# STUDIES ON SOME CENTRAL EFFECTS OF INJECTED DOPAMINE IN YOUNG CHICKS

BY

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A

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# THESIS APPROVAL

This thesis by Obadiah Charles Wambebe meets the requirements governing the degree of Doctor of Philosophy of Ahmadu Bello University and it is approved for its contribution to scientific knowledge and literary presentation.

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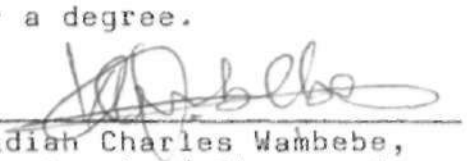
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# DECLARATION

This is to certify that I carried out the work reported in this thesis in the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University under the supervision of Prof. G. E. Osuide. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.



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# DEDICATION.

This thesis is dedicated to my
LORD, JESUS CHRIST, for His boundless
love, faithfulness and enabling grace
and
my dear wife, YEM, for her love, prayers,
perseverance and moral encouragement.

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# LIST OF MY PUBLICATIONS

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The effect of dopamine on maximal electro convulsive seizures in young chicks. Proceedings of the First World Congress on Biological Psychiatry, Buenos Aires.

Osuide, G. & Wambebe, C. (1975).
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Effect of 6-hydroxydopamine on maximal electroshock seizure in young chicks. West African J. Pharmacol. Drug Res. 4, 54 - 56.

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## GLOSSARY OF ABBREVIATIONS.

| | |
|---|---|
| Ach | acetylcholine. |
| CC | cubic centimetre. |
| COMT | catechol- O -methyl transferase. |
| DA | dopamine. |
| DDC | diethyl-dithio-carbamate. |
| DE | dopamine-excitatory mediating receptos. |
| DI | dopamine-inhibitory mediating receptors. |
| dopa/levodopa | 3,4-dihydroxyphenyl alanine. |
| DOPAC | 3,4-dihydroxy-phenyl-acetic acid. |
| DOPS | 3,4-dihydroxy-phenyl-serine. |
| EEG | electroencephaolgram. |
| EMG | electromyograph. |
| FLA-63 | bis (1-methyl-4-homopiperazinyl thiocaruamate) disulphide. |
| g | gramme. |
| HS | hyperstriatum. |
| 5-HT | 5-hydroxytryptamine. |
| HVA | homovanillic acid. |
| i.p. | intraperitoneal. |

| | |
|---|---|
| Kg | killogramme. |
| ml | millilitre. |
| mg | milligramme. |
| NA | noradrenaline. |
| 6-OHDA | 6-hydroxydopamine. |
| OT | optic tectum. |
| pento | pentobarbitone. |
| RF | reticular formation. |
| RO4-4602 | /N' - (DL-seryl)-$N^2$-(2,3,4-trihydroxybenzyl) hydrozine Hcl/. |
| ug | microgramme. |
| V | volume. |
| VMA | vanylmandelic acid |
| W | weight. |

## SUMMARY

The effects of some dopaminoceptor agents were studied on maximal electroshock seizure, chemically-induced seizures, gross behaviour, body temperature, pentobarbitone-induced sleep, EEG and EMG using young chicks.

The susceptibility of chicks to maximal electroshock seizure increased with age within 14 days after hatching. On the other hand, chicks became less susceptible to strychnine-induced seizures with age within 7 days after hatching while the convulsant effect of leptazol was apparently unrelated to age. Low to moderate doses of dopamine, L-dopa and apomorphine significantly protected chicks against electroshock seizure; the protective effect increased with dose of drug and age of chicks. High doses of dopamine, L-dopa and apomorphine apparently enhanced electroshock seizure activity in 1-8 day old chicks; this effect could not be demonstrated in older chicks. Similarly, dopamine protected chicks against both strychnine and leptazol-induced seizures. The

anticonvulsant activity of dopamine against strychnine-induced seizure decreased with age. The effects of dopaminoceptor agonists on maximal electroshock and chemically-induced seizures were antagonised by pimozide and haloperidol.

6- hydroxydopamine, injected intraperitoneally in rats and chicks, did not induce spontaneous seizures but produced significant alterations in the threshold to electroshock seizure in chicks; the particular effects were dose-dependent and time-dependent. Administration of 6-hydroxydopamine once only to 3 day old chicks and once per day into rats on the first and third days post-parturition resulted in an increase in the proportion exhibiting tonic seizure with electroshock when tested 10-12 weeks later. When 6-hydroxydopamine was injected intraperitoneally into adult rats and cocks, there was no significant alteration in seizure threshold.

D-amphetamine offered chicks a dose-dependent protection against electroshock seizure. FLA-63 produced a time-dependent biphasic effect on electroshock seizure. 3, 4-Dihydroxyphenylserine produced significant time-dependent changes in the seizure threshold of chicks.

Dopamine, L-dopa, amantadine and 6-hydroxydopamine produced stereotyped headshaking, jumping and board-pecking in a dose-dependent manner in chicks. Locomotor activity was increased by amantadine and low doses of L-dopa and dopamine. High doses of dopamine and L-dopa reduced locomotor activity. Water intake was increased by 6-hydroxydopamine. 3,4 - Dihydroxyphenyl-serine reduced water intake, pushing and pecking activities while stereotyped headshaking was increased. RO4-4602 increased board-pecking and jumping while pushing and water intake were decreased.

Dopamine, L-dopa, apomorphine and FLA-63 delayed the onset and shortened the duration of pentobarbitone-induced sleep in young chicks. These agents antagonised sleep and EEG synchronization induced by pentobarbitone. In addition, these drugs increased the EMG activity and desynchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation. Pimozide prolonged the duration of pentobarbitone-induced sleep; but had less effect when dopamine was also given.

Both dopamine and L-dopa produced significant dose-dependent hypothermia in young chicks, which was antagonized by haloperidol. In adult fowls, dopamine

did not exhibit any thermal influence but L-dopa still produced profound hypothermia. R04-4602 had no observable thermal effect in chick but potentiated L-dopa-induced hypothermia. Haloperidol produced a significant hypothermia in chicks.

The results obtained with radio-labelled dopamine indicate that most of the intraperitoneally injected drug crossed the blood-brain barrier and accumulated preferentially in the brain. Similarly, using fluorimetric assay technique, the estimated brain concentrations of dopamine after the administration of either dopamine or L-dopa increased profoundly. Interestingly, there was a close parallelism between the distribution of dopamine in different brain regions of chicks pretreated with either dopamine or L-dopa and that of endogenous dopamine in control chicks.

The results of this project show that dopamine and L-dopa injected parenterally accumulates in the brain of young chicks. It is also indicated from the data that dopamine may play significant roles in seizure modulation, sleep mechanism, temperature regulation and behavioural activity of chicks. In addition, some of the agents which are known to interfere with central dopamine neurotransmission produced significant effects

on seizure and behavioural activities. It is concluded that endogenous dopamine may subserve these important physiological activities in the chick.

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# CHAPTER ONE

## 1.1. LITERATURE REVIEW.

### 1.2. General Introduction.

Biochemical, electrophysiological and pharmacological approaches have been used by many workers to study the clinical significance and the physiological role of dopamine (DA) in mammals (Arbuthnott, Crow, Fuxe, Olson & Ungerstedt, 1970; Connor, 1968; Feltz & Albe - Fessard, 1972; Hornykiewicz, 1966; Van Rossum, 1967; Sourkes, 1970; Von Voigtlander & Moore, 1971).

It is now well-established that DA is the immediate precursor of noradrenaline (NA) in chromaffin tissues and noradrenargic nerves in both vertebrate and invertebrate organisms (Blaschko, 1959; Hornykiewicz, 1966; Nagatsu, 1964; Schumann, 1956). Recently, some workers have indicated that DA may possess its own physiological properties in the nervous system independent of its biosynthetic

role (Cools & Van Rossum, 1976; Goldberg, 1972).

1.3. Biosynthesis of DA.

Catecholamines are formed in the brain, chromaffin cells, sympathetic nerves and sympathetic ganglia from their amino acid precursor, L-tyrosine, by a sequence of enzymatic steps first postulated by Blaschko (1939) and confirmed by Nagatsu in 1964 (see fig. 1.1). L-Tyrosine is taken up from the bloodstream and concentrated within the brain and other sympathetically innervated tissues by an active transport mechanism. The rate - limiting step in the biosynthesis of catecholamines is the conversion of L-tyrosine to L-dops which is catalysed by tyrosine hydroxylase enzyme. This enzyme is stereospecific, requires molecular oxygen, ferrous iron radical and a tetrahydropteridine and shows a fairly high degree of substrate specificity.

DA is formed in vivo from L-dopa and catalyzed by L-aromatic acid decarboxylase enzyme. The reaction is very efficient and rapid. Some workers

have reported that the highest enzyme activity is in the caudate nucleus, the hypothalamus and the mesencephalon (Bertler & Rosengreen, 1959; Bogdanski, Weisbach & Udenfriend, 1957; Holtz & Westermann, 1956). Since the limiting step in the biosynthesis of DA and NA is the formation of L-dopa from L-tyrosine by L-tyrosine hydroxylase enzyme, no appreciable amounts of L-dopa can be expected to accumulate in the brain (Levitt, Spector, Sjoerdsma & Udenfriend, 1965).

Large amounts of DA were formed in slices of caudate nucleus of the cat but not in other areas of the brain on incubation in a medium containing L-tyrosine - $^{14}C$ (Masuoka, Schott & Petriello, 1963; McGeer, Ling & McGeer, 1963). In 1965, Glowinski, Iversen & Axelrod confirmed this finding in vivo. These authors showed that after introduction of $^{3}H$-DA into the lateral ventricle of the rat, the amine was easily taken up by brain tissues. However, in contrast to the other brain areas, where 30 to 50% of $^{3}H$ - DA was converted to $^{3}H$-NA, only small amounts of $^{3}H$ - NA were found in the caudate nucleus. These results suggest that the activity of DA - beta -

hydroxylase enzyme (which is responsible for the conversion of DA to NA) is very low in the striatum compared to other brain areas (Hornykiewicz, 1966). The low activity might be a consequence of lack of some factors necessary for full enzymatic function. The same author (1966) suggested an alternative explanation that DA and DA - beta - hydroxylase enzyme might be localized in separate compartments of the stristal cell.

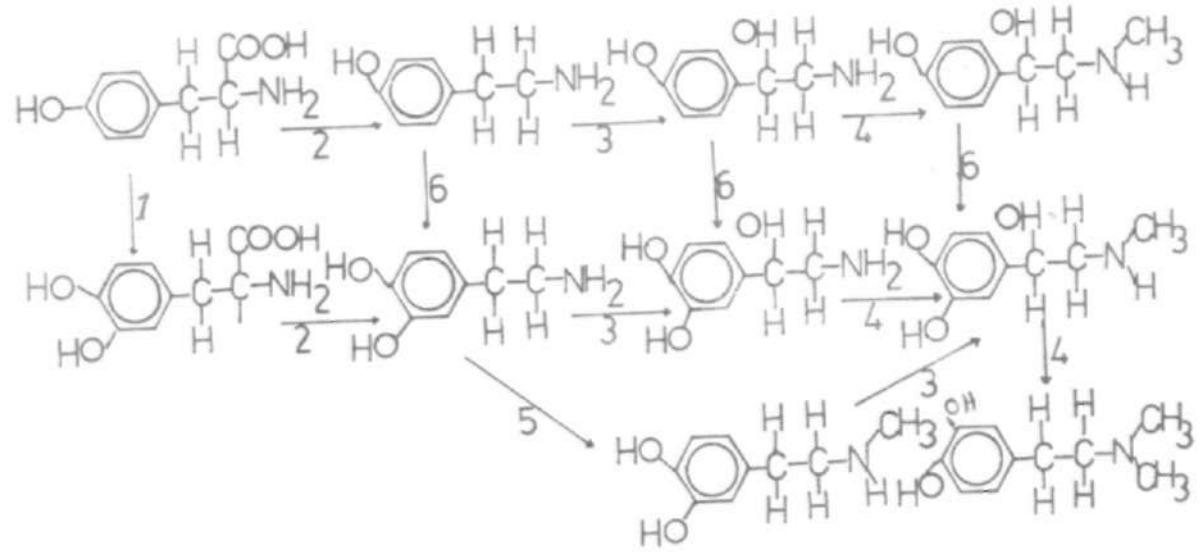


Fig. 1.1. Primary and alternative pathways in the formation of catecholamines. 1 = Tyrosine hydroxylase; 2 = Aromatic amino acid decarboxylase; 3 = Dopamine-beta-hydroxylase; 4=Phenylethanolamine-N-methyl transferase, 5 = Non-specific - N - methyl transferase in lung; 6 = Catechol-O-forming enzyme. A = Tyrosine; B = Tyramine; C = Octopamine; D = Synephrine; E = Dopa; F = Dopamine; G = Noradrenaline; H = Adrenaline; I = Epinine; J = N - methyladranaline. From: Cooper, J.R., Bloom, F.E. & Rothy R.H. (170).

1.4. Catabolism of DA.

The catabolic pathway of DA is schematically shown in fig. 2. Both monoamine oxidase (MAO) and catechol-o-methyl transferase (COMT) enzymes are involved in the formation of homovanillic acid (HVA, 4 - hydroxy - 3 methoxy phenyl acetic acid) from DA in the body (Axelrod, 1959; Blaschko, Richter & Schlossmann, 1937).

Carlsson & Hillarp (1962) reported that most of the DA formed from endogenous L-dopa is first attacked by MAO leading to formation of 3, 4 -

dihydroxyphenylacetic acid (DOPAC) which is subsequently O-methylated by COMT to produce HVA. In 1960, Rosengreen observed that the regional distribution of DOPAC in the brain was similar to that of DA. The occurrence of 3-methoxytyramine in the brain has been reported (Carlsson & Waldeck, 1964) indicating that some brain DA might undergo O-methylation prior to oxidative deamination.

The main brain metabolite of DA is HVA and its distribution roughly parallels that of DA (Bernheimer, 1964; Sharman, 1963). However, there are some quantitative differences between the distribution of HVA and DA in various brain regions. For example, in the striatum,the ratio of DA to HVA varies between 4 in cats and 0.5 in the dog (Laverty & Sharman, 1965). Whereas the corresponding figures for the substantia nigra and the pallidum in man are 0.25 and 0.04 respectively (Bernheimer & Hornykiewicz, 1965). These observations suggest that in spite of the comparatively low actual concentration of DA in

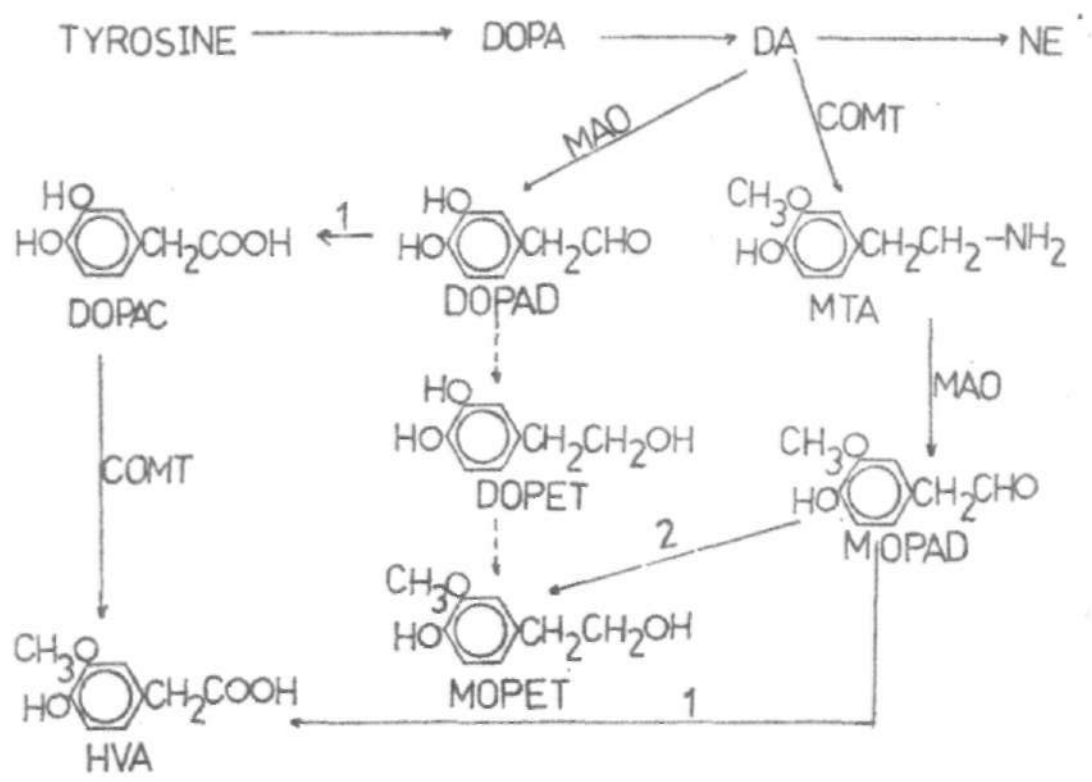



Fig. 1.2. Metabolism of dopamine;

(1) Aldehyde Dehydrogenase enzyme, (2) Aldehyde Reductase - enzyme, COMT - Catechol-O- methyl transferase enzyme, DOPAC - 3,4, - Dihydroxyphenylacetic acid, DOPAD - 3,4, Dihydroxyphenylacetaldehyde, DOPET - 3,4, - Dihydroxyphenylethanol, HVA - Homovanillic acid, MOPAD - 3, Methoxy - 4 - Hydroxyphenylethanol, MTA - 3 - Methoxy-tyramine.
The dashed arrows indicate steps which have not been firmly established.
From: Cooper, J.R., Bloom, F.E. & Roth, R.H. (1970).
The biochemical basis of neuropharmacology. Oxford University Press, Lond. P. 124.

the substantia nigra and pallidum, the amines in these brain regions might be present in physiologically active concentrations comparable to those found in the striatum (Hornykiewicz, 1966).

1.5. Turnover of DA.

The turnover of brain DA is comparable to that of 5-hydroxytryptamine (5-HT), but it is much higher than that of brain NA (Udenfriend, Witkop, Redfield & Weissbach, 1958). In 1965, Vogt correlated the high amounts of HVA in the DA-rich parts of the brain (in contrast to the very low concentration of acid metabolites of NA in the same region) with a faster turnover of DA than NA in the brain. The observation by many workers that several drugs (morphine, oxotremorine, beta - tetrahydronaphthylamine and phenothiazine derivatives) easily elevate HVA and 3-methoxytyramine levels in the caudate nucleus of different species is an additional evidence that the turnover of DA is very fast (Anden, Roos & Werdinius, 1964; Carlsson & Lindqvist, 1963). In addition, Glowinski et al

(1965) found that $^{3}H$ - DA disappeared at a faster rate than $^{3}H$ - NA from striatal tissue after the amines have been taken up from the lateral ventricle *in vivo*. This finding favours a high turnover rate of DA, but not NA, in the striatum.

1.6. Peripheral Physiological Role.

DA displays qualitatively similar peripheral actions as noradrenaline and adrenaline in most test preparations, but DA is comparatively weaker than the other sympathomimetics (Barger & Dale, 1910; Holtz, Heise & Ludtke, 1939). These findings may justify the classification of DA as a weak sympathomimetic amine acting directly or indirectly or both on classical adrenergic receptors.

However, the effects of DA on some blood vessels can not be fully explained on the basis of alpha-adrenoreceptor stimulation. For example, Holtz & Credner (1942) observed that DA caused a marked decrease in arterial blood pressure of guinea - pigs and, to a lesser extent, rabbits, in

contrast to NA. This report has been confirmed by other workers (Burn & Rand, 1958; Dengler, 1957; Schumann, 1956). Recent studies have shown that, in other species, DA has actions on the vascular system which are different from those of NA. Thus, unlike NA, the blood flow through the superior mesenteric vessels and coeliac vessels of dogs and through the kidneys of dog and man, are increased by DA (Ebble, 1964; McDonald & Goldberg, 1963). In addition, these "unusual" actions of DA are not antagonised by classical alpha and beta adrenoreceptor blocking agents (Ebble, 1964; McDonald & Goldberg, 1963). These findings indicate that DA may act directly or indirectly or both on classical adrenoreceptors as well as on specific DA receptors.

In 1972, Barbeau implicated DA in the control of peripheral mechanisms of blood pressure. He also postulated that DA regulates basal renin secretion rate via cyclic adenosine $3^1$, $5^1$ monophosphate (C-AMP). It has been shown that central DA

metabolism may also play a significant role in the regulation of postural blood pressure.

There is increasing evidence that DA may subserve specific physiological actions at various sites in the peripheral nervous system. For example, DA occurs in abundance in adrenergic nerves and ganglia (Von Euler & Lishajko, 1957), blood vessels (Von Euler & Lishajko, 1957), heart (Bertler, Falck & Hillarp, 1959), lung, bronchi (Von Euler & Lishajko, 1957) liver and intestine (Schumann, 1959). In most of these organs DA accounts for over 50% of the total catecholamine content. Infact, it has been reported that DA is the only detectable catecholamine in the lung, liver, intestine and bronchi (Hornykiewicz, 1966). It is tempting to assign a physiological significance to the presence of DA in these organs. In addition, Greengard, McAfee & Kebabian (1972) postulated that DA inhibits transmission at mammalian superior cervical ganglion via an action on C-AMP thereby modulating excitatory cholinergic

influence on the ganglion.

In 1965, Van Rossum observed that the alpha adrenergic receptors in the vas deferens of the rat have a considerably higher affinity for DA than the adrenergic receptors in the rabbit intestine. In a series of intensive studies on smooth muscles Tayo (1977), found that DA was five times more potent than NA in contracting the vas deferens of the rat. The effect of DA on the vas deferens was not blocked by phentolamine while pimozide effectively antagonised this action (Tayo, 1977). Thus, although true peripheral DA neurons have not yet been demonstrated, the "unusual" effects of DA on the blood vessels and vasa deferentia suggest the possible existence of DA receptors in these tissues. Also, the peculiar distribution of DA in different peripheral tissues implicates DA as a transmitter agent possessing specific physiological functions in the periphery.

1.7. Neurotransmitter Status of DA In The Brain.

During the last decade, both direct and indirect evidence have been presented to show that

DA fulfils the neurotransmitter role in the brain (Hornykiewicz, 1966; Cools & Van Rossum, 1976). Here are some of the arguments in favour of DA as a putative neurotransmitter.

In the brain ganglia of molluscs, DA is the only detectable catecholamine and electrophysiological data indicate that DA's effects on nerve cell activity are different from those of NA and Adrenaline (Sweeney, 1963). These findings suggest that DA might be a transmitter substance in the brain of this phyllum.

Secondly, the differential distribution of DA in the mammalian brain further implicates this amine as a neurotransmitter agent. Thus, the highest concentrations of DA in the mammalian brain are restricted to certain regions (i.e. caudate nucleus, putamen, globus pallidum, substantia nigra). It is significant to note that the DA-rich areas contain only very small amounts of NA (Bertler, 1961; Carlsson, 1958; Ehringer & Hornykiewicz, 1960; Laverty & Sharman, 1965). In addition, some workers have reported that DA may be the predominant

catecholamine in the retina (Haggendal & Malmfors, 1963), the tuberculum olfactorium (Anden, unpublished, quoted by Fuxe, 1964) and the infundibulum (Haggendal, unpublished, quoted by Fuxe, 1964).

Also, the fact that in most species DA accounts for about 50% of the total catecholamine content of the entire brain suggests that DA might possess specific physiological effects in addition to its biosynthetic role (Hornykiewicz, 1966).

Fourthly, some workers have used biochemical, histofluorescent and anatomical methods to demonstrate the existence of DA-rich nerve cells in the substantia nigra (especially the pars compacta) which send DA neurons into the nucleus accumbens, corpus striatum, tuberculum olfactorium and median eminence, (Carlsson, Falck & Hillarp, 1962; Fuxe, 1965; Hornykiewicz, 1963).

As a consequence of the demonstration of DA-rich neurons in the brain, many workers using chemical and electrophysiological techniques have identified the neurotransmitter synthesized within the nigro-neostriatal fibres and released within

the caudate nucleus either during rest or electrical stimulation of the presynaptic nerves to be DA (Arbuthnott et al, 1970; Besson, Cheramy, Feltz & Glowinski, 1971; McLennan, 1964; Portig & Vogt, 1969; Von Voigtlander & Moore, 1971). This demonstration confirms earlier suggestions that DA may be a neurotransmitter agent in certain regions of the mammalian brain.

1.8. Physiological Role of DA In The Brain.

York (1967), Connor (1968), and Feltz (1970 a-c) have reported that DA induces characteristic postsynaptic actions - inhibitory postsynaptic potentials in the majority of caudate cells and excitatory postsynaptic potentials in a small number of caudate cells. According to Herz & Zieglgansberger (1966) only orthodromic inhibitory responses of caudate cells to nigral stimulation are mimicked by micro-iontophoretically applied DA. On the basis of this finding, they postulated that DA acts as a postsynaptic inhibitory agent within the caudate nucleus.

Many workers, using pharmacological techniques, have reported that neostriatal DA functions as an essential mediator of processes involved in the genesis and regulation of motor activities. For example, in various animal species charecteristic stereotyped motor behaviours were modified or suppressed by selective interference with intracaudate DA activity (Connor, Rossi & Baker, 1967; Costall, Naylor & Olley, 1972; Ernst & Smelik, 1966; Ungerstedt, Butcher, Butcher, Andén & Fuxe, 1969). Even in man there is a definite correlation between central DA activity and certain psychomotor dysfunctions such as parkinsonism, huntington's chorea and tardive dyskinesias as well as some psychotic disorders (Barbeau & McDowell, 1969; Fog & Pakkenberg, 1970; Randrup & Munkvad, 1972; Stevens, 1973). Recently, Fuxe, Goldstein, Hokfelt, Johnson & Lidbrink (1974) and Olson, Seiger & Fuxe (1972) used combined histochemicel and ontogenetic experiments on the basal ganglia of rats to show that the nucleus accumbens and neostriatum of adult animals contain

two histochemically distinct types of DA-terminal structures. Thus, the possibility of the existence of two types of DA receptors-DA excitatory (DE) and DA inhibitory (DI) mediating receptors, have been suggested (Cools & Van Rossum, 1976). The DE receptor regions were marked by circumscribed islands of a moderate to strong dotted DA fluorescence while the DI receptor regions were marked by the well-known green DA-fluorescence. The DE receptor structures appear at an earlier stage than the DI receptor regions. The same workers observed that the DI receptor areas are less rapidly depleted by the DA - synthesis inhibitor, alpha-methyl-para-tyrosine, than the DE receptor structures. This finding suggests that the DA turnover in the DI regions is slower than in the DE structures. The DI fluorescence is concentrated in the medial part of the caput neostriati and also diffusely distributed throughout the rest of the telencephalic DA-rich structures (Olson et al, 1972). Tennyson, Barrett, Cohen, Heikkila & Mytilineous (1972) have reported similar

findings using the rabbit's caudate nucleus and putamen. Thus, the presence of two different types of DA terminal structures marked by differences in DA-fluorescence, turnover rate, morphology and topography strongly suggest the presence of functionally distinct DA-mechanisms within central DA-rich structures (Cools & Van Rossum, 1976).

Evidence from electrophysiological studies indicates that the putamen receives two types of DA neurons from the substantia nigra. It was observed that some neurons in the putamen are excited by nigral stimulation or by micro-iontophoretically applied DA whereas others are inhibited by nigral stimulation as well as by micro-iontophoretically applied DA (York, 1970). Similar experiments on the caudate nucleus of cats have also shown that this structure contains two electrophysiologically distinct DA-sensitive regions (Bloom, Costa & Salmoraighi, 1965; Connor, 1968; McLennan & York, 1967; Schwartz, 1970). It should be noted that

some inhibitory inputs to the putamen from the substantia nigra are mimicked by micro-iontophoretically applied gamma amino butyric acid (GABA) and blocked by GABA - antagonists - bicuculline and picrotoxin. Also, Feltz & De Champlain (1972 a-b) and Hull, Levine, Buchwald, Heller & Browing (1974) obtained inhibitory responses to nigral stimulation in the absence of DA-innervation of the caudate nucleus. These findings suggest the existence of non-DA inhibitory nigro-caudate fibres in addition to the DA-inhibitory nigro-caudate fibres.

In addition to the electrophysiological findings, pharmacological data show that there may be two distinct types of DA receptors in the brain. In these studies, dopaminoceptor agonists and antagonists were used. The DA agonists include the following agents:- L-3, 4-dihydroxyphenylalanine (L-DOPA) which is converted to DA in vivo and thus increases the amount of DA at the receptor site (Bartholini, Burkard, Pletscher & Bates, 1967; Butcher, Engel & Fuxe, 1970).

Amphetamine is reported to possess direct stimulant activity on DA receptors (Van Rossum & Hurkmans, 1964) in addition to its ability to inhibit monoamine oxidase enzyme and release DA (Besson et al, 1969; Carlsson, Lindqvist, Dahlstrom, Fuxe & Masuoka, 1965; Glowinski, Axelrod & Iversen, 1966). Amantadine is believed to cause the release of DA from extragranular stores in DA-nerves (Farnebo, Fuxe, Goldstein, Hamberger & Ungerstedt, 1971) and to prevent neuronal re-uptake of DA (Heimans et al, 1972). Apomorphine, piribedil, ergometrine and ergocornine have been reported by many workers to stimulate DA receptors directly (Andén, Fuxe, Hokfelt & Rubenson, 1967; Corrdi, Fuxe & Ungerstedt, 1972; Ernst, 1967; Fuxe, 1973; Pijnenburg & Van Rossum, 1973). According to Miller & Iversen, (1974) the metabolite of piribedil, S584, appears to possess dopaminoceptor agonistic activity. Apomorphine produces stereotyped behaviour when administered intra-neostriatally (Ernst & Smelik, 1966), piribedil and ergometrine are less effective in this respect (Pijnenburg & Van Rossum, 1973).

On the other hand, ergometrine produces a long - term increase in locomotor activity when administered into the nucleus accumbens while apomorphine is less effective in this respect (Pijnenburg & Van Rossum, 1973). Thus, there are some striking differences between apomorphine on one hand, and piribedil and the ergot alkaloids on the other hand. For example, apomorphine-induced effects are not affected by alpha-methyl-para-tyrosine whereas the effects of piribedil and the ergot alkaloids are suppressed by alpha-methyl-para-tyrosine (Costall & Naylor, 1973b).

In an attempt to explain these apparent discrepancies, Fuxe, (1973) and Corrodi et al, (1973) postulated an additional presynaptic action for piribedil and the ergot alkaloids.

The suggestion that the caudate nucleus contains two types of DA receptors is supported by behavioural studies using the cat's caudate nucleus. Local application of low doses of L-dopa, DA, apomorphine or amphetamine into the caudate nucleus (the rostromedial part of the nucleus) produces the characteristic contralateral turning syndrome which is reversibly

counteracted by haloperidol or alpha-methyl-para-tyrosine. Both piribedil and ergometrine are completely ineffective when administered into the caudate nucleus (Cools & Van Rossum, 1970; Cools, 1971). On the other hand, when low doses of L-dopa, DA & amphetamine are injected into the caudate nucleus outside the rostromedial area the so-called ipsilateral turning syndrome is elicited whereas piribedil produces contralateral turning syndrome and apomorphine was totally ineffective when injected into these intracaudate sites (Cools et al, 1975a).

Cools, Hendriks & Korten (1975b) reported that the caudate nucleus of rhesus monkey contains two types of DA sensitive receptors. One DA receptor type is involved in the elicitation of skilled manipulatory movements and spasmodic torticollis while the other DA receptor type is implicated in the elicitation of oro-lingual facial dyskinesias and dyskinetic activities in the extremeties. Haloperidol blocks the first DA receptor type but ineffective on the second type of DA receptors (Cools et al, 1975b).

These data clearly suggest the existence of two functional types of DA receptors in the rat, cat and monkeys which are characterised by specific pharmacological and behavioural features.

| | D1 | D2 |
|---|---|---|
| Topography | Cat-caput nuclei caudati restro-medialis.<br>Rat-neostriatum | Scattered throughout the caudate nucleus.<br>Nucleus accumbens |
| Amount | "Small" | "Large" |
| Localization | Presynaptic(?) | Postsynaptic(?) |
| Fluorescence | Diffuse green | Dotted green |
| Ontogeny | "Late" development | "Early" postnatal development. |
| Biochemistry | "High" DA turnover<br>"Sensitive" to AMPT<br>"Insensitive" to reserpine(?) | "Low" DA turnover<br>"Insensitive" to AMPT<br>"Sensitive" reserpine (?) |
| Electro-physiology. | Excitatory | Inhibitory |
| Pharmacology<br>agonists | DA, Apomorphine L-Dopa, Amphetamine. | DA, L-dopa, amphetamine. |
| antagonists | Haloperidol, related butyrophenones unaffected by piriuedil, Ergome-trine, S 584. | Piribedil, ergometrine unaffected by apomor-phine and haloperidol. |

Table 1.1. Characteristic features of D1 - and D2 - receptors (Cools & Van Rossum, 1976).

1.9. Inhibitory Presynaptic DA Receptors.

Some workers have reported that both apomorphine & butyrophenones are capable of interacting with presynaptic sites indicating that some of the DE receptors are presynaptically located (Christiansson & Squires, 1974; Costall & Naylor, 1973a, Seeman, 1974). Langer (1974) observed that activation of presynaptic sites would inhibit release of transmitter stored in that part of the neuron. According to Feltz (1974) it is possible that the presynaptic DI receptors are localized on gabaminergic neurons, which are normally suppressing the excitatory postsynaptic potential (EPSP) induced by excitatory substances like Ach. Infact, GABA is not limited to intracaudate terminal structures but it is also capable of producing inhibitory postsynaptic potential in caudate units which are selectively counteracted by GABA antagonists (Feltz, 1974; Robinson & Wells, 1973). In a preliminary report, Cools & Van Rossum (1976) showed that GABA inhibits the effects induced by local application of DA into the DE receptor areas whereas

picrotoxin (GABA antagonist) suppresses the behaviour induced by haloperidol in this area. According to Cools & Van Rossum (1976) most of the intracaudate units are directly inhibited by GABA and indirectly influenced by DA while the remaining few intracaudate neurons are directly inhibited by DA.

It is therefore understandable that:

(1) intracaudate micro-iontophoretically applied DA does not enhance the excitatory response to nigral stimulation (Feltz, 1970 a-c).

(2) the excitatory responses to nigral stimulation persist in animals with a DA-denervated neostriatum (Feltz & De Champlain, 1972a-b).

(3) haloperidol inhibits the excitatory response to nigral stimulation because it blocks the DA-inhibiting action upon GABA release (Feltz, 1971b).

Recent studies suggest that certain DA-loaded structures within the mammalian brain, especially the caudate nucleus, putamen and nucleus accumbens contain at least two distinct types of DA receptors i.e. presynaptic DE receptors and postsynaptic DI receptors (Cools & Van Rossum, 1976).

From the foregoing account it is obvious that the impairment of the DE-DI balance can disrupt the normal functions of structures containing both types of receptors since the cells in such structures receive afferent fibres from the caudate nucleus which are excited by electrical stimulation of some intracaudate units and inhibited by electrical stimulation of other intracaudate units (Krawthamer, Feltz & Albe-Fessard, 1967; Rogers & McKenzie, 1973). It therefore appears that distinct caudate units are converging on similar output systems. This assumption is compatible with the outcome of several functional studies using different techniques varying from electrical stimulation to lesioning (Buchwald, Pakic, Wyers, Hull & Henser, 1962; Liles & Davis, 1969; Winocur, 1974).

1.10 Pathological Implications.

The data summarized above judiciously suggest that DA may be a neurotransmitter in the brain which can act on two functional types of receptors (i.e. DE & DI receptors). It is therefore conceivable

that an imbalance in the DE-DI receptors in the brain may be significant in the aetiology of certain psychomotor disorders (parkinsonism, torticollis, tardive dyskinesias, huntington's chorea) and schizophrenia.

1.11. Pathophysiology of Parkinsonism.

Parkinson's disease is one of the major neurological disabilities, especially in patients over 60 years of age. James Parkinson first described the "Shaky Palsy" in 1817. The extrapyramidal symptomatology of human parkinsonism (idiopatic parkinsonism & postencephalitic parkinsoniam) is dominated by rigidity, tremor and bradykinesia. The neurophysiological mechanism underlying these symptons have not yet been fully understood but it seems safe to state that both rigidity and tremor are positive symptoms, caused by an over-activity of certain brain areas (Hornykiewicz, 1966). Parkinson's disease and postencephalitic parkirsonism are chronic and degenerative nervous disorders. It follows therefore, that the symptomatology of parkinsonism

is due to the destruction of certain extrapyramidal areas. Thus, both rigidity and tremor might be regarded as "release" symptoms due to lack of inhibition normally controlling an excess of specific motor function.

The most consistent neuropathological finding in human parkinsonism is the degeneration of the melanin-containing nerve cells of the pars compacta of the substantia nigra (Hassler, 1938). The cell loss is most prominent and diffuse in postencephalitic parkinsonism, whereas it is significantly milder in idiopathic parkinsonism (Hassler, 1938). In 1953, Hassler postulated that the degenerative changes in other extrapyramidal regions (striatum, pallidum) are probably irrelevant to the parkinsonian symptomatology.

According to Cooper (1965), surgical destruction of the pallidum or its thalamic projections abolishes rigidity, and to a lesser extent, tremor. From this report, it is conceivable that the substantia nigra (SN) through its nigro-pallidal fibre connections, inhibits pallidal activity. Thus, the degeneration of SN (as occurs in parkinsonism) will lead to an

unchecked pallidal activity resulting in rigidity (Hassler, 1953; Martin, 1959).

With respect to the sympton of akinesia in parkinsonism, it can be assumed that the normal physiological function of SN is to enhance spontaneous motor activity. Two mechanisms have been proposed to explain the excitatory influence of the SN on spontaneous motor activity. In 1953, Hassler proposed that some of the facilitatory motor impulses originating from the cortex are relayed by way of the SN to reach the motor neurons thus enhancing spontaneous movements. According to Hornykiewicz (1966), the SN may modify the influence exerted on motor performance by the other extra-pyramidal regions, particularly the striatum and pallidum. The modification is such that the destruction of SN would lead to a decreased ability of the individual to perform spontaneous voluntary movements. Most workers assumed that the striatum is concerned with elaboration of inhibitory impulses which modify the primitive motor pattern of the pallidum in such a way as to permit the performance of purposeful motor activity of the cortex (Hoff &

Osler, 1957). The inhibition of cortical activity by the striatum seems to be an essential condition for the individual to be able to concentrate on specific motor activities (Jung & Hassler, 1960). However, some authors reported that prolonged chemical stimulation of the caudate nucleus of the cat reduced spontaneous movements and reactivity to external stimuli (Spiegel, 1964; Spiegel & Szekely, 1961). These investigators therefore assumed that the striatum normally exerts an inhibitory influence on spontaneous movements. In support of this hypothesis is the finding that extensive lesion of the striatum induced hyperkinesia in animals and man (Spiegel, 1964).

The proposal that the symptons of parkinsonism are due to DA deficiency in the basal ganglia is substantiated by:-

(a) the observation that parkinsonian patients excrete less DA in the urine than normal subjects (Barbeau, Murphy & Sourkes, 1961).

(b) demonstration of DA deficiency in the extra-pyramidal centres of parkinsonian patients (Ehringer & Hornykiewicz, 1960).

(c) demonstration that drug-induced parkinsonism in experimental animals involve DA depletion.
(d) clinical observation that L-dopa (precursor of DA) is beneficial to parkinsonian patients (Barbeau et al, 1961; Birkmayer & Hornykiewicz, 1961). DA was virtually absent in the striatum while NA and 5-hydroxytryptamine (5-HT) levels in the brain of parkinsonian patients were significantly reduced (Bernheimer, Birkmayer & Hornykiewicz, 1961). These neurochemical findings suggest that there is a marked decrease in the ability of the affected brain tissues to form DA as well as a disturbance in DA storage mechanisms in the striatum of parkinsonian patients. It should be noted that the metabolism of DA in parkinsonian patients differs from that of 5-HT and NA in that monoamine oxidase inhibitors elevate the levels of NA and 5-HT to normal while DA concentration was unaffected (Bernheimer et al, 1963).

In the light of the foregoing, parkinsonism may be defined as a DA deficiency state resulting from disease or injury to the DA neuronal system. The physiological role of this system appears to be that

of an inhibitory modulation of the striatum, which it produces by counter balancing the excitatory cholinergic activity in this region. The level of acetylcholine (Ach) is very high in the striatum and its concentration is unaltered in parkinsonism (Yarh, 1972). The Physiological balance which exists between DA and Ach in a normal individual is disturbed in parkinsonian patient. With loss of DA in the striatum, the cholinergic activity predominates. The basis of present day treatment of parkinsonism is to restore striated DA deficiency by the use of its immediate precursor (L-dopa) or/and reduce cholinergic activity by using anticholinergic agents.

It should be noted that the treatment of parkinsonism is symptomatic and palliative and relies upon a pharmacological manipulation of a pathophysiological state.

1.12. Clinical Management of Parkinsonism

The use of L-dopa by parkinsonian patients is beset with side effects emanating mainly from the peripheral actions of L-dopa metabolites. Some of

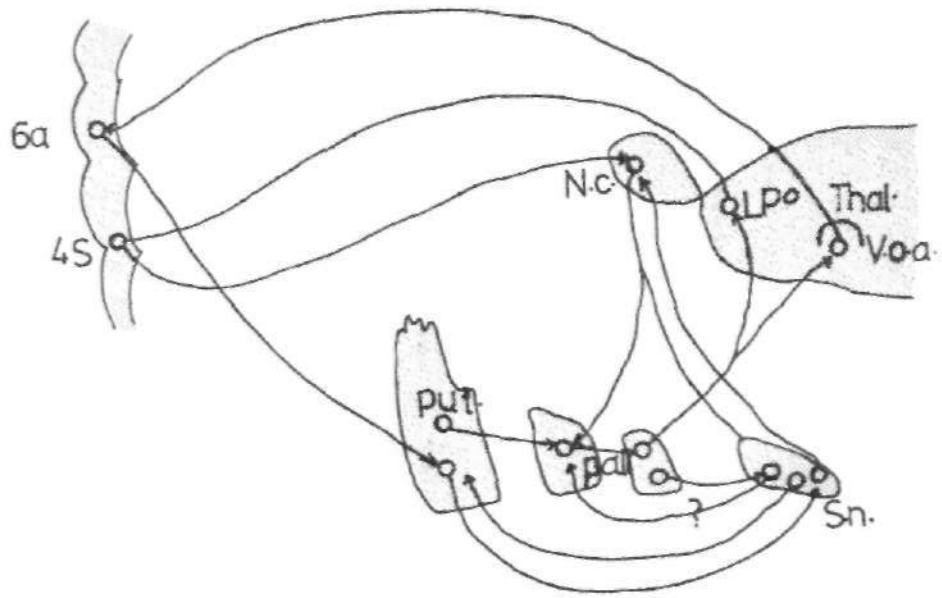



Fig. 1.3. Some fibre connections in the basal ganglia

The diagram illustrates the relationship between the caudate nucleus (N.C.), Putamen (Put.), globus pallidus (Pall.), Substantia nigra (S.n.), the nucleus Ventro Oralis (V.o.a.) and lateroporalis (L.p.o.) - of the thalamus (Thal), ____ Dopamine containing fibres, ? Fibres assumed to contain dopamine.

From: Hornykiewicz, O. (1966). Pharmacological Reviews 18, P. 950.

the side effects include nausea, anorexia, vomiting, nervousness, tension, night mares, etc. The transmission in DA neurons can be enhanced by the following agents:-

(a) DA receptors.

(b) DA catabolic inhibitors.

(c) DA storage & reuptake blockers.

(d) Stimulants at DA receptors.

Both L-tyrosine and L-dopa have been investigated as DA precursors. L-tyrosine was ineffective in parkinsonism possibly because the substrates subserving tyrosine hydroxylase are fully saturated so that the reactive process cannot go in the direction of dopa (Yahr, 1972). On the other hand, most parkinsonian patients benefit greatly from L-dopa. The effective dose range is 2 to 10 G daily by oral administration. Although L-dopa is a highly effective antiparkinsonian agent, it has some limitations. The large daily dose (5-8 G), the delayed onset of therapeutic benefits (in some instances as long as 3 months) and the numerous side effects which keep the daily dose below the amount necessary to alleviate the symptons, are among

the shortcomings of L-dopa. As a consequence of these side effects, peripheral dopa decarboxylase inhibitors are administered concurrently with dopa, so as to accumulate DA in the brain from the exogenous L-dopa. Such a practice drastically quickened the onset of action, reduced the daily dose of L-dopa (a reduction of 80% has been reported) and suppressed most of the side effects of L-dopa to the minimum (Yahr, 1972). Examples of the extracerebral dopa decarboxylase inhibitors include seryl-tri-hydroxyl benzyl-hydrazine (RO4-4602) and alpha-methyldopa-hydrazine (MK486).

Attempts to mobilize endogenous brain DA using drugs which retard its catabolism have met with limited success. The meagre amount of DA in the striatum of parkinsonian patient as well as the availability of alternative enzymatic pathways for its metabolism might explain the poor clinical results with monoamine oxidase inhibitors.

Amphetamine has been known to be beneficial in the treatment of parkinsonism, esperially

postencephalitic parkinsonism with associated somnolence. It enhance DA neurotransmission- directly or indirectly or both.

Another dopaminoceptor agonist which has been investigated clinically is apomorphine. According to Ernst (1967), apomorphine stimulates DA receptors directly. It has only a transient relief on parkinsonian patients. Duby, Cotzias, Papavasilion & Lawrence (1972) reported that L-dopa antagonized apomorphine-induced nausea and sedation while apomorphine antagonised the awakening effect, the involuntary movements and nausea induced by L-dopa. However, since apomorphine has a short duration of action and orally ineffective, it has no practical clinical application in parkinsonism at present.

Agents which block the reuptake and storage of catecholamines presynaptically can be expected to increase the transmitter levels at the receptive sites. The most potent inhibitors of the reuptake of catecholamines are the tricyclic antidepressants.

Unfortunately, they preferentially act on the hypothalamus, thus ineffective in controlling the symptons of parkinsonism (Yahr, 1972).

Amantadine, an antiviral agent, has also been thought to inhibit DA reuptake. When amantadine is administered in a daily dosage of 200 - 300 mg, a rapid modification of parkinsonian symptons occurred. Unfortunately, its effects are not sustained and unwanted side effects (oedema, seizures, visual disturbances, mental confusion) accompany its use.

1.13. Schizophrenia and Hypertension.

Apart from parkinsonism, some investigators have suggested that defects in DA turnover and dissipation may play some role in depression (Barbeau, 1969), Schizophrenia (Stevens, Wilson & Foote, 1974; Horn & Snyder, 1971; Fog, Randrup & Parkkenberg, 1966; Sathananthan, Angrist, & Gershon, 1973) and the aetiology of essential hypertension (Barbeau, 1972).

1.14. DA and Seizures.

In addition to the foregoing pathophysiological implications of DA, electrophysiological and biochemical significance of DA in the body, many workers have implicated DA in seizure mechanisms (De Schaepdryver, Piette & Delaunois, 1962; Jobe, Geiger & Stull, 1974; McKenzie & Scroko, 1972, 1973; Stull, Jobe, Geiger & Ferguson, 1973; Meldrum, Anlezark & Trimble, 1975; Anlezark & Meldrum, 1975; Cox & Lomax, 1975). These workers used mammals to study the influence of DA precursor (L-dopa) and other dopaminoceptor agonists on seizures since the mammalian blood-brain barrier is impermeable to catecholamines. These authors produced conflicting reports on the role of dopamine in seizure mechanisms. Thus, the role of dopamine in seizure mechanisms is still a debated issue.

1.15. DA and Sleep Mechanisms.

There are conflicting reports on the role of dopamine in the mechanisms of sleep in various animal species. For example, Key & Marley (1962) observed

EEG synchronization and behavioural sleep when dopamine was injected into young chicks. Similarly, Wahlstrom (1967) reported that levodopa reduced the threshold hypnotic dose of hexobarbitone in male rats. On the other hand, Spooner & Winters (1965) reported that injected DA produced EEG and behavioural activation in young chicks. In addition, Holtz, Balzer, Westermann & Wezler (1957) observed that L-dopa abolished hexobartitone-induced sleep in iproniazid-pretreated mice. It is therefore obvious that the role of DA in sleep mechanisms is not yet settled.

1.16. DA and Temperature regulation.

In 1972, Fuxe & Sjoqvist proposed that apomorphine-induced hypothermia in mice might be due to direct activation of DA receptors. Similarly, Lal, Puri & Karkalas (1971) attributed withdrawal hypothermia in apomorphine-dependent rats to increased DA neurotransmission. On the other hand, Borbeley & Huston (1972) associated gammabutyrolactone-induced hyperthermia with increased DA neurotransmission. Also, Marley & Stephenson (1969) obtained hyperthermia when DA was infused

into the hypothalamus of young chicks. These conflicting reports indicate that further investigations are necessary in this field.

1.17. My Objectives.

(1) I intend to study the influence of injected DA on chemically and electrically-induced seizures, body temperature, gross behaviour, barbiturate-induced sleep and EEG using young chicks. According to Waelsch (1955) and Key & Marley (1962), the young chick (1 day old to five weeks old) lacks a functional blood-brain barrier to catecholamines. It is pertinent to mention that although the chicks blood-brain barrier is immature at birth, its central nervous system has reached a level of functional organization that allows it to be self-sufficient as a pharmacological preparation (Spooner & Winters, 1966). The results will contribute to a more comprehensive grasp of

the physiological role of DA in the chick. It is assumed that some of the pharmacological actions of injected DA are mainly exagerations of its physiological effects.

(2) According to Spooner (1964), the CNS of chicks responds to neuropharmacological agents in a manner similar to most mammals. Many workers have used L-dopa (precursor of DA) and other drugs which influence DA metebolism to implicate DA in regulatory mechanisms in mammals. In this project, it is intended to study some central effects of some dopaminoceptor agents. Such a data may further our knowledge on the possible role/s of dopamine in the brain.

(3) It is also anticipated that the study of the influence of DA on neurophysiological parameters might implicate (or otherwise) the possible role of DA in the aetiology and/maintenance of diseases involving these functions.

(4) It seems appropriate to study the pharmacology of the agents which are used to modify DA metabolism for the following reasons:-

(a) It is possible that some of the DA agents may possess latent actions which are independent of their effects on DA metabolism.

(b) Since the most potent DA antagonists (i.e. neuroleptics) are used as antipsychotics, it is essential to know their pharmacology very intimately so that their side effects may be better understood and controlled.

(c) Such a pharmacological data may contribute towards a better understanding of the physiological role of DA in the chick. Since the DA agents will only alter endogenous DA levels, the results will portray more closely the physiological role of DA in the chick.

# CHAPTER II

## 2.1. MATERIALS AND METHODS.

### 2.2. Test Animals.

White ranger cockerels and adult cocks (obtained locally from Arewa Agricultural Enterprises Nigerian Ltd., Zaria) and albino rats (inbred in our Animal House) were used for this project.

### 2.3. Drug Solutions.

#### 2.3.1. Preparation of Drug Solutions.

(a) Levodopa (Sigma Chemical Co.).

Levodopa was dissolved in 0.05 M hydrochloric acid solution with the aid of gentle heating over a water-bath. The solution was diluted with normal saline

before injection.

(b) Apomorphine hydrochloride. (British Drug Houses Ltd.).

Apomorphine hydrochloride solution decomposes readily when exposed to air or light. The reaction is accelerated in alkaline medium but retarded by the addition of sodium metabisulphite. Thus, the solutions of apomorphine hydrochloride were prepared with air-free water (i.e. recently boiled and cooled water) and kept in an amber-coloured air-tight container. Also, 0.1% w/w sodium metabisulphite was added to the sample of apomorphine hydrochloride before the preparation of each solution.

(c) Dopamine hydrochloride, (DA, Koch Light and Sigma Chemical Co.), noradrenaline hydrochloride (NA, Sigma Chemical Co.) and 6-hydroxydopamine hydrobromide (Sigma Chemical Co.).

The solutions of DA, NA and 6-OHDA are very susceptible to alkaline hydrolysis. In order to retard the hydrolysis, 0.1% w/w of sodium metabisulphite was added to the solutions. The vehicle (normal saline) was recently boiled and cooled.

(d) FLA-63 (Labkemi AB).

