fall in temperature induced by harmaline.

Iproniazid however behaved differently on two occasions. In one case it shortened the duration of harmaline hypothermia but it provoked two hours after its administration a marked hyperthermia which lasted about 3 hours and fell sharply within one hour (FIG. 34). In the other case it did not appear to modify the habitual course of harmaline when injected alone (FIG. 35).

The administration of an irreversible MAO inhibitor one hour

FIG. 31: Effect of harmaline (30 mg/kg I.P.) followed after one hour by an injection (↓) of pheniprazine (25 mg/kg I.P.)

FIG. 32: Effect of harmaline (30 mg/kg I.P.) followed after one hour by an injection (↓) of tranylcypromine (10 mg/kg I.P.)

FIG. 33: Effect of harmaline (30 mg/kg I.P.) followed after one hour by an injection (↓) of pargyline (50 mg/kg I.P.)

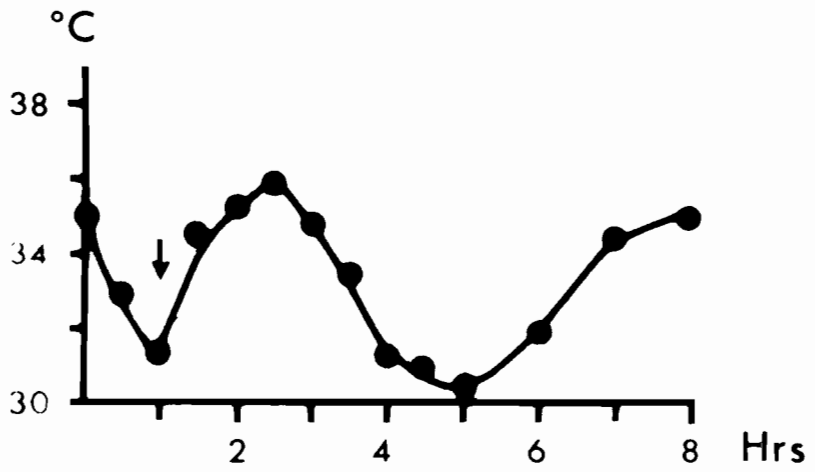


Fig. 31

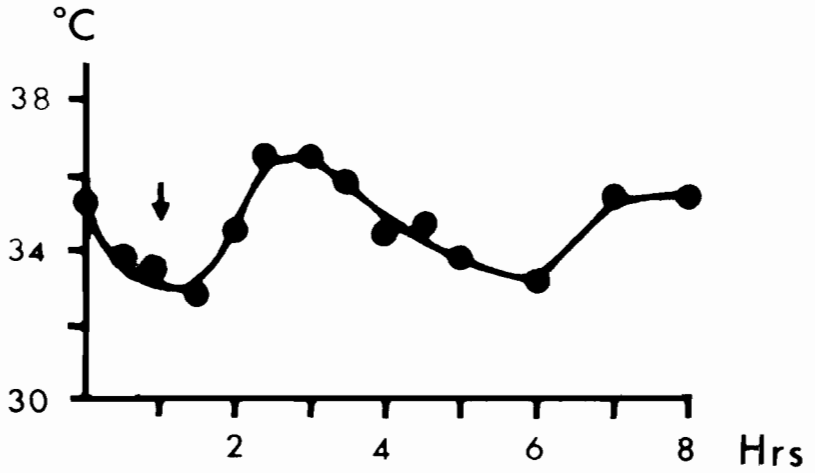


Fig. 32

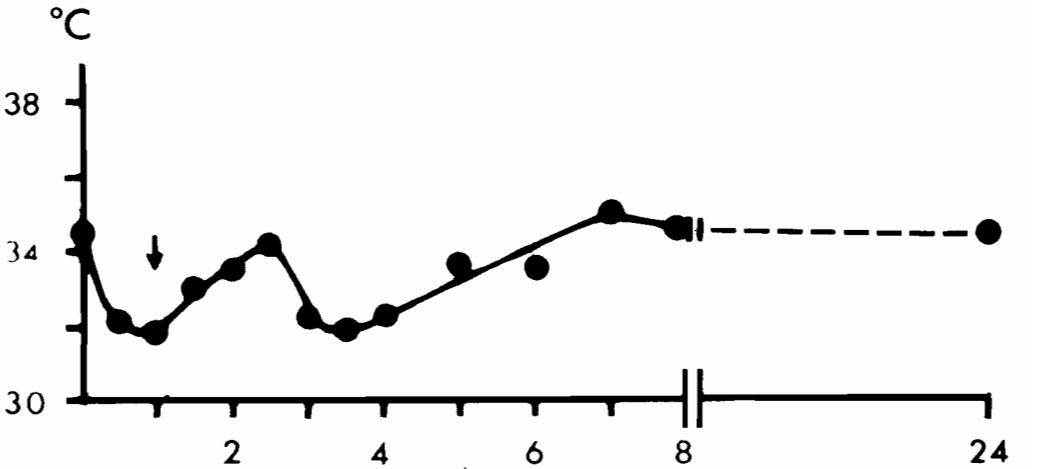


Fig. 33

FIG. 34: Effect of harmaline (30 mg/kg I.P.) followed after one hour by an injection (↓) of iproniazid (100 mg/kg I.P.)

FIG. 35: Effect of harmaline (30 mg/kg I.P.) followed after one hour by an injection (↓) of iproniazid (100 mg/kg I.P.)

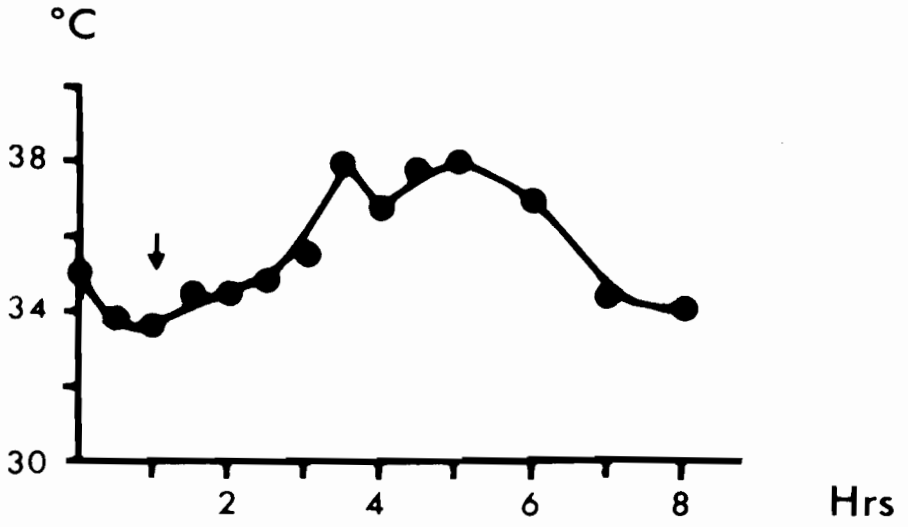


Fig. 34

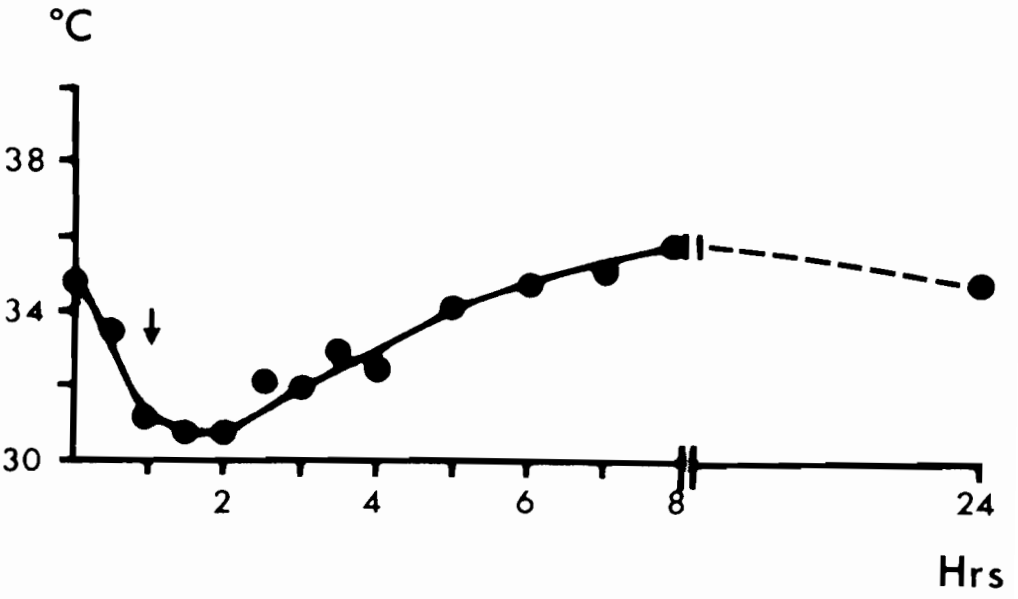


Fig. 35

after the injection of harmaline did not prevent the tremor from continuing its course but did modify its type sometimes. In the two animals to which iproniazid was given it prolonged markedly the duration of the tremor. Pheniprazine and iproniazid had an excitatory action upon the behavior of the animals whereas the rats which received pargyline or parnate did not appear more active than the animals that were given harmaline alone.

No statistical analysis of this experiment was carried out, most data showing a marked deviation from a normal distribution.

(iii) Harmaline and an irreversible MAO inhibitor given simultaneously:

Experiment:

Harmaline 30 mg/kg was injected into the abdominal cavity of 4 rats on one side and one of four irreversible long-acting MAO inhibitors on the other side, at the following dosage:

- tranylcypromine: 10 mg/kg
- iproniazid: 100 mg/kg
- pheniprazine: 25 mg/kg
- pargyline: 50 mg/kg

Results:

In all animals a hypothermic reaction was observed except for the rat that received the combination of drugs, harmaline-tranylcypromine. This animal died (Table 25).

Table 25

Harmaline plus:	Dose mg/kg I.P.	Maximum fall in temperature in ^{o} C*
iproniazid	100	- 2.4 ^{o}
pheniprazine	25	- 2.3 ^{o}
pargyline	50	- 6.0 ^{o}

*Lowest drop recorded deviating from mean initial temperature.

The animal injected with the combination harmaline-tranylcypromine had convulsions associated with marked hyperthermia and died within one hour. With the combination iproniazid-harmaline or pheniprazine-harmaline a triphasic response was obtained (FIG. 36, 37). A drop in temperature occurred at first, but delayed in the case of pheniprazine. It was followed by a return to normal temperature or close to normal and followed again by a second fall, less intense in the case of pheniprazine. At about the seventh hour after the onset of the experiment the body temperature had returned to a normal level for both combinations of drugs. However a third fall in temperature began to take place for the combination pheniprazine-harmaline. With the combination pargyline-harmaline the temperature dropped progressively reaching its lowest point three hours after the injections and returned close to normal seven hours after the drugs had been administered (FIG. 38). The hypothermic effect obtained with the combination pargyline-harmaline was more pronounced than for the others.