FLA-63 was dissolved in 0.05 M hydrochloric acid solution with the aid of gentle heating over a water bath. The solution was diluted appropriately with normal saline before use.

(e) Pimozide (Janssen Pharmaceutica).

Pimozide was suspended in 0.3% v/v Tween 80 and adjusted to the appropriate volume with normal saline.

(f) Haloperidol (Janssen Pharmaceutica) and Reserpine (Ciba).

Both haloperidol and reserpine were supplied in 5 mg ampoules. Appropriate

dilutions were made with normal saline to give reasonable concentrations for parenteral administration.

(g) DL- threo -3, 4 - dihydroxyphenylserine (DOPS, AB Biotec), Nialamid (Pfizer), Strychnine hydrochloride (British Drug Houses Ltd), Leptazol (Sigma Chemical Co), D-amphetamine hydrochloride (British Drug Houses Ltd), Pargyline hydrochloride (Abbott Labs), Amantadine hydrochloride (Ciba - Geigy) and Pentobarbitone Sodium (Abbott Laboratories). Normal saline was used for the preparation of the solutions of the chemical agents mentioned above.

2.3.2. Storage of Drug Solutions.

Due to the instability of some of the drugs I used in this project, fresh drug solutions were prepared for each day's work. The solutions were kept in air-tight, amber-coloured containers and stored in the refrigerator until ready for use.

2.3.3. Volume of Injected Drug Solution.

The volume of drug solution administered to each chick was maintained at a constant proportion (i.e. the weight of the chick in grammes X $10^{-2}$cc) throughout all the experiments, the dose notwithstanding. The doses administered were in the form of the above salts.

2.4. Routes of Administration.

Apart from apomorphine and strychnine which were administered subcutaneously, all the other drugs used in this project were injected intraperitoneally.

2.5. Controls.

The control chicks were injected with the appropriate vehicles used in dissolving the test chemical agents.

2.6. Electroshock Procedure.

The duration of drug pretreatment before

electroshock was 10 minutes for DA, NA and apomorphine, 30 minutes for amphetamine, 1 hour for haloperidol, 3 hours for pargyline, 4 hours for pimozide, 6 hours for FLA-63, 12 hours for nialamid and 21 hours for reserpine. In addition, various pretreatment times (ranging from 10 minutes to hours, days and months) were used for some of the drugs to investigate the influence of duration of drug pretreatment on seizure threshold. Ugo Basile constant current electroshock machine model 7800 with corneal electrodes was used in this project.

The shock duration, frequency and pulse width were maintained at 0.8 second, 100 pulses/second and 0.4 ms respectively throughout the experiments involving chicks. For the rat experiments, the shock duration, frequency and pulse width were 0.2 second, 150 pulses/second and 0.4 ms respectively when testing for anticonvulsant property. On the other hand, when testing for the seizure-potentiating effect of a drug, the shock duration,

frequency and pulse width were 0.2 second, 100 pulses/second and 0.4 ms respectively. The current used for rat's experiments varied between 70 and 100 mA.

In chicks, when testing for anticonvulsant activity the current was chosen which produced 70 - 90% tonic seizure in control animals; while the current which produced 10 - 30% tonic seizure in control animals was used when testing for the seizure-potentiating effect of a drug. On the other hand, when testing for the time-dependent influence of drugs, a current which produced 40 - 60% tonic seizure in the controls was used.

Maximal electroshock seizure was recorded when tonic extension of the forelimbs and hindlimbs was produced. In this project, an increase in the incidence of tonus was regarded as an enhancement of seizure activity while a decrease in the incidence of tonus was regarded as a decrease in seizure activity.

In order to reduce the circardian variation in catecholamine levels of the brain to the minimum, the experiments were performed between 1 pm and 5 pm. In addition, controls were shocked immediately before and after each experiment.

At least 5 chicks were used for each dose of a given drug at any particular time while each experiment was repeated, at least, four times.

Chemically-Induced Seizures.

General.

The chicks were kept singly per compartment for 1 hour before drug injection. Usually 4 chicks were kept under observation during the same period (i.e. 2 chicks which were injected with the convulsant agent while the remaining 2 chicks were pretreated with DA or any other agent prior to the administration of the convulsant agent). The experiments were

performed in a quiet, air-conditioned room (ambient temperature was 22 ± 2°C).

Two doses of leptazol and strychnine were used in this work which produced reasonable levels of tonic seizure in the controls. Each experiment was repeated, at least, ten times (i.e. twenty chicks per dose). Lack of tonic extension of the hindlimbs was regarded as no convulsion. After the administration of the convulsant agent, the chicks were observed for 1 hour.

2.7.2. Leptazol Experiments.

In order to study the enhancing effect of drugs on leptazol-induced seizure, 70 - 80 mg/kg. of leptazol was injected into the chicks which produced tonic convulsions in about 20% of the control chicks. On the other hand, a dose of 80 - 90 mg/kg. of leptazol was injected into chicks when studying the anticonvulsant activity of drugs influencing brain DA levels. Such a dose of

leptazol produced about 60% tonic seizure in control chicks.

The duration of pretreatment before the administration of leptazol was 5 minutes for DA.

2.7.3. Strychnine Experiments.

When studying the potentiating action of drugs on strychnine-induced seizures, 0.6 mg/kg. of strychnine hydrochloride was used. When such a dose was administered into 2 day old chicks about 50% of the control chicks exhibited tonic convulsion.

On the other hand, 0.8 mg/kg. of strychnine hydrochloride was injected into 2 day old chicks when investigating the

anticonvulsant action of drugs against strychnine-induced seizures. Such a dose of strychnine induced tonic seizures in about 55% of control chicks. Generally, a dose of strychnine which produced a reasonable level of convulsion in the control chicks was used for any particular experiment.

The pretreatment times with DA and FLA-63 prior to the injection of strychnine hydrochloride were 5 minutes and 5 hours respectively.

## 2.8. Behavioural Studies.

The chicks were kept in a transparent perspex cage (25cm x 25cm x 32cm dimensions) for 1 hour to acclimatise to the environment before commencing the experiments. Two chicks were observed during the same period (one control, injected with normal saline and the other was treated with the test drug). The chicks were supplied with food and water and warmed by means of an angle poise lamp. The

ambient temperature of the room was maintained at 22 ± 2°C during the periods of observation. The experiments were performed in a quiet room free from disturbances. Each experiment was repeated ten times.

The chicks for a particular experiment were of the same age and approximately the same weight. The experiments were performed within the same period of the day. This precaution was necessitated by the possibility that circardian variation in catecholamine levels might influence the behavioural response of the chicks to psychoactive agents.

The chicks were observed for 4 hours when the following drugs were injected:- DA, L-dopa, pargyline, pimozide, haloperidol, DOPS and 6-OHDA.

The influence of the drugs on locomotor activity, gross behaviour, eating and feeding

habits were carefully studied with the aid of stop clocks and a recording register designed for this study. The register has got different columns for various activities, (e.g. eating, drinking, movement, etc), the appropriate columns were marked when the chick performed a particular activity.

## 2.9. Sleep Studies.

### 2.9.1. General.

White ranger cockerels, 2 to 14 day old and weighing between 37 to 55 grammes, were used in all the experiments. A difference of $\pm$6 and $\pm$10 grammes in weight of chicks was permitted for experiments involving 2 to 7 day old and 8 to 14 day old chicks respectively. The set-up for sleep studies (observational aspect) was the same as described for behavioural studies.

The criteria for sleep in chicks were those used by Fugner and Hoefke (1971). The

chicks were regarded as asleep when with both eyes closed, the chick was:- either crouching with the beak on the floor; or standing with the head leaning against the wall of the cage. A stop clock was used for timing the onset and duration of sleep.

2.9.2. Implantation of Electrocortical and Electromyograph Electrodes.

The chick was anaesthetized with halothane (Fluothane, ICI Ltd.) and implanted with electrodes made from the pointed ends of stainless steel insect pins No. 00, according to the methods of Spooner (1965) and Spooner & Winters (1967).

The optic tectum, hyperstriatum and pontine reticular formation were implanted and secured chronically to the skull with dental cement.

The monopolar electrodes implanted onto the hyperstriatum and optic tectum used an

electrode implanted onto the comb of the chick as reference. The electrodes implanted on the pontine reticular formation were bipolar. The sites of the implanted electrodes were located by the aid of surface markings on the skull in the case of the optic tectum and hyperstriatum while the pontine reticular formation was located histologically.

Electromyograph (EMG) electrodes were made with stainless steel insect pins bent at the ends to make hooks. The electrodes were positioned in the proximity of the head and inserted into the semispinalis capitis muscle of one side of the dorsolateral aspect of the neck. The electrodes were secured chronically to the neck with dental cement.

After the implantation process was completed, the chicks were kept warm using an angle-poise lamp and allowed 24 hours for full recovery from halothane before EEG recording.

2.9.3. EEG Recording.

The chick was placed inside a screened cage and allowed 1 hour to settle down in the new environment before EEG recording. The recording of both the EEG and EMG were done with Grass Polygraph, Model 79D.

2.9.4. Pretreatment Times.

The duration of pretreatment before the administration of pentobarbitone sodium was 5 minutes for dopamine and apomorphine, 30 minutes for levodopa and 4 hours for pimozide. The pretreatment periods were established from preliminary studies in our laboratory.

2.10. Temperature Studies.

2.10.1. Instruments.

The rectal temperature was recorded with the aid of Edale thermistor thermometer model K (Edale instruments, Cambridge Ltd., Toft, Cambridge).

The special thermistor probes were inserted into the chick's rectum to a distance of about 2.5 cm to give reproducible temperature measurements. The experiments were performed in a quiet, air-conditioned room (22 $\pm$ 2$^{o}$C ambient temperature). In view of circadian rhythms of body temperature (Friedman & Walker, 1967), the experiments were conducted between 9 a.m. and 12 noon daily - controls were done concurrently with the test experiments.

2.10.2. Animals.

Male ranger chicks, aged between 3 days and 20 weeks, were used for the experiments. Each experiment was repeated 5 times using 5 chicks per experiment. The temperature recordings were done at regular intervals (every 10 minutes) for one hour before drug administration and continued until the temperature returned to pre-injection level. The chicks were allowed 1 hour to acclimatize to the laboratory conditions before commencing any temperature measurement.

# CHAPTER III

## 3.1. THE EFFECT OF AGE OF CHICKS ON THEIR SUSCEPTIBILITY TO ELECTROSHOCK SEIZURE.

## 3.2. Summary.

3.2.1. A current of 40 mA failed to induce tonic seizure in 1 day old chicks. The same current induced 20%, 60% and 80% tonic seizure in 3, 5 and 7 day old chicks respectively.

3.2.2. The current level required to induce 20% tonic seizure in 1 - 7 day old chicks decreased from 60 mA at 1 day old to 32 mA at 7 day old.

3.2.3. It is concluded that the developing neuronal system might be responsible for the age-dependent increase in seizure susceptibility of young chicks.

## 3.3. Introduction.

According to Spooner & Winters (1966), the central nervous system (CNS) of the chick at birth has attained a reasonable level that permits its use as a pharmacological preparation. However, Osuide (1968, 1972b) reported that the susceptibility of chicks to some convulsant drugs decreased with age. Therefore, it is relevant and important to investigate the effect of age or otherwise on the susceptibility of the chicks to maximal electroshock. I believe that such a data will be useful in the proper execution of this project and may also help future workers in related fields.

## 3.4. Results.

### 3.4.1. The influence of 40 mA current on seizure susceptibilities of 1 - 14 day old chicks (fig. 3.1).

This histogram demonstrates the effect of age on the seizure suceptibilities of 1 - 14 day old chicks.

Both the current and other parameters for electroshock were kept constant throughout these experiments involving various ages of the chicks. A gradual increase in the percentage of tonic seizure was record as the chicks grew older. It can be noted however, that there was no detectable difference between the seizure susceptibilities of 7 and 14 day old chicks.

3.4.2. The variation in current levels necessary to induce 20% seizure in 1 - 7 day old chicks (fig. 3.2.).

The current levels required to induce 20% tonic seizure in 1 - 7 day old chicks were established in our laboratories. The result suggests that the seizure susceptibility of young chicks was less than that of older chicks.

3.4.3. Comparison between the seizure susceptibilities of 7 and 14 day old chicks (fig. 3.3.).

In this case, the seizure susceptibilities of two age groups of chicks were compared to

each other using various current levels. At relatively low current levels, the 7 day old chicks were apparently less susceptible to electroshock seizure than the 14 day old chicks. However, this difference between the two age groups was not observed at a relatively high current level.

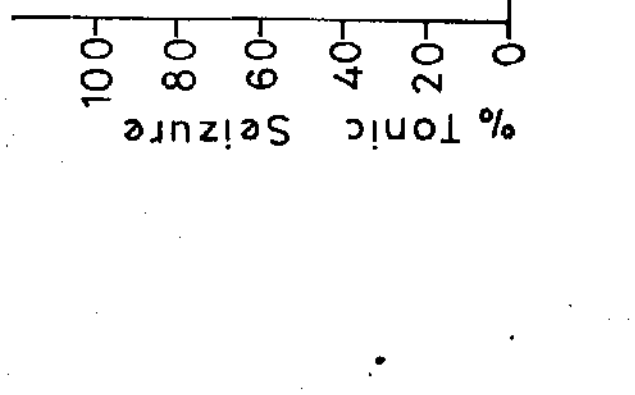



Fig. 3.1. Effect of age on seizure threshold.

Vertical bars represent standard deviations from the mean (n = 40 per histogram). Current = 40 mA.

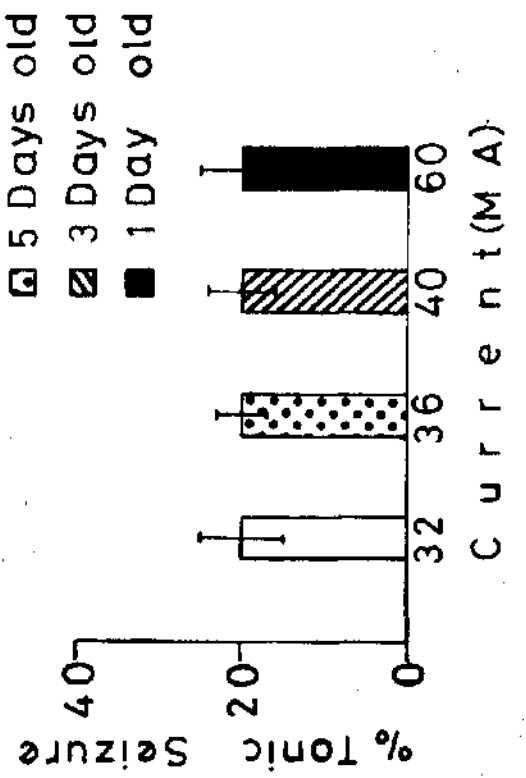



Fig. 3.2. Bar graph showing the various current levels which induced 20% convulsion in chicks aged 1 - 7 days.

Vertical bars represent standard deviations from the means ( n = 30 per histogram).

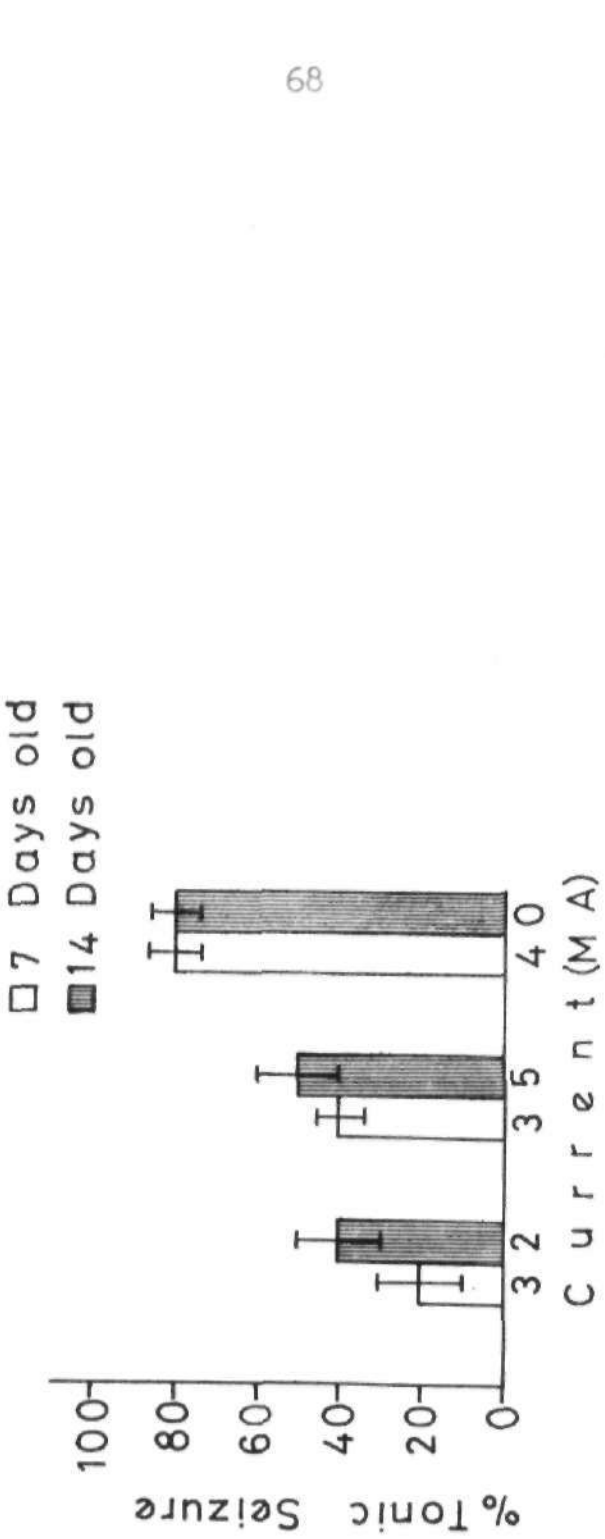



Fig. 3.3. Comparison between the seizure susceptibilities of 7 and 14 day old chicks.

The values used are arithmetic means ± standard deviations (n = 30 per histogram).

3.5. Discussion.

The results shown in figs. 3.1 - 3.3 demonstrate that the susceptibility of chicks to maximal electroshock seizure decreased with age within a fortnight after hatching.

In 1965, Vernadakis & Burkhalter reported that the duration of flexion elicited during electroshock seizures in chicks decreased with age indicating an increase in the severity of the seizures. It is known that myelination confers upon a nerve fibre the properties of lowered threshold, increased conduction velocity and greater ability to carry

repetitive impulses (Vernadakis & Burkhalter, 1965). These authors concluded that the particular pattern and intensity of seizure is related to central nervous system development and may be influenced by the degree of myelination of the neuronal pathways involved in seizure responses. Thus, the results shown in figs. 3.1 and 3.2 might be explained on the basis of a developing neuronal pathway which apparently favoured an enhanced conduction velocity of impulses.

Fig. 3.3 shows the results obtained with 7 and 14 day old chicks, using three current levels. It appears that the difference between the results obtained with 7 and 14 day old chicks became negligible at higher current levels. It can be caustiously suggested that the components of the neuronal development involved in seizure mechanisms of the chick attain a peak stage at about the age of 7 days. After this age, any further neuronal maturity (up to the age of 14 days) did not influence the seizure threshold in any significant manner.

# CHAPTER IV

## 4.1. THE INFLUENCE OF SOME DOPAMINOCEPTOR AGONISTS ON MAXIMAL ELECTROCONVULSIVE SEIZURE IN CHICKS.

## 4.2. Summary.

4.2.1. DA, levodopa and apomorphine were used in this study as dopaminoceptor agonists.

4.2.2. The chicks were significantly protected against electroshock seizure when low to moderate doses of DA, levodopa and apomorphine were used.

4.2.3. The anticonvulsant effect of DA, levodopa and apomorphine was dose-dependent and increased with age of chicks.

4.2.4. High doses of DA, levodopa and apomorphine apparently enhanced electroshock seizure in 1 - 8 day old chicks. However, when 14 day old chicks were used, the seizure-enhancement

by these high doses was absent.

4.2.5. Both pimozide and haloperidol antagonised the effects of DA, levodopa and apomorphine on electroshock seizure.

4.2.6. The seizure threshold of rats to electroshock was not altered by an intraperitoneal injection of DA.

4.2.7. Noradrenaline slightly enhanced seizure activity in young chicks.

4.2.8. The results suggest that stimulation of central DA receptors (directly or indirectly) might be involved in the anticonvulsant activity of the dopaminoceptor agonists used in this project.

4.3. Introduction.

According to Curtis (1969), abnormalities in transmitter function are characteristic to epilepsy.

Thus, disturbances at both presynaptic and postsynaptic sites of central neurons might be responsible for the initiation of an abnormally excessive neuronal activity within the epileptic focus. On the other hand, subsequent development and spread of paroxysmal activity might involve synaptic processes in non-epileptic sites. Generally, electrical stimulation has been used for generating seizures to mimick attacks of epilepsy in man.

It has been suggested by many workers that most of the actions of levodopa are due to dopamine produced from it *in vivo* (Scheel-Kruger & Randrup, 1967; Everett & Borcherding, 1970; Constantinidis, Bartholini, Tissot & Plestcher, 1968; Friedman & Gershon, 1970). It is now generally accepted that apomorphine stimulates central dopamine receptors directly (Ernst, 1967; Ernst & Smelik, 1966).

Recent investigations indicate that dopamine or noradrenaline or both are significant in seizure mechanisms in mammals (Stull et al, 1973). For example, Jobe, Picchoni & Chin (1973a) suggested that noradrenaline may be the modulator of audiogenic seizures. In 1974, Jobe and his coworkers, using rats, suggested that endogenous brain dopamine and noradrenaline may play an attenuating role in electroshock. Also, the anticonvulsant activity of L-dopa in rats has been explained as due to the enhancement of central dopaminergic mechanisms (McKenzie & Soroko, 1972).

The mammalian blood-brain barrier is impermeable to monoamines and direct effects of dopamine can only be produced by injection into discrete brain areas, for example, the corpus striatum (Ernst & Smelik, 1966). The blood-brain barrier of young chicks is permeable to catecholamines (Waelsch, 1955; Key & Marley, 1962). In this study, the

effects of dopaminoceptor agonists on maximal electroshock seizure have been tested using 1 - 14 day old chicks.

## 4.4. Results.

### 4.4.1. Dose-response Relationship.

The anticonvulsant effect of DA (6.25 - 150 mg/kg.), apomorphine (0.1 - 0.5 mg/kg.) and levodopa (6.25 - 25 mg/kg.) increased with dose. However, the seizure-protective effect of these agents became reduced when higher doses were used (fig. 4.1 - 4.4).

### 4.4.2. Variation With Age.

The protective activity of DA, apomorphine and levodopa became more pronounced when older chicks were used. In high doses these dopaminoceptor agents induced an increase in the seizure activity of 1 - 8 day old chicks but lacked such an effect in 14 day old chicks (fig. 4.5 - 4.7).

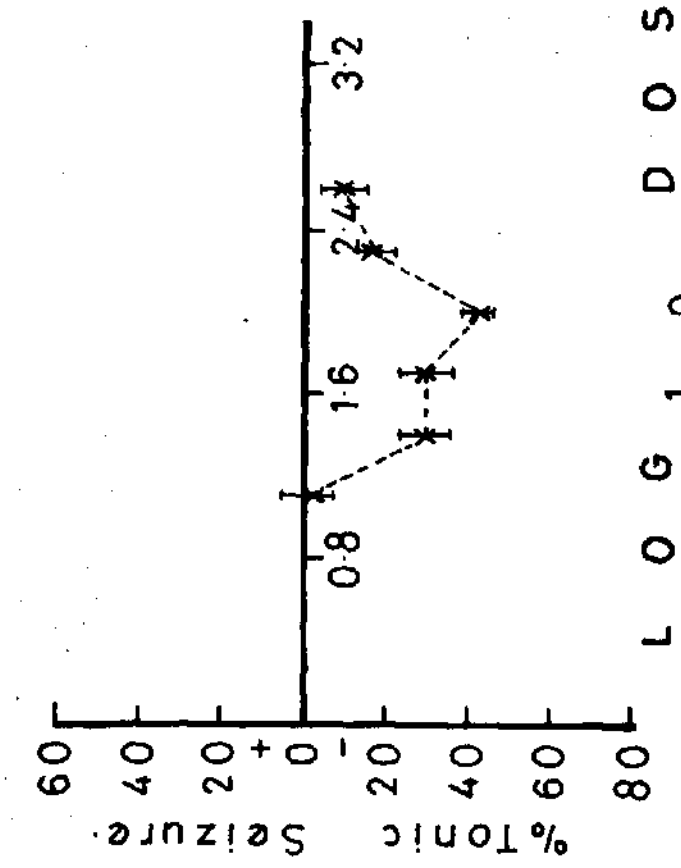



Fig. 4.1. $Log_{10}$ Dose-response relationship of DA against maximal electroshock seizure. The chicks were 14 day old and the doses of DA used were 6.25 to 400 mg/kg. Vertical bars represent standard deviations from the means (n = 40 per point). $P < 0.005$, Chi-Squared test.

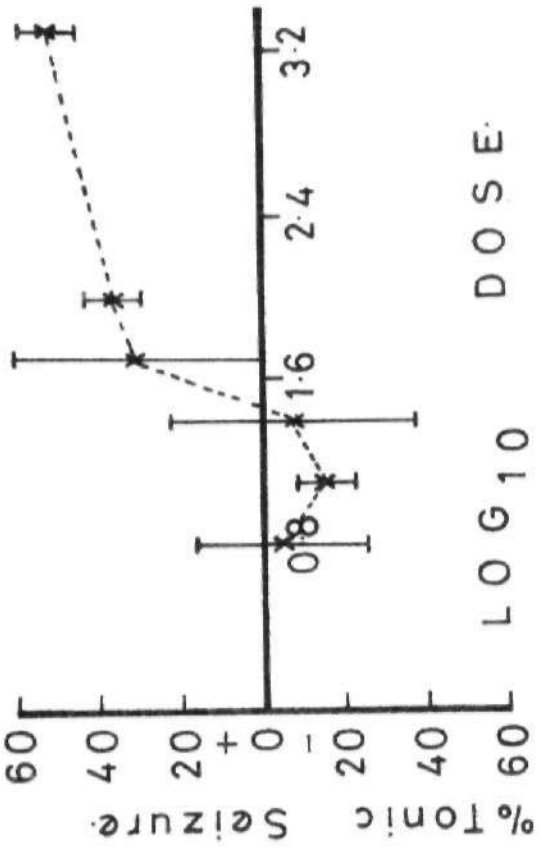



Fig. 4.2. $Log_{10}$ dose-response relationship of L-dopa against maximal electroshock seizure. The chicks were 5 day old and 6.25-400 mg/kg. of L-dopa were used. Vertical bars represent standard deviations from the means (n = 40 per point).

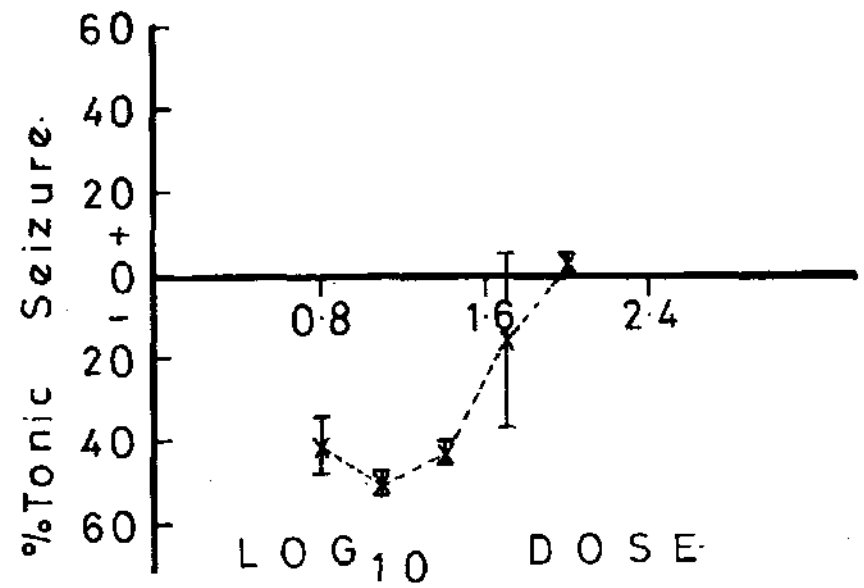



Fig. 4.3. $Log_{10}$ dose-response relationship of L-dopa against maximal electroshock seizure.
The chicks were 14 day old. Doses of L-dopa injected in the chicks varied between 6.25 and 200 mg/kg. Vertical bars represent standard deviations from the means ( n = 30 per point). Statistical analysis was done using Chi-Squared test, $P < 0.005$.

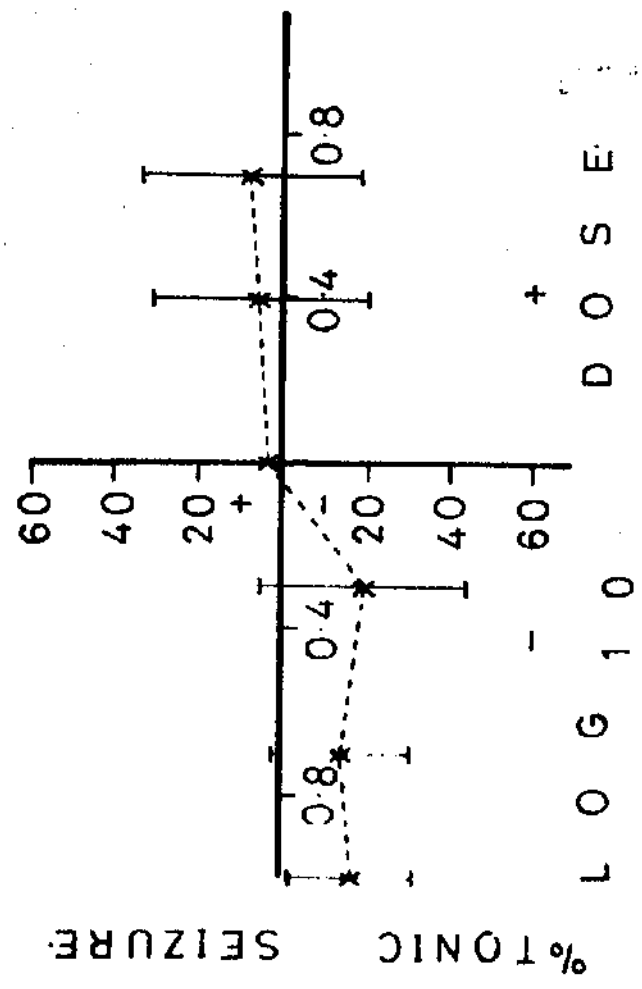



Fig. 4.4. $Log_{10}$ dose-response relationship of apomorphine against maximal electroshock seizure.
The chicks were 5 day old. Doses of apomorphine used were 0.1 to 5 mg/kg.
Vertical bars represent standard deviations from the means ( n = 40 per point).
Chi-Squared test was used to analyse the results ($P < 0.05$).

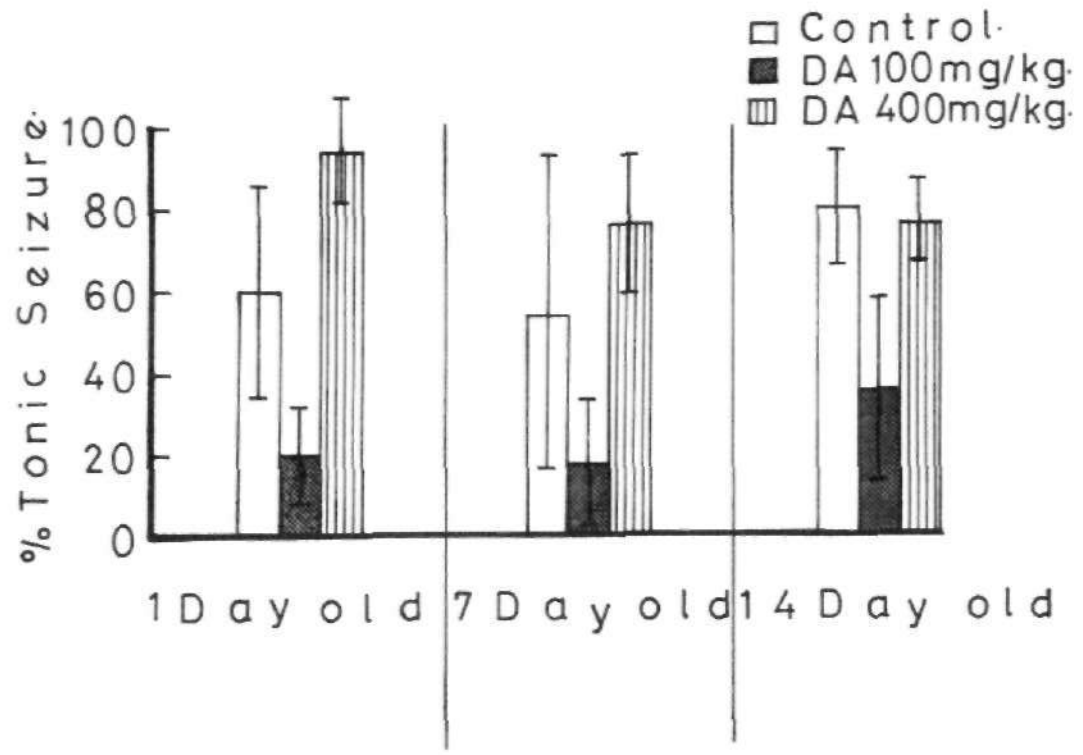



Fig. 4.5. Influence of age of chicks on the effects of DA on their threshold to electroshock seizure.
The electroshock parameters varied according to the age of chicks. Vertical bars represent standard deviations from the means (n = 30 per histogram).

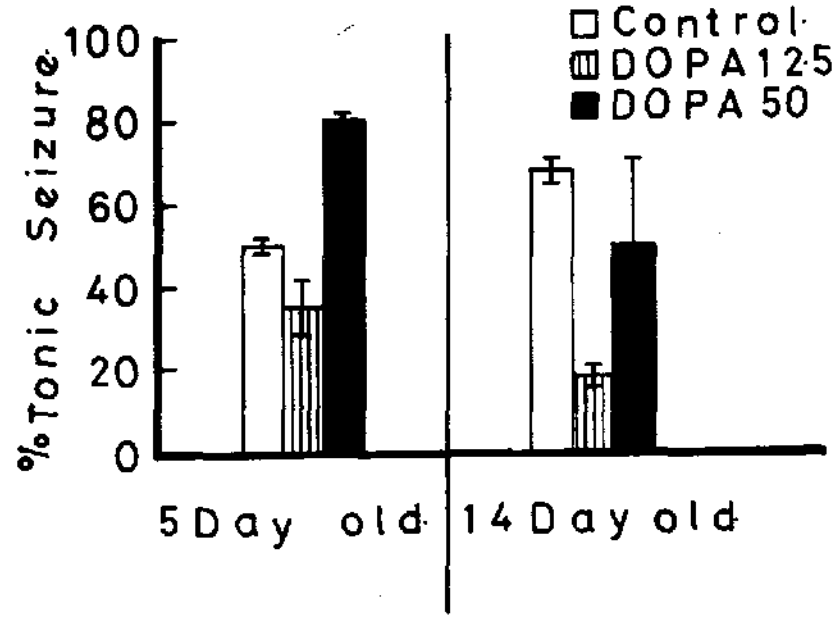



Fig. 4.6. Influence of age of chicks on the effects of L-dopa on their threshold to electroshock seizure.
The electroshock parameters used for 5 day old are different from those use for 14 day old chicks. Vertical bars represent standard deviations from the means (n = 30 per histogram).

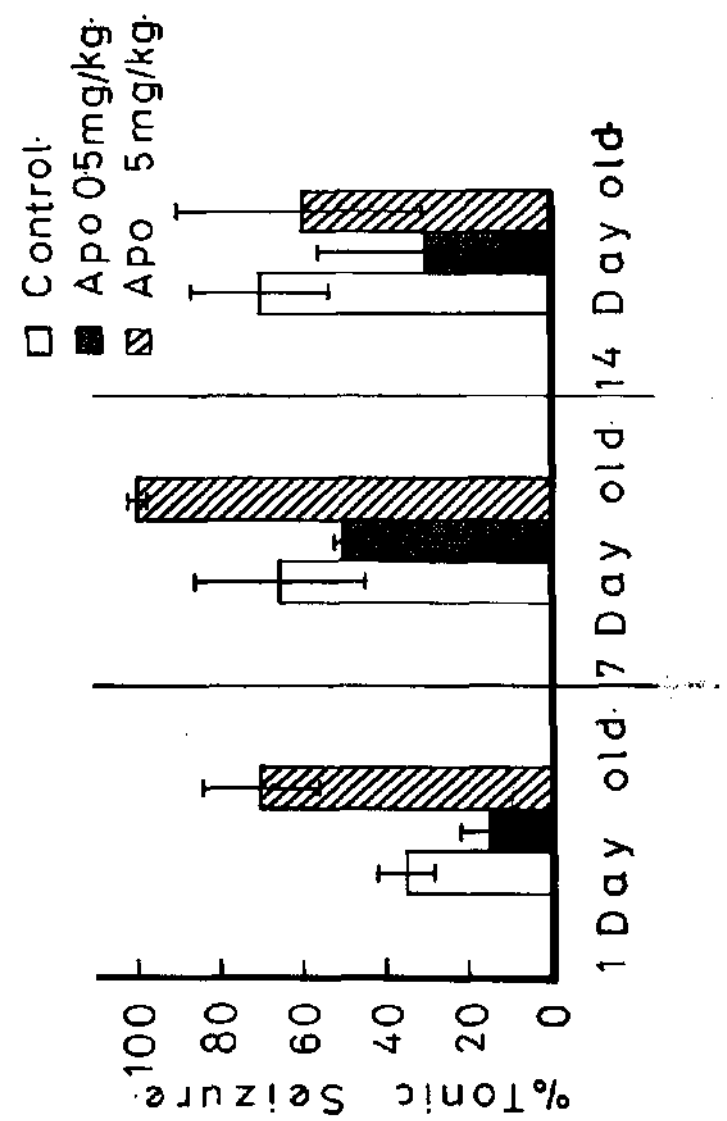



Fig. 4.7. Influence of age of chicks on the effects of apomorphine on their threshold to electroshock seizure. The electroshock parameters were varied according to the age of chicks (n = 30 per histogram).

4.4.3. Interaction With Reserpine.

Both the anticonvulsant activity of DA and its seizure-enhancing effect could still be demonstrated in reserpinized chicks (fig. 4.8). The seizure-potentiating influence of high doses of L-dopa (50 mg/kg.) was also demonstrated using reserpinized chicks (fig. 4.9).

4.4.4. Interaction With Pargyline.

Using pargyline-pretreated chicks, the anticonvulsant activity of low doses of L-dopa was increased while the effects of DA on seizure threshold was not significantly altered (figs. 4.10 & 4.11).

4.4.5. Interaction With FLA-63.

FLA-63 (12.5 mg/kg.) increased the effects of DA on seizure threshold but had no apparent influence on the effects of L-dopa on seizure threshold (4.12 & 4.13).

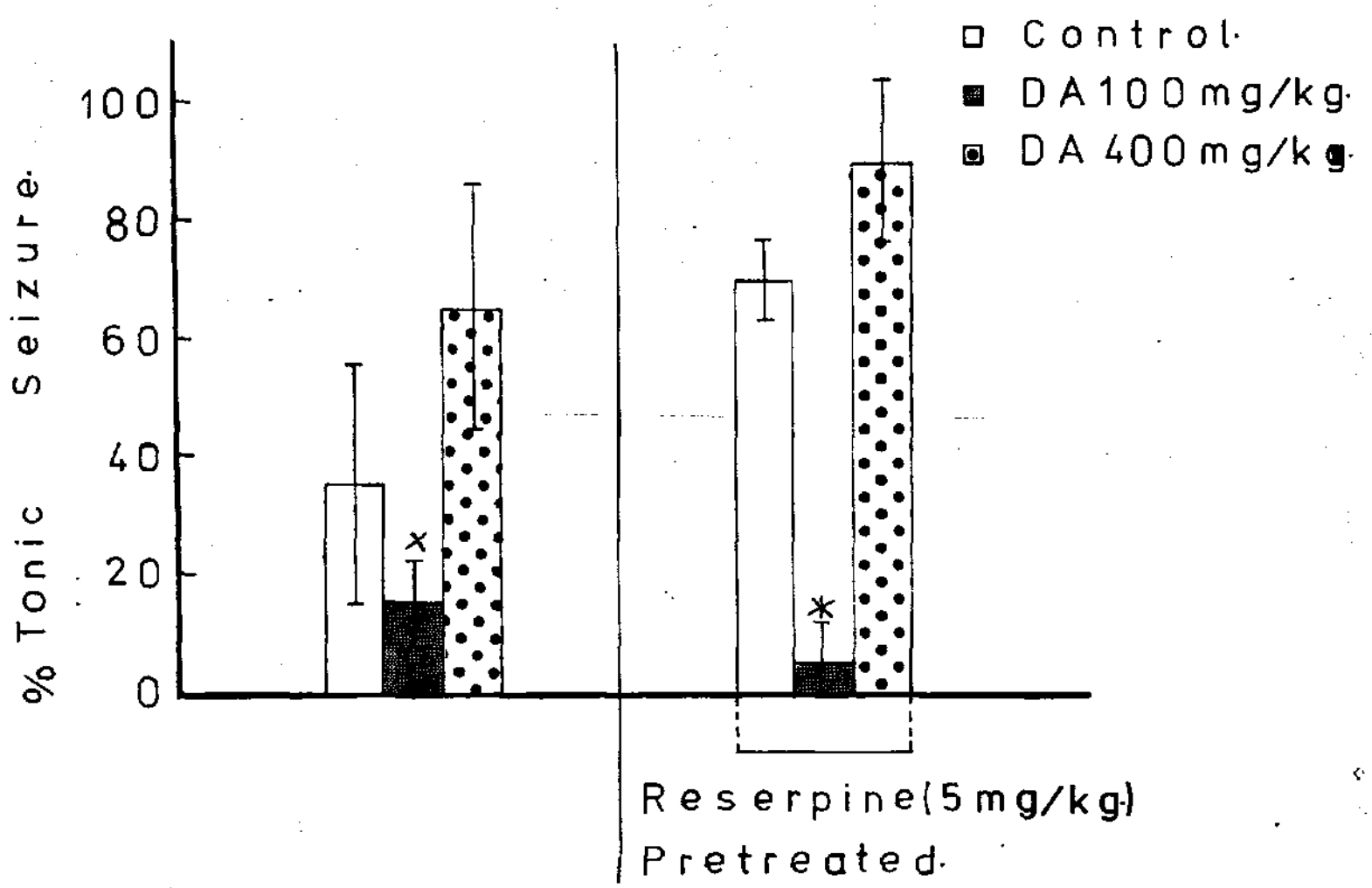



Fig. 4.8. Influence of reserpine on the effects of DA on electroshock seizure. 3 day old chicks were used (n = 40 per histogram). The electroshock parameters were constant for both reserpinized and non-reserpinized chicks. X = $P < 0.05$; * = $P < 0.005$, Chi-Squared test.

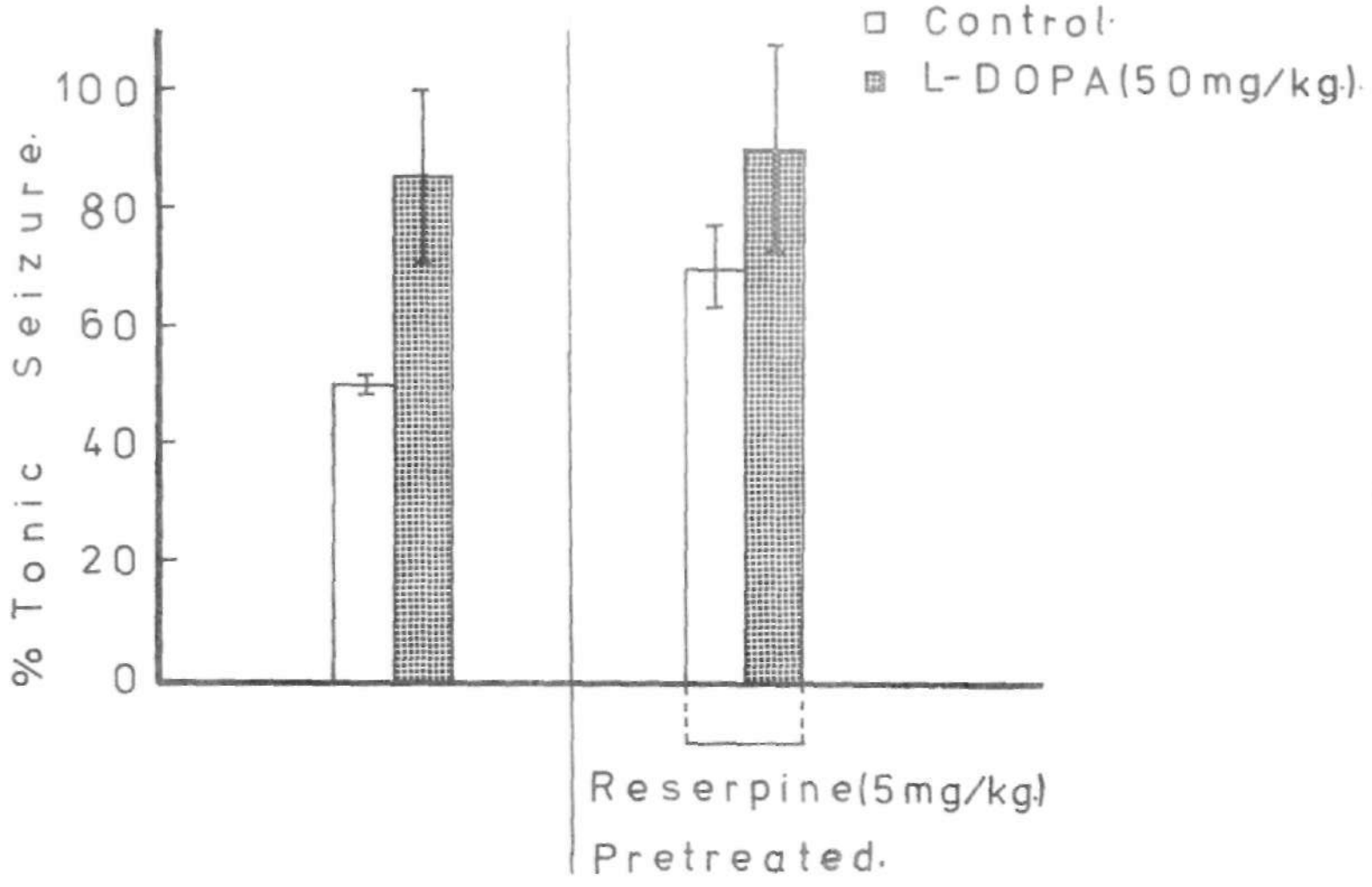



Fig. 4.9. Influence of reserpine on the effect of L-dopa on maximal electroshock seizure. 5 day old chicks were used. The electroshock parameters were the same for both the reserpinized and the non-reserpinized chicks. Vertical bars represent standard deviations from the means (n = 30 per histogram).

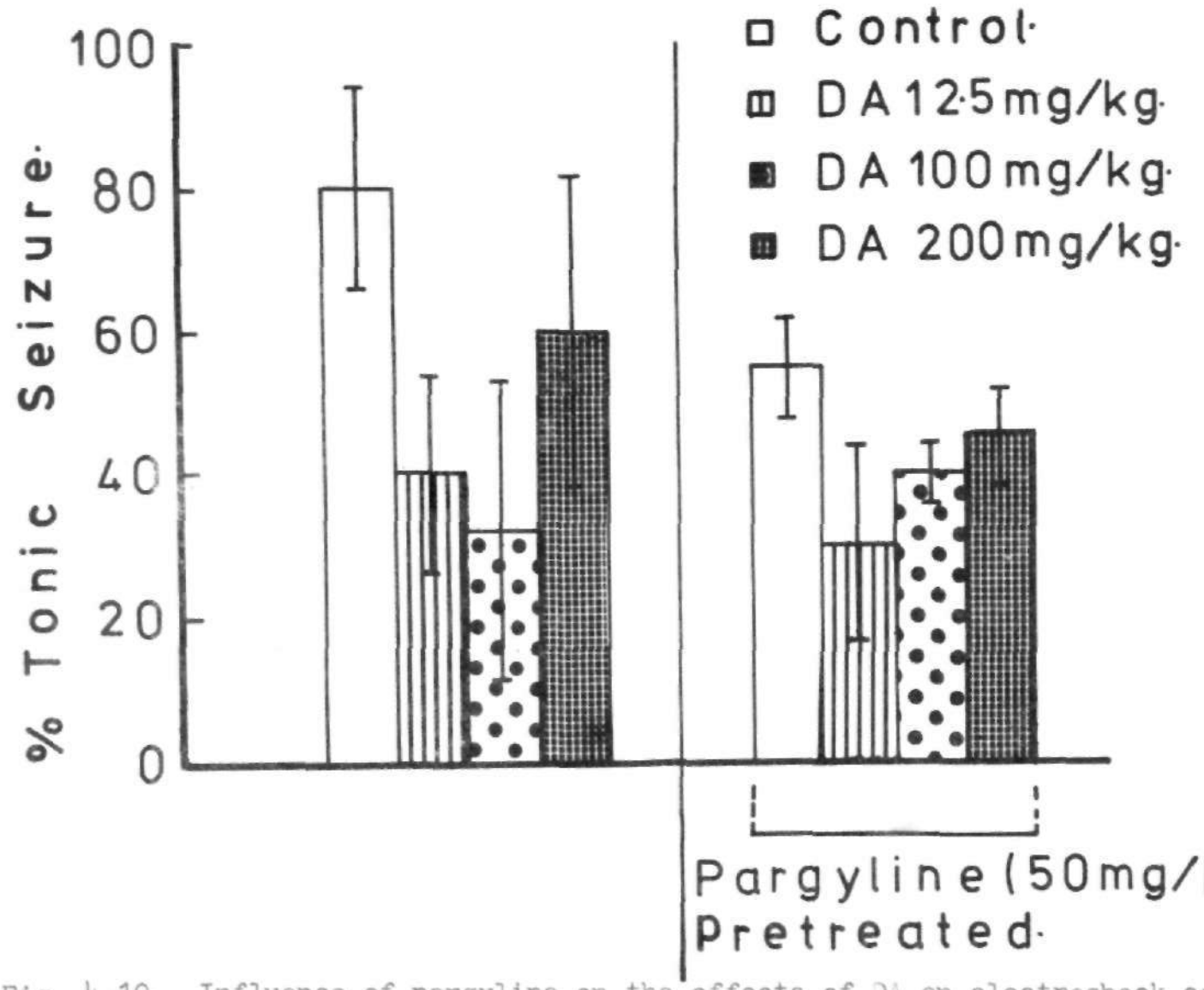



Fig. 4.10. Influence of pargyline on the effects of DA on electroshock seizure. 5 day old chicks were used (n = 30 per histogram). The electroshock parameters were kept constant for both the controls and pargyline pretreated chicks. X = $P < 0.05$.