If we compare the interaction of harmaline followed by a long-acting MAO inhibitor with the interaction of the same drugs administered simultaneously, we notice that there is no relationship between the shape of the temperature curves and the type of drugs. An injection of harmaline followed one hour after by iproniazid did not give a triphasic response while in this case it did. On the other hand, harmaline followed one hour after by pargyline gave a biphasic response while in this case it did not.

The tremor was not prevented by the combinations iproniazid-harmaline and pheniprazine-harmaline but it was minimal and sporadic with the combination pargyline-harmaline.

FIG. 36: Effect of simultaneous administration of harmaline (30 mg/kg I.P.) and iproniazid (100 mg/kg I.P.)

FIG. 37: Effect of simultaneous administration of harmaline (30 mg/kg I.P.) and pheniprazine (25 mg/kg I.P.)

FIG. 38: Effect of simultaneous administration of harmaline (30 mg/kg I.P.) and pargyline (50 mg/kg I.P.)

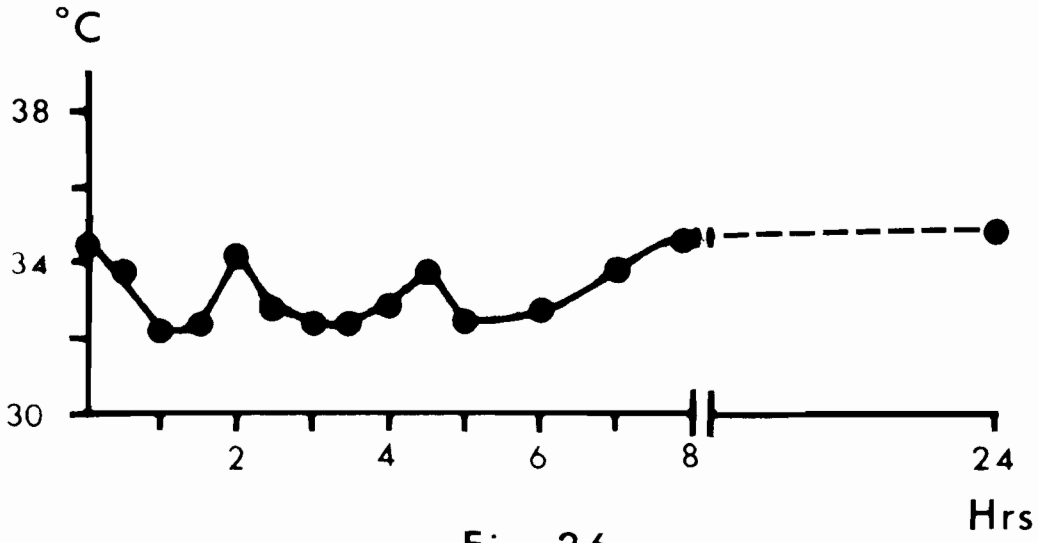


Fig. 36

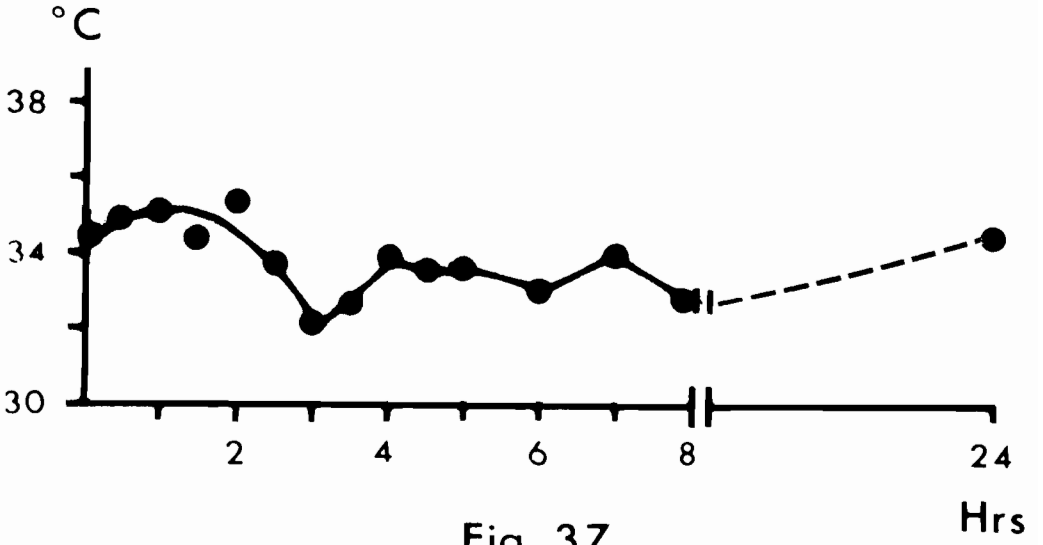


Fig. 37

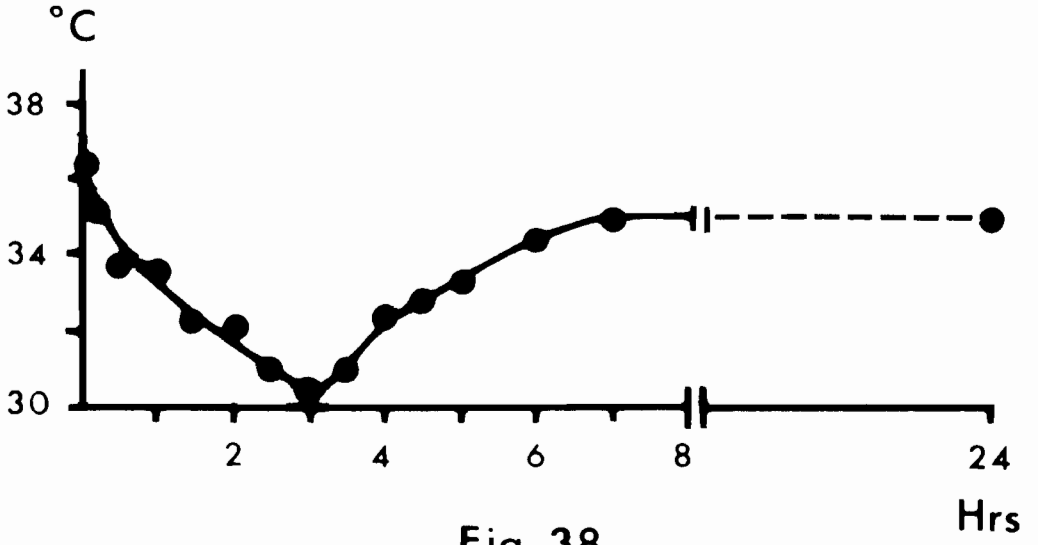


Fig. 38

No statistical analysis of this experiment was done, most data showing a marked deviation from a normal distribution.

d) Interaction between harmaline and amine-depleting agents:

Reserpine, a rauwolfia alkaloid, possesses amongst other pharmacological properties the ability to cause tranquilization and hypothermia (118). It releases serotonin from the central nervous system (119), including the hypothalamus, and also releases catecholamines from central and peripheral sites (120). L-α-Metnyldopa, a synthetic analogue of DOPA (3,4-dihydroxyphenylalanine) and a decarboxylase inhibitor, causes in the rat brain at a dose of 100 mg/kg I.P. a very brief decrease of dopamine and serotonin: the induced decrease in noradrenaline lasts several days (121). MK-781 (L-α-methyl-para-tyrosine), and agent which has no action on brain serotonin, inhibits the conversion of tyrosine to DOPA. It reduces the level of noradrenaline and dopamine in the whole brain of the rat: the maximal reduction in the level of these amines is reached 16 hours after the administration of the drug and the recovery occurs at about 32 hours (122).

Experiment:

A rat was injected by intraperitoneal route either with reserpine 2.5 mg/kg, reserpine 5.0 mg/kg, α-methyldopa 200 mg/kg or L-α-methyl-para-tyrosine. L-α-Methyl-para-tyrosine was given twice in order to obtain with certainty a reduction in the brain level of dopamine and noradrenaline: 150 mg/kg at the onset of the experiment and 100 mg/kg eight hours after. After 24 hours all four rats received harmaline 30 mg/kg I.P.