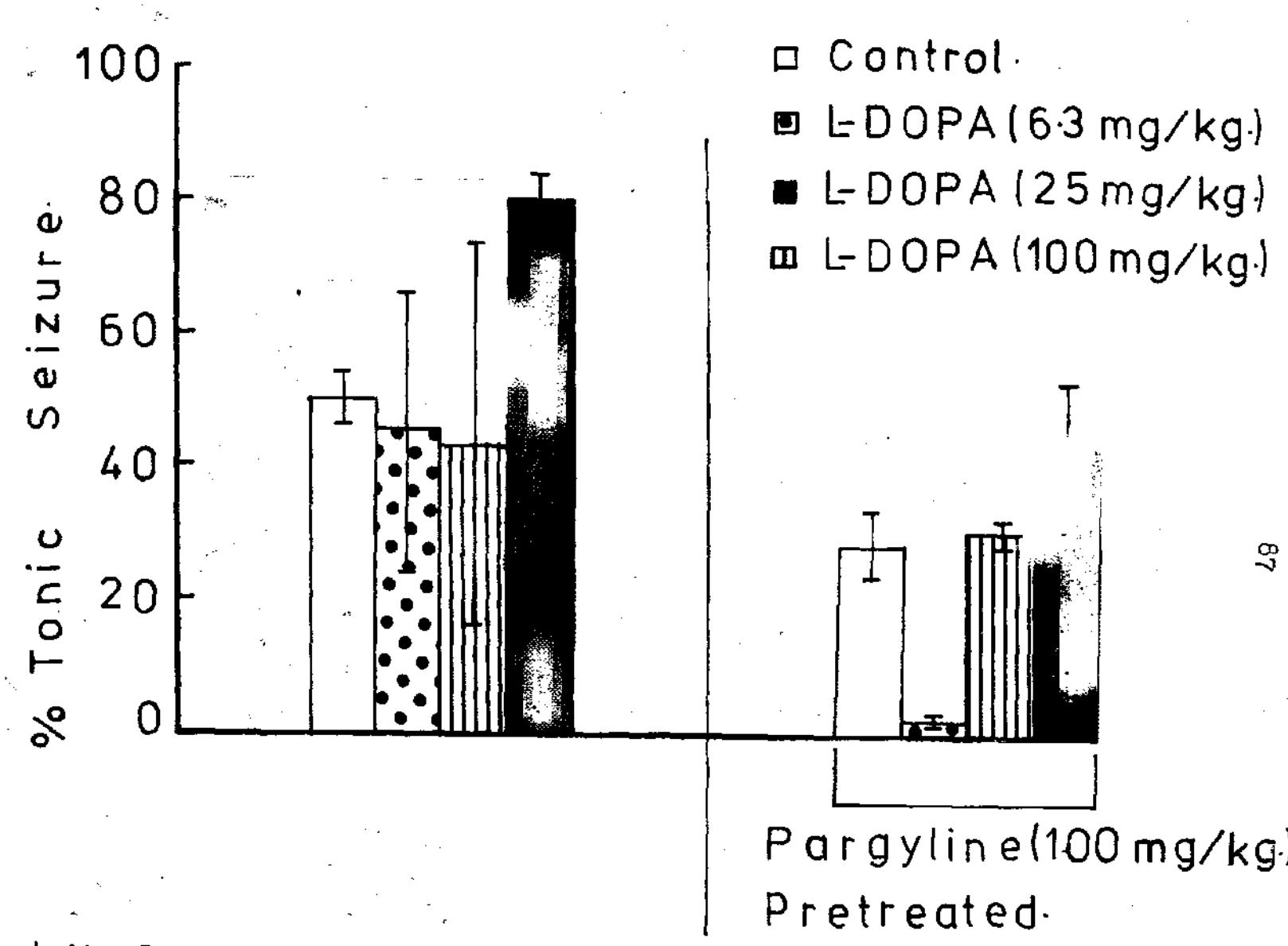



Fig. 4.11. Influence of pargyline on the effects of L-dopa on maximal electroshock seizure. 5 day old chicks were used (n = 30 per histogram). The electroshock parameters were the same for both the controls and pargyline-pretreated chicks.

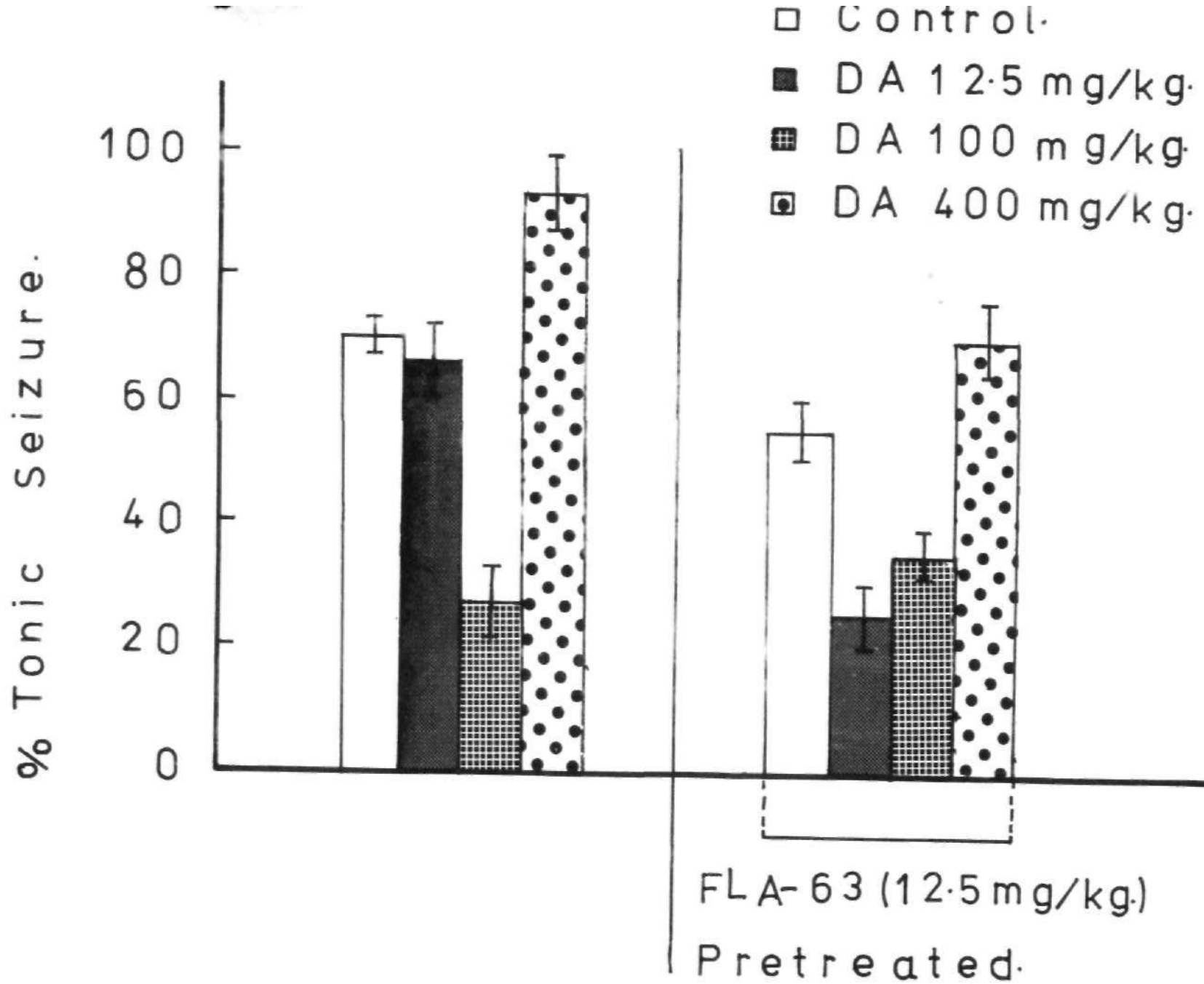



Fig. 4.12. Influence of FLA-63 on the effects of DA on maximal electroshock seizure. 5 day old chicks were used. The electroshock parameters were constant for both FLA-63-pretreated chicks and the untreated controls. (n = 30 per histogram).

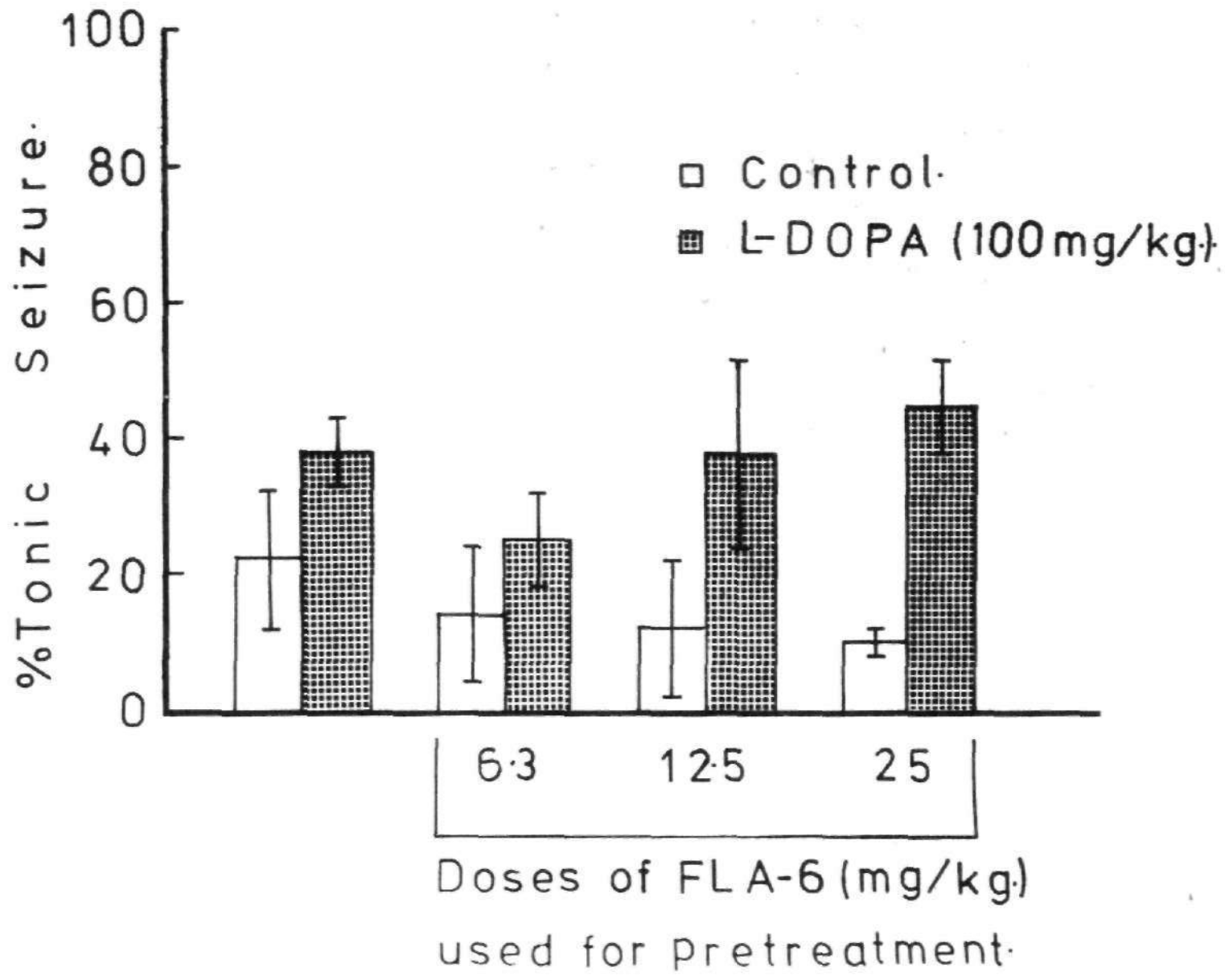



Fig. 4.13. Effect of FLA-63 on the action of L-dopa on electroshock seizure. 6 day old chicks were used. The electroshock parameters were constant for both FLA-63-pretreated chicks and the untreated controls (n = 30 per histogram).

4.4.6. Interaction With RO4-4602.

RO4-4602 (100 mg/kg.) did not influence the effect of L-dopa (100 mg/kg.) on seizure threshold (fig. 4.14).

4.4.7. Interaction With Dopaminoceptor Antagonists.

Both haloperidol (0.4 mg/kg.) and pimozide (4 mg/kg.) effectively antagonised the anticonvulsant effect of DA, L-dopa and apomorphine while the seizure-enhancing influence of both levodopa and dopamine were more resistant to antagonism by pimozide and haloperidol (fig. 4.15 - 4.20).

4.4.8. Effect of Noradrenaline on Seizure Threshold.

Noradrenaline (1 - 40 mg/kg.) slightly increased seizure activity (fig. 4.21). This influence decreased with dose.

4.4.9. Effect of an Intraperitoneally Injected DA on the Seizure Threshold of Rats and Adult Fowls.

DA (12.5 - 400 mg/kg.) did not alter the threshold of rats and adult cocks to electro-shock seizure (Table 4.1).

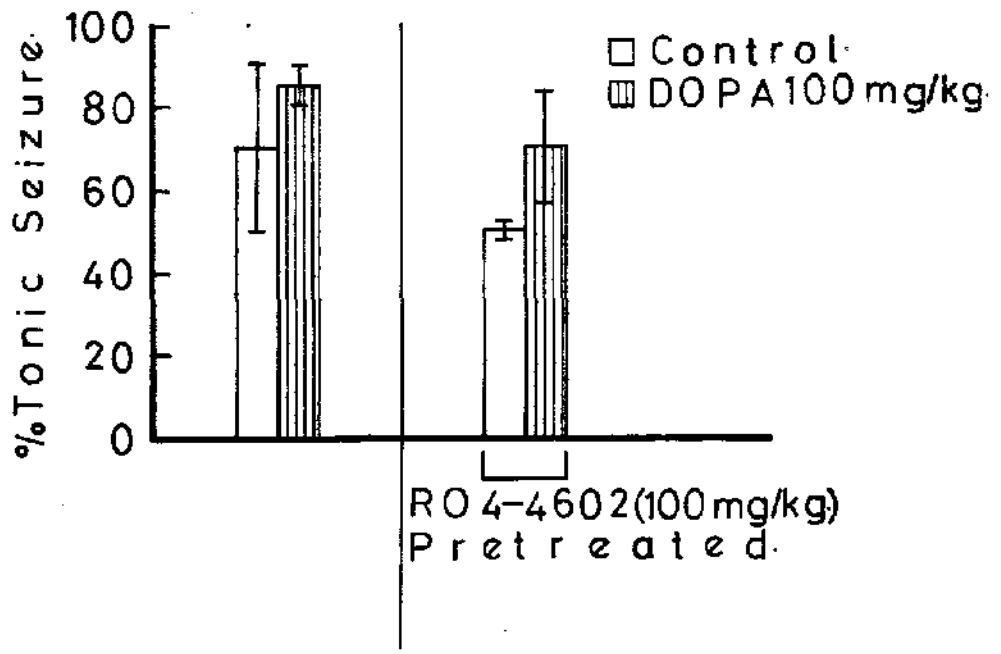



Fig. 4.14. Influence of RO4-4602 on the effect of L-dopa on maximal electroshock seizure. 8 day old chicks were used. The electroshock parameters were the same for both RO4-4602-pretreated chicks and the untreated controls. Vertical bars represent standard deviations from the means (n = 40 per histogram).

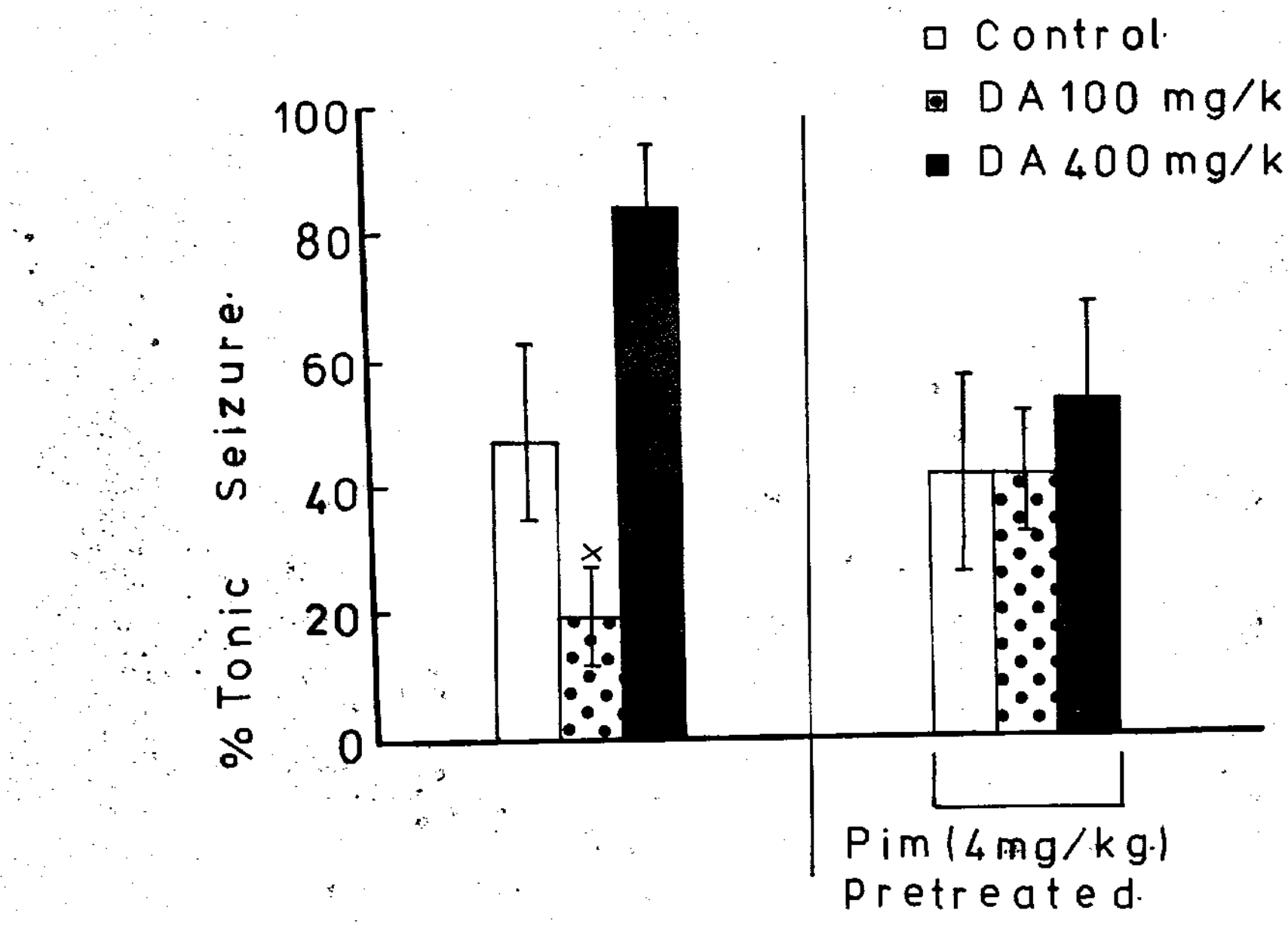



Fig. 4.15. Influence of pimozide on the effects of DA on electroshock seizure. 3 - 7 day old chicks were used (n = 40 per histogram). The electroshock parameters were the same for both the control chicks and those pretreated with pimozide. X = $P < 0.05$, Chi-Squared test.

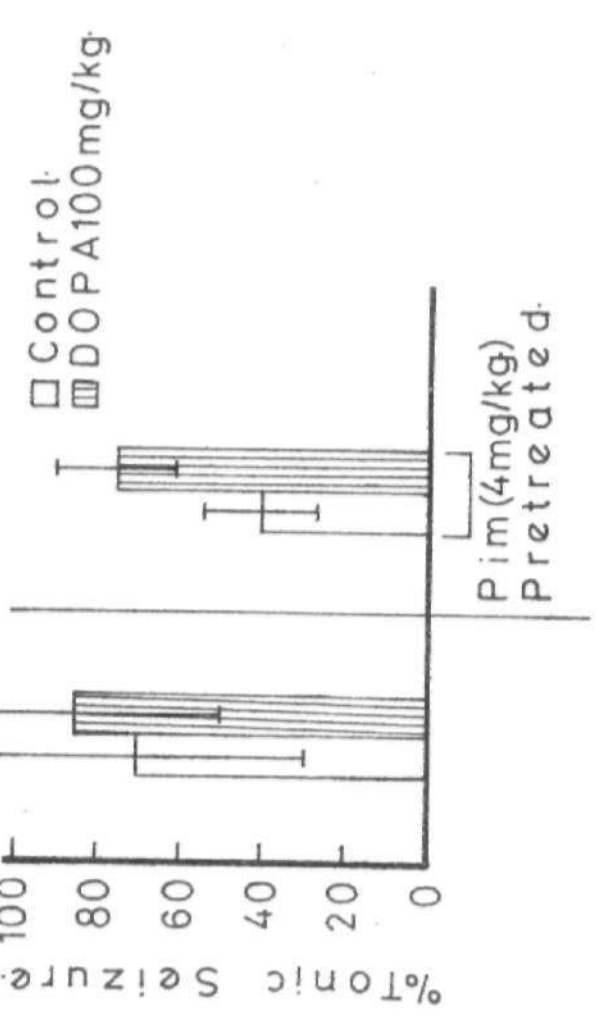



Fig. 4.16. Influence of pimozide on the effect of L-dopa on electroshock seizure. 4 day old chicks were used. The electroshock parameters were kept constant for one both the control and pimozide-pretreated chicks. Vertical bars represent standard deviations from the means (n = 30 per histogram).

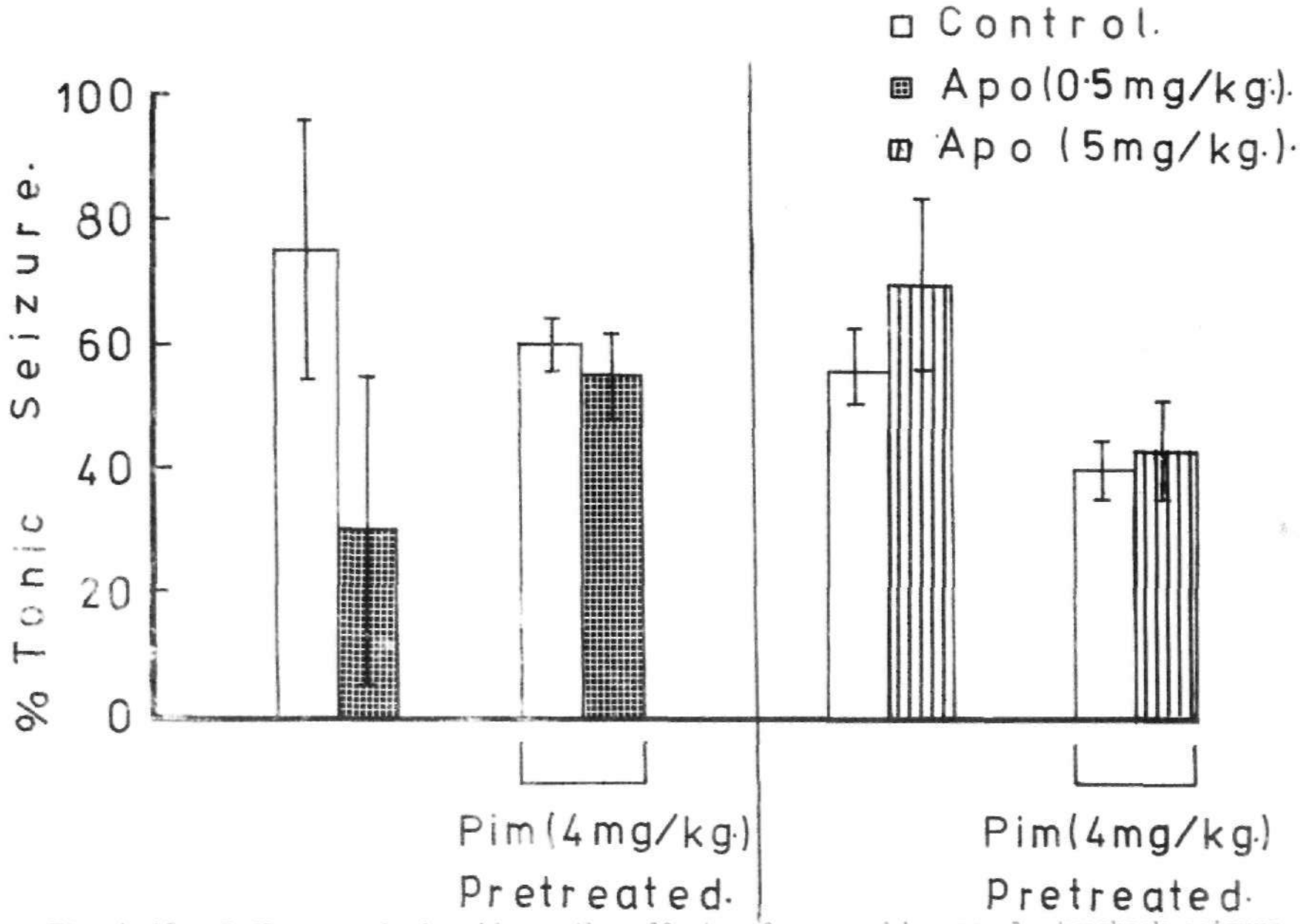



Fig. 4.16. Influence of pimozide on the effects of apomorphine on electroshock seizure. 3-7 day old chicks were used (n = 40 per histogram). The electroshock parameters were the same for both the control chicks and those pretreated with pimozide. The controls on the left-hand panel are different from those on the right-hand panel.

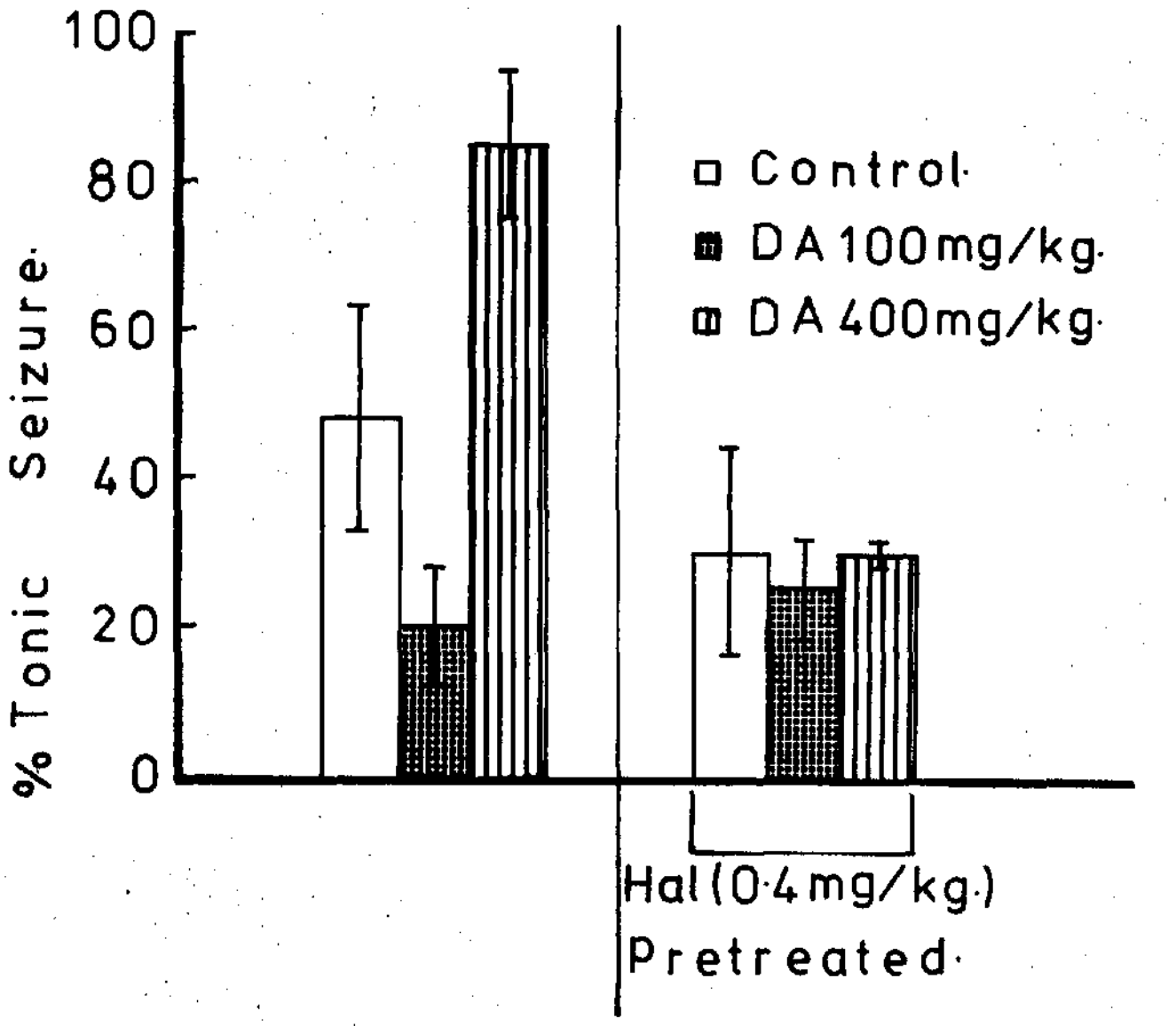



Fig. 4.17. Influence of haloperidol on the effects of DA on electroshock seizure.
3-7 day old chicks were used. The electroshock parameters were kept constant for both control and haloperidol-pretreated chicks (n = 30 per histogram). X = $P < 0.05$, Chi-Squared test.

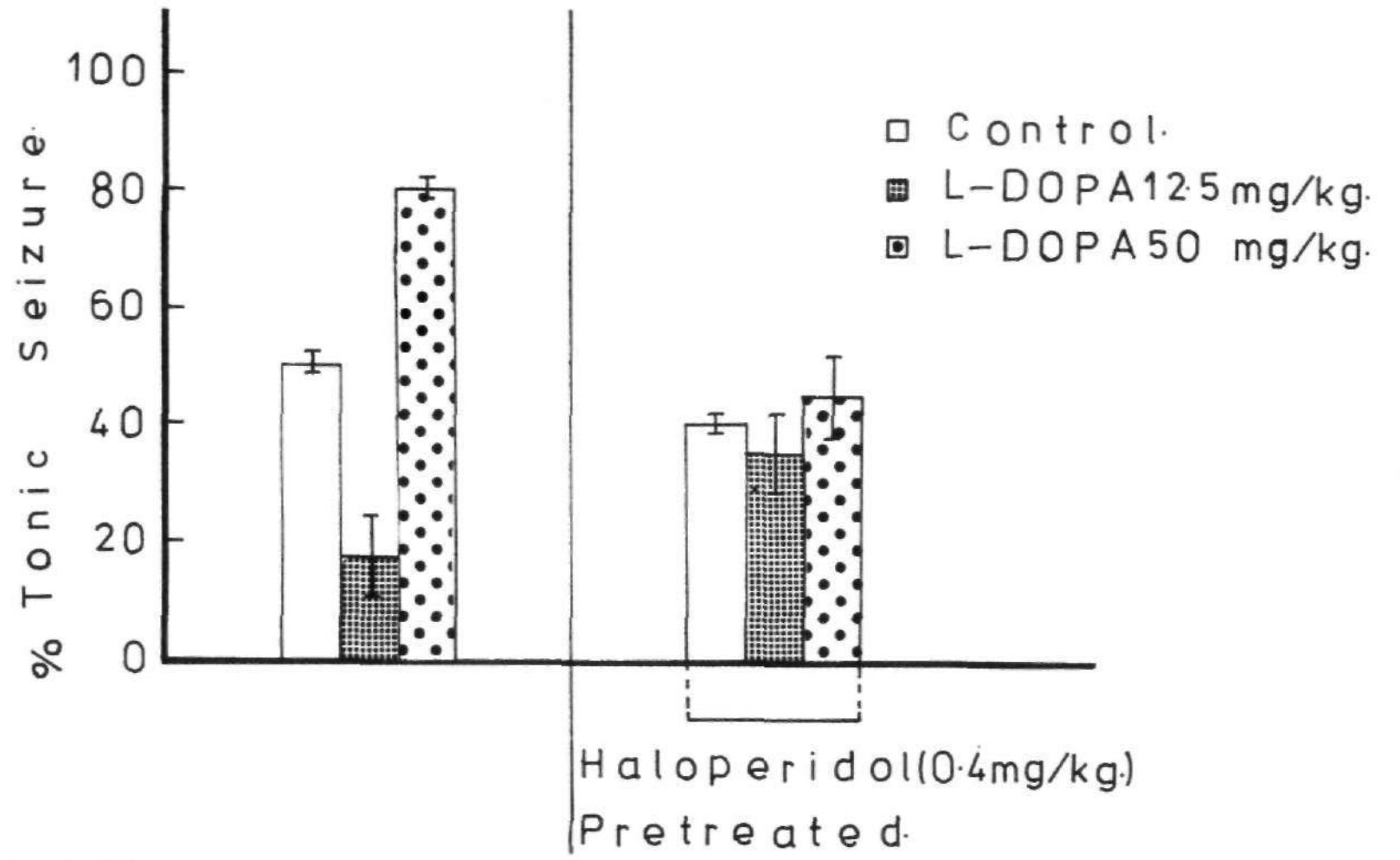



Fig. 4.19. Influence of haloperidol on the effects of L-dopa on electroshock seizure. 3 - 7 day old chicks were used. The electroshock parameters were kept constant for both control and haloperidol - pretreated chicks. Vertical bars represent standard deviations from the means (n = 30 per histogram).

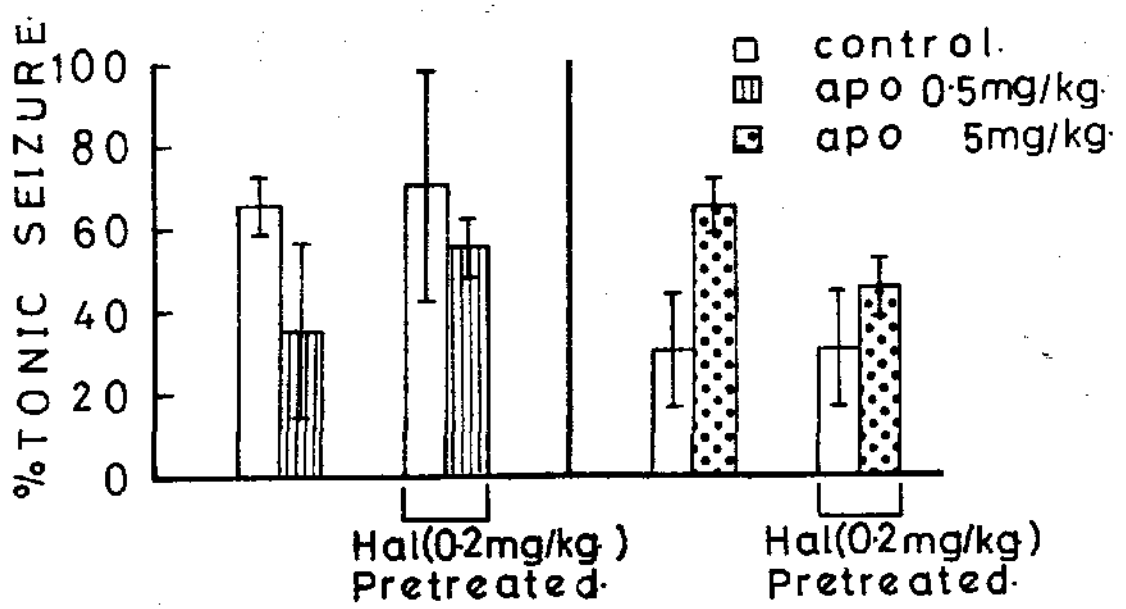



Fig. 4.19. Influence of haloperidol on the effects of apomorphine on electroshock seizure. 3 - 7 day old chicks were used. The electroshock parameters were kept constant for both control and haloperidol-pretreated chicks. Vertical bars represent standard deviations from the means (n = 30 per histogram). The controls on the left panel are different from those on the right-panel.

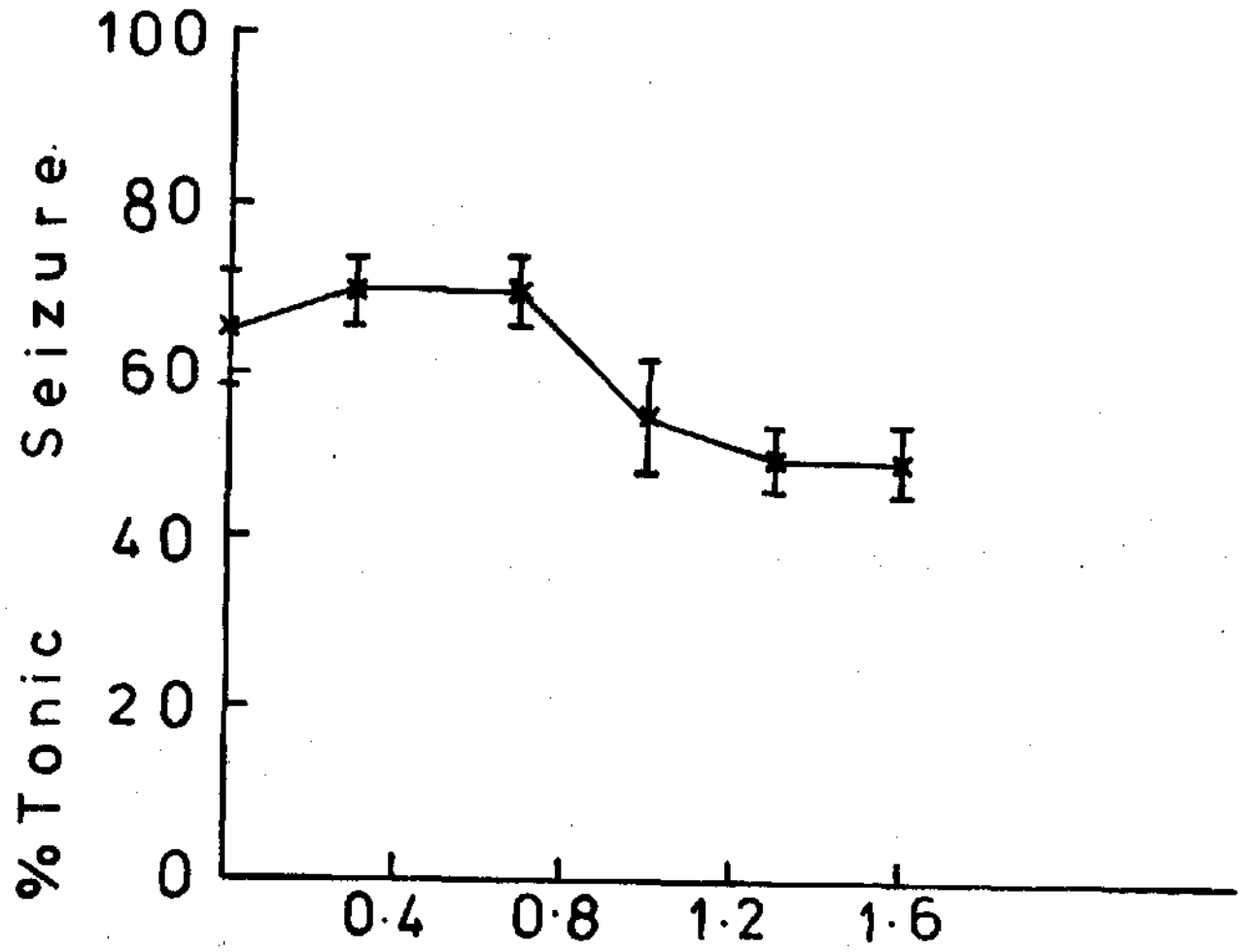



Fig. 4.21. $Log_{10}$ dose-response relationship of noradrenaline against electroshock seizure.
C = 48.0 ± 1.3. 3 day old chicks were used ( n = 30 per point).
Vertical bars represent standard deviations from the means.

| Animal species. | Dose (mg/kg. i.p.) | Number convulsed/ Number used. | Percentage tonic seizure. |
|---|---|---|---|
| Adult albino rats | 0 | 24/30 | 80 |
| | 12.5 | 16/20 | 80 |
| | 25 | 18/20 | 90 |
| | 50 | 14/20 | 70 |
| | 100 | 16/20 | 80 |
| | 200 | 17/20 | 85 |
| Adult cocks (Warren strain) | 0 | 2/10 | 20 |
| | 100 | 2/10 | 20 |
| | 400 | 2/10 | 20 |

Table 4.1. Effect of dopamine on electroshock seizure threshold using adult male albino rats and fowls (Warren strain).

## 4.5. Discussion.

DA, L-dopa and apomorphine exhibited biphasic effects on seizure threshold of 1 - 7 day old chicks. Thus, low to moderate doses of these dopaminoceptor agents significantly decreased the susceptibility of the chicks to electroshock seizure while higher doses increased seizure susceptibility. The results also show that most of the doses of DA, apomorphine and levodopa protected the chicks against electroshock seizure.

Brain monoamines have been implicated in seizure mechanisms in different mammalian species. For example, in 1962, De Schaepdryver et al studied the influence of selective and combined changes in the levels of brain amines on the electroshock threshold in the rabbit[9]. They observed that an increase of 5-HT and of both NA and 5-HT in the brain did not significantly influence the electroshock

threshold. The same authors reported a profound increase of electroshock threshold in presence of selectively increased DA levels in the brain. De Schaepdryver et al (1962) therefore concluded that changes in electroshock threshold are mediated through changes in brain DA levels. Similarly, McKenzie & Soroko (1972) attributed the anticonvulsant activity of L-dopa and apomorphine in rats to enhanced dopamine activity in the brain. More recently, Jobe et al (1974) compared the effects of various treatments on brain NA, DA and 5-HT on electroshock seizure. These workers accumulated data to indicate that both NA and DA exert central nervous system inhibitory effects which limit the spread of electroshock seizure discharge and consequently modulate the intensity of electroshock seizure. Also, Jobe et al (1973b) reported that, following various combination treatments which alter the synthesis, storage and release of catecholamines and/or 5-HT in brain tissue, there is a consistent correlation in rats between increased audiogenic seizure severity and drastically reduced levels of

NA and 5-HT, but not of DA. Thus, they concluded that endogenous NA and 5-HT play an attenuating role in audiogenic seizures in the rat. There are conflicting reports by many investigators indicating that in the rat, NA, DA and/or 5-HT may subserve a modulating function in electroshock seizure (Prockop, Shore & Brodie, 1959; Rudzik & Johnson, 1970; Stull, Jobe, Geiger & Ferguson, 1973). These conflicting reports suggest that it is not yet clear which of the amines are most directly involved in electroshock seizure mechanisms. The issue is further complicated by the fact that these workers could not study the direct effects of these amines in mammals since the monoamines do not cross the mammalian blood-brain barrier.

According to Waelsch (1955) and Key & Marley (1962), the young chick (1 day to 5 week old) lacks a functional blood-brain barrier to catecholamines. Thus, it has been possible to study the direct effects of DA on electroshock seizures using young chicks in this project.

The results suggest that DA is an important modulator of electroshock seizure in chicks while NA

had no significant effect on seizure threshold of chicks. The present data support the concept advanced by De Schaepdryver et al (1962) that changes in electroshock threshold in the rabbit might be mediated through changes in brain DA levels. The results obtained with rats and adult cocks, using an intraperitoneally injected DA,suggest that DA's effect on seizure threshold is mediated primarily via central mechanisms. The results also agree with those of McKenzie & Soroko (1972) who used L-dopa (DA precursor) against electroshock seizures in the rat and explained the anticonvulsant activity of L-dopa in the rat on the basis of L-dopa-induced enhancement of central DA neurotransmission.

It is also indicated from my data that the effects of dopaminoceptor agents on the seizure threshold of young chicks and some mammals might be quite similar. Such a harmonisation would permit a cautious extrapolation of results obtained with the chick to mammals. This argument is in conformity with the original idea of Spooner (1965) that the chick responds to neuropharmacological

agents in a manner similar to most mammals.

It is generally believed that reserpine depletes brain monoamines while pargyline elevates brain levels of monoamines (De Schaepdryver et al, 1962; Jobe et al, 1974). The effects of reserpine and pargyline on seizure threshold are shown in the next chapter. The seizure-enhancing effect of a high dose of DA and L-dopa in 1-7 day old chicks was not significantly affected by reserpinization while the protective activity of DA was potentiated by reserpine. The potentiation of the anticonvulsant activity of DA by reserpine might be due to supersensitivity of postsynaptic DA receptors resulting from the depletion of monoamines from the nerve endings. These results suggest that the biphasic effects of DA on seizure threshold may be due to direct action on DA receptors. As expected, pargyline potentiated the anticonvulsant activity of low doses of L-dopa and reduced the seizure-enhancing effect induced by high doses of L-dopa. Contrary to expectation, the anticonvulsant effect of DA was reduced by pargyline.

This result is anomalous. It is however possible that the activity of monoamine oxidase enzyme in chicks is low at this age (4 day old).

According to Corrodi, Fuxe, Hamberger & Ljungdahl (1970), FLA-63 inhibits dopamine-beta-hydroxylase enzyme, thereby elevating endogenous levels of brain DA while NA levels are drastically reduced. The results obtained with FLA-63 on seizure threshold are discussed in the next chapter. When FLA-63-pretreated chicks were used, the anticonvulsant property of low doses of DA was potentiated while high doses of DA (400 mg/kg.) still enhanced seizure activity. These results suggest that the biphasic effects of DA and levodopa on seizure threshold might not involve NA formed from the injected DA or L-dopa.

In 1966, Carlsson reported that RO4-4602 inhibits L-aromatic acid decarboxylase enzyme peripherally. In this study, RO4-4602 elevated the seizure threshold of young chicks. The effect might be attributed to the elevated brain DA resulting from the accumulation of endogenous levodopa in the brain after treatment with RO4-4602.

The seizure-enhancing effect of high doses of levodopa could still be demonstrated in chicks pretreated with RO4-4602. This observation suggests that the influence of levodopa on seizure threshold might be a central action rather than a peripheral effect.

The effect of DA, levodopa and apomorphine on seizure threshold is significantly influenced by the age of the chicks. Thus, using 1-7 day old chicks these dopaminoceptor agents produced a dose-dependent biphasic effect on seizure threshold (i.e. low doses reduced and high doses enhanced seizure susceptibility). On the other hand, low to high doses of DA (6.25-400 mg/kg.), levodopa (6.25-100 mg/kg.) and apomorphine (0.1-5 mg/kg.) protected 14 day old chicks against electroshock seizure. Thus, the seizure-enhancing influence of DA, levodopa and apomorphine decreased with age within the first week while the anticonvulsant action increased with age during this period. Similar decrease in the effectiveness of some convulsant agents in chicks

has also been reported during this period (Osuide, 1968; 1972b; Osuide & Wambebe, 1975). It can be speculated that the protective activity of DA against electroshock seizure depends partly or wholly on certain neuronal systems which are immature at the time of hatching. As the chick grew older, such neuronal system/s became more developed and thereby permit the mediation of the anticonvulsant activity of dopaminoceptor agonists. Infact, Cools & Van Rossum (1976) have suggested that, at birth, the DA excitatory systems in the mammal are more developed than the DA inhibitory mechanisms. It is possible that the neuronal developments in mammals and birds are generally similar. If part of the anticonvulsant activity of DA is due to enhancement of central inhibitory mechanisms, then such a protective action would become more prominent as the development of the inhibitory system progressed. It is also possible that the age-dependent effect of DA, levodopa and apomorphine on seizure threshold may be due to a gradual maturation of both the

inhibitory and excitatory systems in the chick - making the chick more resistant to seizures and DA more effective in protecting the chick against electroshock seizure.

In 1963, Carlsson & Lindqvist reported that haloperidol competitively blocked DA receptors when administered in low doses. More recently, pimozide has been found to be the most potent and specific DA receptor blocking agent (Andén et al, 1970). The present data show that both haloperidol and pimozide protected the chicks against electroshock seizure. This observation, notwithstanding, the effects of DA, levodopa and apomorphine on seizure threshold were effectively antagonised by both pimozide and haloperidol. The antagonism of the effects of DA, levodopa and apomorphine on seizure threshold by pimozide and haloperidol specifically implicates the involvement of DA receptors in the mediation of these actions.

According to Cools & Van Rossum (1976), there are two functional types of DA receptors in the feline caudate nucleus and the snail, Helix aspersa.

These workers used biochemical, histochemical, electrophysiological and pharmacological techniques to identify excitation-mediating DA receptors (i.e. DE) in the caput nuclei caudati rostro-medialis of the cat and inhibition-mediating DA receptors (i.e. DI) scattered throughout the caudate nucleus of the cat. In the light of this report, it can be explained that the anticonvulsant activity might be mediated via DI receptors while the seizure-enhancing effect of high doses of DA may involve activation of DE receptors or vice versa depending on the functions inhibited or excited by these two DA systems vis-a-vis seizure mechanisms.

In 1972, Kebabian, Petzold & Greengard observed that DA activated adenylate cyclase enzyme in the caudate nucleus of rat brain. They postulated that such DA - sensitive adenylate cyclase might constitute an integral component of brain DA receptors. It is therefore conceivable to ascribe DA-induced activities on seizure threshold to activation of DA - sensitive adenylate cyclase in

the hyperstriatum of the chick. Such an effect might inhibit the spread of nerve impulses in one of two ways:-

(a) Since the DA receptors in the basal ganglia subserve mainly inhibitory role in the extrapyramidal motor system, any increase in activity would enhance its inhibitory influence on the excitatory impulses cruising along the motor pathway. In essence, such an influence would effectively prevent the transmission of nerve impulses through the spinal cord to the motor end-plates of the limbs and neck muscles.

(b) It is also possible that the spread of nerve impulses in the cortex (hyperstriatum of chick) can be prevented by an inhibitory influence of projections from other brain regions to the neurons in the hyperstriatum. Such a hypothesis is compatible with the demonstration of DA and dopaminergic terminals in the cortex (Thierry, Blanc, Sobel, Stinus

& Glowinski, 1973; Hokfelt, Ljungdahl, Fuxe & Johnson, 1974). In addition, it has been reported that micro-iontophoretically applied DA depresses unit activity of cortical neurons (Krnjevic & Phillis, 1963). Possibly, single unit recordings and biochemical assays of various transmitters and their metabolites from different brain areas before, during and after seizures might help to elucidate the mechanisms involved in the effects of dopaminoceptor agonists on seizure threshold.

# CHAPTER V

## 5.1. THE INFLUENCE OF INTRAPERITONEALLY INJECTED 6-HYDROXYDOPAMINE ON ELECTROSHOCK SEIZURE IN CHICKS AND RATS.

## 5.2. Summary.

5.2.1. 6-Hydroxydopamine, injected intraperitoneally in rats and chicks, did not induce spontaneous seizures but produced significant alterations in the threshold to electroshock seizure in chicks; the particular effects were dose-dependent and time-dependent.

5.2.2. Administration of 6-hydroxydopamine once only to 3 day old chicks and once per day into rats in the first and third days after birth resulted in an increase in the proportion exhibiting tonic seizure with electroshock when tested after 10 - 12 weeks.

5.2.3. When 6-hydroxydopamine was injected intra-peritoneally into adult rats and cocks, there

was no significant alteration in seizure threshold.

5.2.4. The results suggest that 6-hydroxydopamine penetrates the central nervous system of young chicks and rats and that adrenergic mechanisms are probably involved in modulating seizure mechanisms in both the chick and rat.

5.3. Introduction.

Many workers have shown that 6-hydroxydopamine causes chemical sympathectomy in mammals (Porter, Totaro & Stone, 1963; Breese & Traylor, 1971; Bartholini, Pletscher & Richards, 1970; Thoenen, Haefely, Gey & Hurlimann, 1967). Brain monoamines have been implicated in seizure mechanisms in different mammalian species (De Schaepdryver et al, 1962; McKenzie & Soroko, 1972; Stull et al, 1977). According to Ungerstedt (1968) and Bloom, Algeri, Groppetti, Revuelta & Costa (1969),

6-hydroxydopamine consistently caused acute degeneration of central noradrenergic nerve terminals thereby causing a marked decrease in brain levels of dopamine and noradrenaline. These workers administered 6-hydroxydopamine directly into the brain because it does not cross the blood-brain barrier. In this project, 6-hydroxydopamine was injected intraperitoneally because the blood-brain barrier to catecholamines in chicks has been shown to be immature at birth (Waelsch, 1955; Key & Marley, 1962). Also, Karczmar (1963) reported that the rat's blood-brain barrier is immature at birth until about six days post-parturition. As a further elucidation of the possible involvement of catecholamines in seizure mechanisms, the influence of 6-hydroxy-dopamine on maximal electroshock seizure in rats and chicks was studied.

## 5.4. Results.

6-Hydroxydopamine produced time-dependent and dose-dependent biphasic effects on the

seizure threshold of young chicks (fig. 5.1). There was an initial anticonvulsant activity followed by a profound seizure-enhancing effect; the former effect was most prominent with low doses of 6-hydroxydopamine and decreased with increase in dose (fig. 5.2 - 5.4). On the other hand, the seizure-enhancing activity induced by 6-hydroxydopamine could be demonstrated in 10 week old cocks which were given a single intraperitoneal injection of 6-hydroxydopamine at the age of 2 days (fig. 5.5). Similarly, there was an enhancement of seizure susceptibility in 12 week old rats which were injected only twice intraperitoneally on the first and third days after birth with 200 micrograms per rat (fig. 5.5). On the other hand, when adult rats and cocks were injected intraperitoneally with 6-hydroxydopamine (20 mg/kg.), one day prior to electroshock delivery, their seizure threshold was not altered (fig. 5.6).

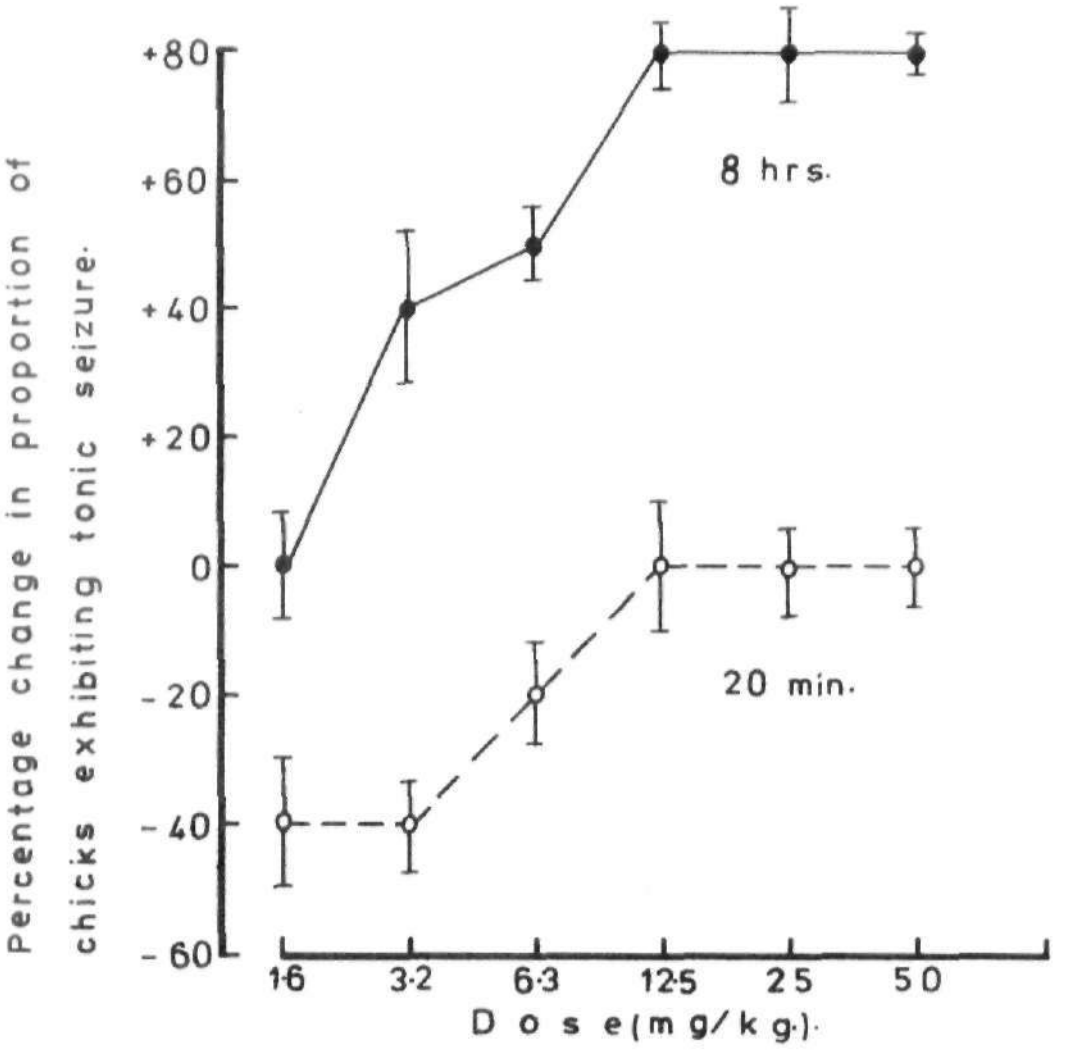



Fig. 5.1. Dose-response relationship of 6-hydroxydopamine on electroshock seizure in 4 day old chicks, 20 min. and 8hr. after intraperitoneal injection. Vertical barsrepresent standard deviations from the means (n = 30 per point).

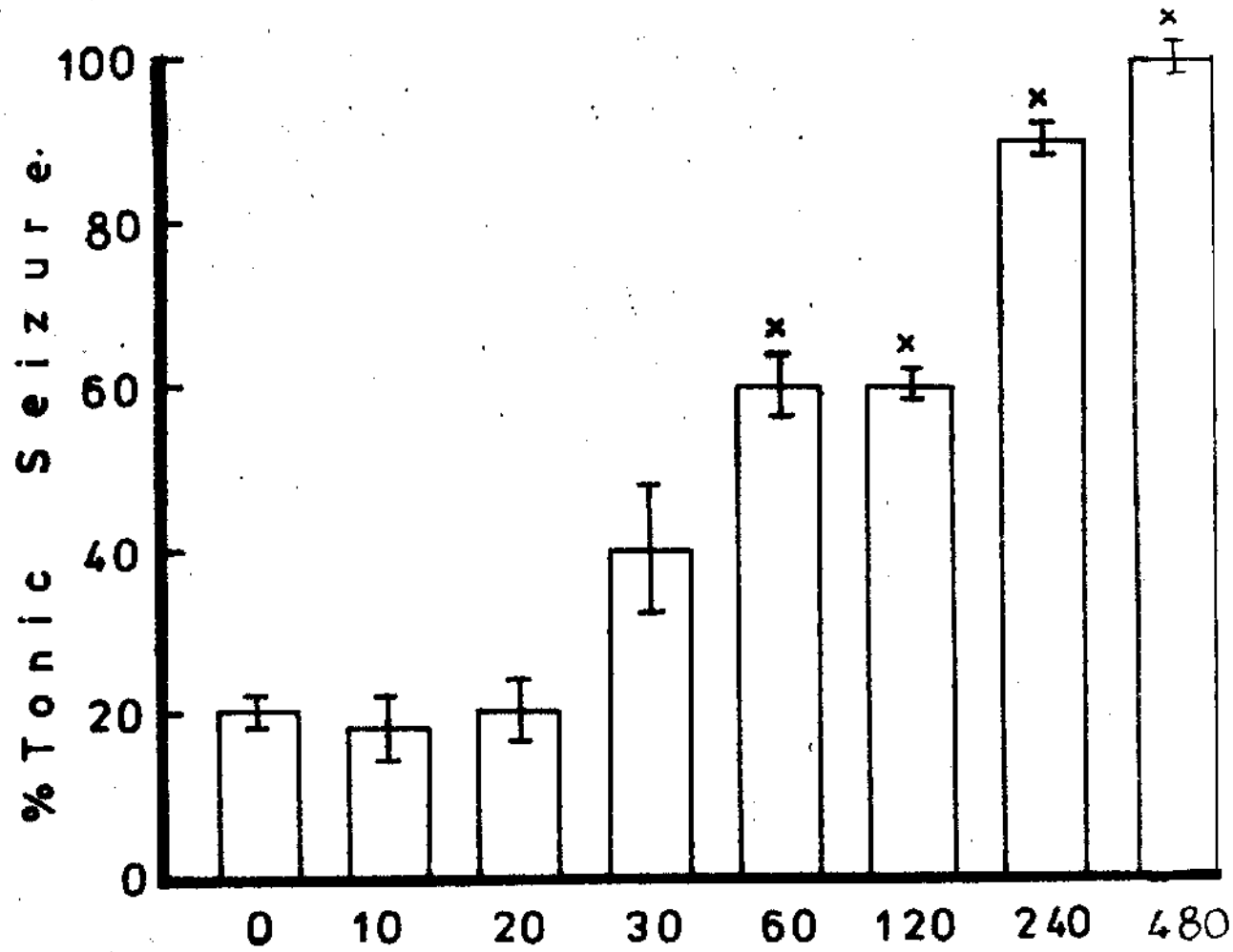



Fig. 5.2. Effect of 25 mg/kg. of 6 - CIDA injected intraperitoneally on electroshock seizure threshold using 4 day old chicks. n = 30 per histogram. x = p<0.001.

Fig. 5.3. Effects of 12.5 mg/kg. of 6-hydroxydopamine injected intraperitoneally on electroshock seizure threshold using 4 day old chicks. n = 30 per histogram. X = P 0.001, Chi-Squared test.




Fig. 5.4. Effect of 6-hydroxydopamine on electroshock seizure using chicks and rats. C = Control chicks and rats were 10 and 12 weeks old respectively (n = 30 per histogram). X = $P \leq 0.005$ (Chi-Squared test). 6-Hydroxydopamine was injected into 2 day old chicks while the rats were injected on the first and third days post-paturition.

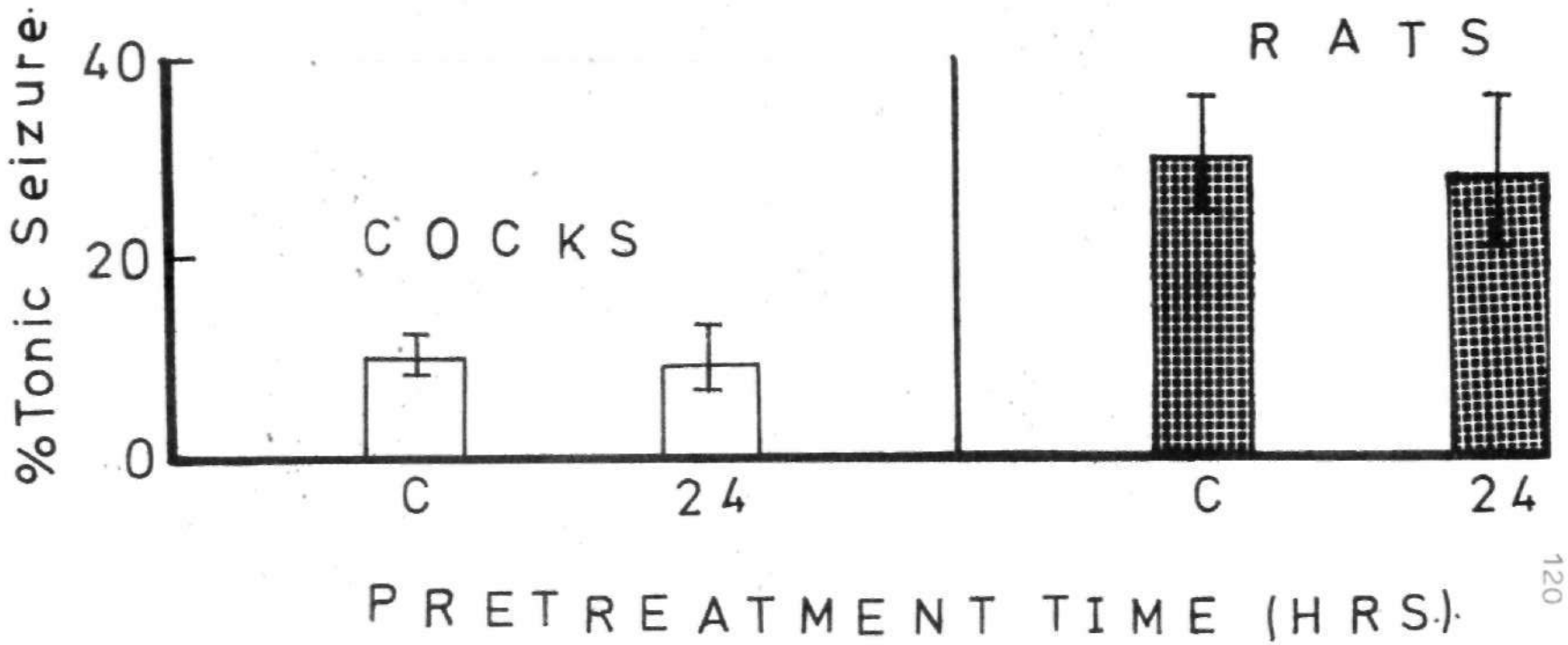



Fig. 5.6. Effect of 6-hydroxydopamine on electroshock seizure using adult fowls and rats. N = 20 per histogram. C = Control adult fowls and rats. Vertical bars represent standard deviations from the means.

## 5.5. Discussion.

The present study in rats and chicks shows that 6-hydroxydopamine injected intraperitoneally produced profound effects on seizure threshold. The effects varied according to dose and drug-pretreatment time prior to electroshock delivery. Thus, low doses initially protected chicks against electroshock seizure but later enhanced seizure activity. According to Laverty, Sharman & Vogt (1965) 6-hydroxydopamine initially releases catecholamines from sympathetic nerves before producing neuronal degeneration. It is therefore possible that the initial anticonvulsant property of low doses of 6-hydroxydopamine might be due to the released catecholamines. The anticonvulsant property of 6-hydroxydopamine has also been observed against post-decapitation convulsion and clonic phase of electroshock seizure by Suenaga, Yamada & Fukuda (1977). However, Suenaga et al (1977) did not report a subsequent increase in seizure intensity by 6-hydroxydopamine.

It is interesting that the initial anticonvulsant effect of 6-hydroxydopamine decreased significantly with dose. This observation may be due to a very rapid neuronal degeneration following the initial catecholamine release or it may be that at high doses the neuronal degeneration is not preceeded by catecholamine release.These results support the general concept that brain catecholamines may play an attenuating role in seizure mechanisms. For example, McKenzie & Soroko (1972) explained the anticonvulsant activity of levodopa in rats as due to enhancement of central dopamine mechanisms. Similarly, Stull et al (1977) reported that intracerebroventricular administration of RO4 - 1284, which depletes brain dopamine and noradrenaline, enhanced electroshock seizure intensity in the rat. The same workers (1977) observed that intracere-broventricular injection of dopamine or noradrenaline antagonised RO4 - 1284-induced seizure potentiation.

Administration of a single dose of 6-hydroxydopamine to young chicks and two doses to

young rats appeared to cause a permanent alteration in their threshold to electroshock seizure. For example, when chicks and rats pretreated with 6-hydroxydopamine were tested 10 weeks later, their threshold to electroshock seizure was profoundly reduced. Lehman (1977) similarly observed a decrease in the threshold of mice to audiogenic convulsion following an intraventricular administration of 6-hydroxydopamine. According to Karczmar (1963) the blood-brain barrier is present in newborn rats but inefficient when compared to adult rats. It is, therefore, probable that 6-hydroxydopamine injected intraperitoneally penetrated the incompletely formed blood-brain barrier of newborn rats and chicks to induce chemical sympathectomy. Thus, neuronal degeneration may explain the apparent permanent alteration of seizure threshold by 6-hydroxydopamine. This suggestion agrees with the report of Liew & Laverty (1975) that 6-hydroxydopamine injected intraperitoneally into newborn rats on days 1 and 2, after birth, caused a permanent depletion of noradrenaline in the cortex,

hippocampus and spinal cord.

The similarity between the effect of 6-hydroxydopamine in both the rat and chick might suggest that the mechanisms underlying seizure activities in these species are quite similar and involve adrenergic system. Studies involving drug-interactions between antidepressants and 6-hydroxydopamine coupled with biochemical assays of catecholamines in the brain may yield useful data which may improve our understanding of the mechanisms of 6-hydroxydopamine-induced alterations in seizure activity.

# CHAPTER VI

## 6.1. EFFECTS OF MISCELLANEOUS AGENTS ON ELECTROSHOCK SEIZURE THRESHOLD.

## 6.2. Summary.

6.2.1. D-amphetamine offered chicks a dose-dependent protection against electroshock seizure. This action was blocked by pimozide.

6.2.2. FLA-63 produced a time-dependent biphasic effect on electroshock seizure; an initial enhancement of seizure followed by an anticonvulsant effect.

6.2.3. DOPS produced significant time-dependent changes in the seizure threshold of chicks.

6.2.4. Both pargyline and nialamid significantly protected chicks against electroshock seizure.

6.2.5. Reserpine produced a time-dependent biphasic effect on seizure threshold of chicks.

6.2.6. Haloperidol protected chicks against electroshock seizure.

6.2.7. Pimozide produced a time-dependent biphasic effect on seizure threshold.

6.2.8. These results suggest that agents interferring with DA neurotransmission have significant effects on the threshold of chicks to electroshock seizure.

6.3. Introduction.

There is increasing evidence to support the hypothesis that the actions of amphetamine in the central nervous system are both indirect, being due to release or inhibition of uptake of DA at terminals of

the nigro-neostriatal pathway (Randrup & Munkvad, 1966; Stolk & Rech, 1970; Traylor & Snyder, 1971) and direct (Feltz & De Champlain, 1969). In 1972, McKenzie & Soroko suggested that the anticonvulsant activity of amphetamine in rats might be due to the enhancement of central dopaminergic transmission.

In 1970, Corrodi and his co-workers reported that FLA-63 is an inhibitor of dopamine-beta-hydroxylase enzyme and thus accumulates endogenous DA while brain NA levels are drastically reduced. On the other hand, 3, 4-dihydroxyphenylserine (DOPS) converts levodopa directly to NA without the intermediary - DA (Thorn & Ludvig, 1962). Such an agent reduces brain levels of DA and accumulates NA.

Both pimozide and haloperidol are used conventionally as DA receptor antagonists (Carlsson & Lindqvist, 1963; Janssen, 1967; Anden et al, 1970).

It is generally believed that reserpine depletes brain monoamines while pargyline and nialamid elevate brain levels of monoamines (De Schaepdryver et al, 1962).

All the agents mentioned above influence DA neurotransmission either directly or indirectly. To our knowledge, there is no report on the effects of these agents on the electroshock seizure threshold of chicks. It was therefore intended, in this project, to study the effects (if any) of amphetamine, FLA-63, DOPS, pargyline, nialamid, reserpine, haloperidol and pimozide on seizure threshold of chicks to electroshock

## 6.4. Results.

### 6.4.1. D-amphetamine (0.5 - 16 mg/kg.).

D-amphetamine protected the chicks against electroshock seizure. This effect was dose-dependent and statistically significant (fig. 6.1). The anticonvulsant effect of d-amphetamine was effectively antagonised by pimozide (Table 6.1).

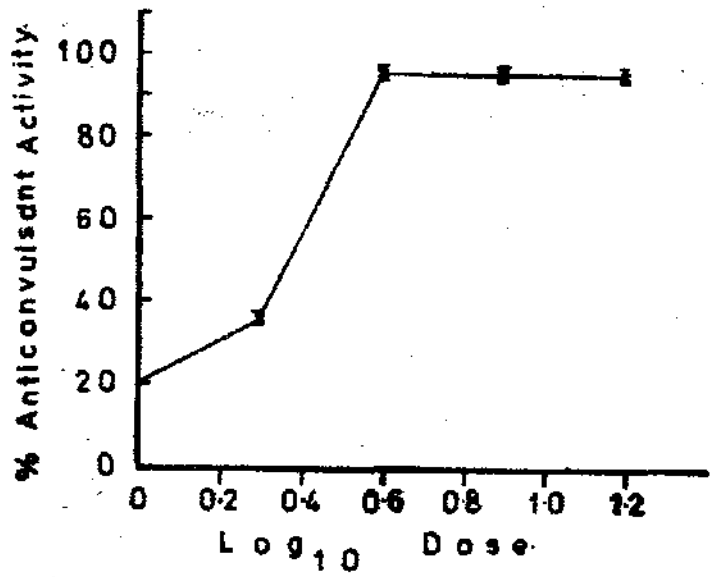



Fig. 6.1. $Log_{10}$ dose response relationship of amphetamine against electroshock seizure. 3 day old chicks were used. Vertical bars represent standard deviations from the means ( n = 20 per point). Statistical analysis was done using Chi-Squared test, $P<0.005$.

| Drug | Dose (mg/kg.) | No convulsed/ No used | % Tonic seizure | $P$ Value |
|---|---|---|---|---|
| 0 | 0 | 16/20 | 80 | - |
| D-amphetamine | 4 | 1/20 | 5 | $< 0.0001$ |
| Pimozide | 8 | 12/20 | 60 | NS |
| Pimozide + D-amphetamine | 8<br>4 | 8/20 | 40 | NS |

Table 6.1. Antagonism of the anticonvulsant action of d-amphetamine by pimozide. NS = non-significant. Chi-Squared test was used to analyse the results (with Yate's correction).

6.4.2. FLA-63 (6.3 - 50 mg/kg.).

The effects of FLA-63 on the incidence of tonic seizure was time-dependent; an initial increase in the incidence of seizure activity (maximal at 30 min. after injection) followed by a decrease in the incidence of tonic seizure (4-8 hours after injection). Both actions were statistically significant (fig. 6.2).

6.4.3. DOPS (1.25 - 10 mg/kg.).

After 1 hour of injection of DOPS, the seizure activity was slightly increased. In addition, there was a profound decrease in seizure activity, 24 hours after injection. This effect was dose-dependent and highly significant (fig.6.3).

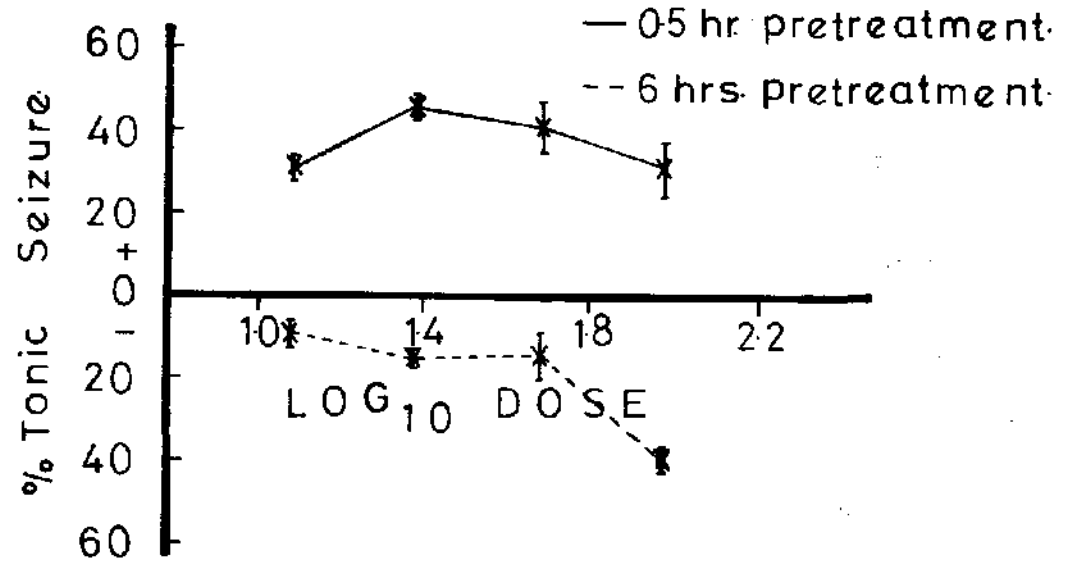



Fig. 6.2. $Log_{10}$ dose-response relationship of FLA-63 against electroshock seizure. 4 day old chicks were used. Vertical bars represent standard deviations from the means ( n = 20 per point). Chi-Squared test was used to analyse the results, $P < 0.05$ for the biphasic effects.

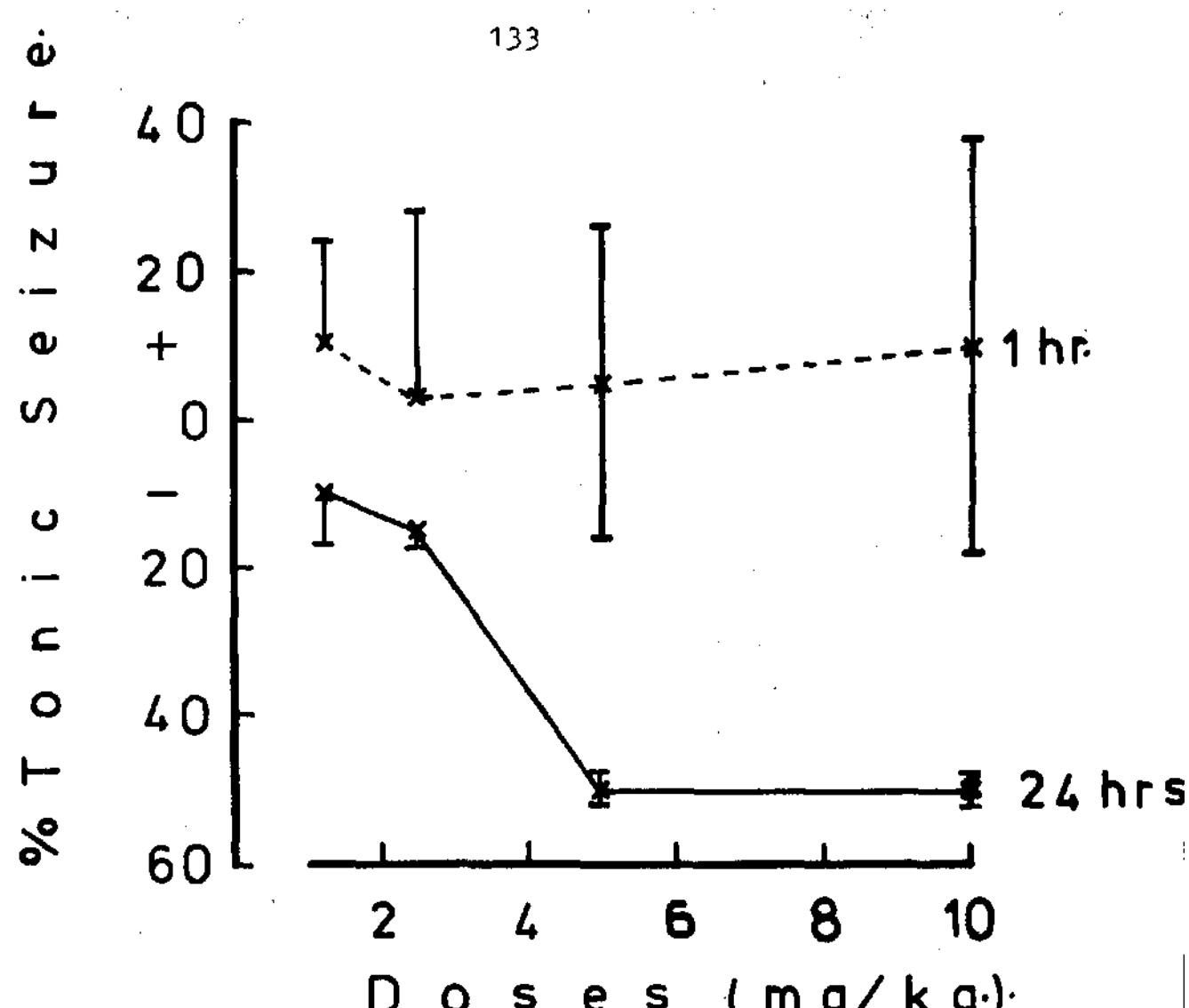



Fig. 6.3. Dose-response relationship of DOPS against electroshock seizure. 4-6 day old chicks were used (n = 20 per point). Vertical bars represent standard deviations from the means. $P<0.001$ (Chi-Squared test) for its anticonvulsant effect while the seizure-enhancing influence was nonsignificant.

6.4.4. Pargyline (25-200 mg/kg.) and Nialamid (250-800 mg/kg.).

Both pargyline and nialamid protected the chicks against electroshock seizure in a dose-dependent manner (Tables 6.2 & 6.3).

6.4.5. Reserpine (2.5 - 10 mg/kg.).

After 4 hours of injection of low doses of reserpine (2.5 - 5 mg/kg.) there was a decrease in seizure activity. This initial effect was not observed with high doses (7.5 - 10 mg/kg.). All the doses used in this work increased the incidence of seizure activity after 21 hours pretreatment time (fig. 6.4).

| Doses (mg/kg.) | No convulsed/ No used | % Tonic seizure | $p$ Value. |
|---|---|---|---|
| 0 | 22/30 | 73 | - |
| 25 | 22/30 | 73 | NS |
| 50 | 22/30 | 73 | NS |
| 100 | 20/30 | 67 | NS |
| 200 | 20/30 | 67 | NS |
| 300 | 21/30 | 70 | NS |
| 400 | 9/30 | 30 | $<$ 0.001 |
| 800 | 0/30 | 00 | $<$ 0.0001 |

Table 6.2. Dose-dependent anticonvulsant action of nialamid against electroshock seizure in young chicks.

Pretreatment time prior to electroshock delivery was 12 hours.

NS = non-significant.

Statistical analysis was done using Chi-Squared test (with Yate's correction).

| Doses g/kg.) | No convulsed/ No used | % Tonic seizure | *P* Value. |
|---|---|---|---|
| 0 | 26/40 | 65 | - |
| 25 | 22/40 | 55 | NS |
| 50 | 10/40 | 25 | $< 0.05$ |
| 100 | 10/40 | 25 | $< 0.05$ |
| 200 | 16/40 | 40 | $< 0.05$ |

Table 6.3. Dose-dependent anticonvulsant action of pargyline against maximal electroshock seizure in young chicks.
Pretreatment time prior to electroshock delivery was 3 hours.
NS = non-significant.
Statistical analysis was done using Chi-Squared test (with Yate's correction).

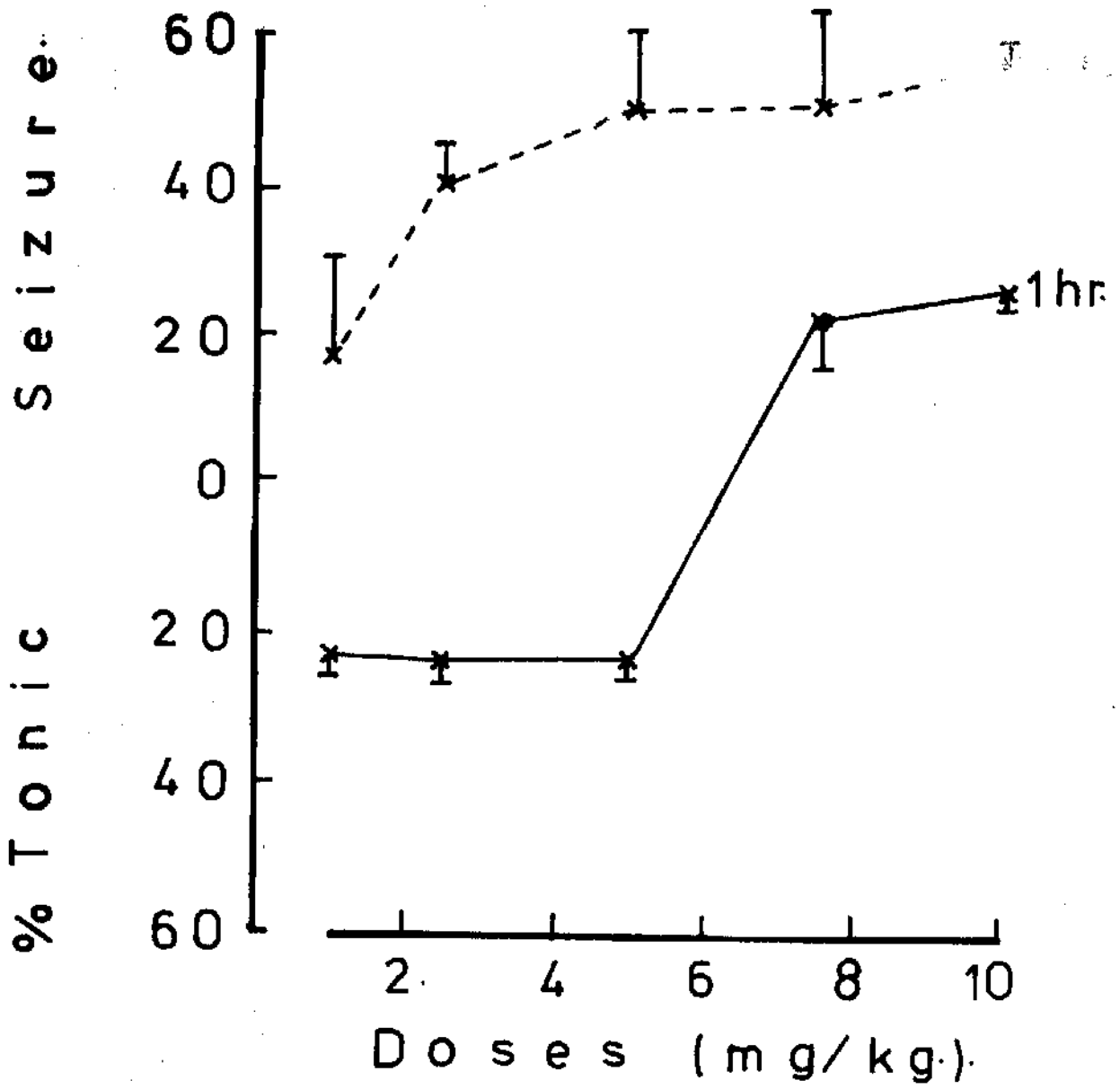



Fig. 6.6. Dose-response relationship of reserpine against electroshock seizure. 5 day old chicks were used (n = 20 per point). $P < 0.005$ (Chi-Squared test) for the seizure-enhancing effect while its anticonvulsant effect was non-significant. Vertical bars represent standard deviations from the means.

6.4.6. Pimozide (2 - 16 mg/kg.).

Pimozide produced a time-dependent biphasic effect on seizure threshold. There was an initial enhancement of seizure activity which decreased with dose. After 4 hours of drug pretreatment, pimozide protected the chicks against electroshock seizure. The anticonvulsant activity of pimozide was highly significant and increased with dose (fig. 6.5).

6.4.7. Haloperidol (0.1 - 10 mg/kg.).

Haloperidol protected the chicks against seizure activity, the drug-pretreatment time notwithstanding. The anticonvulsant activity of haloperidol was highly significant and increased with dose (fig. 6.6).

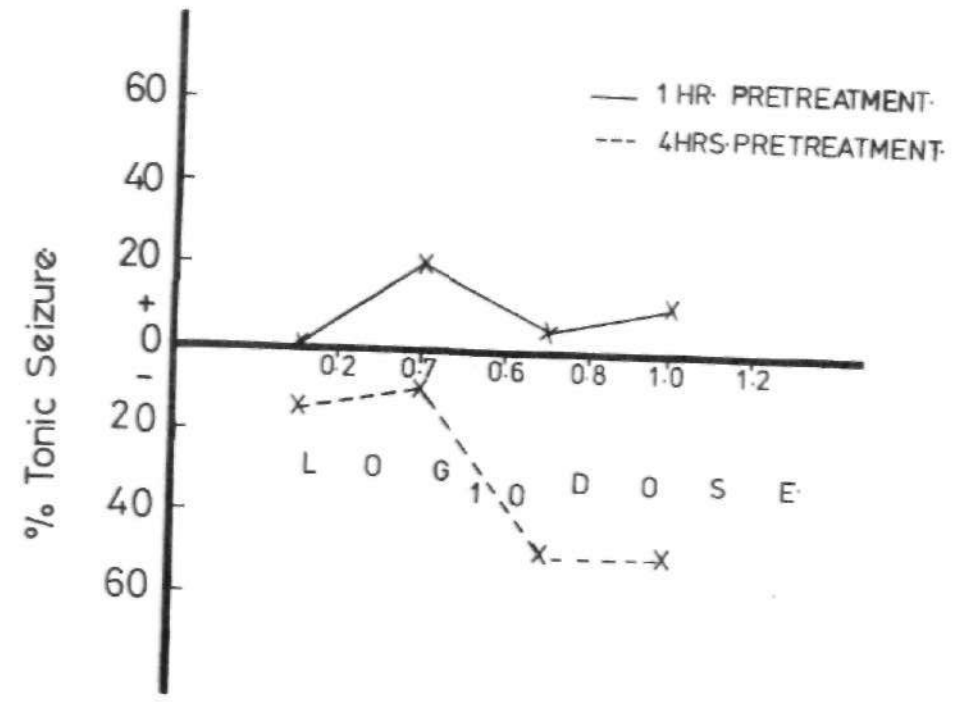



Fig. 6.7. $Log_{10}$ dose-response relationship of pimozide against electroshock seizure.
5 day old chicks were used. Vertical bars represent standard deviations from the means ( n = 20 per point). Chi-Squared test was used to analyse the results, $P < 0.05$ for the anticonvulsant effect while the initial seizure-enhancing effect was non-significant.

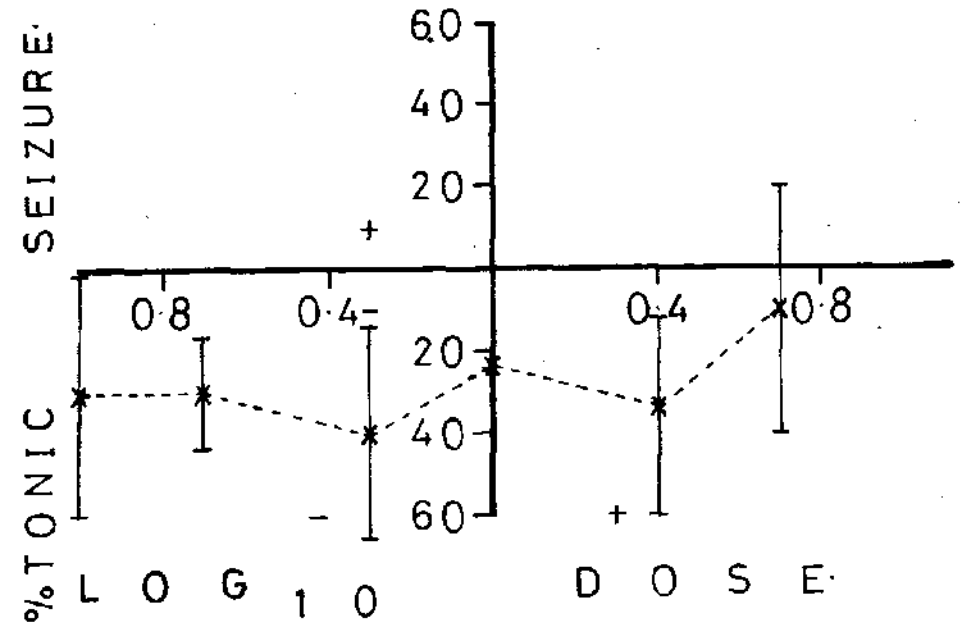



Fig. 6.8. $Log_{10}$ dose-response relationship of haloperidol against electroshock seizure. 5 day old chicks were used. Drug pretreatment time prior to electroshock delivery was 1 hr. Vertical bars represent standard deviations from the means (n = 20 per point), $P < 0.005$, Chi-Squared test.

## 6.5. Discussion.

The results obtained with d-amphetamine show that it is a potent anticonvulsant agent. This observation agrees with the report by McKenzie & Soroko (1972) that d-amphetamine protected rats against electroshock seizure. This similarity indicates that the mechanisms underlying seizure activity in both the chick and the rat may be quite similar. The antagonism of the anticonvulsant action of d-amphetamine by pimozide implicates the involvement of DA receptors in the mediation of this seizure protection. It is possible that d-amphetamine produced its action via direct activation of DA receptors or indirectly by increasing the availability of DA at its receptor sites or both. The mechanism of action of d-amphetamine on seizure threshold will be further elucidated when neurochemical studies are done.

The initial enhancement of seizure activity induced by FLA-63 may be unrelated to its inhibition of dopamine-beta-hydroxylase enzyme. In view of the rapid onset of action (30 min.), which may be too short for effective enzyme inhibition, it is suggested that the increase in seizure activity induced by FLA-63 may be due to its own direct effect or its interference with other neuronal system/s rather than its conventional action on dopamine-beta-hydroxylase enzyme. On the other hand, the protective action of FLA-63 observed 4 hours after pretreatment may be attributed to elevated brain DA resulting from the inhibition of dopamine-beta-hydroxylase enzyme. This proposal is compatible with the results obtained with some dopaminoceptor agonists (cf. chapter IV). Biochemical assays of brain monoamines after administration of FLA-63 may help to

explain the mechanisms underlying the biphasic effects of FLA-63 on seizure threshold.

The initial decrease in seizure threshold observed with DOPS is non-significant and may be due to normal variations between experiments. On the other hand, this effect may be due to the direct action of DOPS on some neuronal systems involved in seizure mechanisms. It is, however, interesting to note that 8 hours after the injection of DOPS, the seizure activity was profoundly decreased (peak effect was at 24 hours post-injection). This anticonvulsant effect is most probably due to NA which accumulated in the brain as a result of DOPS. It will be necessary to conduct neurochemical studies to confirm (or otherwise) the above proposal.

The results show that both pargyline and nialamid protected the chicks against electroshock seizure. This protection may be attributed to the increased brain levels of monoamines. This hypothesis is compatible

with the generally accepted concept that both pargyline and nialamid inhibit monoamine oxidase enzyme thereby elevating endogenous concentrations of monoamines. However, this assertion is based upon results obtained with mammalian species, it will therefore be relevant to conduct neurochemical studies after administration of either pargyline or nialamid to chicks.

The initial decrease in seizure activity obtained with low doses of reserpine (1.5 - 2.5 mg/kg.) may be attributed to the initial increase in available monoamines at their receptive sites induced by reserpine resulting from the inhibition of reuptake mechanism. Some of the available monoamines may escape the destructive effect of MAO and thereby produce the anticonvulsant effect. This initial action was latter followed by an increase in seizure activity which may be due to a decrease in the level of brain monoamines resulting from their depletion by reserpine. When higher doses of reserpine were used (7.5 - 10 mg/kg.), there was no biphasic effect - only a

profound increase in seizure activity was obtained. It is possible that the rate of depletion of monoamines with such high doses of reserpine was too rapid for any initial increase in available monoamines to be observed. These speculations need neurochemical support to justify their validity.

Pimozide, an antipsychotic agent, is an established, highly potent and specific antagonist of DA receptors (Anden et al, 1970). The initial enhancement of seizure activity by pimozide might be due to its own action which might not be related to the blockade of dopamine receptors. It is equally possible that this initial effect is due to dopamine receptor blockade. Accordingly, stimulation of dopamine receptors should protect the chicks against seizure activity. This explanation conforms with the results shown in Chapter IV. The protective effect of pimozide might result from a feedback increase in dopamine transmission

which may be a consequence of dopamine receptor blockade (Carlsson, 1975).

Haloperidol is a widely used neuroleptic which blocks dopamine receptors competitively (Carlsson & Lindqvist, 1963). The profound protective activity of haloperidol might be due to its own effect on other systems (e.g. noradrenaline) or a result of a feedback mechanism on dopamine transmission. Infact, Carlsson (1975) reported an increase in the turnover of DA in rat's corpus striatum after treatment with haloperidol. A similar neurochemical study, using the chick, is thus indicated.

# CHAPTER VII

## 7.1. INFLUENCE OF DOPAMINE ON STRYCHNINE- AND LEPTAZOL-INDUCED SEIZURES.

## 7.2. Summary.

7.2.1. The susceptibility of chicks to strychnine-induced seizures decreased significantly with age while leptazol-induced seizures were not significantly affected by the age of the chicks.

7.2.2. Dopamine protected the chicks against strychnine-and leptazol-induced convulsions.

7.2.3. The anticonvulsant activity of DA against strychnine seizure decreased with age.

7.2.4. FLA-63-pretreated chicks were protected against strychnine seizure by DA.

7.2.5. Pimozide effectively blocked the anticonvulsant effect of DA against both strychnine-and leptazol-induced seizures.

7.2.6. It is concluded that the anticonvulsant activity of DA against both strychnine- and leptazol-induced seizures might be mediated via activation of dopamine receptors.

7.3. Introduction.

Leptazol is known to produce generalized, asynchronous clonic movements of the limbs which are usually superceded by flexion and tonic extension of the forelimbs in laboratory animals. Such a pattern of convulsion is similar to that observed after electrical stimulation of the brain using supramaximal current (Esplin & Zablocka-Esplin, 1970). On the other hand, the pattern of convulsion

induced by strychnine in laboratory animals is characterised by tonic extension of the forelimbs without any flexion. It is generally believed that the pattern of convulsion induced by leptazol is akin to that observed in patients suffering from grand mal epilepsy while the strychnine-induced seizure is similar to human cases of tetanus convulsion (Franz, 1975). In view of the implicated role of DA in the modulation of electroshock seizure in chicks (cf chapter IV), it became necessary to investigate the influence of DA on both leptazol-and strychnine-induced seizures in young chicks.

## 7.4. Results.

### 7.4.1. The influence of age on strychnine-induced seizure.

Fig.7.1. shows clearly that

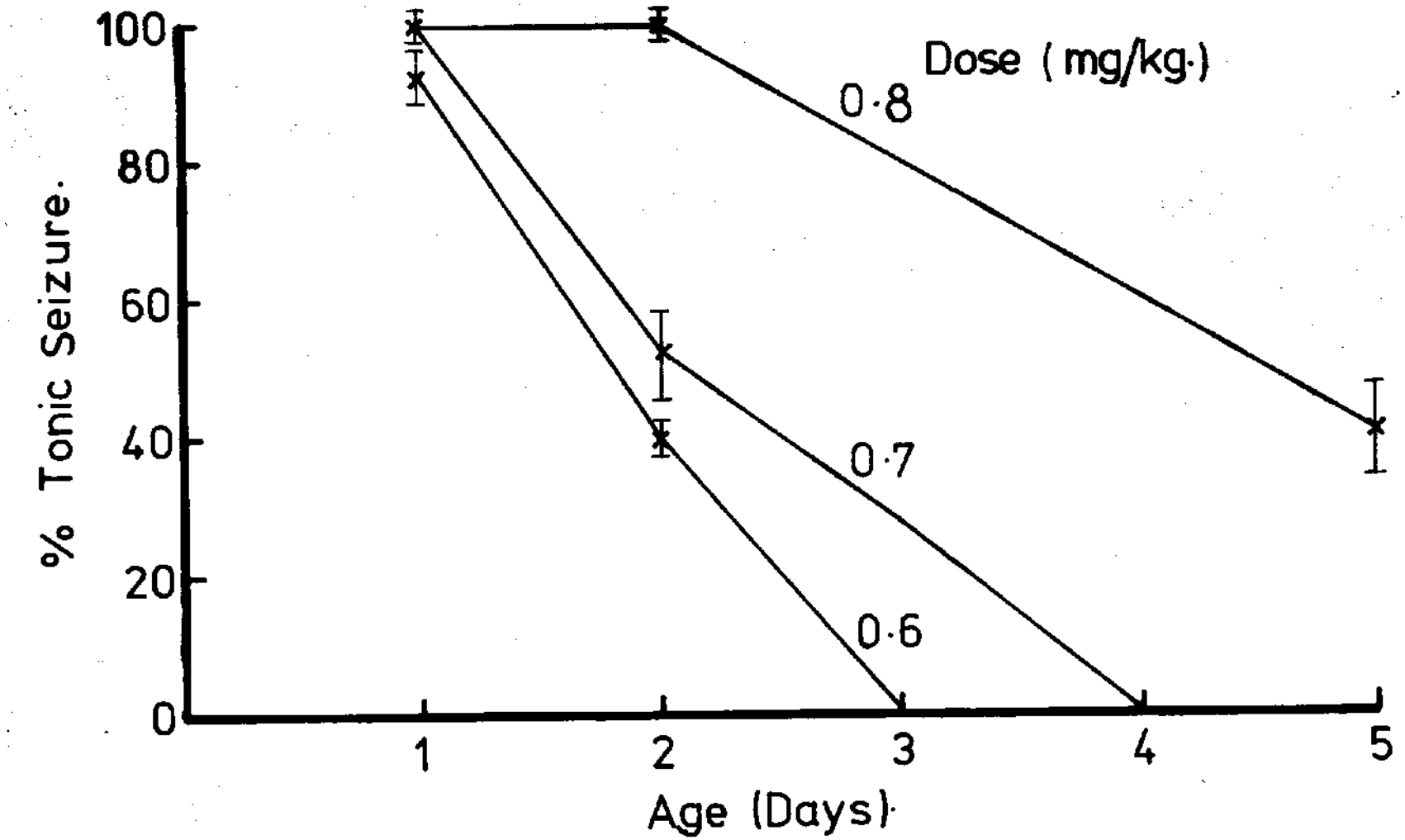



Fig. 7. . Effect of age on strychnine-induced seizures. Vertical bars represent standard deviations from the means. Chi-Squared test was used to analyse the data by comparison of the results obtained with 1 day old and those of 3/4/5 day old chicks, $P < 0.005$.

the convulsant activity of strychnine decreased with age. The decrease in the seizure susceptibility of the chicks to strychnine with age was highly significant.

7.4.2. Dose-response relationship of dopamine against strychnine - induced seizure.
The anticonvulsant effect of DA against strychnine seizure was dose-dependent (fig. 7.2.). 50 mg/kg. of DA was the best anticonvulsant dose.
It can be noted that 200 mg/kg. of DA potentiated strychnine-induced seizure.

7.4.3. The effect of FLA-63 on the action of DA against strychnine-induced seizure.
FLA-63 (25 mg/kg.) apparently increased the threshold of the chicks to strychnine seizure. Although the anticonvulsant activity of DA against strychnine seizure

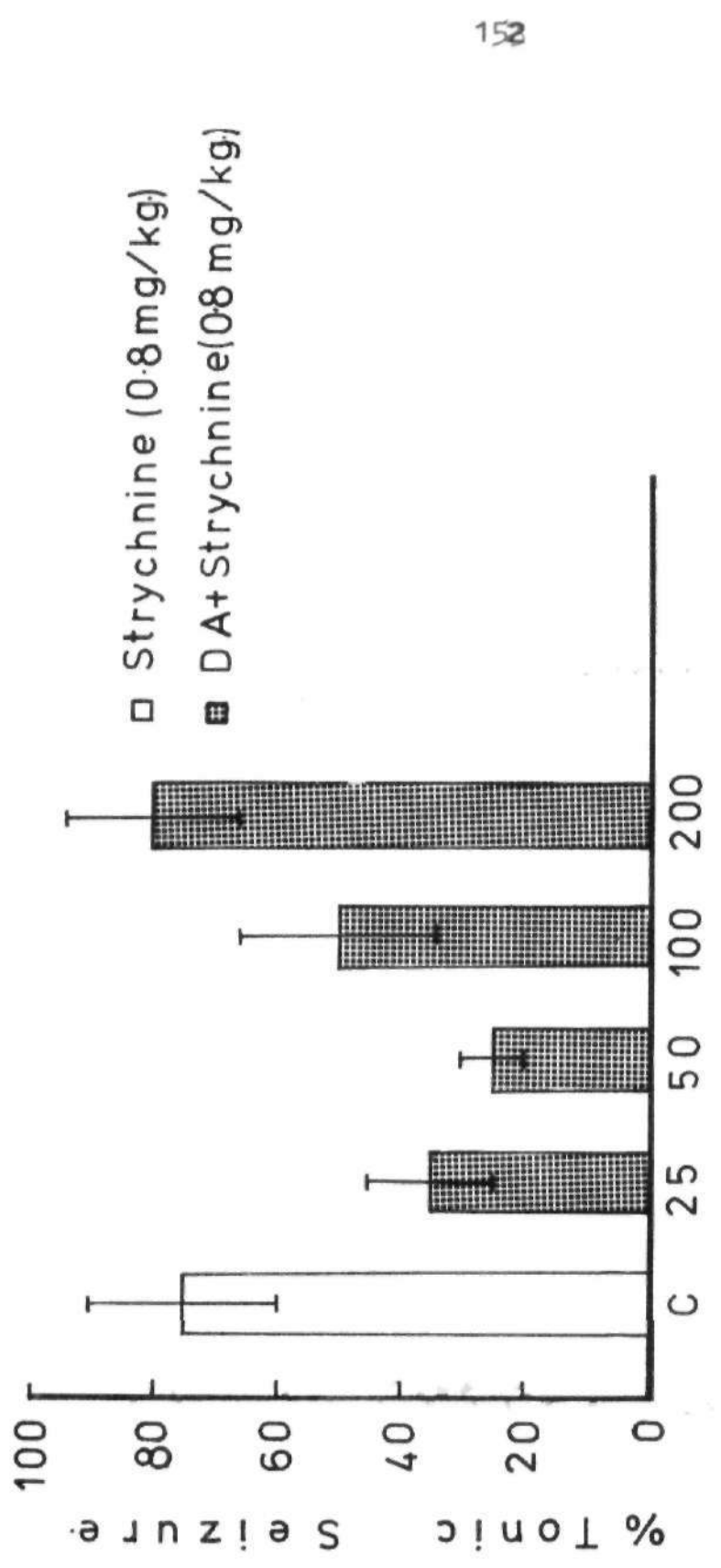



Fig. 7.2. Effect of DA on strychnine-induced seizures. Vertical bars represent standard deviations from the means. P < 0.005 (Chi-Squared test) for the anticonvulsant effect while the seizure-enhancing effect of DA was non-significant (n = 30 per histogram).