At 24 hours, the brain amine level was in theory as follows:

Drug	Serotonin	Noradrenaline	Dopamine
Reserpine	↘	↘	↘
α-Methyldopa	Normal	↘	Normal
L-α-methyl-para-tyrosine	Normal	↘	↘

Results:

During the first day the reserpine-treated rats showed the usual signs of reserpinisation; eyelid ptosis, diarrhea and tranquilization. The last feature was more pronounced in the animal given 5 mg/kg of reserpine. No behavioral change was observed in the other animals.

The first day the rats that received either reserpine 2.5 mg/kg, α-methyldopa or L-α-methyl-para-tyrosine showed a transitory fall in temperature of 1 to 2° C with subsequent return towards normal level. The body temperature of the animal injected with 5 mg/kg of reserpine, after a slight elevation at the first hour, decreased during the next two hours to 29.8° C: this was a drop of 4.8° C from the mean initial temperature. It remained around this level until the injection of harmaline at the 24th hour of the experiment. Despite this hypothermia no shivering appeared.

After the injection of harmaline at 24 hours, that is, on the second day, the following changes were observed (Table 26). Harmaline completely reversed in 3 hours the reserpine-induced hypothermia in the rat that had received 5 mg/kg of reserpine. Within one hour the temperature increased from 29.5° C to 33° C and remained at that level for approximately one and a half hours. It increased again within one and a half hours to 36.2° C and finally returned towards a normal level 6 hours after

the injection of harmaline. A slight and progressive elevation of the temperature occurred thereafter; it was still lasting when the last measurement was taken (FIG. 39).

The animal pretreated with reserpine, 2.5 mg/kg, showed the usual response to harmaline as did the rat pretreated with α-methyldopa (FIG. 40).

The animal pretreated with L-α-methyl-para-tyrosine showed an abrupt drop in body temperature following the injection of harmaline. It fell to 29° C which represented a deviation of 5.2° C from the mean initial temperature. It remained there for two hours and progressively returned to the normal temperature of the animal during the next four hours (FIG. 41).

All these changes in temperature were statistically significant at the .001 level of probability (Table 26).

Table 26

Harmaline injected into a rat pretreated with:	Change in temperature in ° C*	P
Reserpine 2.5 mg/kg	- 3.5°	< .001
Reserpine 5.0 mg/kg	+ 7.1°	< .001
α-Methyldopa	- 3.0°	< .001
L-α-methyl-para-tyrosine	- 5.2°	< .001

*Lowest drop or elevation recorded deviating from mean initial temperature.

The harmaline-induced tremor was present in all animals, very intense for the rat pretreated with reserpine 5 mg/kg and quite slight for the one pretreated with L-α-methyl-para-tyrosine. For the other animals it did not differ from the rat which received harmaline alone.

FIG. 39: Effect of harmaline (30 mg/kg I.P.) in a rat pretreated 24 hours before with reserpine (5 mg/kg I.P.)

FIG. 40: Effect of harmaline (30 mg/kg I.P.) in a rat pretreated 24 hours with L-α-methyl-dopa (200 mg/kg I.P.)

FIG. 41: Effect of harmaline (30 mg/kg I.P.) in a rat pretreated 24 hours before with L-α-methyl-para-tyrosine (250 mg/kg I.P.)

↓ = injection of harmaline.

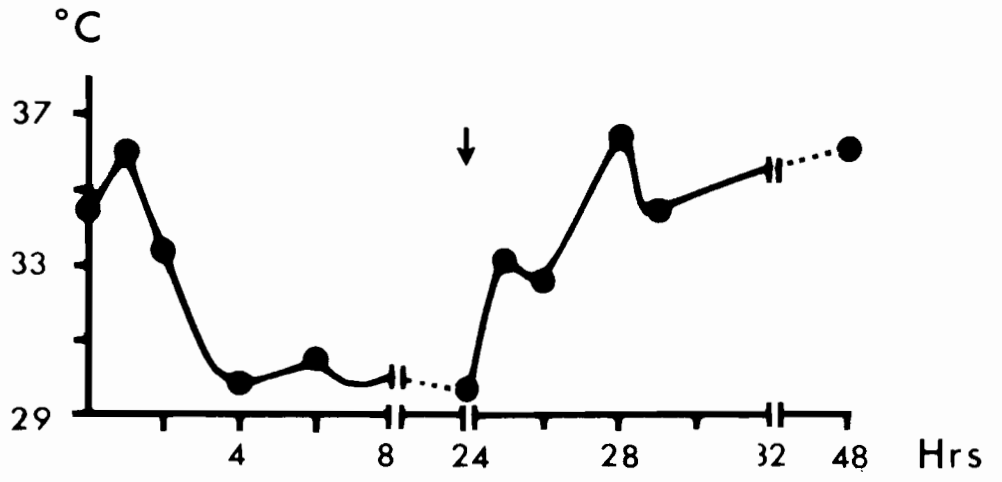


Fig. 39

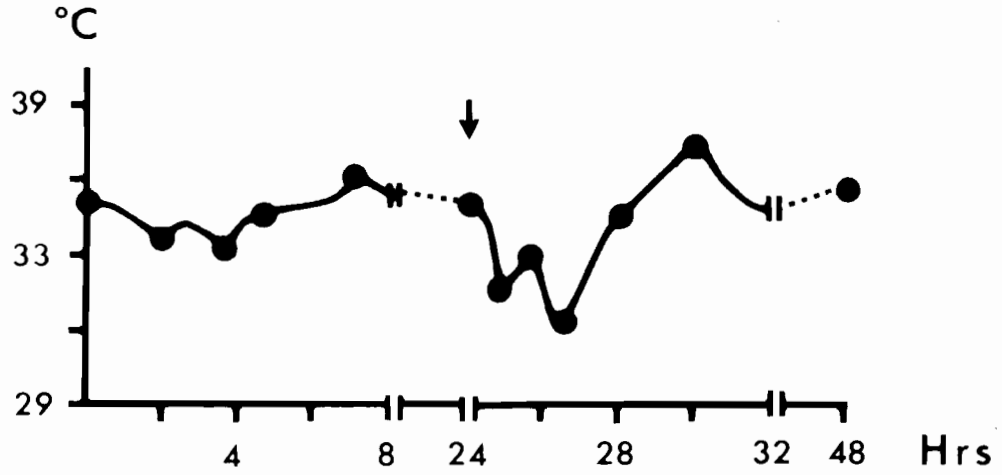


Fig. 40

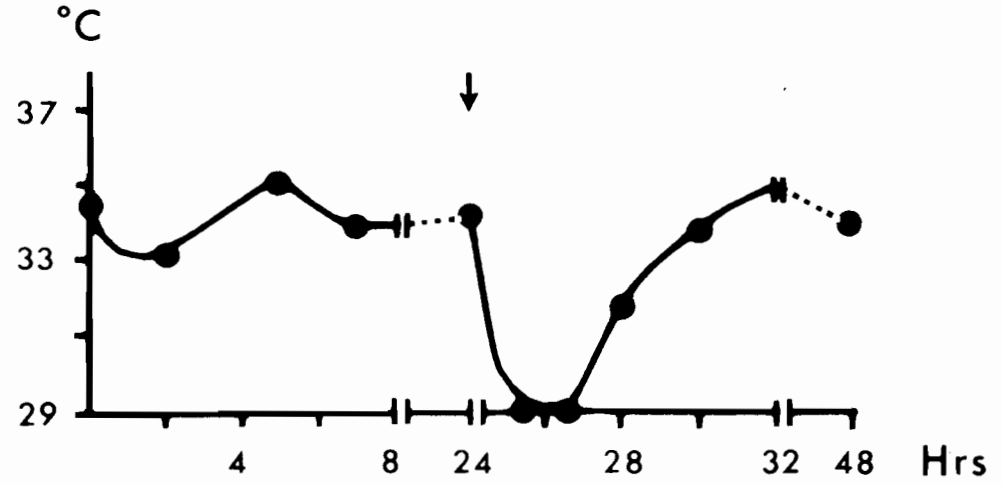


Fig. 41

6. Discussion:

Thermoregulation is a very complex phenomenon. Many pharmacological agents of different nature affect the body temperature and all cannot be expected to act by the same mechanism. Any complete explanation of their effect would necessitate taking every part of the intricate mechanism of thermoregulation into account (FIG. 42); it would also require a detailed knowledge of all the biochemical properties of these drugs. For example, the detailed mechanism of action of MAO inhibitors has not yet been established (123) and the fact that MAO inhibitors possess many pharmacological properties unrelated to MAO inhibition is acknowledged (94).