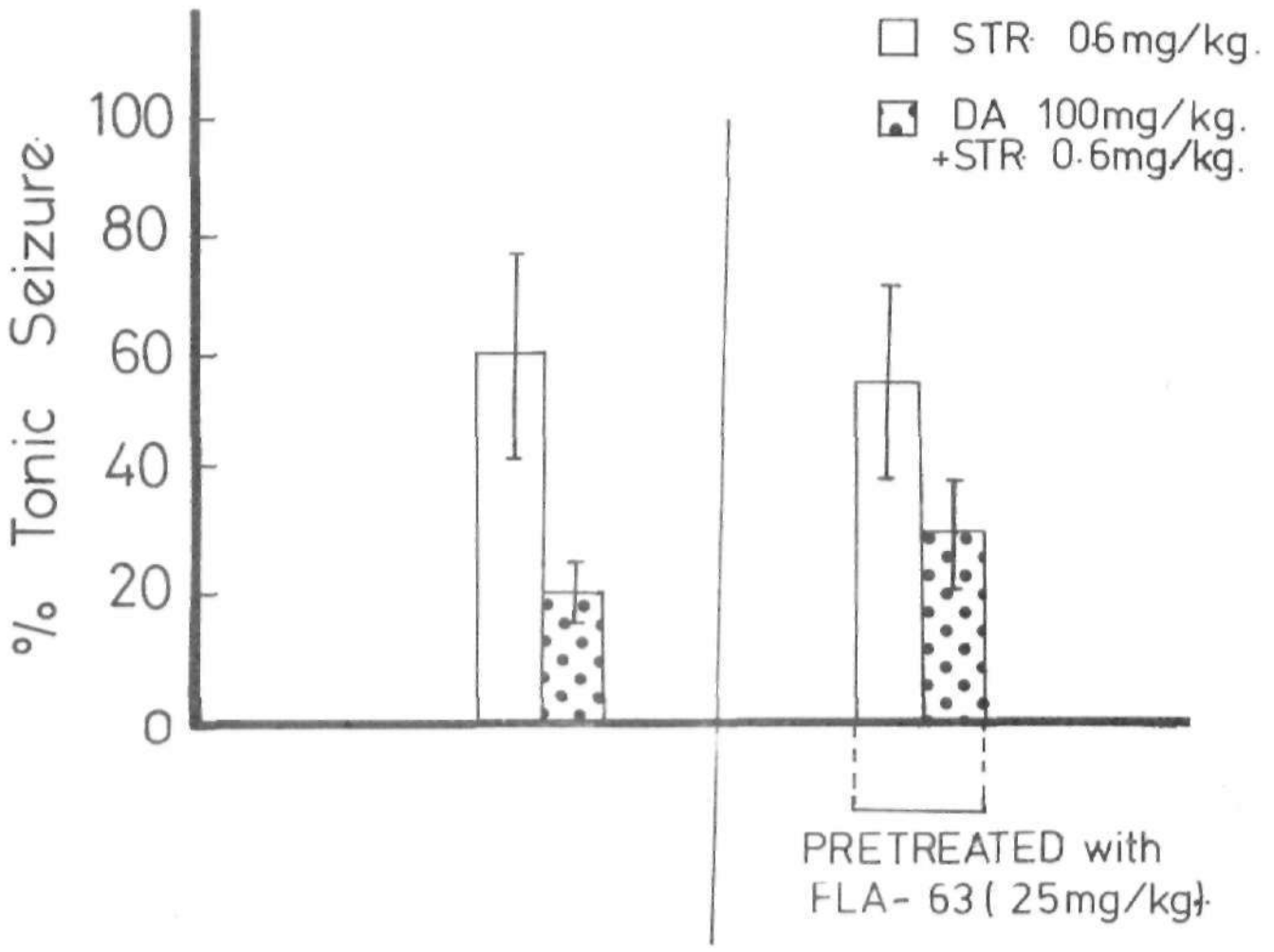



Fig. 7.3. Effect of FLA-63 on the action of DA against strychnine-induced seizure. Vertical bars represent standard deviations from the means (n = 30 per histogram). The pretreatment-time with FLA-63 prior to injection of DA and strychnine was 4 hrs. X = $P < 0.005$, Chi-Squared test.

was apparently reduced in FLA-63-pretreated chicks the protection was, nonetheless, highly significant (fig. 7.3.).

7.4.4. The effect of age on the action of DA against strychnine-induced seizure.
As shown on Table 7.1, the protective activity of DA against strychnine-induced seizure decreased with age of the chicks.

7.4.5. The effect of pimozide on the anticonvulsant activity of DA against strychnine-induced seizure.
The protective action of DA against strychnine seizure was effectively antagonized by pimozide (fig. 7.4.).

7.4.6. The influence of age on the convulsant activity of leptazol.
Fig. 7.5, shows that the convulsant effect of leptazol was not significantly affected by the age of the chicks.

| Age (Days) | Doses (mg/kg) | | No convulsed/ No used. | % Tonic seizure | P Value |
|---|---|---|---|---|---|
| | Strychnine | DA | | | |
| 1 | 0.6<br>0.6 | 0<br>100 | 38/40<br>12/40 | 95<br>30 | -<br>$<0.0001$ |
| 2 | 0.8<br>0.8 | 0<br>100 | 22/40<br>13/40 | 55<br>33 | -<br>0.05 |
| 5 | 1<br>1 | 0<br>100 | 28/40<br>20/40 | 70<br>50 | -<br>0.1 |

Table 7.1. The P Values were obtained by using the result obtained with Strychnine alone as the control for each day.

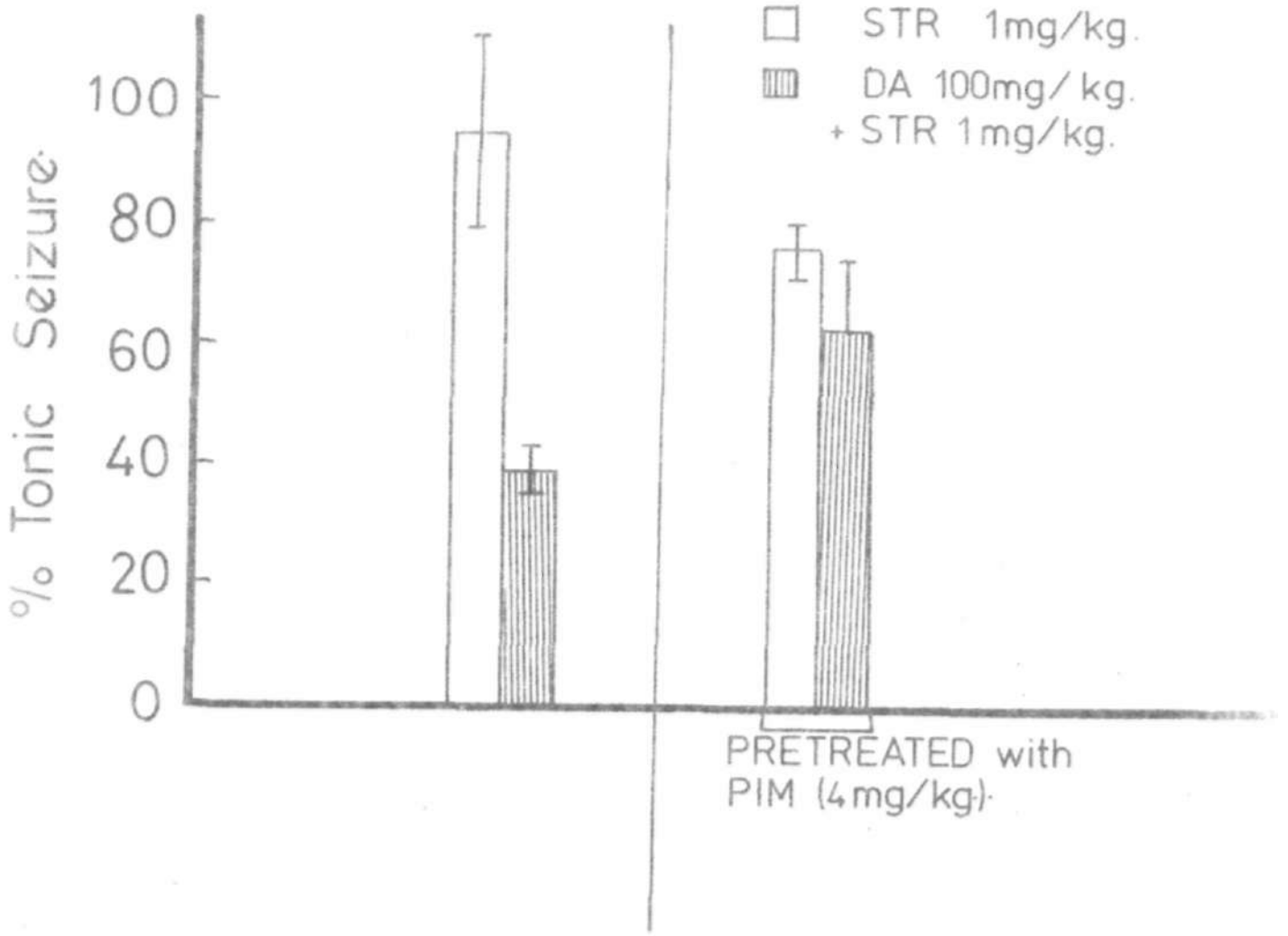



Fig. 7.4. Effect of pimozide on the anticonvulsant activity of DA against strychnine-induced seizure. 4 day old chicks were used (n = 30 per histogram). The pretreatment time with pimozide was 4 hours. Vertical bars represent standard deviations from the means.

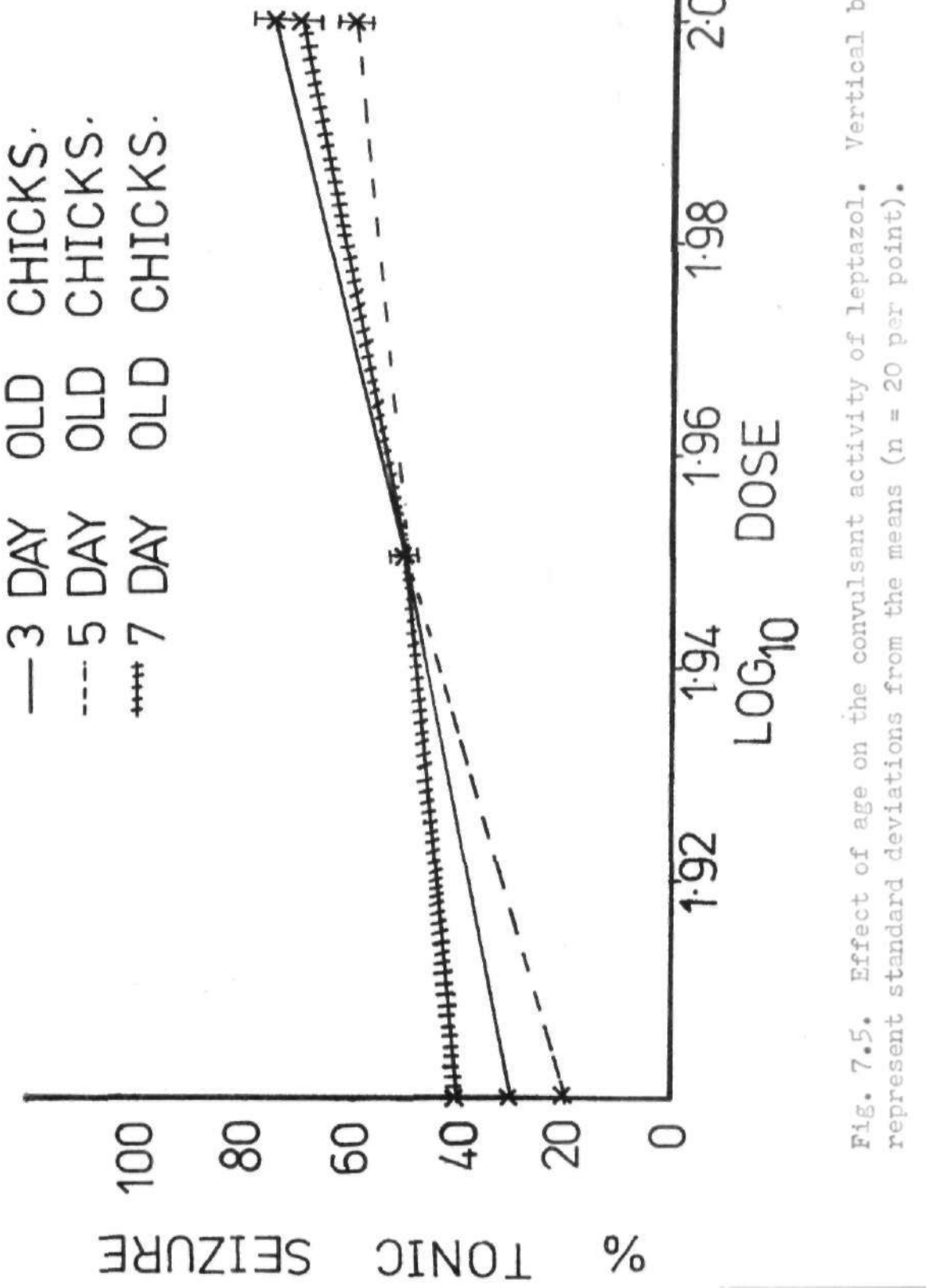



Fig. 7.5. Effect of age on the convulsant activity of leptazol. Vertical bars represent standard deviations from the means (n = 20 per point).

7.4.7. The influence of DA on leptazol-induced seizure.

Moderate doses of DA (25-100 mg/kg.) protected chicks significantly against leptazol-induced seizure (Table 7.2.). On the other hand, high doses of DA (200-400 mg/kg.) apparently potentiated leptazol-induced seizure (fig. 7.6.).

7.4.8. The influence of pimozide on the anticonvulsant activity of DA against leptazol-induced seizure.

Table 7.3. shows that the protective activity of DA against leptazol-induced seizure was effectively antagonised by pimozide.

| Dose of leptazol (mg/kg.) | Dose of DA (mg/kg.) | No convulsed/ No used. | % Tonic seizure |
|---|---|---|---|
| 70 | 0 | 10/20 | 50 |
| 70 | 25 | 6/20 | 30 |
| 70 | 50 | 5/20 | 25 |
| 70 | 100 | 4/20 | 20 |
| 80 | 0 | 13/20 | 65 |
| 80 | 25 | 8/20 | 40 |
| 80 | 50 | 3/20 | 15* |
| 80 | 100 | 6/20 | 30 |
| 90 | 0 | 16/20 | 80 |
| 90 | 25 | 12/20 | 60 |
| 90 | 50 | 6/20 | 30* |
| 90 | 100 | 7/20 | 35* |

Table 7.2. Effect of DA on leptazol-induced seizur

* $p < 0.05$, Chi-Squared test (with Yate's correction) and was obtained by using chicks given only leptazol as controls.

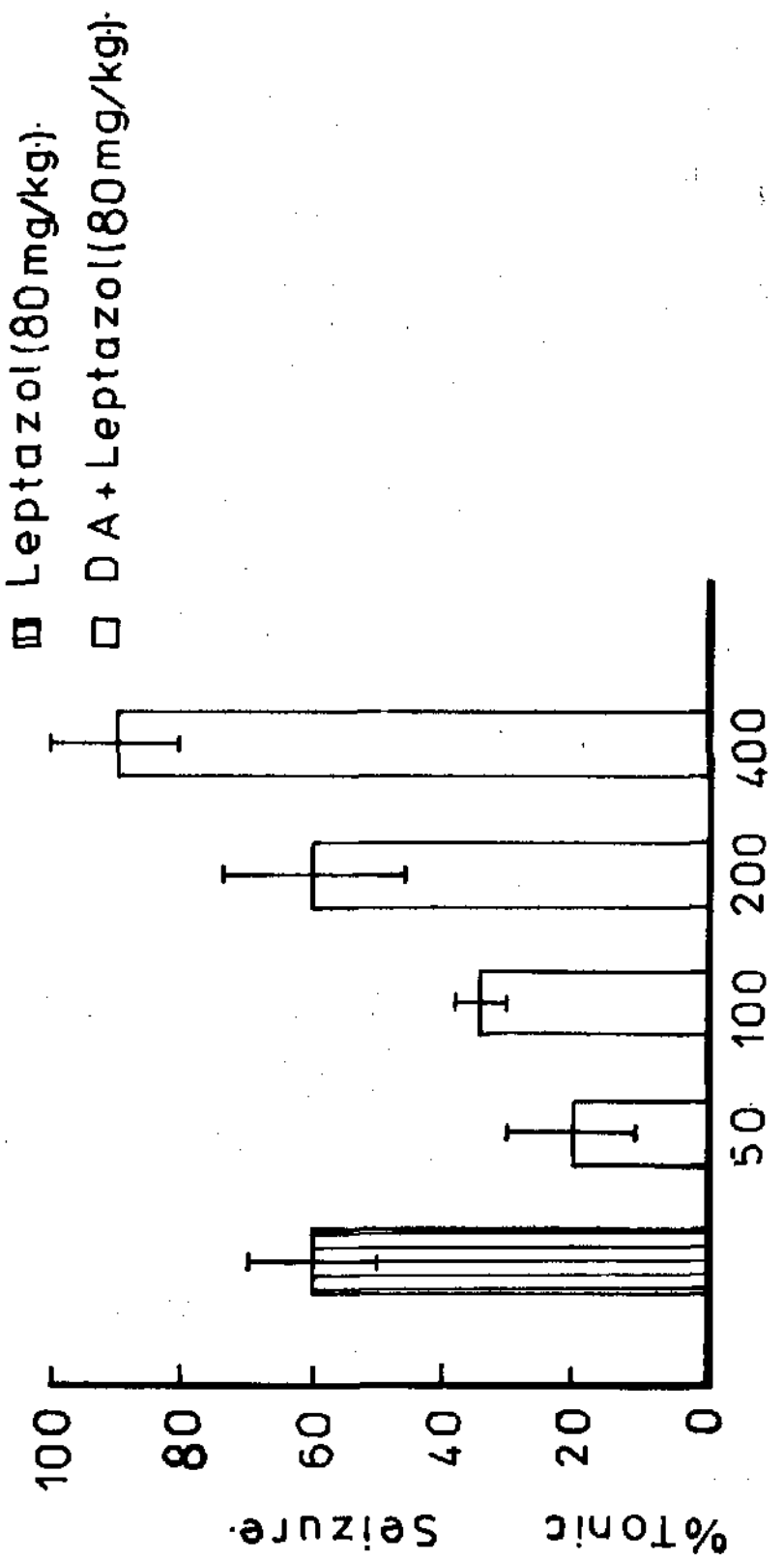



Fig. 7.6. Influence of DA on leptazol-induced seizures. 4 day old chicks were used. Vertical bars represent standard deviations from the means (n = 20 per histogram). X = P<0.05, Chi-Squared test.

| Dose of leptazol | Dose of DA | Dose of pimozide | No convulsed/ No used. | % Tonic seizure |
|---|---|---|---|---|
| 80 | 0 | 0 | 13/20 | 65 |
| 80 | 50 | 0 | 3/20 | 15* |
| 80 | 50 | 4 | 10/20 | 50 |

Table 7.3. Effect of pimozide on the **protective action of DA against** leptazol-induced seizure.

5 day old chicks were used.

* $P < 0.05$, Chi-Squared test with Yate's correction and was obtained by using chicks given only leptazol as controls.

## 7.5. Discussion.

According to Eyzagiurre & Lilienthal (1949) and Lewin & Esplin (1961), leptazol produces generalized excitation of the brain possibly by reducing the neuronal recovery time. In 1972, Gross & Woodbury reported that leptazol increased the permeability of toad bladder to potassium ions. They, therefore, speculated that similar permeability changes in the brain could increase extraneuronal potassium levels, which would consequently depolarise neuronal membranes partially. The anticonvulsant activity of DA against leptazol-induced seizure is compatible with the results obtained with dopaminoceptor agents against electroshock seizure (cf Chapter IV). It is, therefore, possible that the mechanisms involved in both electroshock and leptazol-induced seizures in the chick might be quite similar.

In 1953, Bradley, Easton & Eccles reported that subconvulsive doses of strychnine blocked spinal inhibition. It is now generally accepted that strychnine interferes mainly with postsynaptic inhibition. According to Kuno & Weakly (1972), strychnine is a competitive antagonist of the inhibitory sites in the spinal cord.

The threshold of the chicks to strychnine-induced seizures increased with age of the chicks. The elevated seizure threshold with age was highly prominent. Either enzymatic or neuronal maturational factors or both might be responsible for the elevated seizure threshold with age. For example, Vernadakis & Woodbury (1965) reported that, in the rat, the excitatory neuronal mechanisms were more developed than the inhibitory neuronal mechanisms at birth. Since strychnine is a competitive inhibitor of the inhibitory transmitter, the effects observed

after the injection of strychnine might be an algebraic sum of the action of strychnine and that of the natural inhibitory transmitter (most likely, glycine). Thus, as the inihibitory neuronal mechanisms develop with age, the antagonistic influence of the natural inhibitory transmitter against the effect of strychnine becomes more prominent which results in a decrease in the susceptibility of chicks to strychnine. It is noteworthy that the protection of chicks against strychnine seizure by DA decreased with age. This result is a contrast to the effect of DA on electroshock seizure (cf. chapter IV). The development of the neuronal systems might explain such a result. The result seems to indicate that DA is more effective in controlling seizures emanating from the brain than those originating from the spinal cord. Such a postulation would suggest that, in the chick, the primary site of action of DA is the brain and not the spinal cord vis-a-vis seizure modulation.

According to Corrodi et al (1970), FLA-63 inhibits dopamine-beta-hydroxylase enzyme thereby elevating the endogenous levels of DA. When FLA-63-pretreated chicks were latter injected with strychnine, their seizure threshold was found to be elevated-suggesting that endogenous DA possesses anticonvulsant activity. Also, the exogenous administration of DA to FLA-63-pretreated chicks significantly protected the chicks against strychnine-induced seizure. This result suggests that the anticonvulsant effect of injected DA might be due to DA rather than NA formed from it in vivo. In 1970, Anden et al reported that pimozide was a highly specific and potent dopaminoceptor antagonist. The anticonvulsant activity of DA against both strychnine-and leptazol-induced seizures was effectively antagonised by pimozide. This antagonism further implicates the involvement of dopamine receptors in the anticonvulsant activity of DA against both strychnine-and

leptazol-induced-seizures in the chick. It is significant that high doses of DA (200 - 400 mg/kg.i.p.) increased both electrically-induced and chemically-induced seizures in the chick. It is therefore possible that similar mechanisms might underlie such observations (see chapter IV for the explanations tendered).

# CHAPTER VIII

## 8.1 THE BEHAVIOURAL EFFECTS OF SOME AGENTS WHICH INTERFERE WITH DOPAMINE TRANSMISSION.

## 8.2 Summary.

8.2 1. DA and levodopa produced stereotyped pecking of board, chirping, headshaking and raising of wings dose-dependently. Locomotor activity was also increased by low doses but reduced by high doses of both DA and levodopa.

8.2 2. Amantadine increased locomotor activity. Chicks injected with amantadine exhibited stereotyped pushing, jumping and head-shaking in a dose-dependent manner. 200 mg/kg. of amantadine killed the chicks after clonic convulsion. Apart from stereotyped pushing and headshaking, DDC abolished all the other effects of amantadine.

8.2.3. 6-OHDA produced stereotyped headshaking, pushing, jumping and board-pecking dose-dependently. Water-intake was also increased by 6-OHDA.

8.2.4. DDC sedated the chicks and reduced water-intake, pushing and pecking activities while stereotyped headshaking was increased.

8.2.5. Low doses of RO4-4602 increased board-pecking while higher doses decreased this activity. Pushing and water-intake were reduced while jumping activity was enhanced.

8.2.6. It appears that some of the behavioural effects induced by these agents are not mediated via increased DA activity while stereotyped pecking, jumping, pushing and headshaking might involve an enhanced central DA neurotransmission.

## 8.3. Introduction.

Various behavioural patterns have been induced in mammalian and avian species by dopaminoceptor agents. For example, Anden & Jackson (1975) reported that the administration of DA into the nucleus accumbens of reserpine-nialamid pretreated rats produced a dose-dependent increase in locomotor activity. Also, DA has been implicated in the induction of experimental catalepsy (failure to correct externally imposed abnormal postures) usually observed after the administration of neuroleptics (Courvoisier, Ducrot and Julou, 1957). Some workers have shown that neuroleptics (phenothiazines and butyrophenones) increase the acid metabolite of DA in the brain via a negative feeback mechanism leading to increased DA release (Andén et al, 1964; Carlsson & Lindqvist, 1963). Similarly, drug-induced stereotyped behaviour in

laboratory animals have been attributed primarily to enhanced activity of central DA mechanisms (Randrup & Munkvad, 1970; Stolk & Rech, 1970). To our knowledge, a comprehensive study of the behavioural effects of agents that influence DA activity has not been undertaken in chicks. Since most of these agents primarily influence endogenous DA levels, the results might indicate the possible behavioural role of physiological levels of DA in avian species. The study might also show the behavioural effects (if any) of these agents which might be unrelated to their influence on DA activity. Recently, the mesolimbic DA system has been implicated in the aetiology of psychosis, thus it is highly imperative to have a behavioural spectrum of agents influencing central DA activity in various animal species.

Such a data might be useful in the choice of a standard and reliable laboratory model of psychosis.

8.4. Results.

8.4.1. Pargyline.

Low doses (12.5 - 25 mg/kg.) increased locomotor activity, chirping and pecking behaviours after 10 minutes of drug administration; peak activity was noted at about 20 minutes after the injection. The chicks resumed their normal behavioural activity 1 hour after the injection of low doses of pargyline. Moderate to high doses (50 - 200 mg/kg.) produced an initial sedation. Infact, all the 10 chicks injected with 200 mg/kg. of pargyline slept (means of the onset and duration of sleep were 3.36 $\pm$ 0.6 min. and 21.1 + 4.4 min. respectively.

These chicks exhibited hyperactive behaviours (increased locomotor activity, chirping and pecking) 3 hours after the injection of pargyline. A peculiar behavioural fanning of the wings was also observed after 3 hours of pretreatment with pargyline. Head-shaking and cloacal evacuation were observed in these chicks at about 1 hour after the injection of 200 mg/kg. of pargyline (fig. 8.1 - 8.4).

8.4.2. Nialamid.

High doses of nialamid (400 - 800 mg/kg.) induced 100% hypnosis in young chicks. For example, the means of the onset and duration of sleep when 800 mg/kg. of nialamid was injected into the chicks were 5.8 ± 2 min. and 3 hours respectively. Moderate doses (50 - 300 mg/kg.) sedated the chicks for some hours.

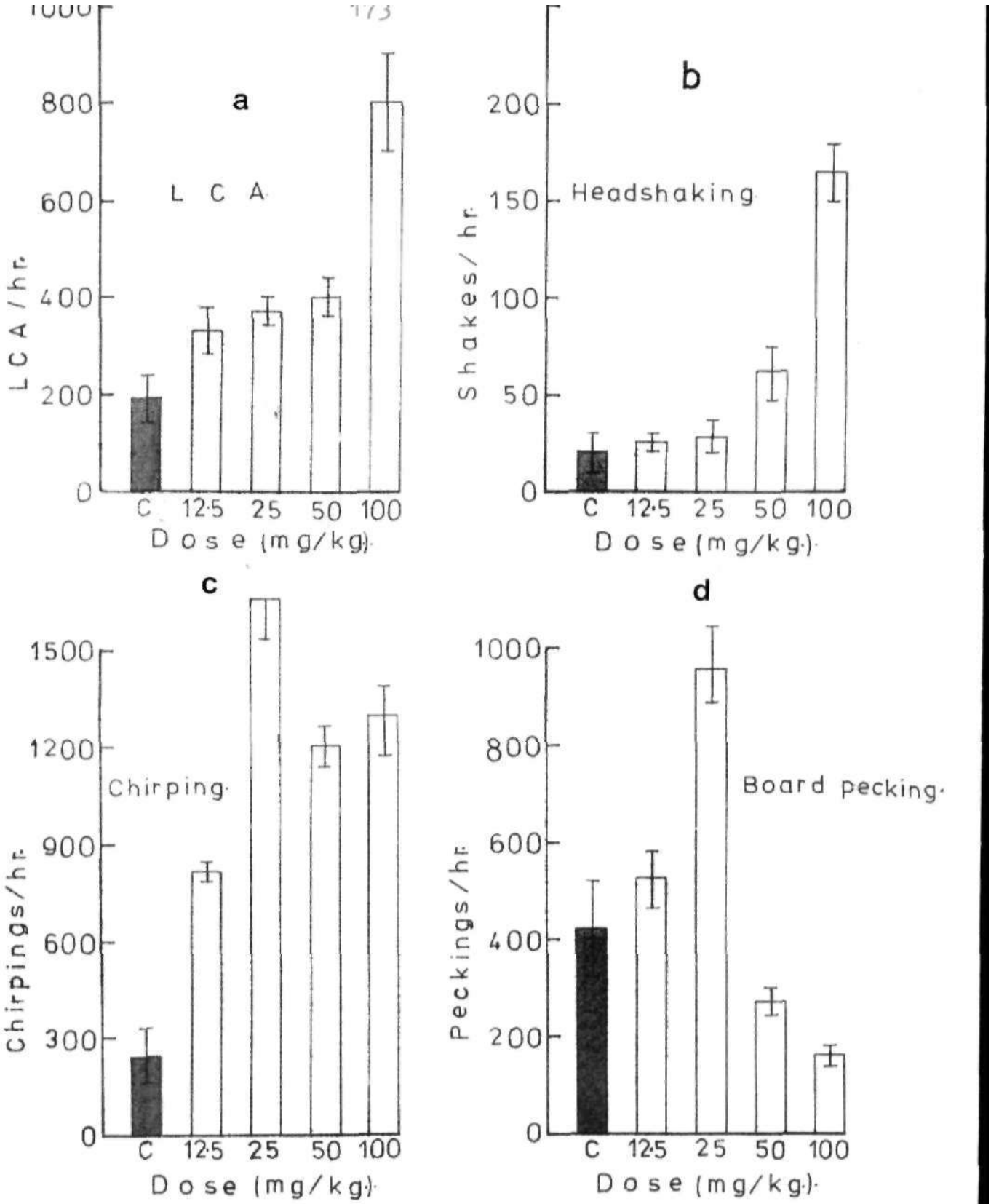



Fig. 8.1-8.4. Effects of pargyline on chick's behaviour. LCA=locomotor activity. A,B,C & D represent figs. 8.1,8.2,8.3 & 8.4.

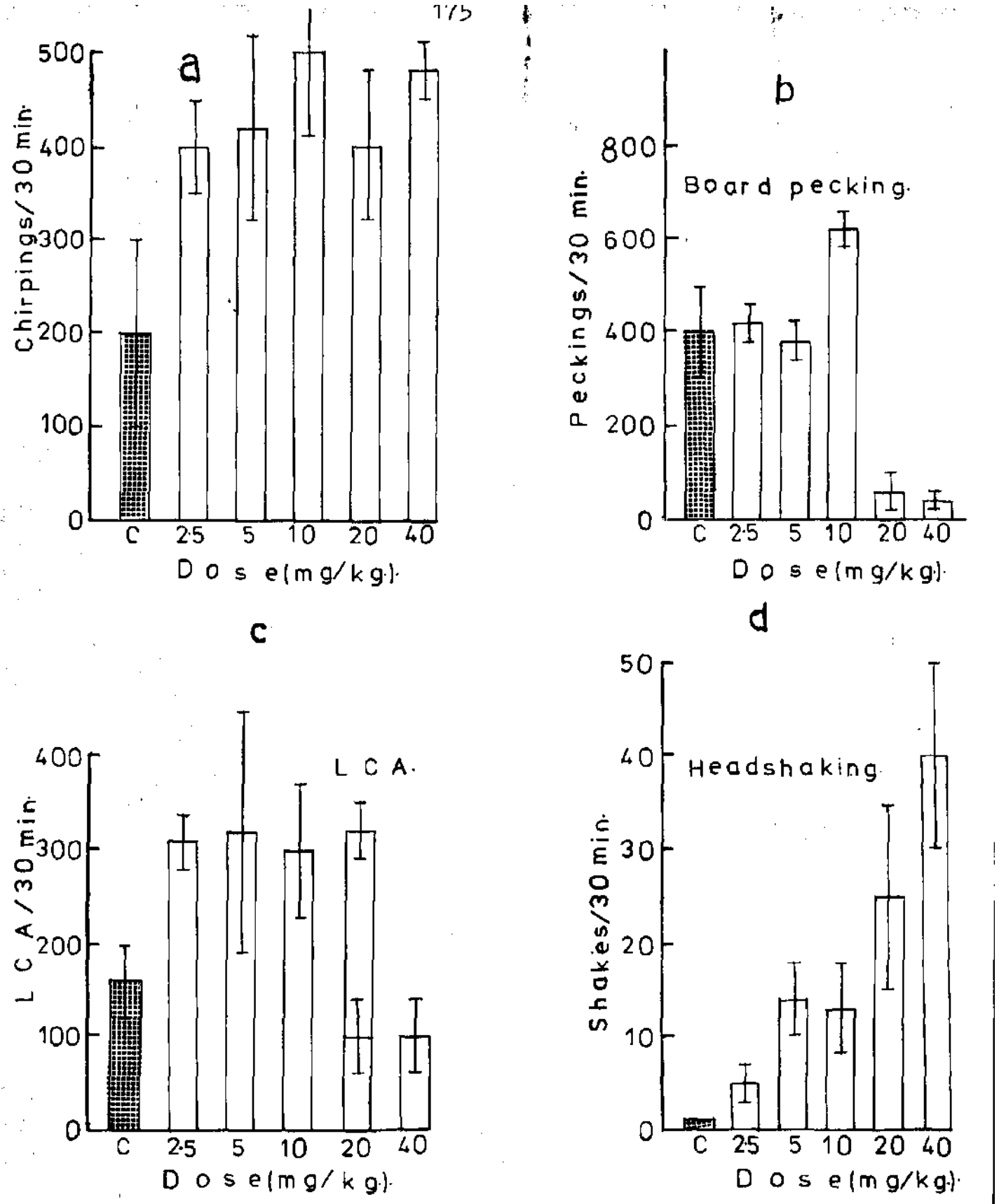
On the other hand, low doses (12.5 - 25 mg/kg.) significantly increased locomotor activity, chirping, stereotyped pecking and pushing. The means of the onset and duration of the hyperactivity were 8.1 ± 1.8 min. and 51.3 ± 11.1 min. respectively.

8.4.3. DA.

DA (2.5 - 20 mg/kg.) increased both locomotor activity and stereotyped pecking of the board (fig. 8.5 & 8.7) while a dose of 40 mg/kg. decreased both activities. Continuous chirping and headshaking was enhanced by DA (2.5 - 40 mg/kg.) in a dose-dependent manner (fig. 8.6 & 8.8). Stereotyped fanned wings was exhibited by chicks injected with DA (12.5 - 40 mg/kg.). No aggressive behaviour was observed.

8.4.4. L-Dopa.

12.5 mg/kg. of L-dopa increased locomotor activity while higher doses




**Figs. 8.5-8.8.** Effects of DA on chick's behaviour. LCA = locomotor activity. A,B,C & D represent figs. 8.5,8.6,8.7 & 8.8. respectively.

(25 - 100 mg/kg.) reduced locomotion (fig. 8.9). Chirping activity was similarly increased by low doses of L-dopa (12.5 - 25 mg/kg.) while higher doses (50 - 100 mg/kg.) reduced the chirping activity (fig. 8.10). Stereotyped pecking of the board was exhibited by chicks injected with 12.5 - 50 mg/kg. of L-dopa (fig. 8.11). The stereotyped headshaking observed in chicks which were injected with L-dopa (12.5 - 100 mg/kg.) increased with dose (fig. 8.12). Levodopa induced stereotyped fanning of wings. No aggressive behaviour was observed in chicks injected with levodopa.

8.4.5. Amantadine.

Amantadine (2.5 - 80 mg/kg.) increased locomotor activity and produced stereotyped activities characterised by

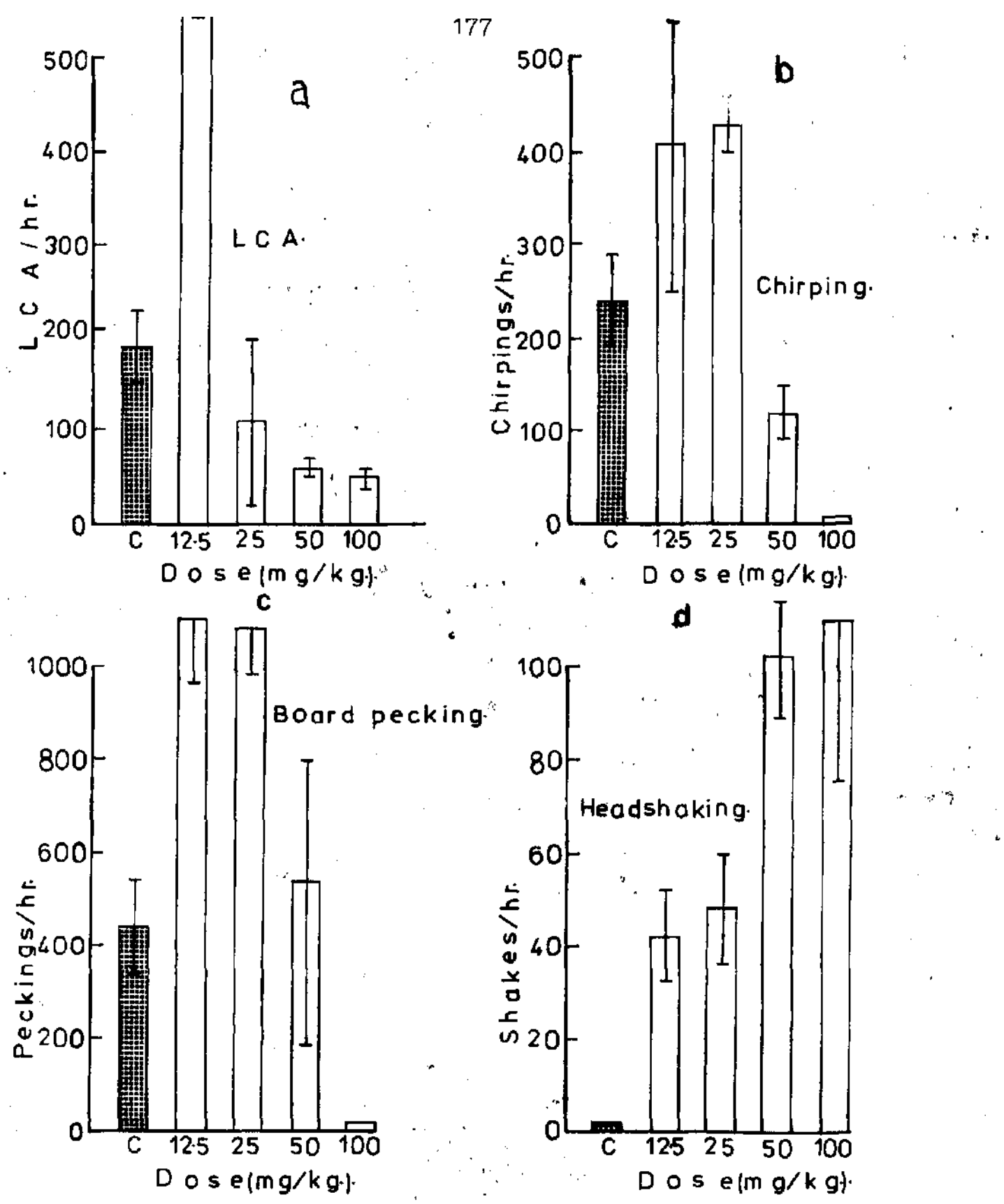
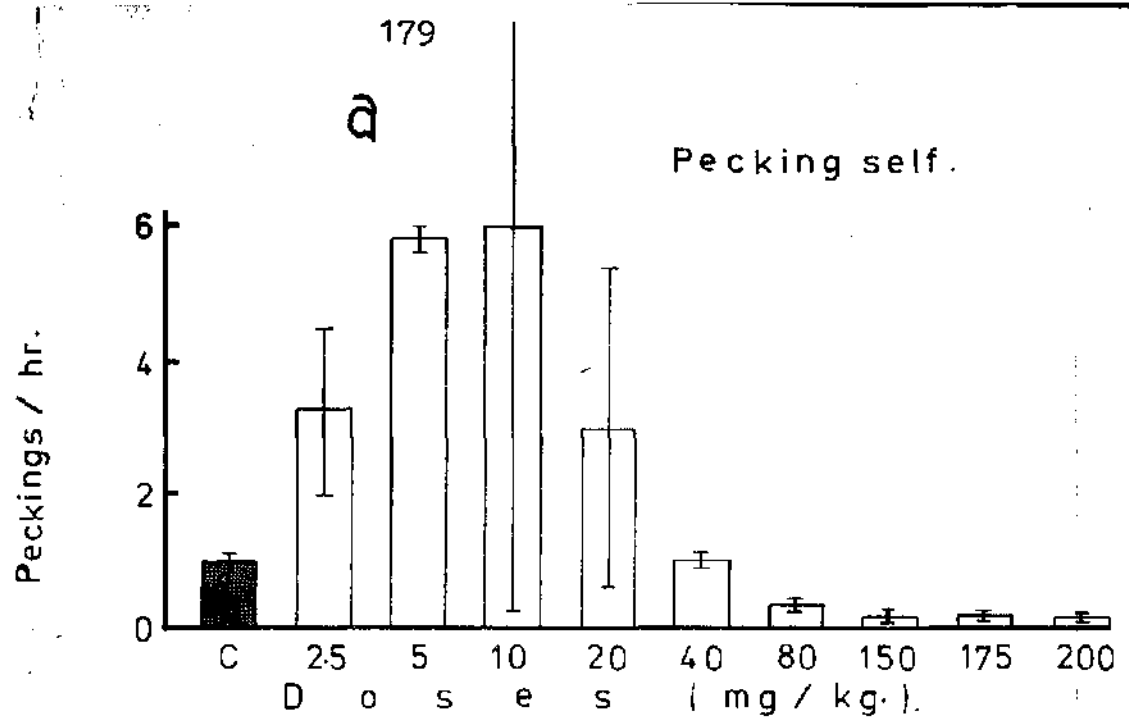


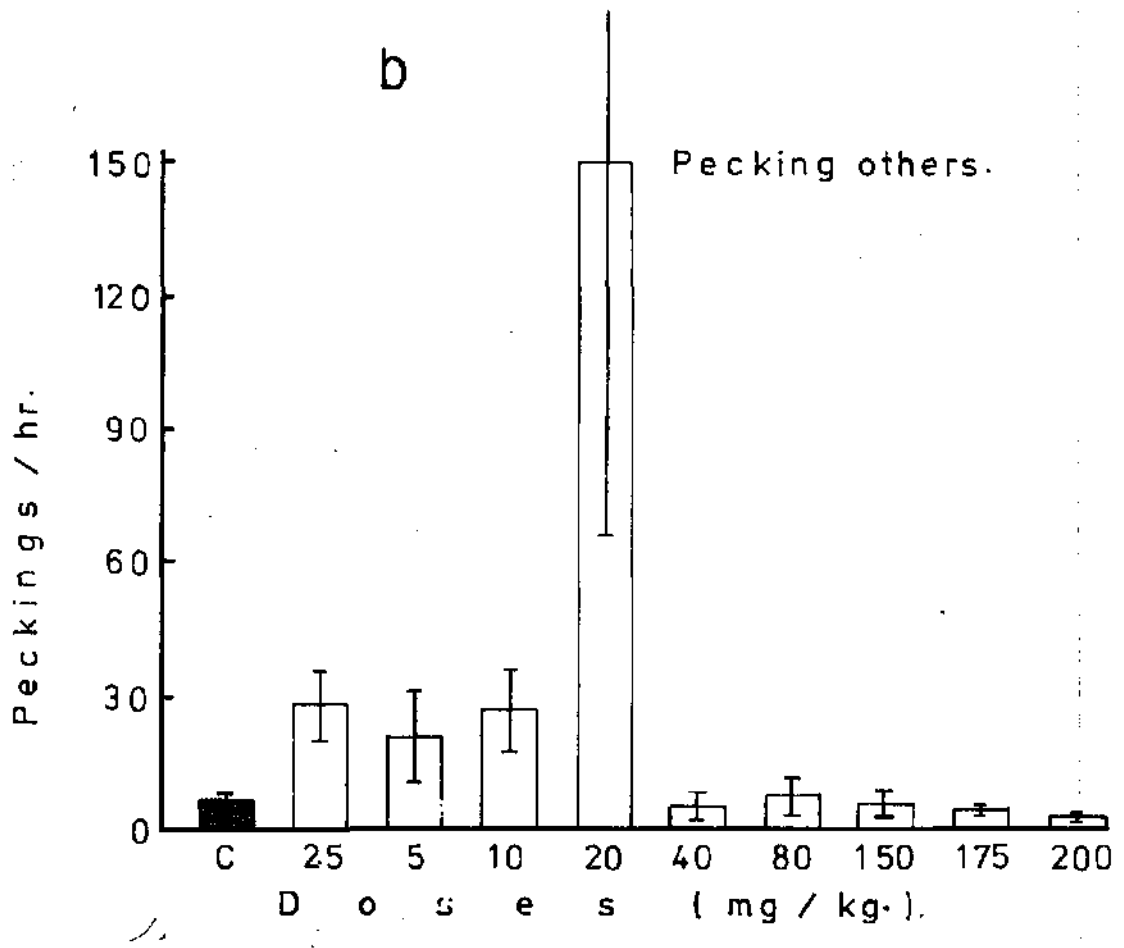

Fig. 8.9-8.12. Effects of l-dopa on the behaviour of chicks. A,B, C & D represent figs. 8.9,8.10, 8.11,8.12,respectively.

pecking (self and others), pushing, jumping and headshaking (fig. 8.13 - 8.17). These stereotyped activities were dose-dependent. 150-200 mg/kg. of amantadine did not exhibit any abnormal behaviour in chicks. 150 mg/kg. of amantadine produced tremors while 175 mg/kg. convulsed (clonic) all the chicks without any mortality. On the other hand, 200 mg/kg. convulsed all the chicks (clonic) and produced a 100% mortality. Some abnormal behaviours induced by amantadine were blocked by DDC (200 mg/kg.) while pushing and headshaking were unaffected.

6-OHDA.

5 to 160 mg/kg. of 6-OHDA induced abnormal behaviour characterised by headshaking, pushing, jumping and pecking (fig. 8.18 - 8.23).




Figs. 8.13 & 8.14. Effects of amantadine on pecking. A. & B represent figs. 8.13 & 8.14.