Before we discuss our results on the interaction of harmaline with other compounds, we must comment that most of our experiments have been made on single animals and that the intensity of the changes in temperature of the treated animals is, to a certain extent, subject to biological variation. The tremor, if it could not be quantified exactly in time of duration or in intensity, was a readily observed phenomenon. From our experiments no generalizations can be made at this point but only speculations.

In the discussion of the results, hypothermia and harmaline-induced tremor both need to be taken into account. Indeed, both overlap in time of appearance and duration; in addition this tremor is, we believe, thermogenic.

Our experiments with irreversible long-acting MAO inhibitors seem to indicate that harmaline could produce hypothermia and tremor independently of MAO inhibition.

The first and main reason for stating this is that pretreatment

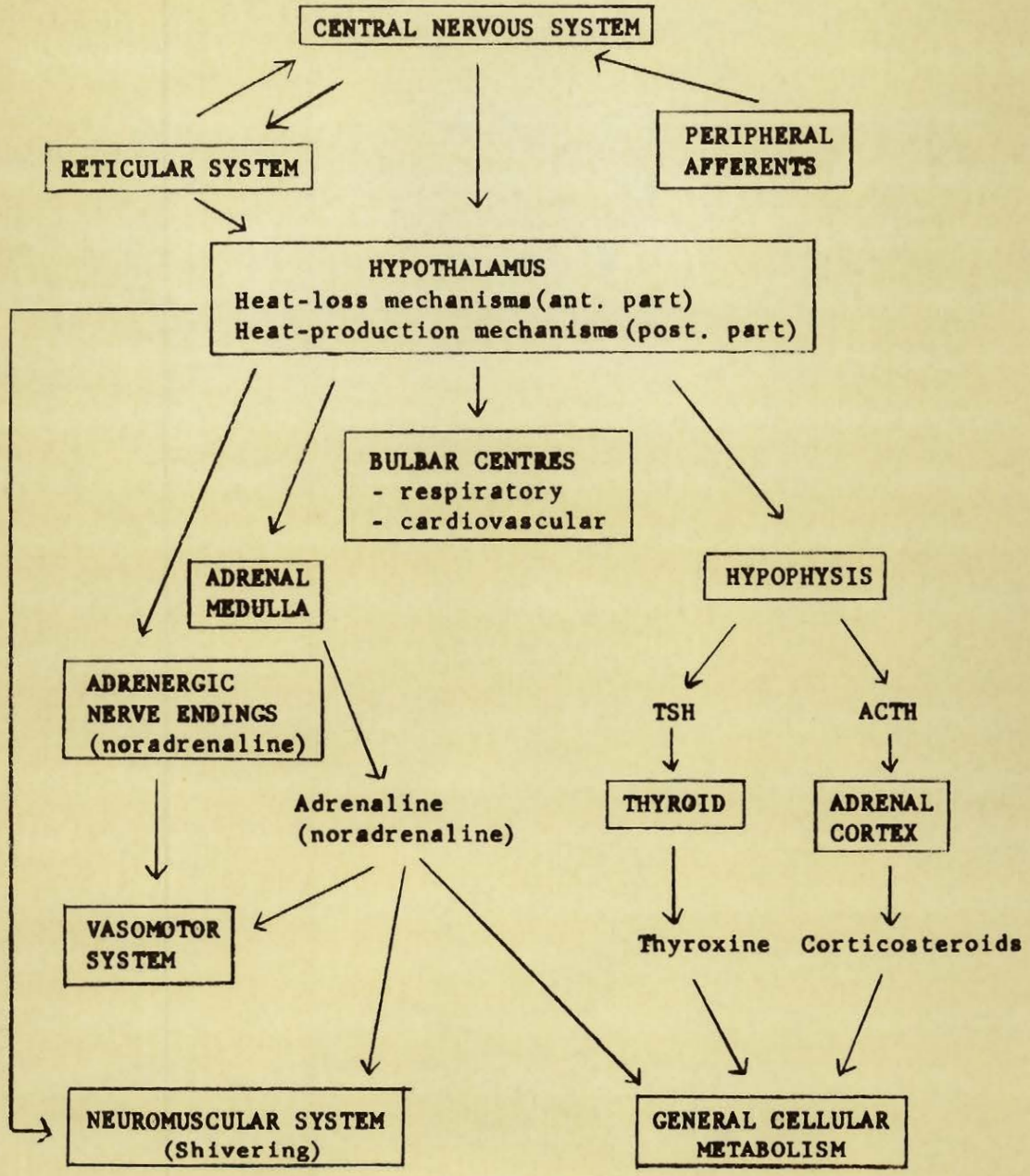


FIG. 42 GENERAL DIAGRAM OF THERMOREGULATION

Adapted from H. Laborit (110)

with a long-acting MAO inhibitor never prevented harmaline from inducing tremor and, usually, some degree of hypothermia. The long-acting MAO inhibitor, because it already occupied the active site on MAO and could not be displaced, should have prevented both effects of harmaline from appearing if they were due to MAO inhibition. A statistically significant fall in temperature was seen in the tranylcypromine-pretreated rat. Although a negligible fall in temperature occurred in the pargyline-pretreated animal, a more pronounced one may have been forestalled by the heat produced by the very intense tremor. Indeed the harmaline tremor was enhanced in the animals pretreated either with pheniprazine or pargyline and in these two cases a less considerable hypothermia occurred.

The second reason is based on the observation that, when two doses of the same irreversible long-acting MAO inhibitor were administered 24 hours apart, in the cases of iproniazid and pargyline, the second dose could again lower slightly the body temperature in spite of the fact that the first dose had already inhibited MAO. It is felt that this drop of temperature brought about by the second injection of these drugs, although not statistically significant, has nevertheless some importance and that this drop was unrelated to MAO inhibition. Harmaline, like these inhibitors, induced some degree of hypothermia after prior inhibition of MAO and in addition it provoked tremor.

Likewise, Schmidt and Fühse have suggested that the effect of MAO inhibitors on the body temperature was unrelated to MAO inhibition. Indeed they noted that the effect of different MAO inhibitors on the body temperature varied qualitatively and quantitatively. They found that harmaline, pargyline and pheniprazine caused hypothermia. On the other hand, they observed that nialamid had no effect on the body temperature

while phenelzine increased it. On that basis, they concluded that the effect of different MAO inhibitors on the temperature of the rat was not related to the inhibition of MAO or to the basic chemical structure of the inhibitors (52). Like them, we found that harmaline, pargyline and pheniprazine induced hypothermia; moreover we noted that iproniazid also had the same property.

The interpretation of the data on the interaction of harmaline with irreversible MAO inhibitors administered simultaneously or one hour after is a difficult one.

No explanation can be offered at this time as to why harmaline hypothermia is temporarily reversed when tranylcypromine, pheniprazine or pargyline were administered one hour after harmaline. A mechanism other than MAO inhibition has to be involved if the active site on the enzyme is already occupied by harmaline. A hyperthermic toxic effect should not be set aside in this case; the irreversible MAO inhibitors unable to attach to MAO could have acted as toxic agents on some other part of the temperature-regulating mechanism. This does not explain however the erratic action of iproniazid.

A similar mechanism can hardly be invoked to explain the interaction of harmaline and long-acting MAO inhibitors administered simultaneously. In this type of experiment alternation of rises and falls in temperature are observed when pheniprazine or iproniazid is given in conjunction with harmaline, and many factors may be operating here: variations in the intensity of the tremor and heat produced by the latter, changes in activity of the animal and differences in the rate of absorption and transport of the two mutually competitive inhibitors. In the case of pargyline given concomitantly with harmaline, the minimal tremor

could partly explain why an even fall of temperature occurred instead of an irregular one.

Our provisional explanation of the differential effect of harmaline in its interaction with amine-depleting agents will be based on the hypothesis of a critical level and balance of amines in the brain. If the brain is profoundly depleted of serotonin, noradrenaline and dopamine, harmaline could then act as an MAO inhibitor and reverse quickly, with the help of the intense tremor, the prolonged hypothermia brought about by a dose of 5 mg/kg of reserpine. It would restore rapidly the critical level of the amines necessary for thermal maintenance. If however only an insufficient or selective depletion of these biogenic amines in the brain is accomplished by a low dose of reserpine, by α-methyldopa or α-methyl-para-tyrosine, harmaline would induce hypothermia by a mechanism independent of MAO inhibition, the latter mechanism having relatively minor significance under those conditions.

The MAO inhibitors, W3207A and phenelzine, have also been reported to reverse completely reserpine-induced hypothermia in mice but the reversal took place mostly between 6 to 24 hours following the injection of the drugs and it was attributed only partially to their MAO inhibitory property (124). However, the mouse may not react in the same way as the rat and in addition the drugs may take longer to inhibit MAO in that species.

The tremor can be dissociated from the effect of harmaline on body temperature and seems independent of MAO inhibition, and of the level of brain amines. When pargyline and harmaline were administered simultaneously, a marked drop in temperature occurred while the tremor was minimal. Other investigators have also tended to separate motor and

thermic effects in their studies with the related alkaloid harmine (41, 43). For example, Zetler reported tremor and increase in temperature in the mouse but a fall in temperature in the rat. With regard to brain amines, not only did harmaline produce tremor while the reversal of the reserpine hypothermia took place, but the tremor was also present when harmaline induced hypothermia in the animals insufficiently or selectively depleted of some brain amines.

III. GENERAL DISCUSSION

1. Harmaline metabolism:

Because of the close similarity in chemical structure and effects of harmaline and harmine, the metabolism of harmaline has been compared to that of harmine. The quantitative determination of harmaline and harmine showed that both drugs reached their maximum concentration in the organs analyzed about 30 minutes following their intraperitoneal injection. The tissue concentration of harmine had fallen to a very low level one hour after administration of the drug while that of harmaline declined progressively over a period of several hours.