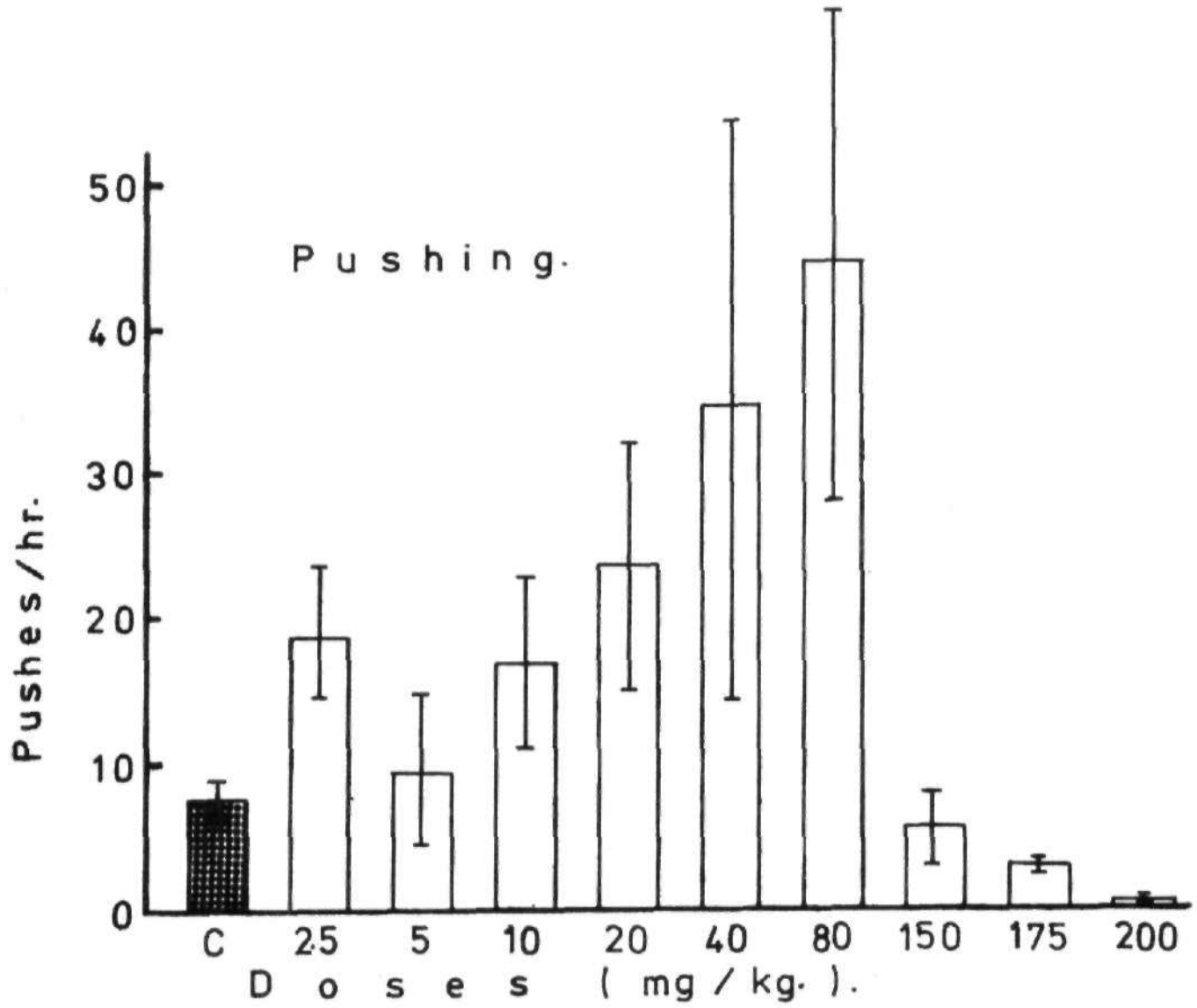
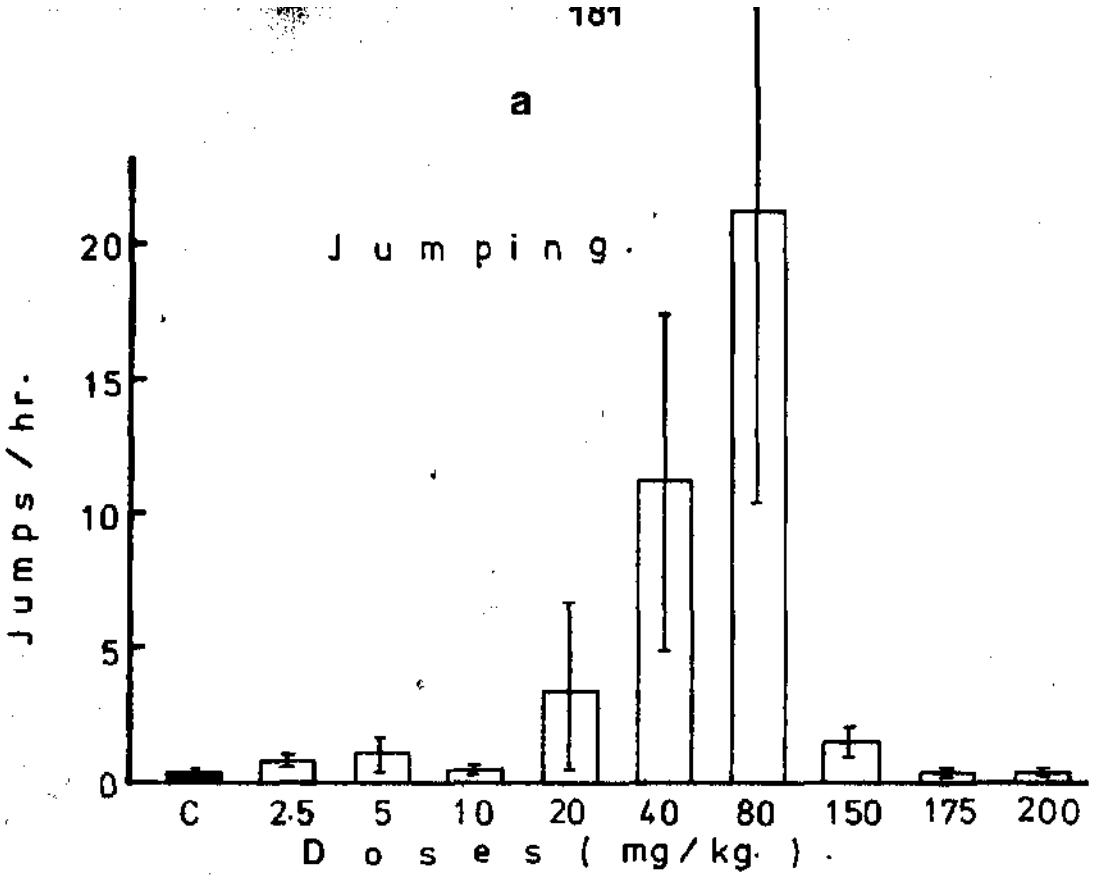
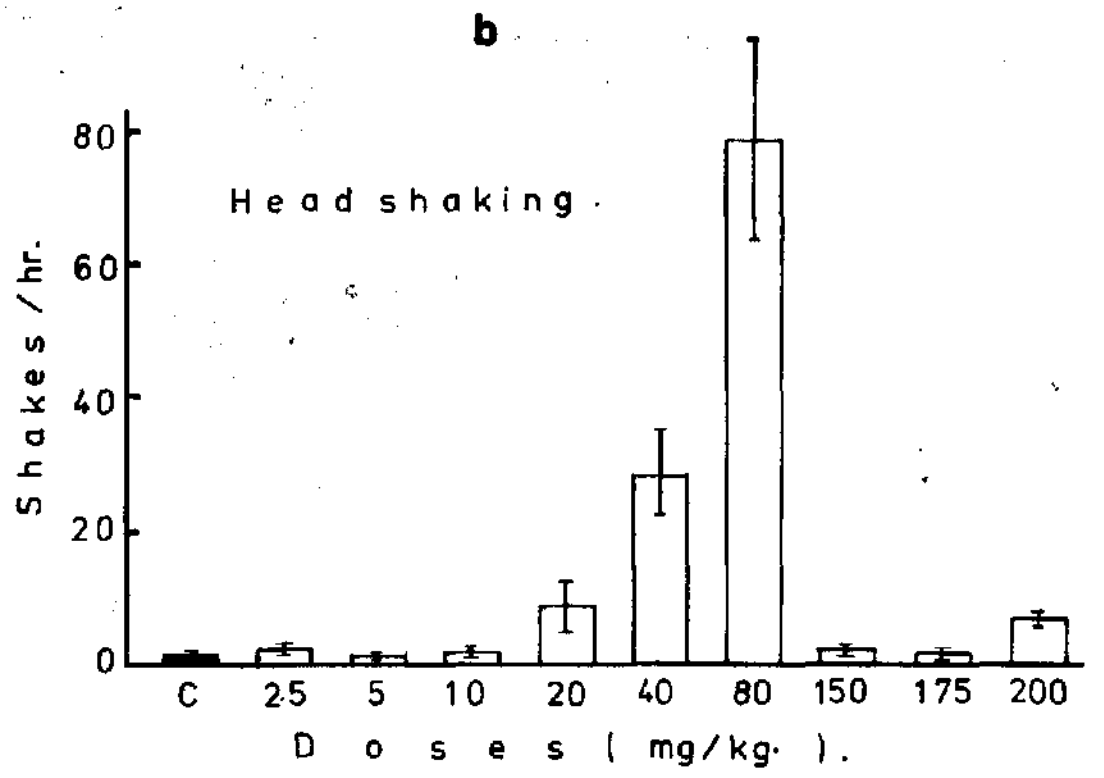
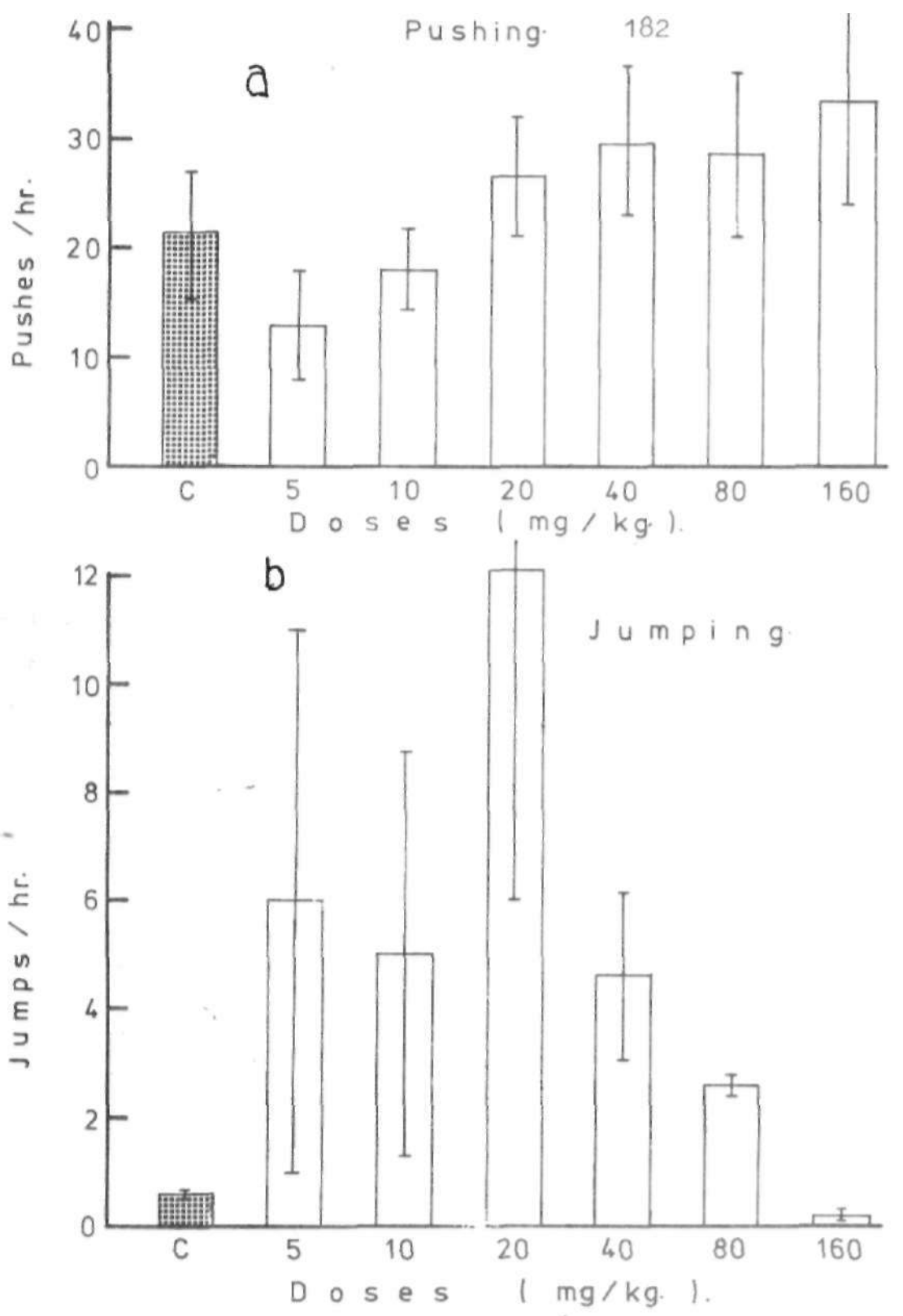



Fig. 8.15. Effects of amantadine on pushing activity of chicks. Standard deviations are represented by vertical bars ( n = 20).




Figs. 8.16 & 8.17. Effects of amantadine on jumping and headshaking.
A&B represent figs. 8.16 & 8.17.




Figs. 8.18 & 8.19. Effects of 6-OHDA on pushing and jumping activities in the chick. A & B represent figs. 8.18 & 8.19.

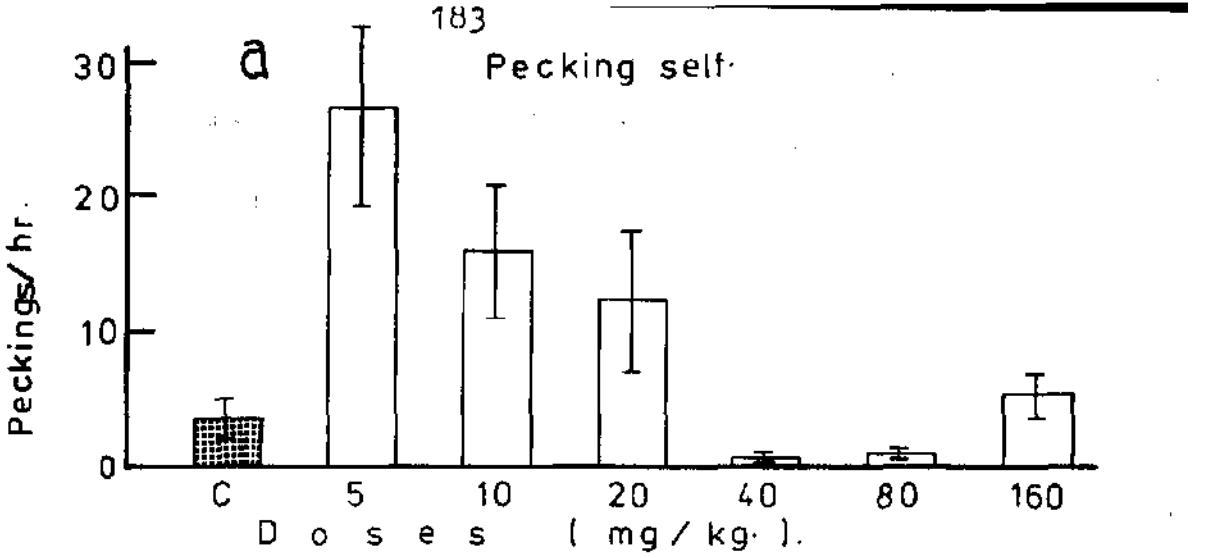



Fig. 8.20. Effect of 6-OHDA on chicks' self-pecking activity.

b

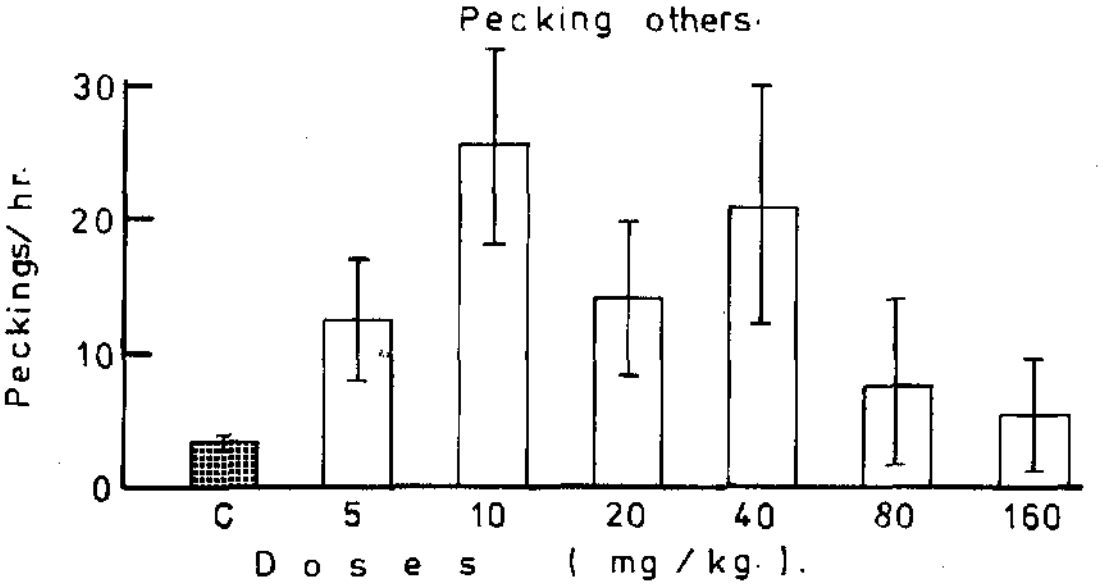
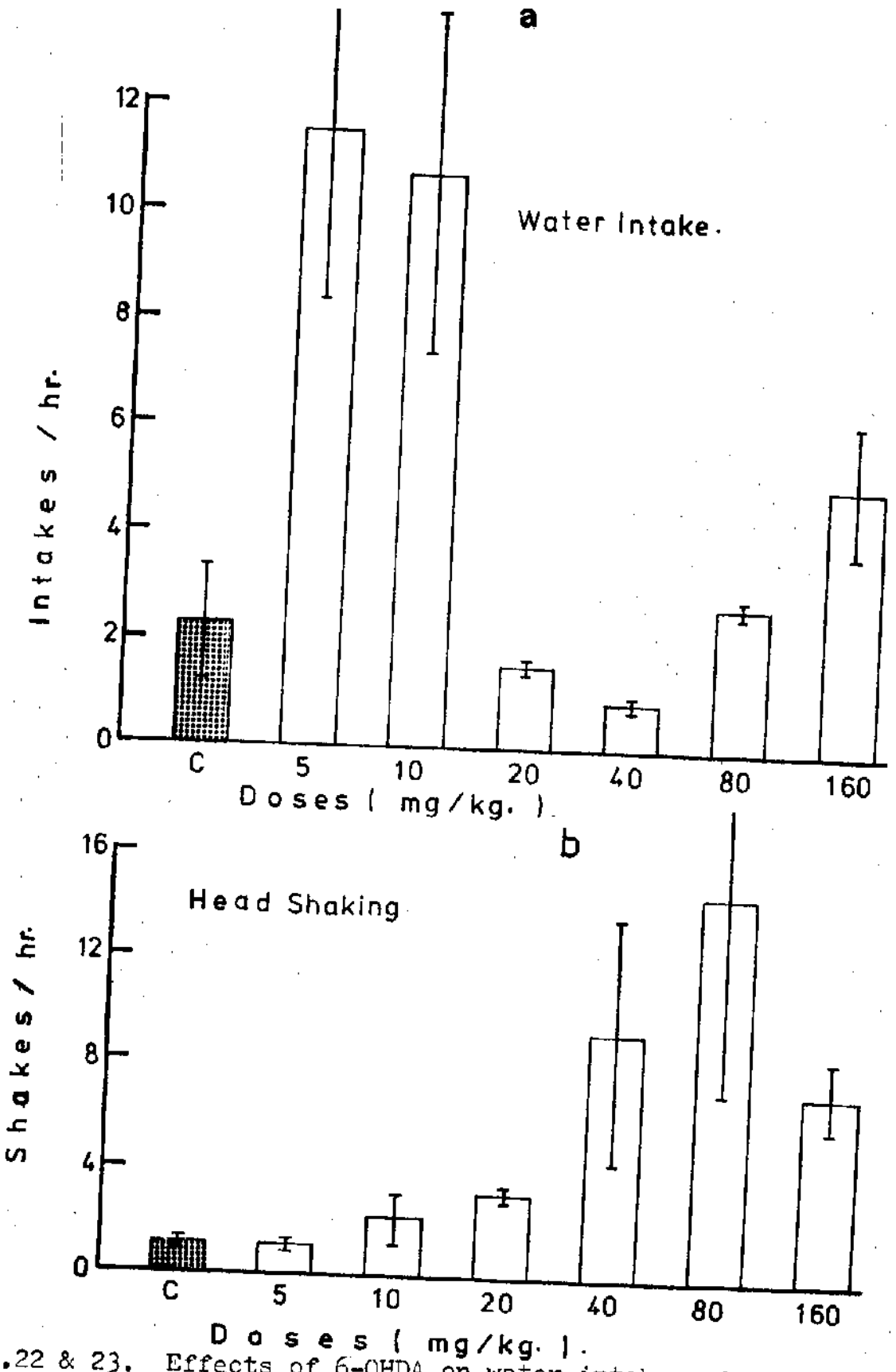



Fig. 8.21. Effect of 6-OHDA on pecking of other chicks.




Figs. 8.22 & 23. Effects of 6-OHDA on water intake and headshaking. A & B represent figs. 8.20 & 21.

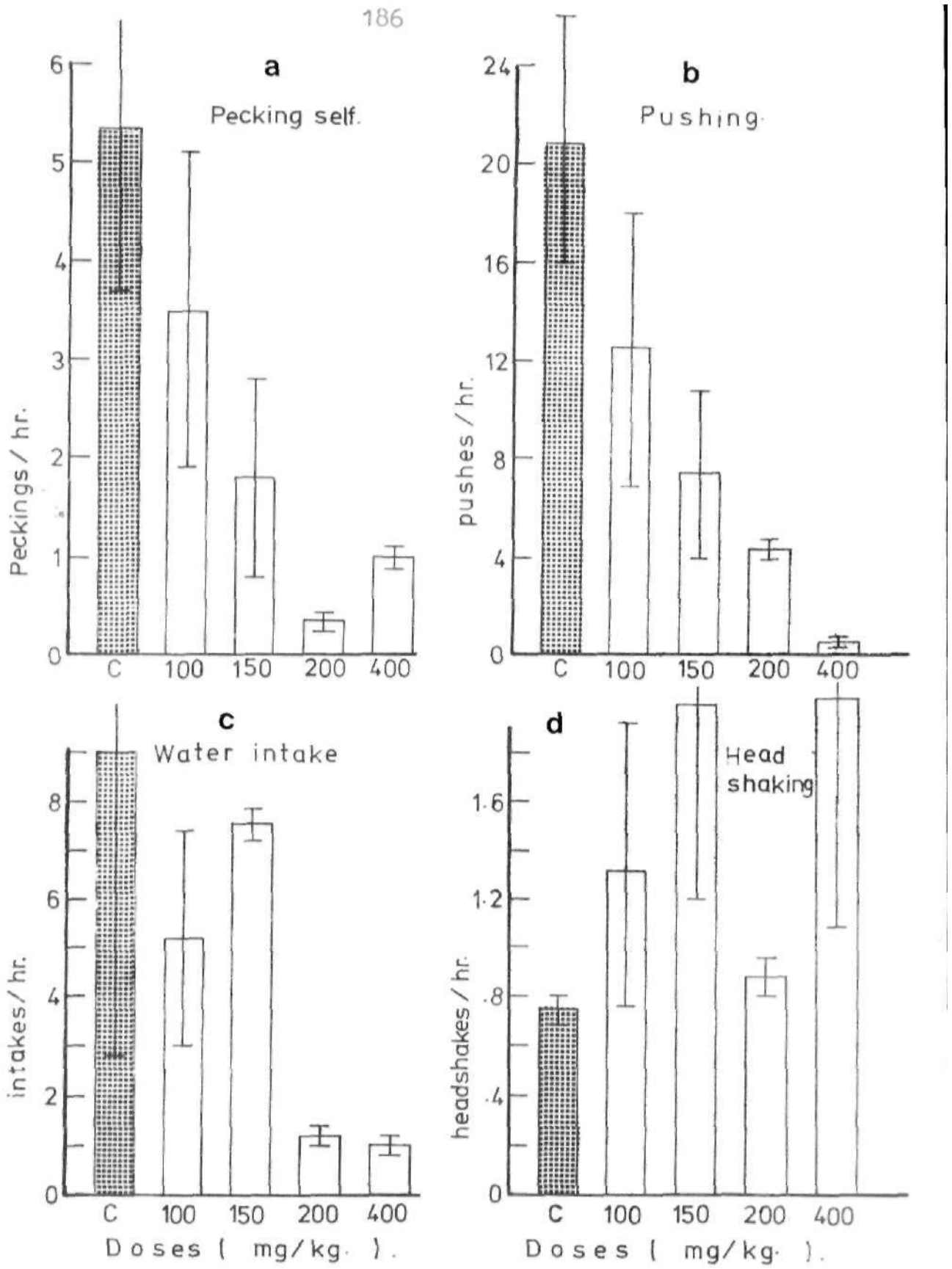
The chicks injected with 6-OHDA drank water more often than the controls (fig. 8.23). The effects of 6-OHDA were dose-dependent. Except, the effects of 6-OHDA on headshaking and pushing which increased sequentially with dose, the other effects did not follow any simple dose-response pattern.

8.4.7. DDC.

DDC (100 - 400 mg/kg.) dose-dependently reduced water-intake, pushing and pecking activities while stereotyped headshaking was increased (fig. 8.24 - 8.27). The chicks were sedated by DDC.

8.4.8. R04 - 4602.

25 mg/kg. of R04 - 4602 increased board-pecking activity but higher doses (50 - 75 mg/kg.) decreased this activity. Jumping activity was increased by a dose




Figs. 8.24-8.27. Effects of DDC on chick's behaviour. A,B,C & D represent figs. 8.24,8.25,8.26 & 8.27 respectively

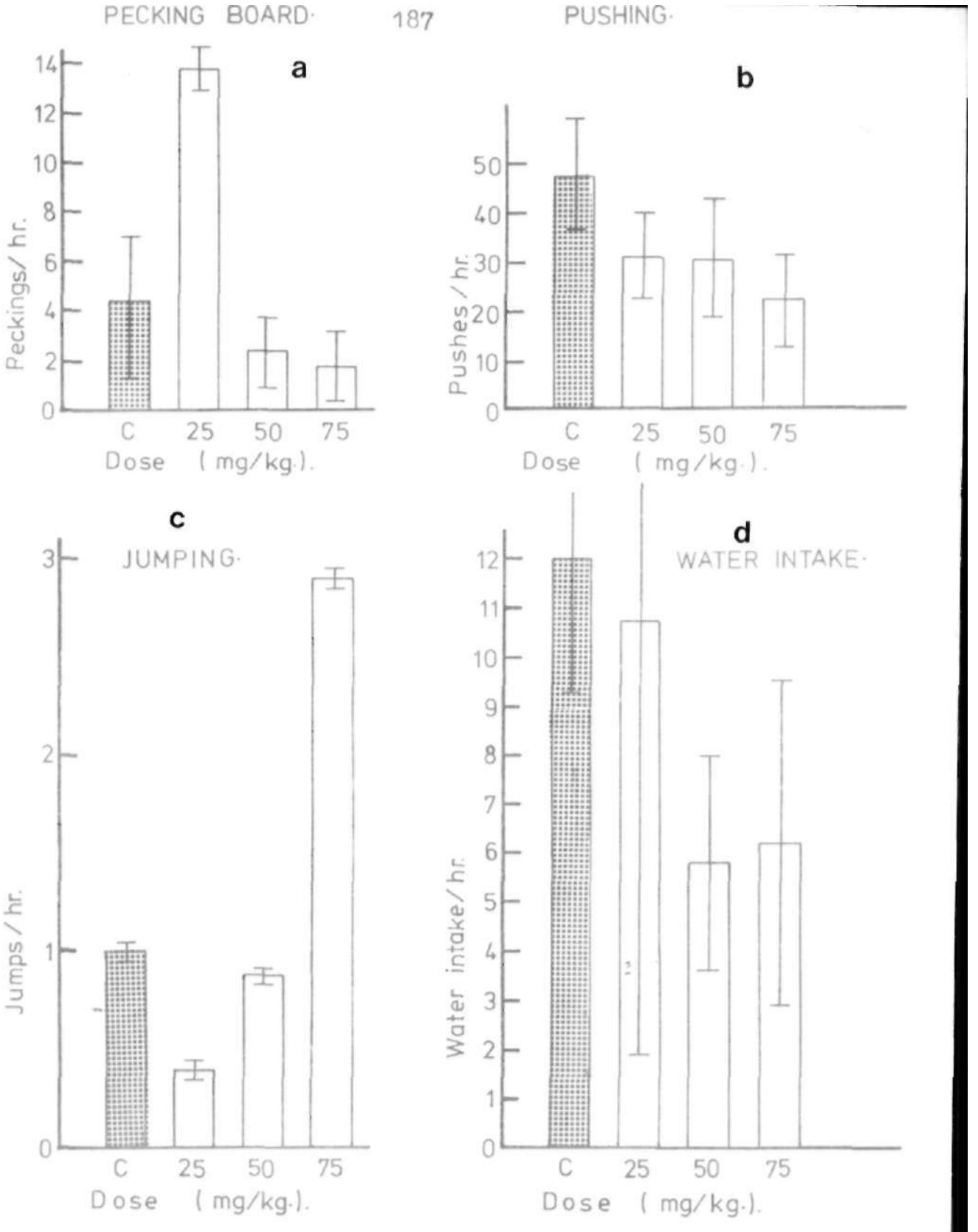



Fig. 8.28-8.31 Effects of RO4-4602 on chick's behaviour.
A,B, C & D represent figs. 8.28,8.39,8.30 & 8.31 respectively.

of 75 mg/kg. while lower doses reduced this behavioural manifestation. Both pushing and water-intake were reduced dose-dependently by RO4-4602 (fig. 8.29 - 8.32).

## 8.5. Discussion.

It is now generally accepted that drug-induced stereotyped behaviours in various animal species are mediated primarily via activation of central DA receptors (Munkvad & Randrup, 1966; Fog, Randrup & Pakkenberg, 1966; Hornykiewicz, 1966; Traylor & Snyder, 1971). Although behavioural effects of dopaminoceptor agonists and antagonists have been studied by many workers, the influence of agents influencing DA neurotransmission has not been undertaken in the chick.

Many authors have reported that L-dopa increases locomotor activity in mice,

rats, rabbits, cats and monkeys (Blaschko & Chruschiel, 1960; Everett & Wiegand, 1962; Monnier & Tissot, 1958; Smith, 1963; Sourkes, 1964). Smith & Dews (1962) observed that DL-dopa suppressed locomotor activity in the normal mice while pargyline-pretreated animals exhibited a marked increase in locomotor activity. In these results, both DA ( 5 - 25 mg/kg.) and L-dopa (12.5-100 mg/kg.) increased locomotor activity which parallels the results obtained using mammalian species. This parallelism supports Spooner's (1965) proposal that the chick responds to neuropharmacological agents in a manner similar to most mammals. Both DA and L-dopa exhibited stereotyped chirping, board-pecking, headshaking and fanned wings. Unlike apomorphine, these dopaminoceptor agonists failed to induce aggressive behaviour in the chick.

It is possible that DA receptors involved in aggressive behaviour are different from those mediating stereotyped chirping, headshaking and fanning of wings. Thus, while apomorphine is more potent on the former type of DA receptors, the latter type of DA receptors might be more sensitive to injected DA and DA formed from injected L-dopa. Such a proposal is compatible with the hypothesis advanced by Cools & Van Rossum (1976) that there might be two functional types of DA receptors in the snail, rat and cat.

Both pargyline and nialamid are known to inhibit monoamine oxidase enzyme thereby elevating endogenous levels of all the monoamines (De Schaepdryver et al, 1962). Various doses of both pargyline and nialamid, after 3 hours pretreatment time, induced some abnormal activities characterised by continuous chirping, pecking, fanned wings, headshaking and cloacal evacuation. These abnormal behavioural effects might be due to an enhanced dopamine neurotransmission (CF. DA and L-dopa results).

On the other hand, moderate to high doses of pargyline (50 - 200 mg/kg.) and nialamid (50 - 800 mg/kg.) initially hypnotised most of the chicks. The sedative effect of these agents might be due to elevated NA or 5-HT in the brain since Osuide & Wambebe (unpublished) have observed hypnosis in chicks injected with NA or 5-HT. The possibility of an initial direct effect of these agents on brain mechanisms mediating sleep can not be totally ignored.

According to Carlsson (1966), RO4-4602 inhibits L-aromatic acid decarboxylase enzyme peripherally thereby increasing brain levels of DA. RO4-4602 decreased water-intake and pushing activity while board-pecking and jumping activities were enhanced by some doses. It is interesting to note that 25 mg/kg. of RO4-4602 increased board-pecking while stereotyped jumping was reduced. On the other hand, 75 mg/kg. decreased board-pecking but increased jumping activity. Such a data suggests that

the receptors (DA/NA/5-HT) involved in the mediation of these activities might be different functionally. It is possible that the neuronal system/s responsible for the reduction in pushing activity and water-intake are similar.

In 1962, Thorn & Ludwig reported that high doses of DDC inhibited dopamine-beta-hydroxylase enzyme in the rat, thereby elevating endogenous levels of DA. The sedation observed in animals treated with DDC is too rapid in onset to be due to enzyme inhibition alone (unpublished observation of Svensson & Waldeck, 1969). In this project, DDC initially hypnotised the chicks, 12 hours after injection while water-intake, pecking and pushing activities were reduced dose-dependently. Stereotyped headshaking was increased. On the basis of this data, it can be speculated that the stereotyped headshaking may be due to the enhanced DA levels in the brain while the influence on other behavioural parameters may be due to non-specific activity of DDC on other neuronal systems.

Amantadine has been reported to antagonise stereotyped behaviours induced by apomorphine (Cox & Tha, 1973) and bromocriptine (Schnieden & Cox, 1975). On the basis of this antagonism, these authors suggested that amantadine may be acting indirectly. In 1971, Svensson & Stromberg reported that amantadine might produce its antipankinsonian effect by releasing DA from its nerve terminals. Furthermore, amantadine has been reported to inhibit neuronal reuptake of DA (Heimans, Rand & Fennessy, 1975). The results show that doses ranging from 20 to 80 mg/kg. increased headshaking, pushing and jumping activities while higher doses had no effect on these stereotyped activities. On the other hand, 2.5 - 20 mg/kg. increased stereotyped peckings (self and others) but higher doses failed to induce these stereotypies. The antagonism of amantadine-induced jumping, pushing and increased locomotion by DDC suggests that DA might not be intimately involved in the mediation of these activities but rather NA. It is equally possible

that the antagonism of these effects by DDC might not be related to inhibition of DA-beta hydroxylase enzyme but rather due to the general sedation induced by DDC.

There are conflicting reports on the effect of 6-OHDA on water-intake (Bloom et al, 1971; Uretsky & Iverssen, 1970). In this project, water-intake, peckings (self and others) and jumping activities were increased by low doses of 6-OHDA (5-10 mg/kg.) while higher doses (20-160 mg/kg.) decreased stereotyped jumping, pushing and headshaking. These effects were observed within 6 hours after intraperitoneal injection of 6-OHDA. The chicks appeared normal behaviourally after 12 hours of drug administration except that they were generally less sensitive to tactile stimulation when compared to control chicks. The stereotyped activity may be due to catecholamines (especially DA) released by injected 6-OHDA (Porter et al, 1963). It appears that the receptors mediating stereotyped pecking are functionally different from those responsible for jumping, pushing and

headshaking (CF; amantadine results). These results seem to indicate that there might be two types of DA receptors in the chick which mediate two distinct stereotyped activities. In order to understand the mechanisms involved in these behavioural manifestations, it is necessary to study the interactions between tricyclic antidepressants (protect DA neurones against 6-OHDA-induced sympathectomy) and 6-OHDA on various behavioural parameters as well as EEG effects of such interactions. Also, assays of monoamines and their metabolites in the brain may help in the elucidation of the mechanisms underlying the behavioural effects of the various agents used in this project.

# CHAPTER IX

## 9.1. ANTAGONISM OF PENTOBARBITONE SLEEP BY DOPAMINE, LEVODOPA AND APOMORPHINE IN CHICKS.

## 9.2. Summary.

9.2.1. The influence of dopamine, levodopa and apomorphine on sleep produced by pentobarbitone was studied in 1 to 14 day old chicks.

9.2.2. Pentobarbitone-induced sleep was associated with synchronization of the electroencephalogram (EEG) of the hyperstriatum, optic tectum and pontine reticular formation.

9.2.3. Moderate doses of DA produced desynchronization of the EEG and antagonized pentobarbitone-induced EEG synchronization.

9.2.4. High doses of DA apparently produced a delayed sleep which was potentiated by pimozide and antagonised by adrenoreceptor blocking agents.

9.2.7. It is concluded that dopamine might be involved in the process of wakefulness and maintenance of consciousness in the chick.

9.3. Introduction.

Dopamine has been reported to produce electrocortical slow wave activity and behavioural sleep in 1 to 28 day old chicks (Key & Marley, 1962). Similarly, levodopa has been reported to reduce the threshold hypnotic dose of hexobarbitone in male rats (Wahlstrom, 1967). However, Spooner & Winters (1966 ) demonstrated that dopamine caused increased motor activity and an EEG alerting reaction in 5 to 14 day old chicks. Holtz, Balzer & Westermann (1957) reported that levodopa abolished hexobarbitone-induced sleep in iproniazid-pretreated mice. Thus, the role of dopamine in sleep mechanisms is not yet fully understood. In this chapter, the influence of dopamine, levodopa, apomorphine and pimozide on pentobarbitone-

induced sleep using young chicks is reported.

## 9.4. Results.

### 9.4.1. Effect of age on pentobarbitone sleep.

Saline had no hypnotic effect on the chicks. The hypnotic effect of pentobarbitone sodium decreased with age (Table 9.1 & fig.9.1).

### 9.4.2. Influence of dopamine on pentobarbitone-induced sleep.

Dopamine (25 - 100 mg/kg.) significantly delayed the onset and shortened the duration of pentobarbitone-induced sleep (Table 9.2). The antagonism of pentobarbitone-induced sleep by dopamine was most prominent in 14 day old chicks (Table 9.2). Table 9.2 also shows that dopamine-induced antagonism of pentobarbitone sleep increased with dose of dopamine (25 - 100 mg/kg.). On the other hand, high doses of dopamine (200 - 400 mg/kg.) delayed the onset but prolonged the duration of pentobarbitone sleep (Table 9.2).

| Age (Days) | Dose (mg/kg.) | No asleep/ No used. | Onset of sleep (min.) | | Duration of sleep (min.) | |
|---|---|---|---|---|---|---|
| | | | Mean | S.D. | Mean | S.D. |
| 2 | 7 | 10/10 | 4.3 | 0.6 | 28.2 | 3.9 |
| 4 | 7 | 0/10 | - | - | - | - |
| 4 | 10 | 10/10 | 5.0 | 0.4 | 22.0 | 2.4 |
| 5 | 10 | 6/10 | 8.6+ | 0.5 | 13.7+ | 2.8 |
| 6 | 10 | 2/10 | 11.5+ | 0.2 | 8.9+ | 0.6 |
| 2 | 15 | 10/10 | 3.05 | 1.4 | 109.1 | 14.4 |
| 3 | 15 | 10/10 | 4.2 | 1.0 | 87.2+ | 22.4 |
| 4 | 15 | 10/10 | 5.3 | 0.6 | 36.2+ | 6.89 |
| 5 | 15 | 9/10 | 4.5 | 1.0 | 31.5+ | 6.2 |
| 6 | 15 | 9/10 | 4.9 | 1.4 | 18.9+ | 7.2 |
| 14 | 15 | 8/10 | 5.0 | 0.3 | 12.9+ | 1.6 |

Table 9.1. The effect of age on the hypnotic action of pentobarbitone sodium in chicks.

\+ Significantly different from the controls (same age and dose of pentouarbitone).

$p < 0.001$, $t$- test.

Pentobarbitone sleeping time was significantly reduced with age. Also, the percentage hypnosis decreased while the onset of sleep was delayed with age.

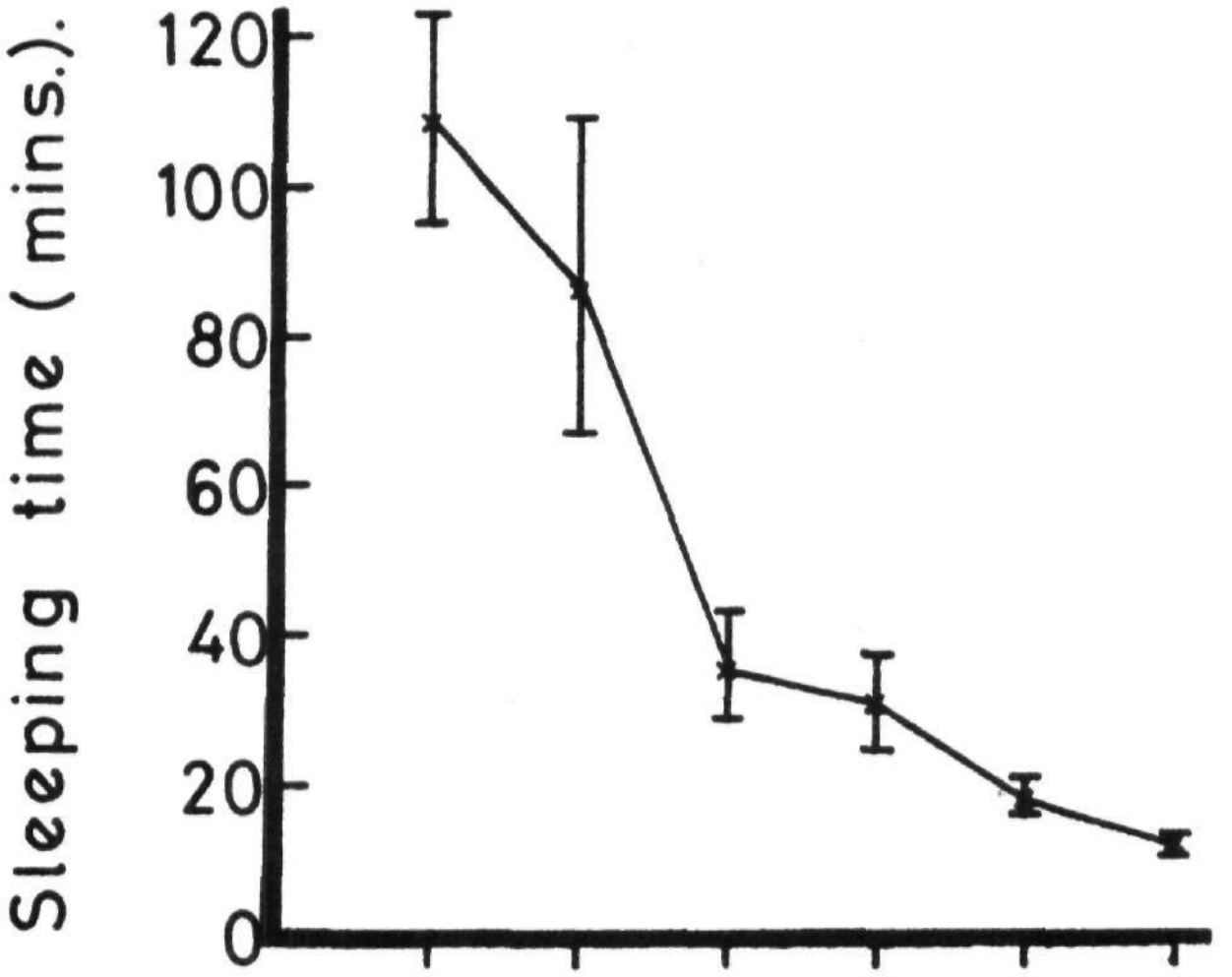



Fig. 9.1. Effect of age on pentobarbitone-induced sleep in young chicks. Dose of pentobarbitone = 15 mg/kg. N = 20 per point.

ANALYSIS OF VARIANCE

| Source | Degree of freedom | Sum of squares | Mean squares | F. Ratio | F. Probability. |
|---|---|---|---|---|---|
| Between Groups | 5 | 7337.4916 | 14675.4983 | 102.231 | 0.001 |
| Within Groups | 50 | 7177.6076 | 143.5522 | | |
| Total | 55 | 8055.0993 | | | |

| Age (Days) | Dose (mg/kg. i.p.) Pento. | DA. | No asleep/ No used. | Onset of sleep (min.) Mean | S.D. | Duration of sleep.(min.) Mean | S.D. |
|---|---|---|---|---|---|---|---|
| 2 | 7 | 0 | 10/10 | 3.3 | 0.7 | 28.2 | 3.9 |
| 2 | 7 | 100 | 4/10 | 8.6 | 1.7 | 8.0+ | 0.8 |
| 4 | 10 | 0 | 10/10 | 5.0 | 0.2 | 21.9 | 2.0 |
| 4 | 10 | 25 | 10/10 | 5.5 | 0.6 | 15.3+ | 1.5 |
| 4 | 10 | 50 | 6/10 | 13.2 | 2.5 | 17.2+ | 3.2 |
| 4 | 10 | 100 | 1/10 | 17.2 | - | 16.5+ | - |
| 4 | 10 | 200 | 8/10 | 11.3 | 3.0 | 22.3 | 2.8 |
| 4 | 10 | 400 | 10/10 | 9.7 | 0.8 | 66.4+ | 3.6 |
| 4 | 15 | 0 | 10/10 | 4.8 | 0.7 | 33.6 | 4.2 |
| 4 | 15 | 100 | 3/10 | 7.0 | 0.5 | 22.1+ | 3.4 |
| 5 | 15 | 0 | 9/10 | 3.4 | 0.7 | 28.7 | 6.8 |
| 5 | 15 | 100 | 6/10 | 5.5 | 0.9 | 22.6+ | 3.9 |
| 14 | 15 | 0 | 8/10 | 4.8 | 1.0 | 13.1 | 1.3 |
| 14 | 15 | 100 | 1/10 | 12.5 | - | 18.4 | - |

Table 9.2. The influence of DA on pentobarbitone sleep.

\+ Significantly different from the controls (same age and dose of pentobarbitone), $p < 0.001$, $t$ - test.

DA delayed the onset and shortened the duration of sleep. Also, the percentage hypnosis was less in DA-pretreated chicks than the controls (i.e. given only pentobarbitone).

9.4.3. The Influence of Pimozide (4 mg/kg.), Phenoxybenzamine (10 mg/kg.) and Propranolol (10 mg/kg.) on the Sedation Induced by High Doses of DA (400 mg/kg.).

Apparently, the sedation induced by high doses of DA was potentiated by pimozide but antagonised by both phenozybenzamine and propranolol (Table 9.3).

9.4.4. Effect of Pimozide on Pentobarbitone sleep.

Pimozide (4 mg/kg.) significantly shortened the onset and prolonged the duration of pentobarbitone sleep ( Table 9.4). Using pimozide - pretreated chicks, dopamine delayed the onset and shortened the duration of pentobarbitone-induced sleep (Table 9.4).

9.4.5. Influence of Levodopa on Pentobarbitone-induced Sleep.

Levodopa (25-200 mg/kg.) profoundly antagonised pentobarbitone-induced sleep (Table 9.5).

| Dose (mg/kg.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dopamine | Pimozide | Phenoxybenzamine | Propranolol | No asleep/ No used. | Onset of sleep (min.) | | Duration of sleep (min.) | |
| | | | | | Mean | S.D | Mean | S.D |
| 400 | 0 | 0 | 0 | 2/20 | 10.3 | 0.3 | 15.8 | 3.6 |
| 0 | 4 | 0 | 0 | 0/20 | - | - | - | - |
| 0 | 0 | 10 | 0 | 0/20 | - | - | - | - |
| 0 | 0 | 0 | 10 | 0/ | - | - | - | - |
| 400 | 4 | 0 | 0 | 8/20 | 5.4 | 1.8 | 21.5 | 9.0 |
| 400 | 0 | 10 | 0 | 0/20 | - | - | - | - |
| 400 | 0 | 0 | 10 | 1/20 | 11.9 | - | 14.3 | - |
| 400 | 0 | 0 | 10 | 1/20 | 11.9 | - | 14.3 | - |

Table 9.3. The influence of pimozide, phenoxybenzamine and propranolol on the sedation induced by high doses of DA in 5 day old chicks. The pretreatment times with pimozide, phenoxybenzamine and propranolol prior to the injection of DA were 4hours, 8 hours and 2 hours respectively. The chicks injected with DA alone were quiet and immobile while those injected with phenoxybenzamine/propranolol plus DA were behaviourally excited. On the other hand, pimozide apparently potentiated the sedation induced by high doses of DA.

| Dose (mg/kg.i.p.) | | | No asleep/ No used. | Onset of sleep (min.) | | Duration of sleep (min.) | |
|---|---|---|---|---|---|---|---|
| Pento. | DA. | Pimozide | | Mean | S.D. | Mean | S.D. |
| 15 | 0 | 0 | 19/20 | 4.0 | 0.6 | 30.0 | 3.4 |
| 15 | 100 | 0 | 7/20 | 6.9 | 1.2 | 21.5+ | 3.5 |
| 15 | 0 | 4 | 20/20 | 2.3 | 1.6 | 59.4+ | 15.3 |
| 15 | 100 | 4 | 14/20 | 7.3 | 1.0 | 38.0+ | 1.5 |

Table 9.4. Antagonism of pimozide-induced potentiation of pentobarbitone sleep by DA in 5 day old chicks.

\+ Significantly different from the controls (same dose of pentobarbitone), $p < 0.001$, $t$ - test.

++ Significantly different from the chicks given pimozide and pentobarbitone, $p < 0.001$, $t$ - test.

Pimozide reduced the onset and prolonged the duration of pentobarbitone-induced sleep. The effect of pimozide on pentobarbitone sleep was significantly reduced when the chicks were also given DA prior to the administration of pentobarbitone.

| Age (days) | Dose (mg/kg.) | | No asleep/ No used. | Onset of sleep (min.) | | Duration of sleep (min) | |
|---|---|---|---|---|---|---|---|
| | Pento. | L-dopa. | | Mean | S.D. | Mean | S.D. |
| 2 | 7 | 0 | 10/10 | 4.3 | 0.6 | 28.0 | 3.9 |
| 2 | 7 | 25 | 0/10 | - | - | - | - |
| 2 | 7 | 50 | 0/10 | - | - | - | - |
| 3 | 10 | 0 | 10/10 | 3.3 | 0.5 | 54.8 | 3.9 |
| 3 | 10 | 50 | 4/10 | 5.4 | 1.6 | 49.1 | 2.5 |
| 3 | 10 | 100 | 0/10 | - | - | - | - |
| 4 | 10 | 0 | 10/10 | 5.1 | 0.5 | 22.0 | 1.3 |
| 4 | 10 | 25 | 0/10 | - | - | - | - |
| 4 | 10 | 50 | 0/10 | - | - | - | - |
| 4 | 15 | 0 | 10/10 | 4.8 | 0.6 | 33.6 | 3.0 |
| 4 | 15 | 25 | 6/10 | 5.0 | 1.0 | 44.7 | 6.5 |
| 4 | 15 | 50 | 1/10 | 6.3 | - | 53.1 | - |
| 4 | 15 | 100 | 0/10 | - | - | - | - |

Table 9.5. The influence of levodopa on pentobarbitone-induced sleep in young chicks.
Pretreatment of the chicks with levodopa significantly reduced the proportion of chicks which slept following the administration of pentobarbitone. Also, levodopa delayed the onset but prolonged the duration of pentobarbitone-induced sleep.

9.4.6. Influence of Apomorphine on Pentobarbitone sleep.

Apomorphine (0.25 - 0.5 mg/kg.) antagonized pentobarbitone-induced sleep (Table 9.6). Apomorphine significantly delayed and shortened the onset of pentobarbitone-induced sleep.

9.4.7. Effect of FLA-63 on Pentobarbitone-induced Sleep.

Pretreatment of chicks with FLA-63 (12.5 mg/kg.) prior to administration of pentobarbitone, reduced the percentage hypnosis and significantly shortened the duration of pentobarbitone sleep (Table 9.7).

9.4.8. Effects of Dopamine, Levodopa and Apomorphine on the EEG and EMG.

Dopamine (100 - 400 mg/kg.) increased the EEG activity and desynchronized the EEG of both the hyperstriatum and optic tectum. The effect of dopamine on the EEG of the pontine reticular formation

| Dose (mg/kg.) | | No asleep/ No used. | Onset of sleep (min.) | | Duration of sleep (min.). | |
|---|---|---|---|---|---|---|
| Pento. | Apomorphine | | Mean | S.D. | Mean | S.D. |
| 10 | 0 | 10/10 | 4.6 | 1.8 | 20.0 | 4.2 |
| 10 | 0.5 | 0/10 | - | - | - | - |
| 15 | 0 | 10/10 | 4.0 | 1.5 | 28.3 | 3.0 |
| 15 | 0.25 | 4/10 | 12.0 | 2.9 | 15.4 | 1.6 |
| 15 | 0.5 | 0/10 | - | - | - | - |

Table 9.6. The effect of apomorphine on pentobarbitone-induced sleep using 4 day old chicks. Pretreatment of the chicks with apomorphine profoundly reduced the proportion of chicks which slept following the administration of pentobarbitone. Apomorphine also delayed the onset and shortened the duration of pentobarbitone sleep.

| Dose (mg/kg.i.p.) Pento. | FLA-63 | DA | No asleep/ No used. | Onset of sleep (min.) | | Duration of sleep (min.) | |
|---|---|---|---|---|---|---|---|
| | | | | Mean | S.D. | Mean | S.D. |
| 10 | 0 | 0 | 10/10 | 5.0 | 0.7 | 31.9 | 5.4 |
| 10 | 12.5 | 0 | 7/10 | 6.0 | 1.5 | 22.5+ | 6.5 |
| 10 | 12.5 | 100 | 3/10 | 8.3 | 1.2 | 17.1+ | 5.5 |
| 10 | 12.5 | 400 | 3/20 | 8.5 | 1.5 | 16.0+ | 3.0 |

Table 9.7. The effect of FLA-63 on pentobarbitone-induced sleep using 3 day old chicks.
+ $p < 0.025$ (the chicks given only pentobarbitone were used as controls), student - $t$ - test.

FLA-63 (12.5 mg/kg.) reduced the number of chicks which slept and significantly shortened the duration of pentobarbitone sleep when FLA-63-pretreated chicks were used.

was not quite obvious (figs.9.2 and 9.3). Similarly, levodopa (100 mg/kg.) and apomorphine (0.25 mg/kg.) profoundly increased the EMG activity and desynchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation (figs. 9.4 & 9.5 respectively).

9.4.9. Effects of Pentobarbitone and DA on the EEG and EMG.

Pentobarbitone (10 mg/kg.) reduced muscle activity and synchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation (fig. 9.6). Dopamine (100 mg/kg.) antagonised the EEG effect of pentobarbitone on the hyperstriatum, optic tectum and pontine reticular formation. On the other hand, 40 mg/kg. of dopamine apparently potentiated pentobarbitone-induced synchronization of the hyperstriatum while the EEG activity of the optic tectum and pontine reticular formation were slightly

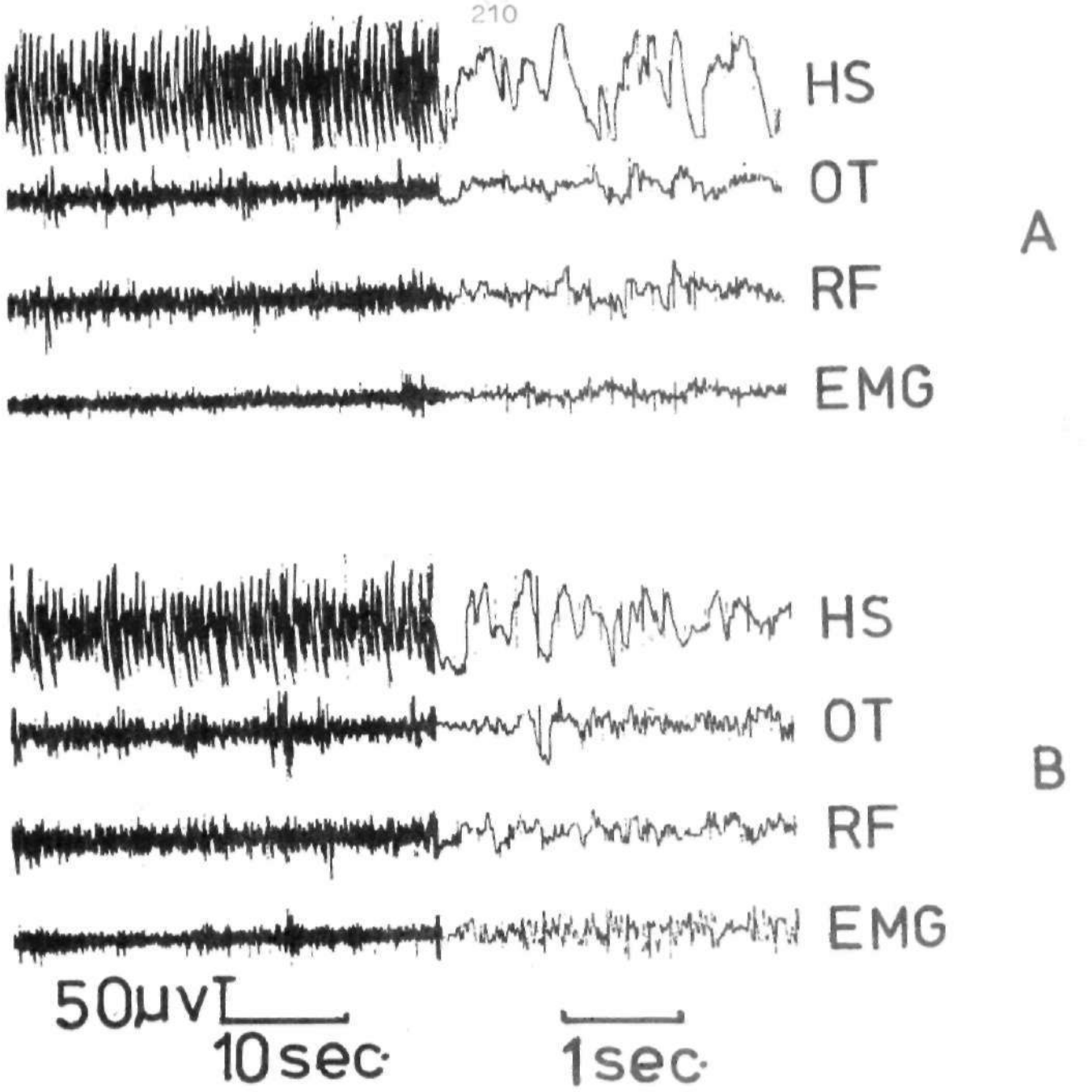



Fig. 9.2. Effect of DA (100 mg/kg.i.p.) on chick EEG. and EMG. using 5 day old chick. Left-hand panels are at one-tenth the chart speed of right-hand panels. Upper panels (A) are controls while the lower panels (B) are treated with DA (10 min. after injection). HS = hyperstriatum; OT = optic tectum; RF = reticular formation; EMG = electromyograph (similar abbreviations are used in figs. 9.2-9.9).

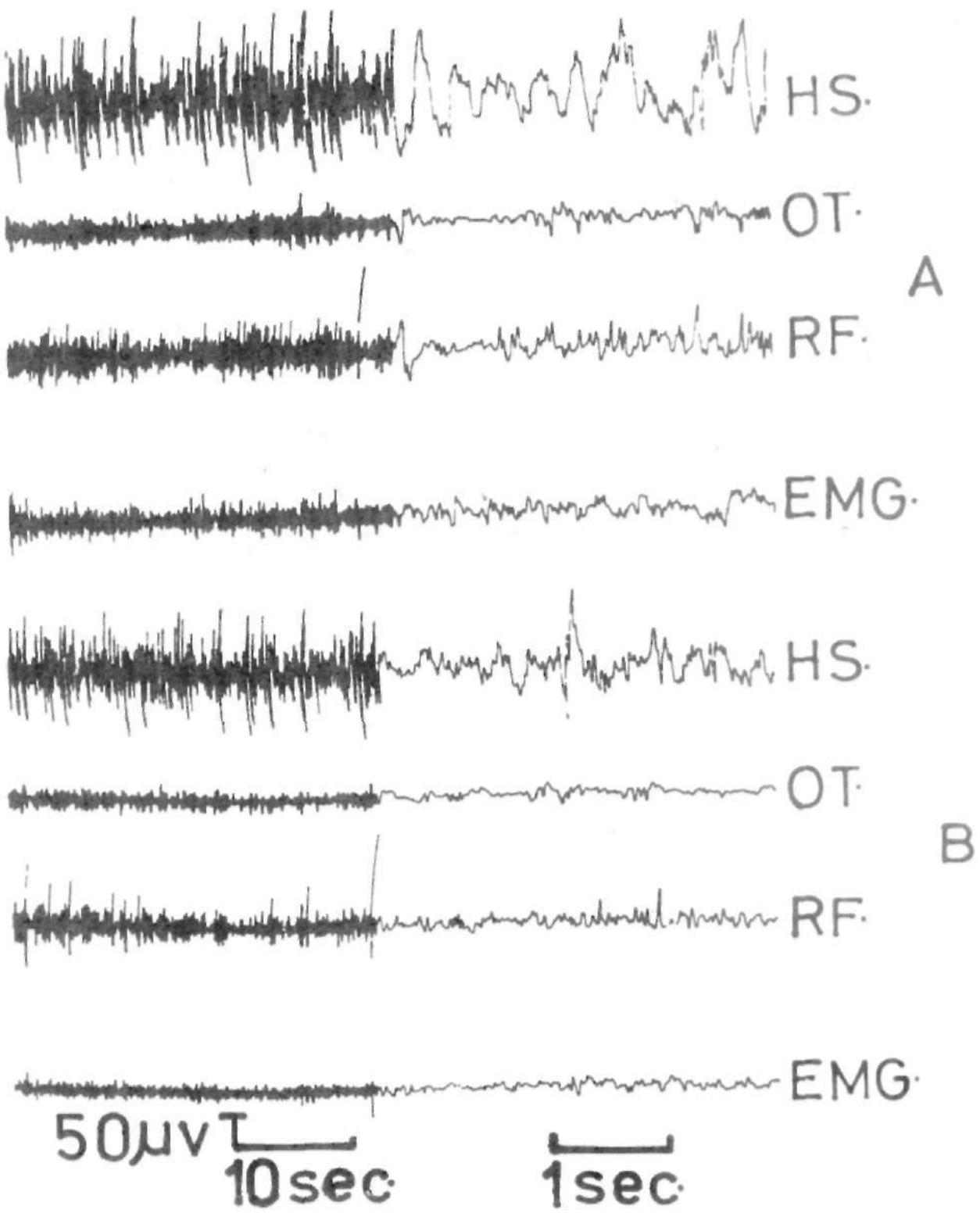



Fig. 9.3. Effects of DA (400 mg/kg.i.p.) on the chick EEG and EMG using 4 day old chick. Left-hand panels are one-tenth the chart speed of the right-hand panels. Upper panels (A) are controls, lower panels (B) are DA-treated (5 min. after injection).

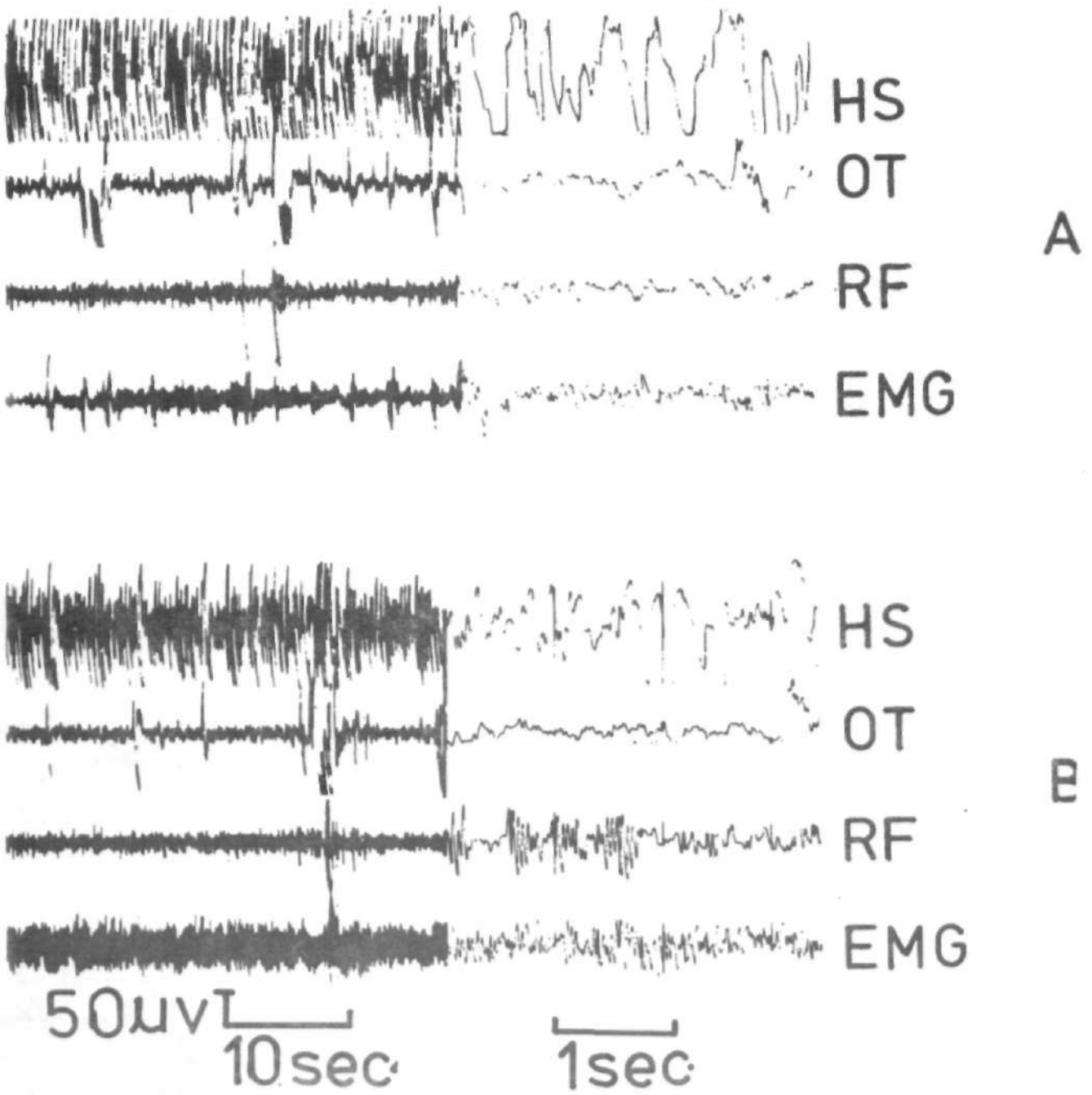



Fig. 9.4. Effect of L-dopa (25 mg/kg.) on the chick EEG and EMG using 4 day old chick. Left-hand panels are one-tenth the chart speed of the right-hand panels. Upper panels (A) are controls, lower panels (B) are Dopa-treated (30 min. after injection).

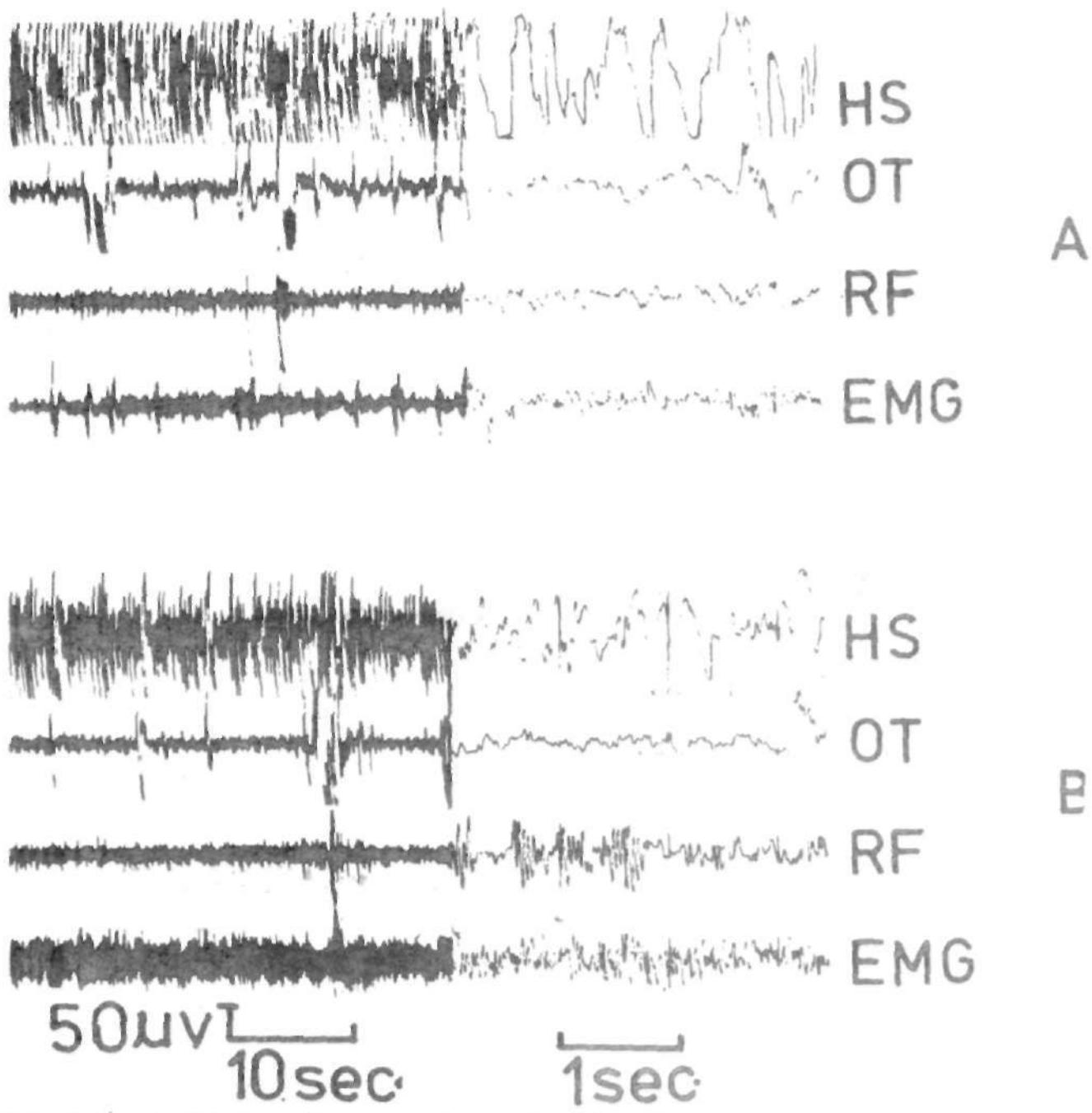



Fig. 9.5. Effects of apomorphine (0.25 mg/kg.s.c.) on the chick EEG and the EMG using 4 day old chick. Upper panels (A) are controls, lower panels (B) are apomorphine treated (10 min. after injection).

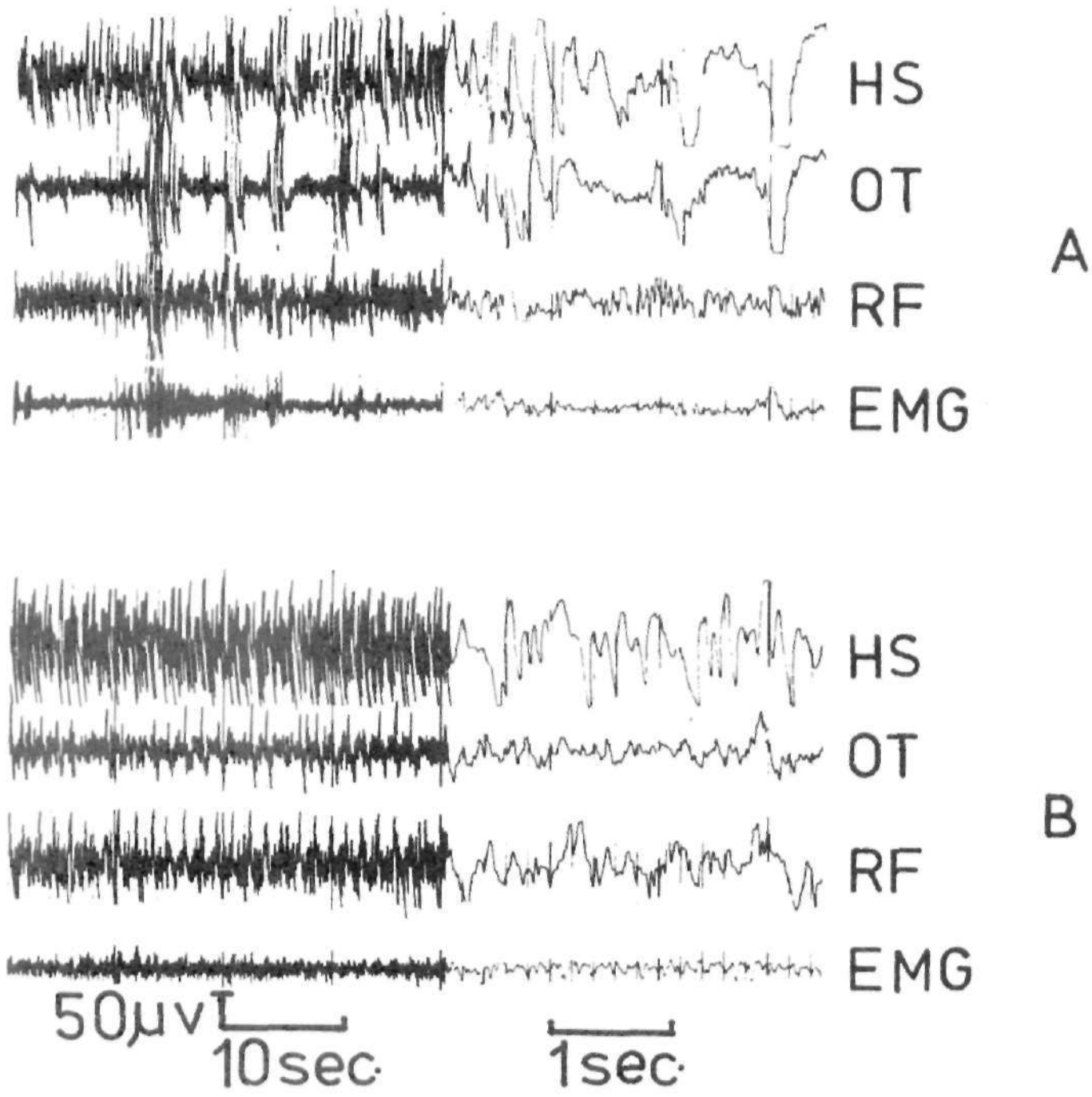



Fig. 9.6. Effect of pentobarbitone (10 mg/kg.i.p.) on the chick EEG. and the EMG. using 4 day old chick. Left-hand panels are at one-tenth the chart speed of right-hand panels. Upper panels (A) are controls, lower panels (B) are pentobarbitone-treated (10 min. after injection).

desynchronised and the EMG activity was enhanced (figs.9.7 & 9.8).

9.4.10. Effect of FLA-63 On The EEG and EMG.

FLA-63 (12.5 mg/kg.) increased muscle activity and desynchronized the EEG of the hyperstriatum, optic tectum and reticular formation. Using FLA-63-pretreated chicks, injected DA (400 mg/kg.) enhanced the EMG activity and further desynchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation (fig. 9.9).

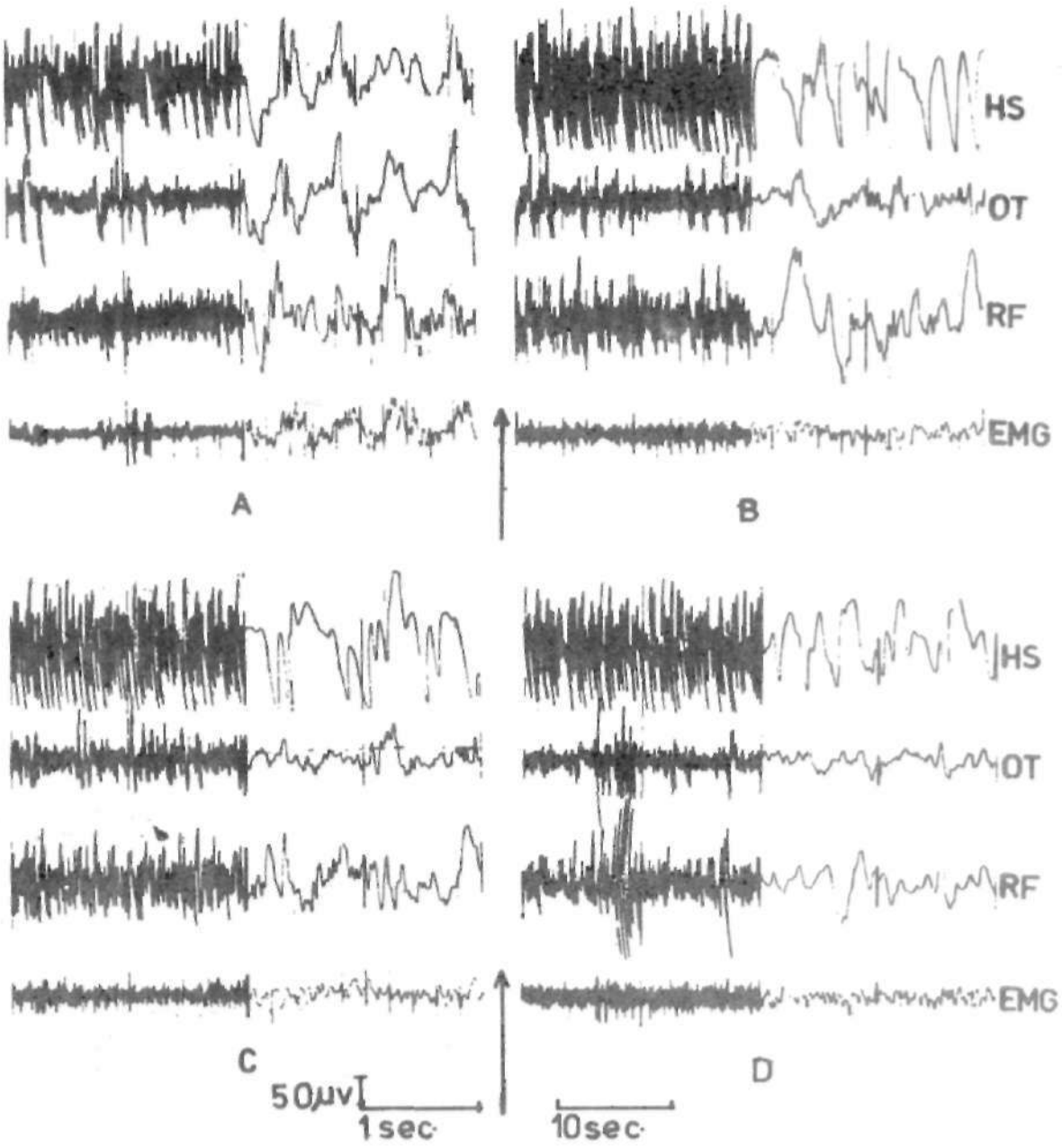



Fig. 9.7. Effects of DA (100 mg/kg.i.p.), 5 min. latter plus pentobarbitone (10 mg/kg.i.p.) on the chick EEG. and the EMG. using 4 day old chicks. Upper panels are controls with saline (A) and pentobarbitone (B), lower panel (C) is control with saline and D is DA + pentobarbitone (10 min. after injection of pentobarbitone).

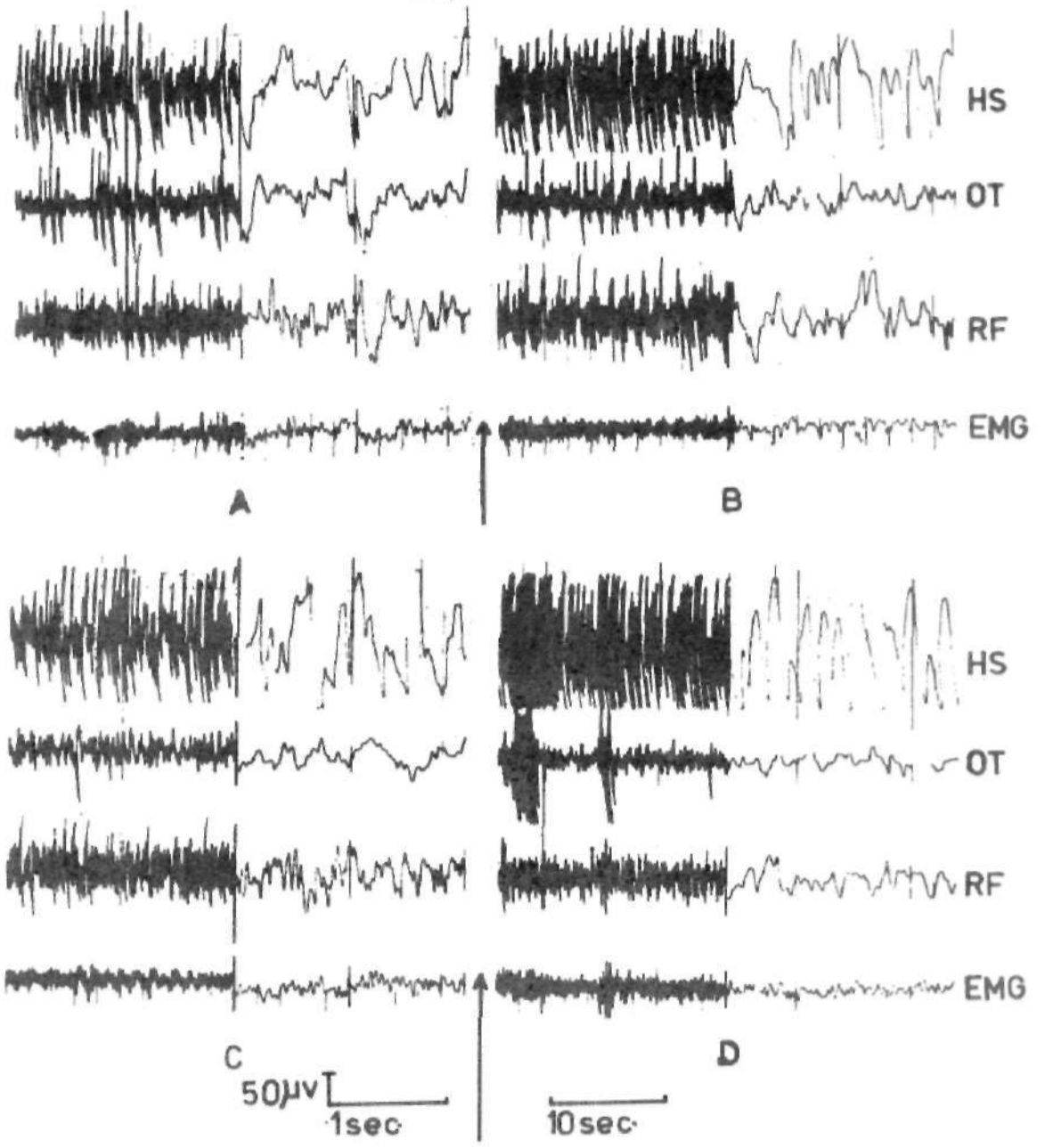



Fig. 9.8. Effects of DA (400 mg/kg.i.p.) 5 min. latter plus pentobarbitone (10 mg/kg.i.p.) on the chick EEG. and the EMG. using 4 day old chicks. Upper panels are controls with saline (A) and pentobarbitone (B), C is control with saline and D is DA + pentobarbitone (10 min. after injection of pentobarbitone).

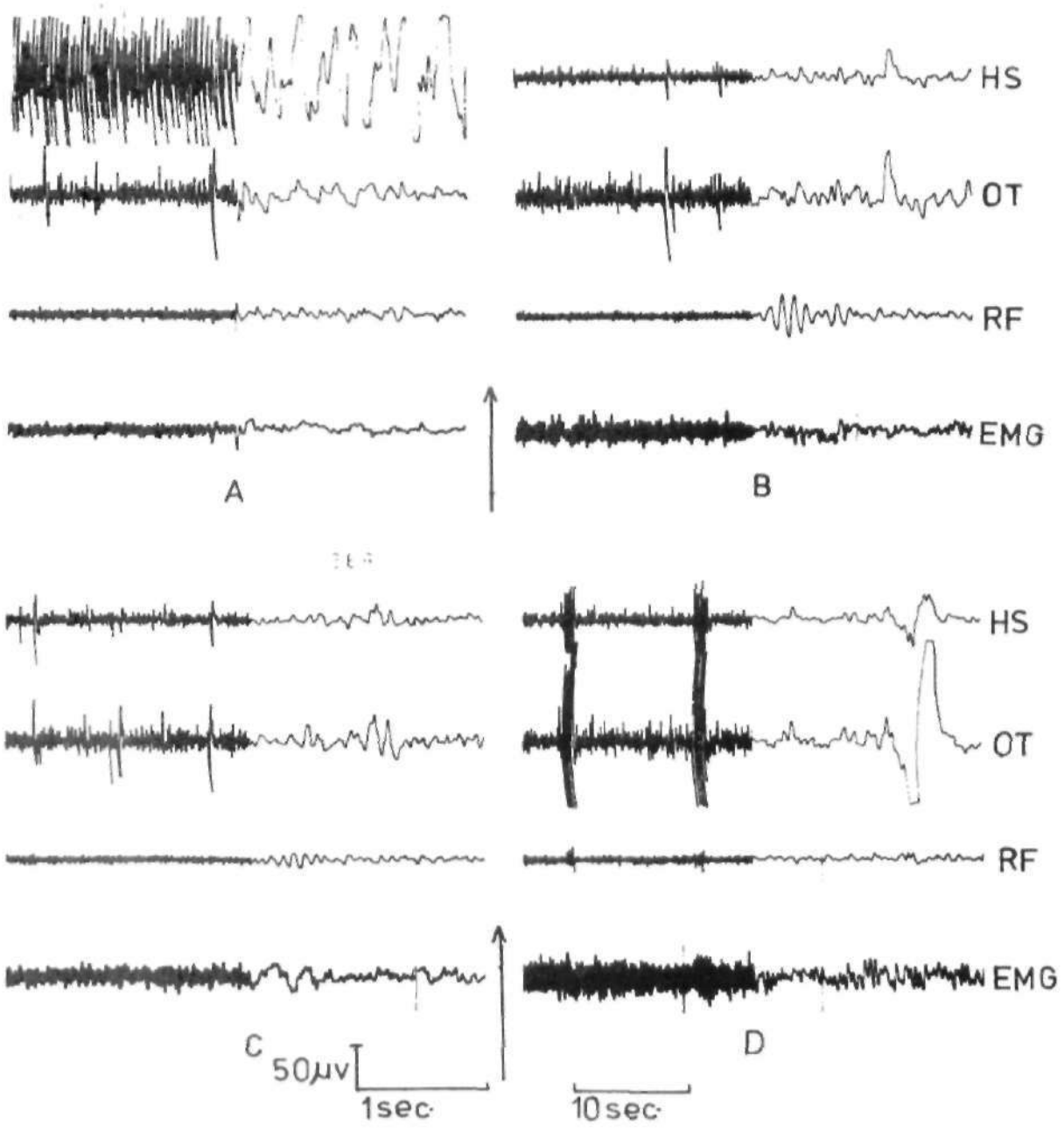



Fig. 9.9. Effects of FLA-63 (12 mg/kg.), 6 hours latter plus DA (400 mg/kg.) on the chick EEG. and the EMG. using 5 day old chicks. Upper panels are controls with saline (A) and FLA-63 (B), C is control with FLA-63 and D is FLA-63 (8 hours latter) + DA (10 min. after injection of DA).

9.5. Discussion.

As shown in fig. 1, the duration of sleep induced by the same dose of pentobarbitone decreased with the age of the chicks. Similar decrease in activity with age of chicks have been observed for tremorine (Bowman & Osuide, 1967), amino oxyacetic acid (Osuide, 1972b) and strychnine (Osuide & Wambebe, unpublished observation). Both metabolic and maturational factors might contribute to produce this result. The metabolism of hexobarbitone is negligible at birth in the mouse, guinea-pig, rat and human (Jondorf, Maickel & Brodie, 1958; Fouts & Adamson, 1959; Vesell, 1968). The increase in the amount of the hepatic drug-metabolising microsomal enzymes with age would progressively decrease the amount of pentobarbitone available for action and therefore reduce its hypnotic effect.

The behavioural actions of levodopa are thought to be due to DA produced from it in vivo upon decarboxylation (Scheel-Kruger & Randrup,

1967; Everett & Borcherding, 1970; Friedman & Gershon, 1971) and the behavioural actions of apomorphine have been attributed to direct stimulation of central dopaminergic receptors (Ernst, 1967; Roos, 1969; Ungerstedt et al, 1969; Anden, Fuxe, Hokfelt & Rubenson, 1967). Both levodopa and apomorphine were used in this study as dopaminoceptor agonists for comparative purpose with observations with dopamine.

According to Key & Marley (1962) and Waelsch (1955), the avian blood-brain barrier is permeable to catecholamine until 4 weeks after hatching. In this work, 2 - 14 day old chicks were used which permitted a more direct study of the effects of DA on pentobarbitone sleep. The results indicate that apomorphine, levodopa and dopamine produced EEG arousal in chicks and significantly antagonised pentobarbitone-induced sleep. This pharmacological antagonism suggest that these dopaminoceptor agonists induce behavioural

arousal. Lidbrink (1974) reported that behavioural waking from sleep can be markedly supressed by the destruction of the ascending nigro-neostriatal DA system. Wallach & Gershon (1971) have observed EEG desynchronization in adult cats following the injection of levodopa. Thus,the results with DA, levodopa and apomorphine are compatible with the finding of Lidbrink (1974) and Wallach & Gershon (1971); since these workers used mammalian species in their studies, the role of endogenous DA in sleep mechanisms might be quite similar in chicks and mammals.

Many workers have suggested that DA may be a natural inhibitory neurotransmitter in the mammal (Bloom et al, 1965; York, 1967; McLennan & York, 1967; Herz & Zieglgansberger, 1966). While others have suggested inhibition and excitation of different parts of the brain (Hornykiewicz, 1966; Cools & Van Rossum, 1976).

In the chick, Key & Marley (1962) are of the opinion that DA induces sleep. In the

present data, however, DA and levodopa did not produce sleep but rather aroused the chicks as previously noticed by Spooner & Winters (1967). Antagonism of barbiturate-induced sleep further confirms the excitatory effects of DA. High doses of DA, however, induced sleep in some of the chicks and potentiated barbiturate sleep, probably due to metabolism to noradrenaline (Spooner & Winters, 1967). FLA-63 is believed to inhibit dopamine-beta-hydroxylase enzyme and thus accumulates endogenous DA while NA levels are drastically reduced (Corrodi et al, 1970). The antagonism of pentobarbitone-induced sleep by FLA-63 agrees with the speculation that endogenous brain dopamine may be intimately involved in the mechanisms of wakefulness. According to Andén et al (1970), pimozide is a potent and specific dopaminoceptor blocking agent. As a neuroleptic, pimozide potentiated barbiturate sleep in the chick. Interestingly, moderate doses of DA reduced pimozide-induced potentiation of pentobarbitone sleep.

Such a result indicates that increased central dopamine receptor activity may be involved in the mechanism of wakefulness in the chick. Although 400 mg/kg. of DA produced behavioural sleep according to the criteria used, there were EEG and EMG signs of initial excitation (fig. 9.3). Also, this high dose delays the onset of pentobarbitone sleep (Table 9.2). These results suggest that the behavioural sleep might possibly be due to immobilisation, following a phase of excitation as suggested by Spooner & Winters (1965, 1967) producing the same behavioural effects as central nervous system depression. It is equally possible that the potentiation of pentobarbitone sleep observed with high doses of DA might be due to NA formed from the injected DA. Osuide & Wambebe (unpublished observation) have observed that NA injected to young chicks hypnotised them within 5 min.

The results further show that the sedative effect of high doses of dopamine was antagonised by adrenoreceptor blocking agents suggesting that noradrenaline, formed from the injected dopamine, might be involved in this action (Spooner & Winters, 1965). Lastly, the data indicate that when the chicks were pretreated with FLA-63, there was no potentiation of pentobarbitone sleep with high doses of DA. Infact, under such treatments, pentobarbitone sleep was significantly antagonised. However, the possibility of the existence of two types of DA receptors (inhibitory and excitatory) subserving the mechanisms of consciousness and sleep can not be ignored (cf. chapter 4). Further electrophysiological and neurochemical studies may help to elucidate the mechanisms underlying the dose - dependent biphasic effects of DA on pentobarbitone sleep.

# CHAPTER X

## 10.1. HYPOTHERMIC EFFECT OF DOPAMINE AND L-DOPA IN THE CHICK.

### 10.2. Summary.

10.2.1. The thermal influence of DA and L-dopa was studied in young chicks and adult fowls using rectal thermometer.

10.2.2. DA produced a significant dose-dependent hypothermia in young chicks but did not exhibit any thermal effect in adult fowls.

10.2.3. The hypothermic effect of L-dopa in young chicks was highly significant and dose-dependent. The hypothermic effect of L-dopa in adult fowls was not as pronounced as in young chicks.

10.2.4. High doses of haloperidol profoundly lowered the rectal tempterature of young chicks.

10.2.5. The hypothermic effect of DA and L-dopa in young chicks was effectively antagonised by haloperidol.

10.2.6. R04-4602 had no observable thermal effect in chicks but potentiated L-dopa-induced hypothermia.

10.2.7. The results suggest that the hypothermic influence of DA and L-dopa might be mediated via activation of central DA receptors.

10.3. Introduction.

In 1964, Feldberg & Myers implicated monoamines in the regulation of mammalian body temperature. It was also proposed by the same workers (1964) that 5-HT is released within the anterior hypothalamus to activate heat production while at the same site NA is released to deactivate the

heat production systems to cause heat loss. The role of the individual monoamines in temperature regulation is still controversial. For example, Fuxe & Sjoqvist (1972) and Barnett, Goldstein & Taber (1973) associated apomorphine-induced hypothermia in mice to activation of DA receptors. Similarly, Lal, Puri & Karkalas (1971) attributed withdrawal hypothermia in morphine-dependent rats to increased DA neurotransmission. On the other hand, Borbéley & Huston (1972) attributed gammabutyrolactone-induced hyperthermia in rats to increased DA neurotransmission. Also, Marley & Stephenson (1975) obtained hyperthermia when DA was infused into the hypothalamus of young chicks. It is therefore apparent that the specific involvement of DA in temperature regulation, both in mammals and birds, is not yet fully understood.

In this project, I intended to accumulate data which may further elucidate the role of DA in temperature regulation by studying the effect of DA and L-dopa on rectal temperature of young chicks and adult fowls.

## 10.4. Results.

### 10.4.1. Normal Saline.

Since the drugs used in this study were administered in normal saline, the control chicks were injected with the same vehicle. Normal saline produced a slight decrease (0.4$^{o}$C) in rectal temperature (fig. 10.1.). The means of the onset and duration of this effect were 15 $\pm$ 7 and 23.3 $\pm$ 12.3 minutes respectively. In some of the experiments, there was either a slight rise (0.2$^{o}$C) or no effect at all in rectal temperature.

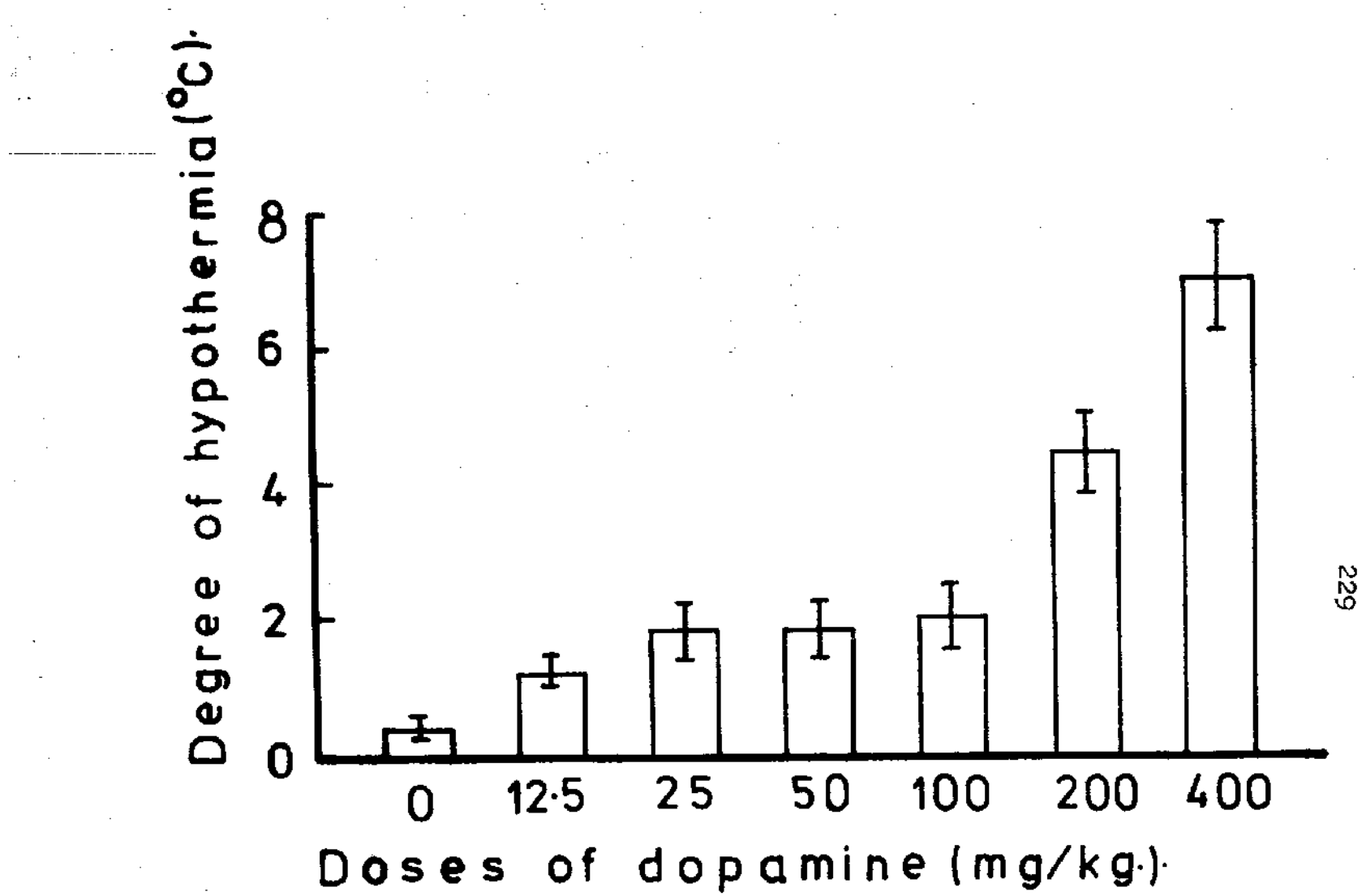



Fig. 10.1. Dose-response relationship of DA against rectal temperature. 3 - 7 day old chicks were used. Standard deviations from the means were represented by vertical bars ( n = 20 per histogram).

The difference between the controls and the results obtained with the chicks treated with drugs are the values presented under "results".

10.4.2. DA.

DA (12.5 - 400 mg/kg.) induced hypothermia in chicks in a dose-dependent manner (figs. 10.1. & 10.2). The effect was apperent after 10 minutes of drug administration and attained peak effect at about 30 minutes after administration. The effect was still observed after 2 hours of drug administration. DA lowered the temperature of adult fowls by 0.7$^{o}$C which was the same as observed in saline-treated fowls (fig. 10.3). Haloperidol (0.4 mg/kg.) antagonised the thermal effect of DA (fig. 10.4).

The doses of DA (1 mg/kg.) and FLA-63 (25 mg/kg.) used in these experiments

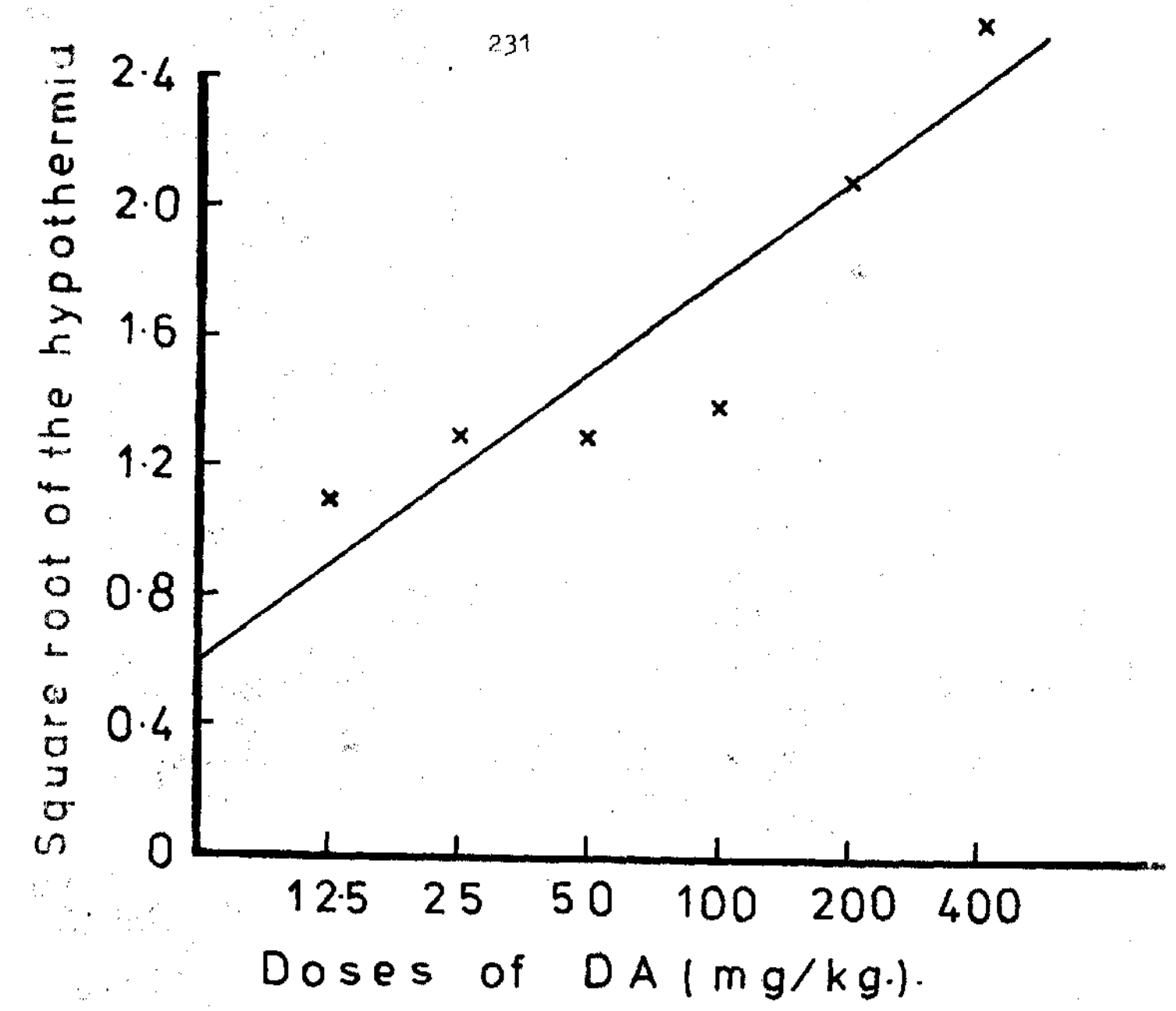



Fig. 10.2. Dose-response (square root) relationship of DA against rectal temperature.
The data used to draw the graph are the same as in fig. 10.1.

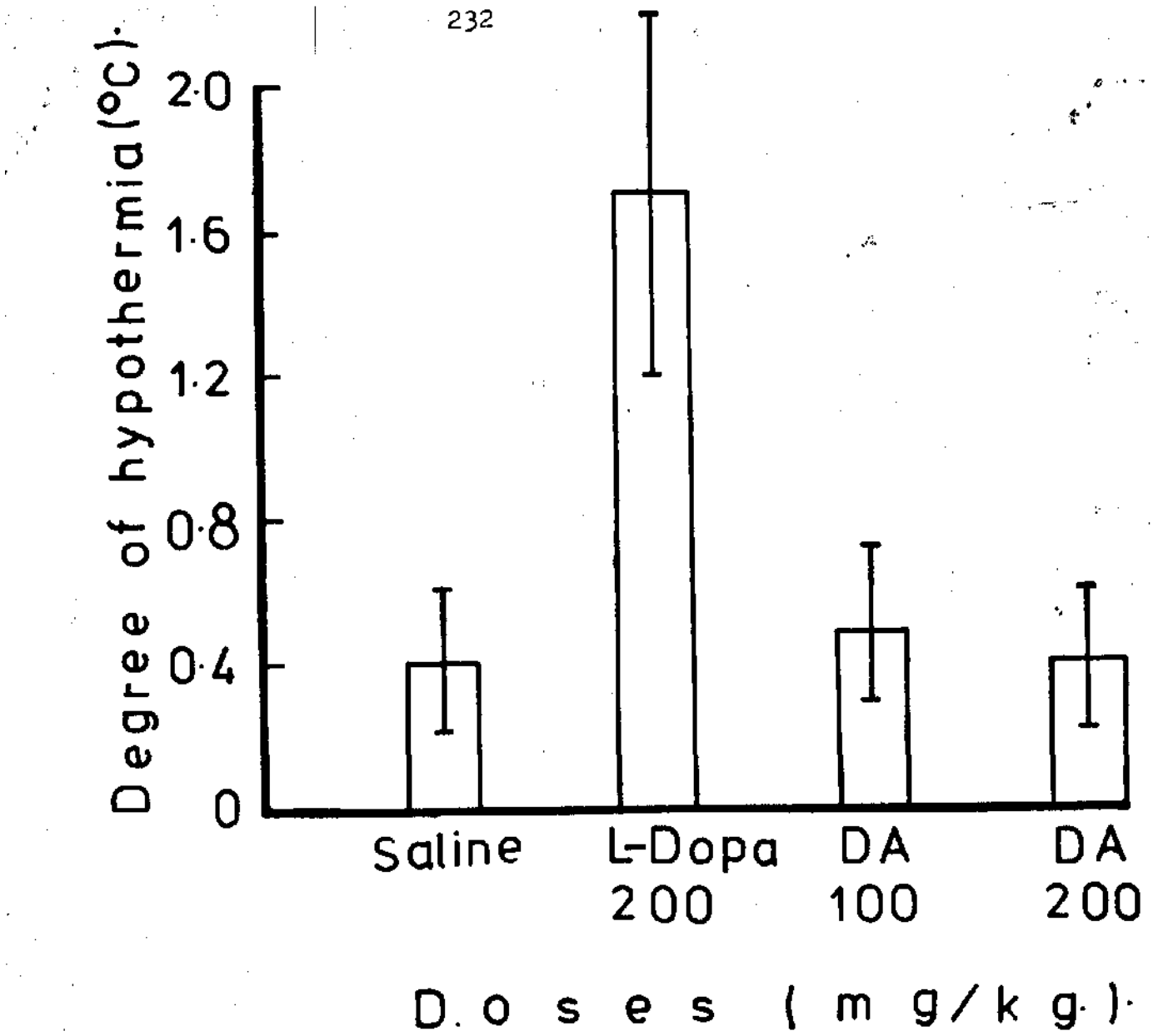



Fig. 10.3. Influence of DA on rectal temperature of adult fowls. The fowls were 20 weeks old. Vertical bars represent standard deviations from the means (n = 10 per histogram).