The quantitative determination of harmaline has demonstrated that the rapid but short-lasting activity of harmaline as an MAO inhibitor and the short duration of the elevation in brain amine levels, for example serotonin (95), was proportional to the concentration of harmaline in this tissue. Such a correlation also exists between tissue concentration of the related alkaloid harmine and the increased dopamine in the rat brain (101).

The thin-layer chromatography of some organs gave results that agreed with the above studies of tissue concentration as a function of time after injection. It showed in addition the more extensive and slower metabolism undergone by harmaline as compared to harmine. This technique also demonstrated that no residue of harmaline or harmine is left in the tissues after 24 hours. For the former, this had been shown indirectly by other investigators in measuring MAO activity in rat liver (95) and mouse brain (98); no residual MAO inhibition had been found 24 hours after the injection of harmaline.

While none of the metabolites of harmaline has yet been con-

clusively identified, there is reasonable evidence on the basis of color of fluorescence and Rf to suggest that harmalol is one of them. It implies thereby a process of demethylation occurring probably in the liver. Demethylation at position 8, transforming harmaline to a hydroxyharman, would indeed abolish its excitatory action on the central nervous system and its potent MAO inhibitory activity. Such a process of inactivation would also apply to harmine, (37, 51).

The presence in the brain, in important amount, of only harmaline would lead to think first that it can pass easily the blood-brain barrier, secondly that it acts directly on the central nervous system, and thirdly that its action is not mediated through a metabolite. This would also be true of harmine.

The reported inefficacy of methoxyharmans taken by oral route could be due not only to poor absorption by the gut (101) but also to the rapid inactivation of the amount absorbed.

2. Harmaline: effect on the body temperature and on the motor system.

Our investigation of the effect of harmaline on the body temperature confirmed its hypothermic action and tremor-inducing property. In addition it was observed that SKF-525A could potentiate and prolong its action. It was also noted that SKF-525A like many other compounds tested could itself lower the body temperature.

Hypothermia could be induced by harmaline despite pretreatment with an irreversible long-acting MAO inhibitor. This would indicate that harmaline works, probably at the hypothalamic level, by a mechanism other than MAO inhibition. Such a mechanism unrelated to MAO inhibition had been postulated by Schmidt and Fühse to explain the different effect of harmaline and various other MAO inhibitors on the body temperature (52).

Hypothermia was also provoked by harmaline in rats whose brains were depleted selectively of noradrenaline by intraperitoneal injection of α-methyldopa or of dopamine and noradrenaline by administration of L-α-methyl-para-tyrosine.

Under all conditions tested, namely pretreatment with long-acting irreversible MAO inhibitors and amine-depleting agents, harmaline induced tremor. Because of this we feel, like previous investigators (42, 45, 46), that harmaline acts directly on the extrapyramidal system to produce tremor. This direct action on the extrapyramidal system would explain why Sourkes and Poirier obtained a greatly exaggerated tremor in their brain-lesioned monkeys and transformation of choreiform and ballistic movements to a coarse tremor after injection of harmaline or harmine (49).

Tremor and effect on body temperature can be dissociated as proved for example by the concomitant presence of tremor during the reversal of reserpine hypothermia by harmaline. This last phenomenon illustrates the existence of two different mechanisms of action that can operate simultaneously. One, believed to depend on MAO inhibition, would bring about the reversal of the hypothermia in a rat markedly depleted of brain amines by a large dose of reserpine. The other, independent of MAO inhibition and the more important, would produce in this case the tremor which contribute by heat-production to raise the temperature.

Blum and his co-workers have noted the efficacy of harmine in relieving reserpine-induced parkinsonism in man but not that induced by chlorpromazine (69). The ability of harmaline not only to prevent reserpine hypothermia (81) but also to reverse it may have some relation to the above clinical observation.

IV. SUMMARY

The results of our studies of harmaline can be summarized as follows:

(1) Twenty-four hours after administration of harmaline no trace of this compound or closely related compounds can be found in tissues.

(2) The metabolism of harmaline is slower and more extensive than that of harmine.

(3) No metabolite of harmaline has yet been conclusively identified.

(4) There is some evidence for the occurrence of harmalol, formed from harmaline, and for some conversion of harmaline to the harmine series of compounds.

(5) Harmaline appears to induce tremor and hypothermia by a mechanism other than MAO inhibition. This mechanism probably involves a direct action on the extrapyramidal system and the hypothalamus. The relationship between harmaline hypothermia and a specific amine or brain amine levels is not clear.

(6) Harmaline tremor and hypothermia are two effects that can be dissociated and the former seems independent both of MAO inhibition and brain amine levels.

V. CONCLUSION

We are left with the paradox of a class of drugs capable of producing extrapyramidal signs but used in the past in the treatment of parkinsonian symptoms, of a compound inducing itself hypothermia but yet able to reverse or prevent reserpine hypothermia.

The complexity of the interaction between harmaline and other MAO inhibitors or reserpine raised, in the mind of the psychiatrist who did these experiments, the thought of the difficulties involved in evaluating the real significance of the results obtained by administering combinations of psychoactive drugs to patients. The interaction of one compound with another one, each of them possessing its own pharmacological and biochemical properties, renders this task complex because many variables are operating simultaneously. The integration of all information gathered from basic and clinical research will be needed to help us reach a clearer understanding of the action of the psychoactive drugs in clinical use.

Finally, during this work in basic research I was reminded of a premonitory statement written by Freud, shortly before his death; "the future may teach us how to exercise a direct influence, by means of particular chemical substances upon the amounts of energy and their distribution in the apparatus of the mind" (125).

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