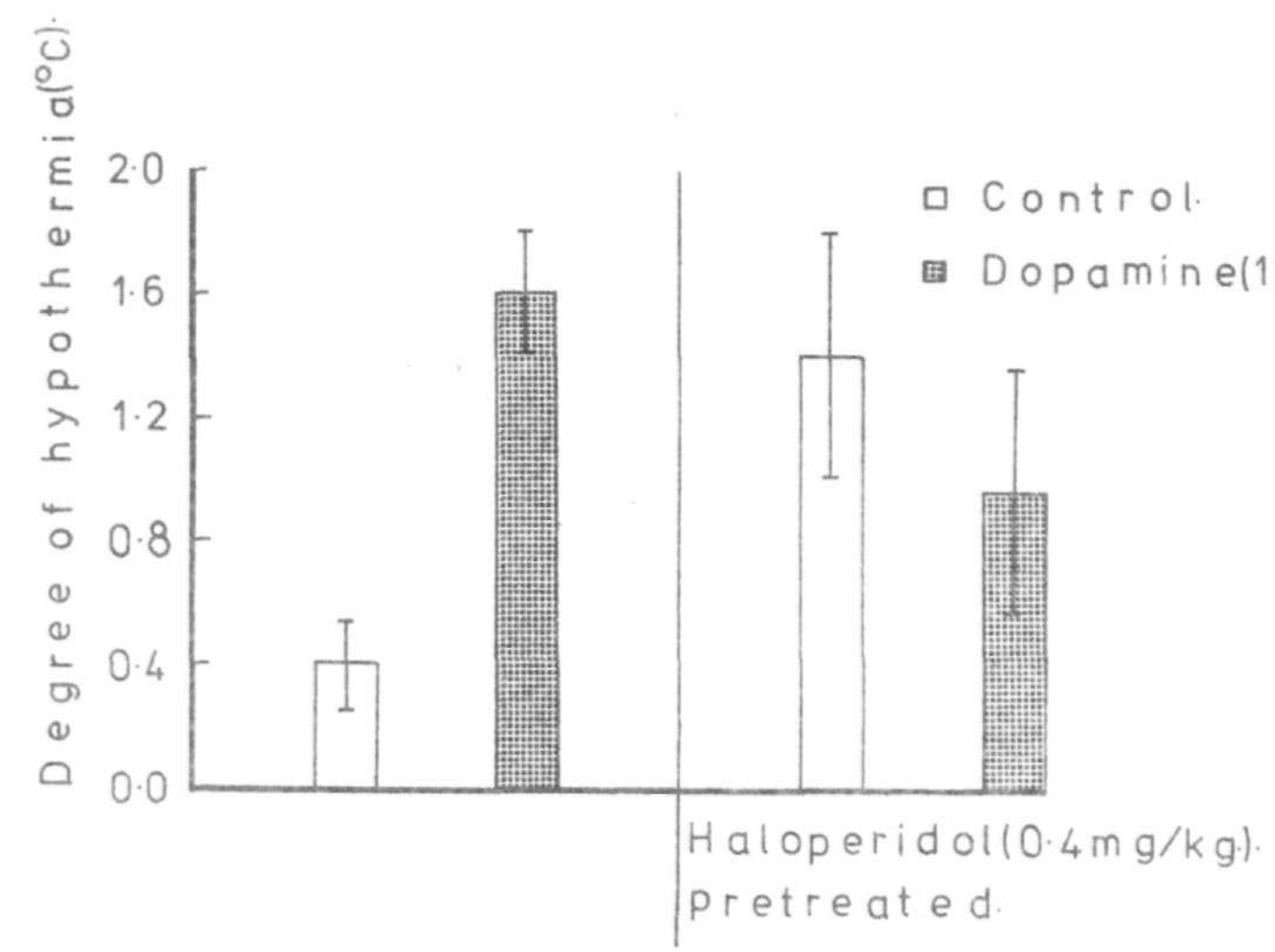



Fig. 10.4. Effect of haloperidol on the hypothermic action of DA. 3 day old chicks were used. Vertical bars represent standard deviations from the means (n = 30 per histogram).

lowered the body temperature to the same extent as saline-treated chicks (i.e. 0.3°C). However, when FLA-63-pretreated chicks were injected with DA (1 mg/kg.), the hypothermic effect observed was 1.6 ± 0.5°C. Indicating a significant decrease in temperature (Table 10.1).

10.4.3. L-dopa.

L-dopa (25-400 mg/kg.) induced profound hypothermia in chicks. The effect became apparent after 10 minutes of administration and reached peak effect at about 30 minutes. The hypothermia was still observed after 2 hours of drug administration (fig. 10.5 & 10.6). L-dopa (100 mg/kg.) lowered the body temperature of adult fowls, but to a lesser extent when compared to 3 day old chicks (fig. 10.7). Haloperidol (0.4 mg/kg.) antagonised the thermal effect of L-dopa (fig. 10.8).

| Drug | Dose (mg/kg.i.p.) | Hypothermia ($^{o}C$) |
|---|---|---|
| Normal saline | 0.4 ml | 0.3 ± 0.1 |
| DA | 1.0 | 0.3 ± 0.1 |
| FLA-63 | 25.0 | 0.5 ± 0.1 |
| DA + FLA-63 | 1.0<br>25.0 | 1.6 ± 0.5 |

Table 10.1. Effect of FLA-63 on DA-induced hypothermia.

3 day old chicks were used.

N = 80 chicks.

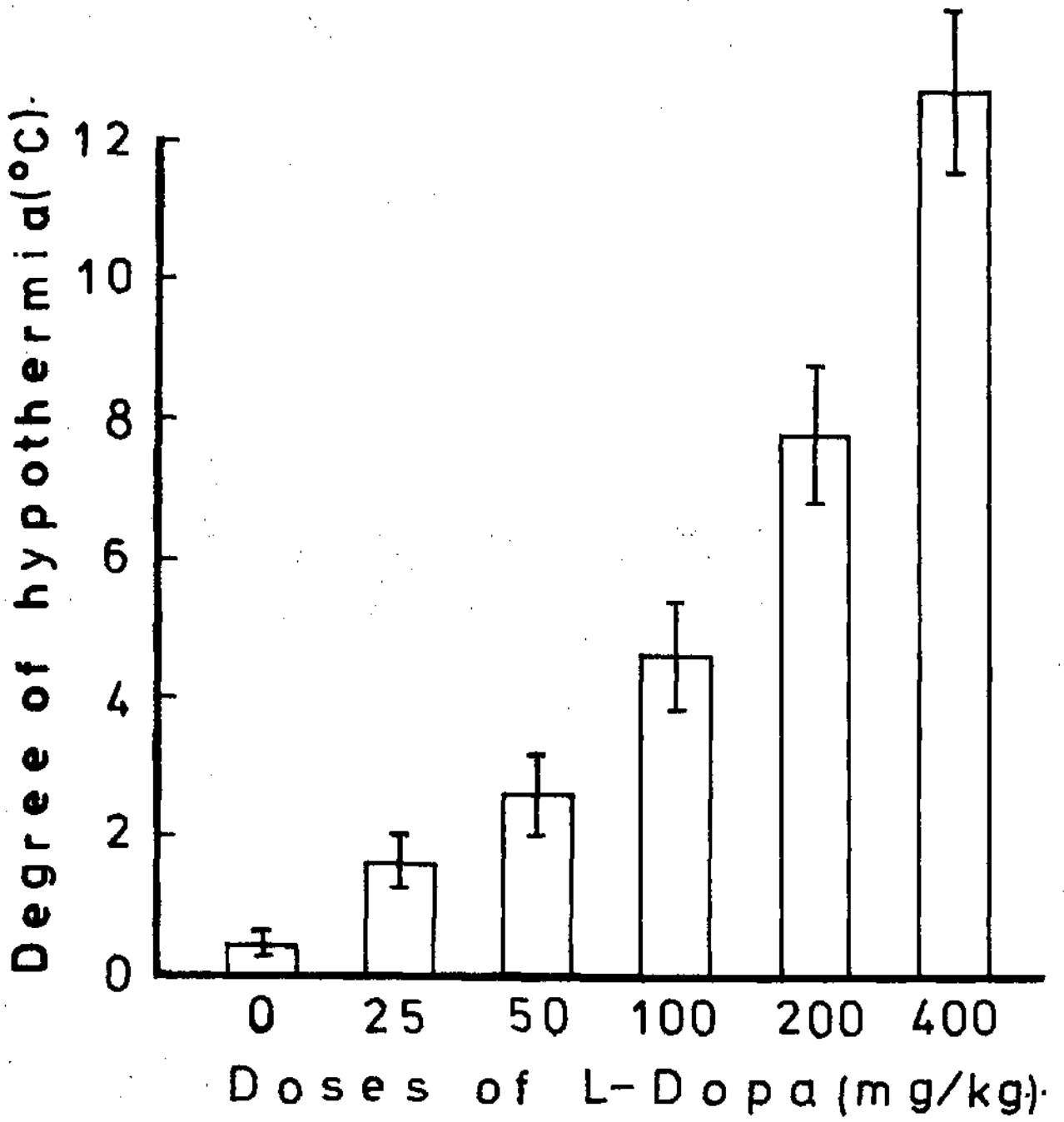



**Fig. 10.5.** Dose-response relationship of L-dopa against rectal temperature.
3 day old chicks were used. Vertical bars represent standard deviations from the means ( n = 20 per histogram).

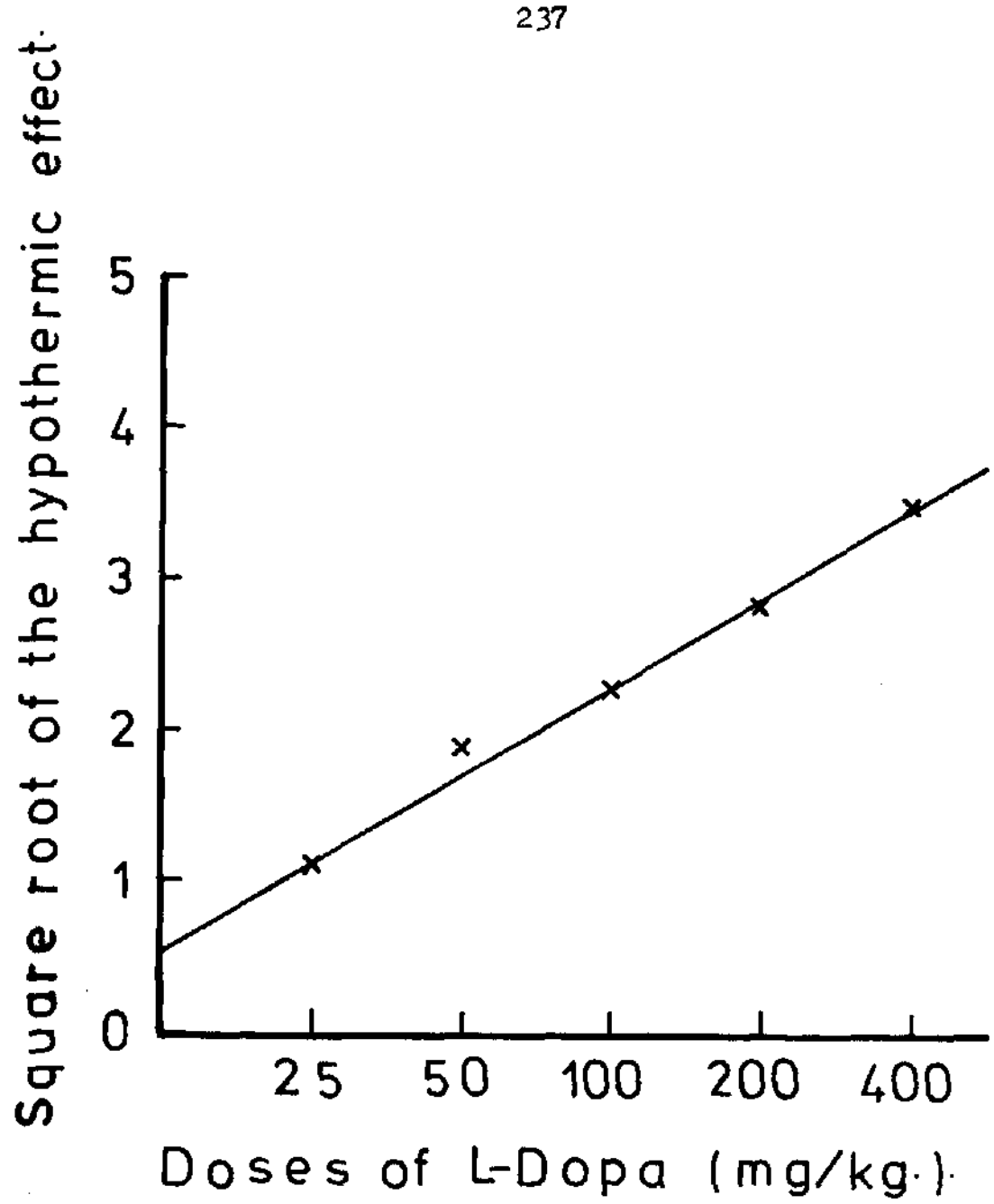



Fig. 10.6. Dose-response (square root) relationship of L-dopa against rectal temperature. The data used to draw this graph are the same as in fig. 10.5.

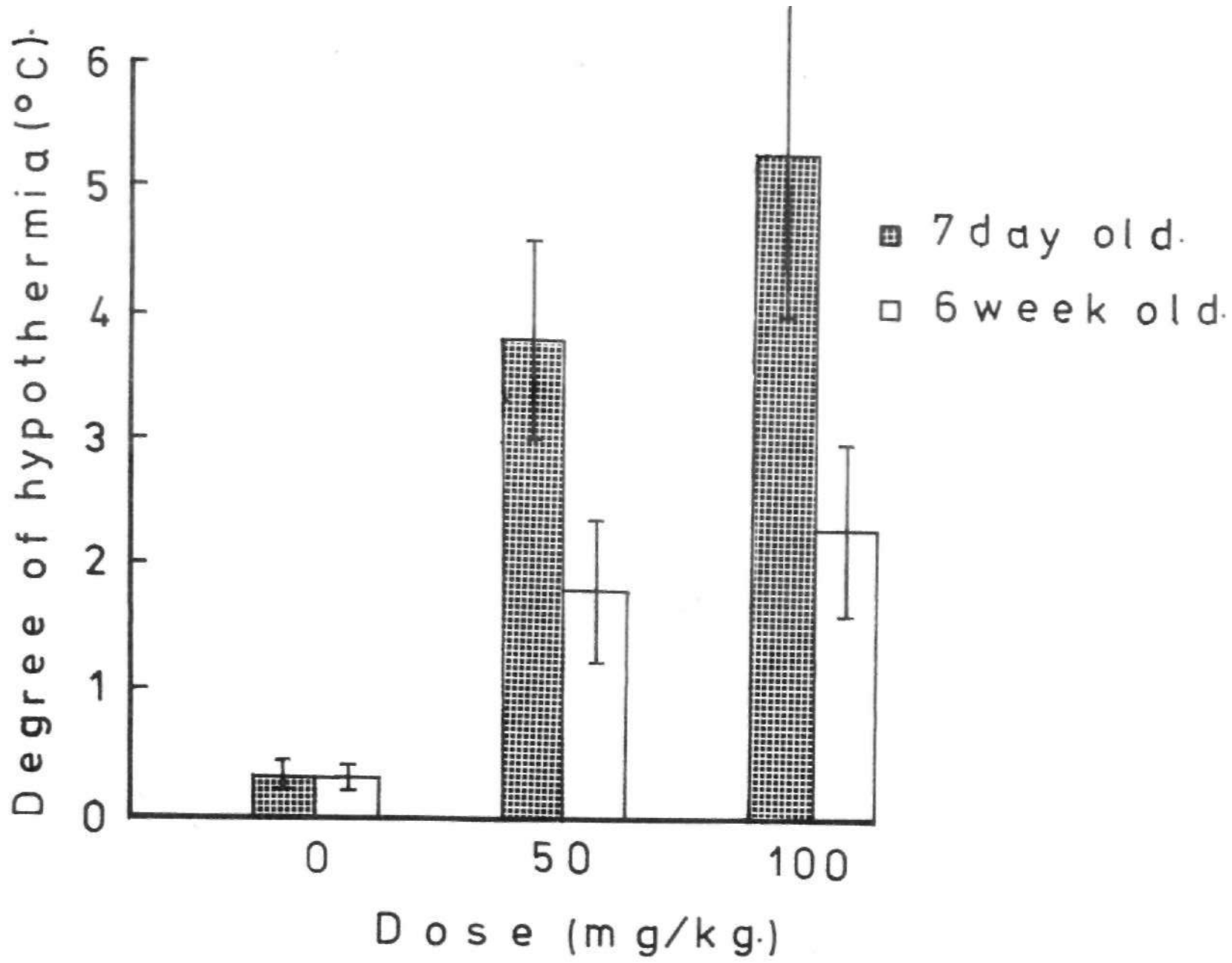



Fig. 10.7. Influence of L-dopa on rectal temperature using adult fowls. 20 week old fowls were used. Vertical bars represent standard deviations from the means (n = 10 per histogram).

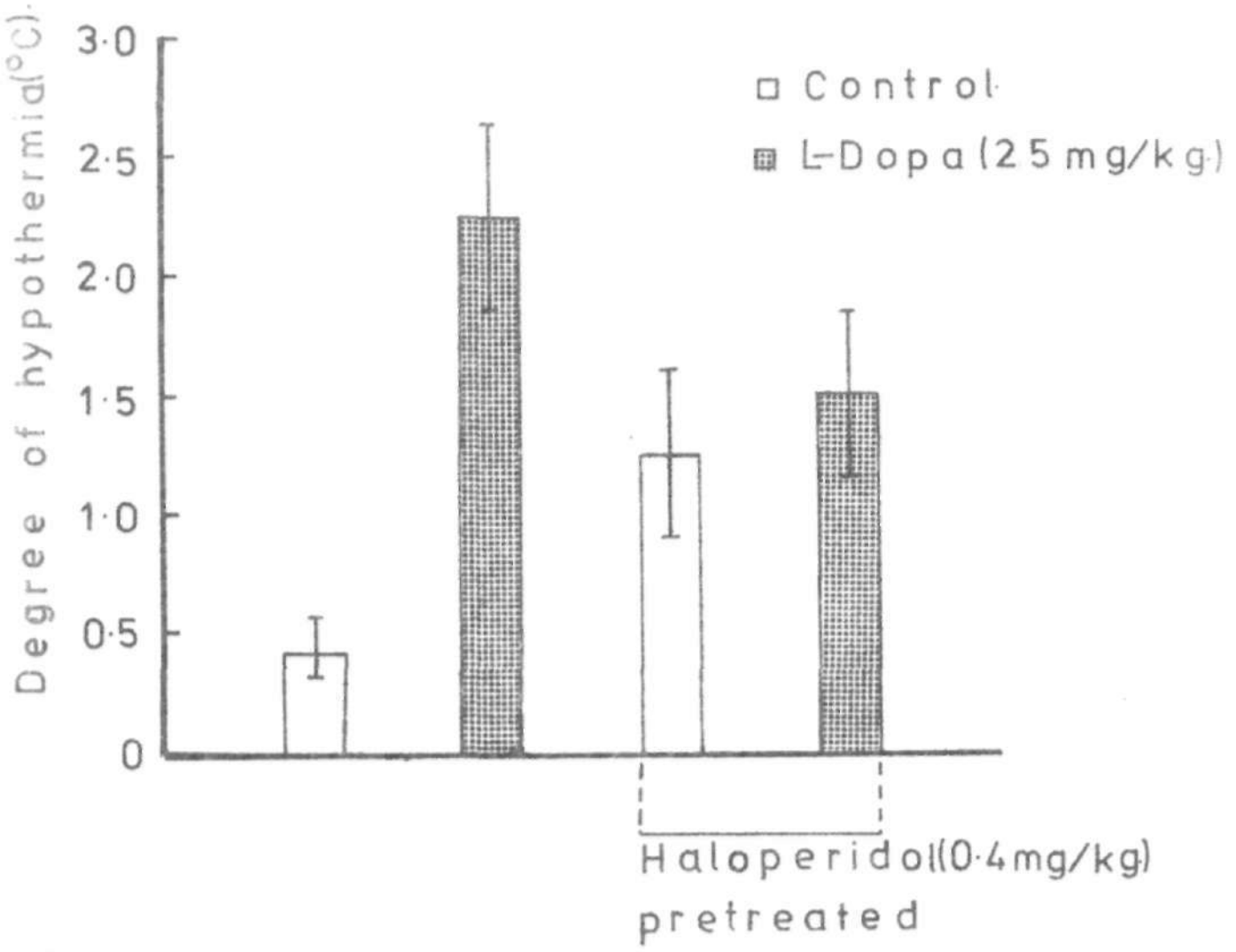



Fig. 10.8. Effect of haloperidol on the hypothermic action of L-dopa.
3 day old chicks were used. Vertical bars represent standard deviations from the means (n = 20 per histogram).

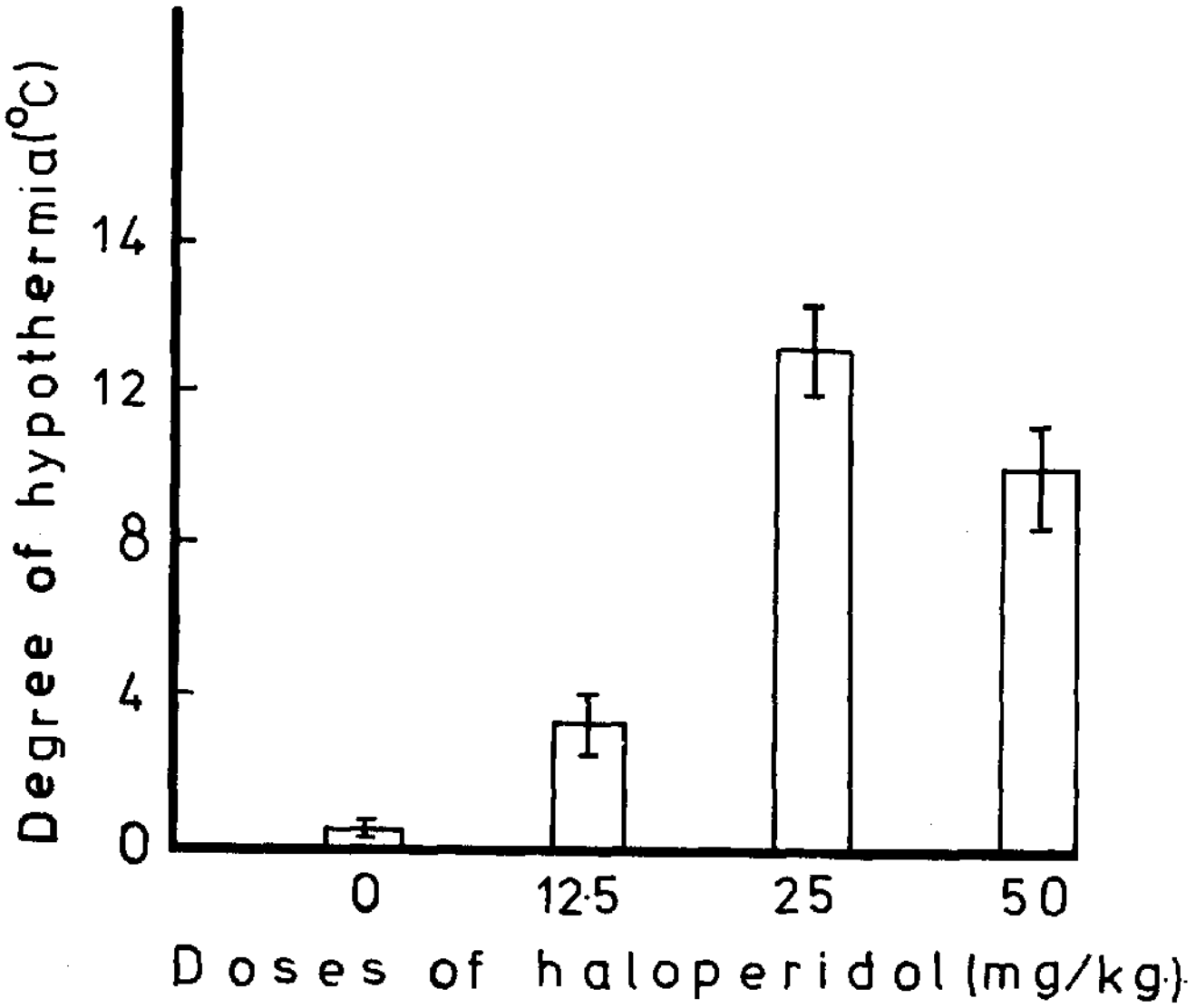



Fig. 10. 9. Effect of haloperidol on rectal temperature. 3-5 day old chicks were used. Standard deviations from the means are represented by vertical bars ( n = 20 per histogram).

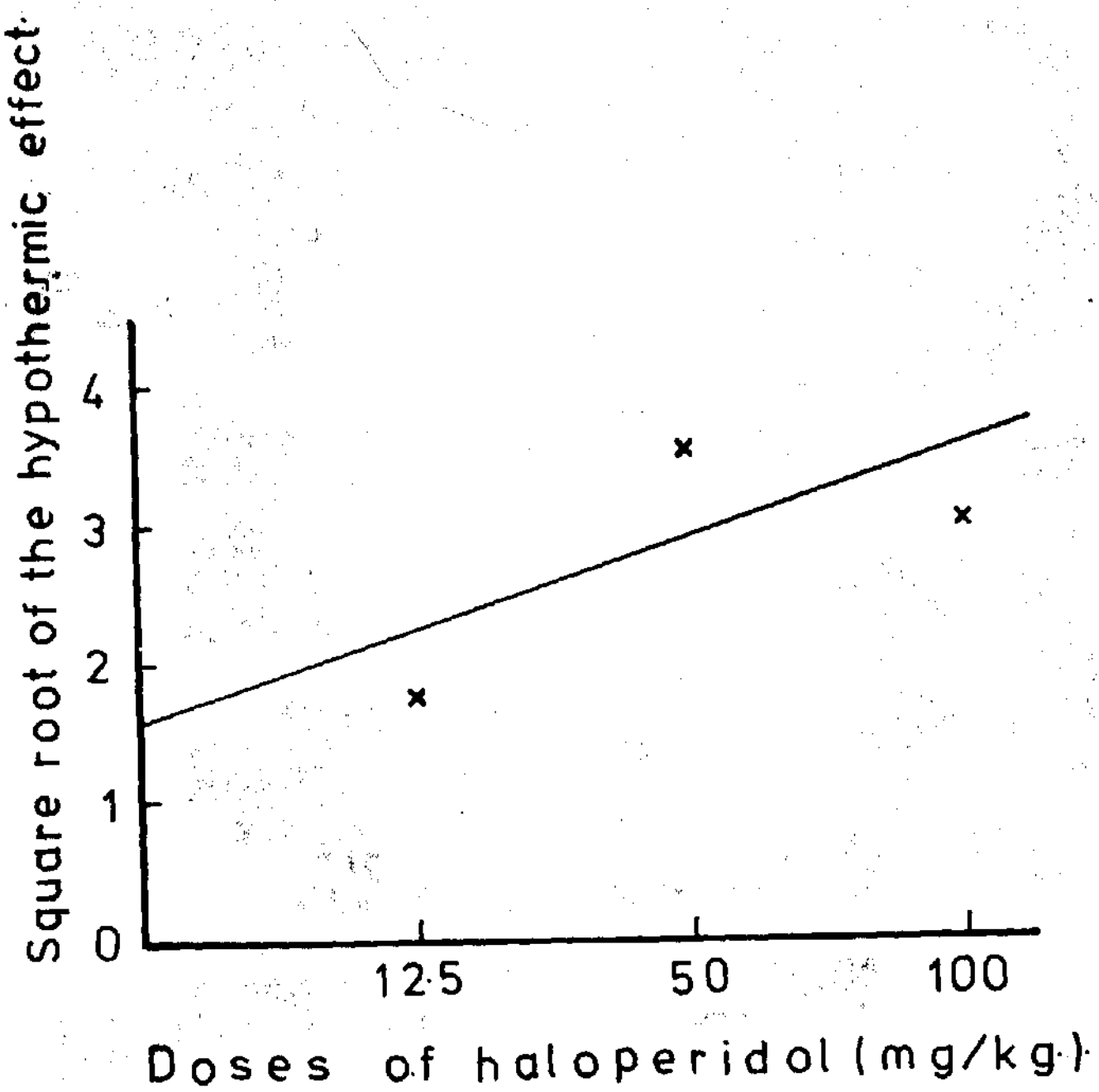



Fig. 10.10. Dose-response (square root) relationship of haloperidol against rectal temperature.

The data used to draw this graph are the same as in fig. 10. 9.

10.4.4. Haloperidol.

Haloperidol (12.5-50 mg/kg.) significantly lowered the body temperature of young chicks (figs. 10.9 & 10.10). The hypothermic effect was apparent after 10 minutes of drug administration and attained peak effect at about 30 minutes of drug administration. The hypothermic effect lasted for over 2 hours.

10.5. Discussion.

The results show that DA lowered the rectal temperature of young chicks in a dose-dependent manner. Haloperidol is a conventional DA receptor antagonist (Carlsson & Lindqvist, 1963) which effectively blocked DA-induced hypothermia in the chick. Such an antagonism shows that specific activation of DA receptor may be essential in the mediation of

hypothermia by injected DA. It is significant to note that the dose of haloperidol used to antagonise DA in this project (i.e. 0.4 mg/kg.) lowered the rectal temperature per se. The hypothermic effect of haloperidol notwithstanding, it effectively antagonised DA-induced hypothermia. Since DA does not cross the blood-brain barrier of adult fowls (Waelsch, 1955; Key & Marley, 1962), the observation that DA had no effect on the rectal temperature of adult fowls may suggest that the hypothermic action of DA in young chicks was mediated primarily via a central mechanism. In 1970, Corrodi and his co-workers reported that FLA-63 is an inhibitor of DA-beta-hydroxylase enzyme thereby accumulating endogenous brain DA. The results show significant potentiation of DA-induced hypothermia by FLA-63 suggesting that the hypothermic influence of DA might not be due to its biosynthetic product - NA.

The hypothermic effect of L-dopa was more

pronounced than that induced by DA. This may be due to rapid enzymatic destruction of DA peripherally which consequently lowered the quantity of injected DA that reached the brain as DA. As a result of the partial blood-brain barrier in young chicks, it is possible that more L-dopa would reach the brain than DA after parenteral administration of equivalent doses. It is assumed that the immaturity of the blood-brain barrier of young chicks is not absolute. Any of the above proposals (or both) may explain the higher potency of L-dopa over DA in the induction of hypothermia in the chick. It is generally believed that most of the actions of injected L-dopa are due to DA formed from it in vivo after its enzymatic decarboxylation (Everett & Borcherding, 1970; Friedman & Gershon, 1972; Scheel-Kruger & Randrup, 1967). In this project, the hypothermic effect of L-dopa was significantly reduced by haloperidol which consequently implicates the

involvement of DA receptors in this activity. The thermal effect of L-dopa was greater in young chicks (less than one week old) than old chicks. Generally, hypothermia can be induced either by increasing the mechanisms of heat loss or by reducing the mechanisms of heat generation. According to Cox, Ary & Lomax (1976) the withdrawal hypothermia in morphine-dependent rats may involve a downward setting of the thermostat by DA. It is possible that a similar mechanism applies to hypothermia induced by DA and L-dopa in the chick. It can however be speculated that the decrease in L-dopa-induced hypothermia in chicks with age may be due to neuronal development involved in heat generation mechanisms. It is however, interesting to note that there is no significant difference between 6 week old and adult fowls via-a-vis L-dopa-induced hypothermia. This observation may suggest that the mechanisms involved in temperature regulation in the chick does not develop further

after the age of six weeks. The potentiation of L-dopa-induced hypothermia by RO4-4602 (an extracerebral dopa decarboxylase enzyme inhibitor) indicates that this hypothermic action might be mediated centrally.

High doses of haloperidol profoundly lowered the rectal temperature of chicks. It is difficult to reconcile the results obtained with DA receptor agonists (i.e. hypothermia by DA and L-dopa) with those obtained with DA receptor antagonist (i.e. hypothermia by haloperidol). According to Carlsson & Lindqvist (1963), high doses of haloperidol blocks alpha adrenergic receptors. Thus, if it is assumed that NA is physiologically involved in mechanisms of heat generation in the chick, then blockade of alpha adrenergic receptors by haloperidol would lead to hypothermia. However, involvement of other mechanisms (e.g. feedback processes at DA nerve terminals) can not be ignored. It is

anticipated that neurochemical studies may improve our understanding of the mechanisms underlying haloperidol-induced hypothermia in the chick.

# CHAPTER XI

## 11.1. RADIO-ASSAY AND FLUORIMETRIC ASSAY.

## 11.2. Summary.


11.2.1. In vitro studies showed that avian brain tissue, cardiac and skeletal muscles took up $^{3}$H-DA from phosphate buffer medium.

11.2.2. Radioactivity could not be demonstrated in cardiac and skeletal muscles after an intraperitoneal injection of tritiated DA into young chicks.

11.2.3. The results indicate that 90% of $^{3}$H-DA injected intraperitoneally to young chicks crossed the blood-brain barrier and accumulated preferentially in the brain.

11.2.4. Using fluorimetric assay technique, the endogenous brain content of DA in the

chick was found to be about 7.6 µg. while the estimated brain levels of DA after injections of DA (100 mg/kg.) and L-dopa (25 mg/kg.) were 17.3 µg and 87.3 µg. respectively.

11.2.5. There was a close parallelism between the distribution of DA in different brain regions of chicks pretreated with either DA or L-dopa and that of endogenous DA in the controls.

11.2.6. It is concluded that some of the pharmacological effects observed following the parenteral administration of either DA or L-dopa may be mediated via the brain.

11.3. Introduction.

The results discussed in chapters IV to IX indicate that an intraperitoneally injected DA significantly influenced gross behaviour, seizure threshold, sleep

mechanism and body temperature of young chicks. Since some of these effects may be mediated peripherally or centrally, it became necessary to inject labelled DA intraperitoneally and measure radioactivity from both peripheral and central tissues. However, since the routine experiments were performed using unlabelled DA and L-dopa, it was relevant to estimate the brain levels of DA after injection of critical doses of DA and L-dopa (i.e. doses which produced significant pharmacological effects).

## 11.4. Materials and Methods.

### A. Radio-assay.

The reagents and chemicals used include absolute ethanol (British Drug Houses Ltd.), 10% w/v hyamine hydroxide (British Drug Houses Ltd.) in NE 233

(Liquid Scientillation, Nuclear Enterprises Ltd., Sighthill, Edingbugh), 10% w/v trichloroacetic acid (British Drug Houses Ltd.), 0.05M phosphate buffer ($p^{11}$ 7.0) and [ethylamine-1,2-$^{3}$H] Dopamine hydrochloride (specific activity = 8.3 Ci/mmol; radioactive concentration=1 mCi/ml; mw = 190; The Radiochemical Centre, Amersham).

0.2 ml of labelled $^{3}$H - DA was injected into young chicks intraperitoneally. Ten minutes after the injection, the chick was killed by decapitation. The brain, cardiac muscle or skeletal muscle was quickly removed, transferred into a white porcelain mortar, weighed and homogenised manually in cold phosphate buffer (0.5 M, pH 7.0). After a thorough homogenisation, the homogenate was diluted serially using cold phosphate buffer solution (PBS) to obtain various concentrations (e.g. 1 mg/ml, 100 µg/ml, 50 µg/ml, 20 µg/ml, 10 µg/

ml, 1 µg/ml). In some of the estimations, small amounts of the homogenate were weighed (e.g. 1 mg, 5 mg, or 10 mg), further homogenised prior to serial dilution with cold phosphate buffer solution. The sampling manifold (Millipore Corporation, Bedford, Massachussetts, 01730) was prepared for use and three volumes from each concentration (e.g. 0.1 ml, 0.2 ml, 0.4 ml) were sampled onto the sampling manifold. The samples were washed with cold phosphate buffer solution and their protein content precipitated with 10% w/v trichloroacetic acid, washed again with cold phosphate buffer solution and dried with absolute ethanol. The samples were then transferred into the harvesting tray, left in a hot air oven at 160$^{o}$C for 1 hour and kept in the fridge (4$^{o}$C) overnight before counting. The samples were transferred into small disposable.

containers and 3 ml of a mixture of 10% w/v hyamine hydroxide (100 ml) and NE 233 (300 ml) was added to each sample. The samples were then counted using Scaler Ratemeter SR3 (Nuclear Enterprises Ltd.). The control experiments were done similarly except that the chicks were injected with 0.4 ml normal saline (0.9% w/v of Nacl). Each experiment was repeated three to six times using different chicks. Total radioactivity was obtained by substracting the blank reading (using brain homogenate of control chicks) from the activity using chicks treated with labelled DA.

11.4. B. Fluorimetric assay.

The method used in this project is based essentially on that of Anton & Sayre (1962, 1964, 1968, 1972).

11.4.1. Chemicals.

The following chemicals were used

during the fluorimetric assay: aluminium oxide (British Drug Houses Ltd.), absolute ethanol (analar grade, May & Baker), citric acid (May & Baker), disodium ethylene-diamine tetraacetic acid (British Drug Houses Ltd.), hydrochloric acid (analar grade, British Drug Houses Ltd.), perchloric acid (analar grade, British Drug Houses Ltd.), phosphoric acid (analar grade, British Drug Houses Ltd.), sodium hydroxide (May & Baker), sodium periodate (analar grade, British Drug Houses Ltd.), sodium sulphite (analar grade, British Drug Houses Ltd.).

11.4.2. Activation of aluminium oxide.

The activation of aluminium oxide was done as follows: 100 gm aluminium oxide in 500 mls of 2 M hydrochloric acid was heated to 90$^{o}$C for 45 min. with rapid stirring using a hot plate and an electronic stirrer (Griffin & George Ltd.).

The heavy particles were allowed to settle for about 1 min. and the supernatant, including fine alumina, poured off. The precipitate was washed twice with fresh 250 ml 2 M hydrochloric acid at 70°C for 10 min., allowed to settle as before and decanted each time. The precipitate was further washed twice with 250 ml 2 M hydrochloric acid and decanted. The aluminium oxide was then washed free from acid (to pH 3/4) with repeated 200 ml portions of doubled distilled water, decanting fine particles at each step. The aluminium oxide was dried at 120°C for 1 hour followed by activation at 200°C for 2 hours and stored in a dessicator over silica gel.

11.4.3. Extraction of DA from brain samples.

The chick was decapitated 10 min. after injection of DA or 30 min. after injection of L-dopa. The whole brain or a

specific region of the brain (i.e. the pons and medullary oblongata/cerebellum/optic tectum/hyperstriatum) was quickly removed, put in 10 ml of cold perchloric acid solution (0.4N) to which 200 mg EDTA has been added and homogenised using either a hand homogeniser or an electronic homogeniser (Sorvall Omni-mixer). The homogenate was diluted to 25 ml with cold perchloric acid solution (0.4 N), poured into a centrifuge tube and centrifuged for 20 min. at 18,000 rpm, $0^{o}C$, using MSE High Speed 18 refrigerated centrifuge. The supernatant was then poured carefully into a 250 ml beaker containing 1.2 gm of EDTA (to protect the amines from decomposition at high pH values) and 400 mg of activated aluminium oxide (to adsorb the amines). A small quantity of 10 M sodium hydroxide solution was added to the mixture prior to gradual addition of 1 M of sodium hydroxide solution. The suspension was

shaken after each addition of sodium hydroxide solution. This procedure was continued until the pH of the suspension rose to 8.5. The pH was monitored by the use of three types of pH paper. According to Anton & Sayre (1962), the pH giving maximum adsorption of catecholamines with least oxidation is about 8.6. The suspension was then stirred for 5 min. using an electronic stirrer with a two-bladed glass paddle. After 5 min., the stirrer was stopped and rinsed into the beaker. The catecholamines should now be adsorbed into the aluminium oxide particles. The aluminium oxide was allowed to settle for 1 min. after which the supernatant was poured off. The tendency for some of the aluminium oxide to float on the surface was prevented by the addition of 0.5 ml of absolute ethanol. The aluminium oxide was rinsed twice with about 10 ml doubled

distilled water. The aluminium oxide was then transferred into a 10 ml amber coloured bottle with the aid of 2 ml of 0.05 M hydrochloric acid. The catecholamines were eluted into the acid medium by shaking the mixture vigorously for 15 min. with an electronic shaker (Griffin flask shaker). After the elution, the mixture was transferred into a 10 ml glass centrifuge tube and centrifuged for 2 min. using an ordinary bench centrifuge (Gallenkamp Junior Centrifuge). The acid eluate (containing DA) was then transferred to a 10 ml amber-coloured bottle. Hydrochloric acid was used to elute the amine because it increases the efficiency of DA elution preferentially (Anton & Sayre, 1962).

11.4.4. Oxidation of DA.

Although the catecholamines possess native fluorescence, it is usual to oxidise them in order to increase their fluorescence

sensitivity as well as improve their specificity (Anton & Sayre, 1972). The fluorescent dihydroxyindole derivative was formed with sodium periodate. In 1972, Anton & Sayre reported that sodium periodate was superior to other oxidising agents with respect to the oxidation of DA. The procedure of oxidation was essentially as follows: To about 2 ml of the acid eluate, 1 ml of alcoholic phosphate buffer (made by mixing 1 ml 70% ethanol with 9 ml phosphate buffer pH 7.0, 0.5 M) was added. After the addition of each reagent, the mixture was mixed thoroughly for 15 seconds using an electronic shaker (Griffin flask shaker). Then 0.2 ml of 0.5% sodium periodate was added, 1 min. latter 1 ml of alkaline sulphite reagent was added. The alkaline sulphite reagent was prepared by dissolving 125 mg anhydrous sodium sulphite in 0.5 ml of water and then adding 4.5 ml

of 5 M sodium hydroxide. 3.6 ml of citrate buffer (0.15 M, pH 4.0) was quickly added to the mixture prior to the addition of 1.7 ml of phosphoric acid (3 M). The citrate buffer was prepared by adjusting a solution of 1.0 M citric acid ($C_6H_8.H_2O$) to pH 4.0 with sodium hydroxide pellets and diluting with double distilled water to 0.15 M. The reagents were freshly prepared for each day's assay. 3 ml of the oxidised DA was then transferred to a cuvette and allowed to stand in a room at about 22°C ambient temperature for 30 min. before reading at 333 mμ and 397 mμ uncorrected excitation and emission wavelengths respectively. Perkin-Elmer fluorescnece spectrophotometer, model MPF 44A was used to read the fluorescence. Each assay was repeated at least thrice.

11.4.5. Controls.

For the negative controls, a chick

injected with doubled distilled water was decapitated and the whole brain or a specific region of the brain was immediately removed and placed in 10 ml of cold perchloric acid. DA was extracted from the sample following the same procedure as outlined above. The sequence of addition of the reagents to the extracted DA was also the same as stated above except that sodium periodate was omitted. Thus, the fluorescence observed was not due to the oxidised DA but rather to some other endogenous substances extracted along with DA as well as the reagents per se. The relative fluorescence was recorded at all the sensitivities used in this project. Such values were substracted from the observed fluorescence using oxidised DA extracted from control chicks (i.e. without any drug treatment). The resultant fluorescence was regarded as that due to

the endogenous DA. Similarly, the fluorescence values obtained using control chicks at various sensitivities were substracted from values obtained using DA extracted from test chicks (i.e. injected with drugs) at corresponding sensitivities. The resultant fluorescence was attributed to DA which accumulated as a result of the injected drug.

11.4.6. Calibration curves.

Various concentrations of authentic sample of DA were oxidised following the same procedure as outlined above. The concentrations used ranged between 2 µg and 100 µg and gave reasonable fluorescence to cover all the sensitivities used in this project (the coarse sensitivities were 0.3, 1, 3 & 10 as well as fine sensitivities of 1 to 10 for each coarse sensitivity).

The experiments were repeated thrice and the arithmetic means of such values were used to draw the graphs of the relative fluorescence/concentration. Such graphs were used to estimate the concentrations of DA extracted from chicks.

11.5. Results.

A. Radio-assay.

a) In vitro studies.

Radioactivity of 0.1 ml of $^{3}H$ - DA = 1,200,000 $\pm$ 1,400.

Radioactivity of 0.1 ml of $^{3}H$ - DA added to 4 µg/ml of brain homogenate = 682,700 $\pm$ 1,733.

% Quenching by brain tissue = 43.0.

Radioactivity of 0.1 ml $^{3}H$ - DA added to 4 µg/ml of skeletal muscle tissue = 669,530 $\pm$ 1,669.

% Quenching by skeletal muscle = 44.0.

Radioactivity of 0.1 ml $^{3}H$ - DA added to 4 µg/ml of myocardial tissue = 649,350 ± 1,792.

% Quenching by myocardial tissue = 46.0.

b) In vivo studies.

i) Brain.

Volume of $^{3}H$ - DA injected to chicks = 0.2 ml.

Expected total radioactivity from brain tissue = $1.37 \times 10^{6}$.

Observed total radioactivity from brain tissue = $1.23 \times 10^{6}$.

% of $^{3}H$ - DA injected i.p. which entered the brain = 90.0.

ii) Cardiac muscle.

Volume of $^{3}H$ - DA injected to chicks = 0.2 ml.

Expected total radioactivity from cardiac muscle = $5.25 \times 10^{6}$.

Observed total radioactivity from cardiac muscle = 0.

% of $^{3}H$ - DA injected i.p. which accumulated in cardiac tissue = 0.

iii) Skeletal muscle.

Volume of $^{3}H$ - DA injected to chicks = 0.2 ml.

Expected total radioactivity from skeletal muscle = $1.54 \times 10^{8}$.

Observed total radioactivity from skeletal muscle = 0.

% of $^{3}H$ - DA injected i.p. which accumulated in skeletal muscle = 0.

**B.** Fluorimetric assay.

a) % Recovery.

In order to evaluate the degree of success of the extraction process of DA

from brain tissue, it is necessary to estimate the % recovery; i.e. comparison between the peaks obtained with authentic samples of DA and the same amount of DA added to brain tissue prior to extraction of DA. The experiments were repeated thrice. Here are the results:-

Quantity of DA used = 100 µg.

Peak obtained with authentic samples of DA = 75 $\pm$ 18.4.

Peak obtained with extracted DA = 70.5 $\pm$ 23.3.

(Note that the fluorimetric parameters were maintained at the same levels for both recordings).

$$\% \text{ Recovery} = \left(\frac{70.5}{75} \times \frac{100}{1}\right) = \underline{94}.$$

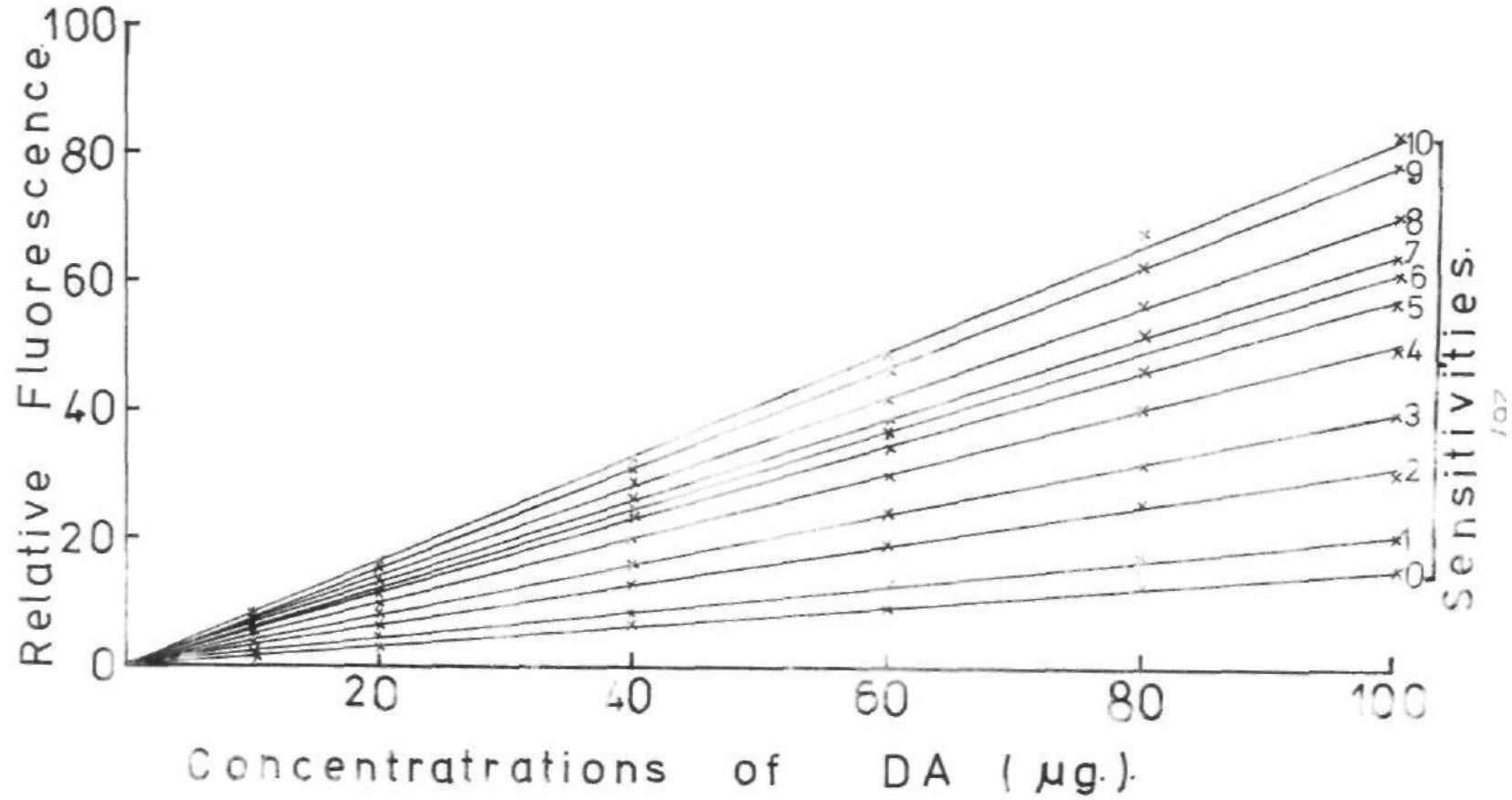



Fig. 11.1. Calibration Curve.
Coarse sensitivity of the amplifier = 0.3. Fine sensitivities of the amplifier are shown on the right-hand panel of the figure (0.10).

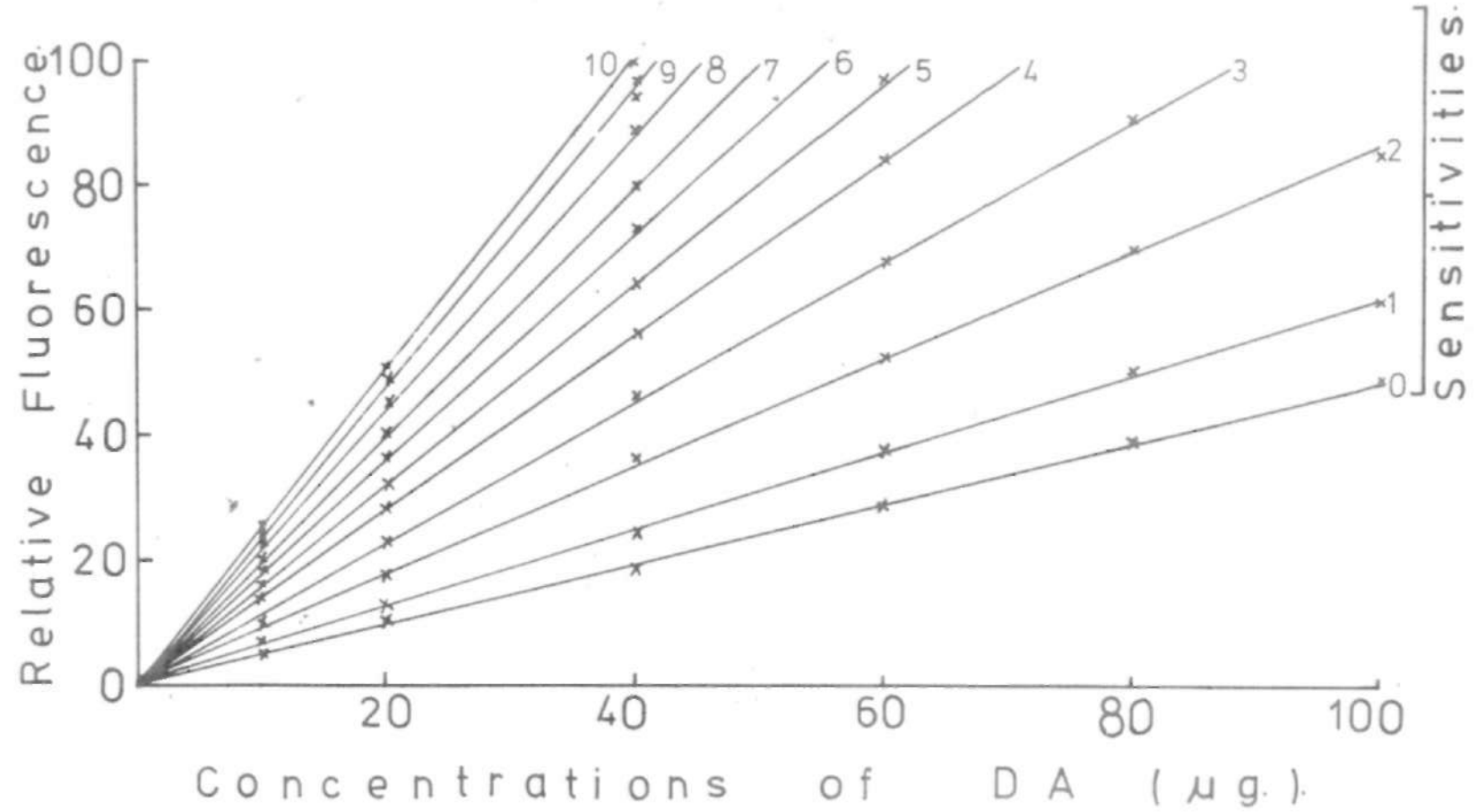



Fig. 11.2. Calibration Curve.
Coarse sensitivity of the amplifier = 1. The fine sensitivities of the amplifier are shown on the right-hand panel of the figure (0.10).

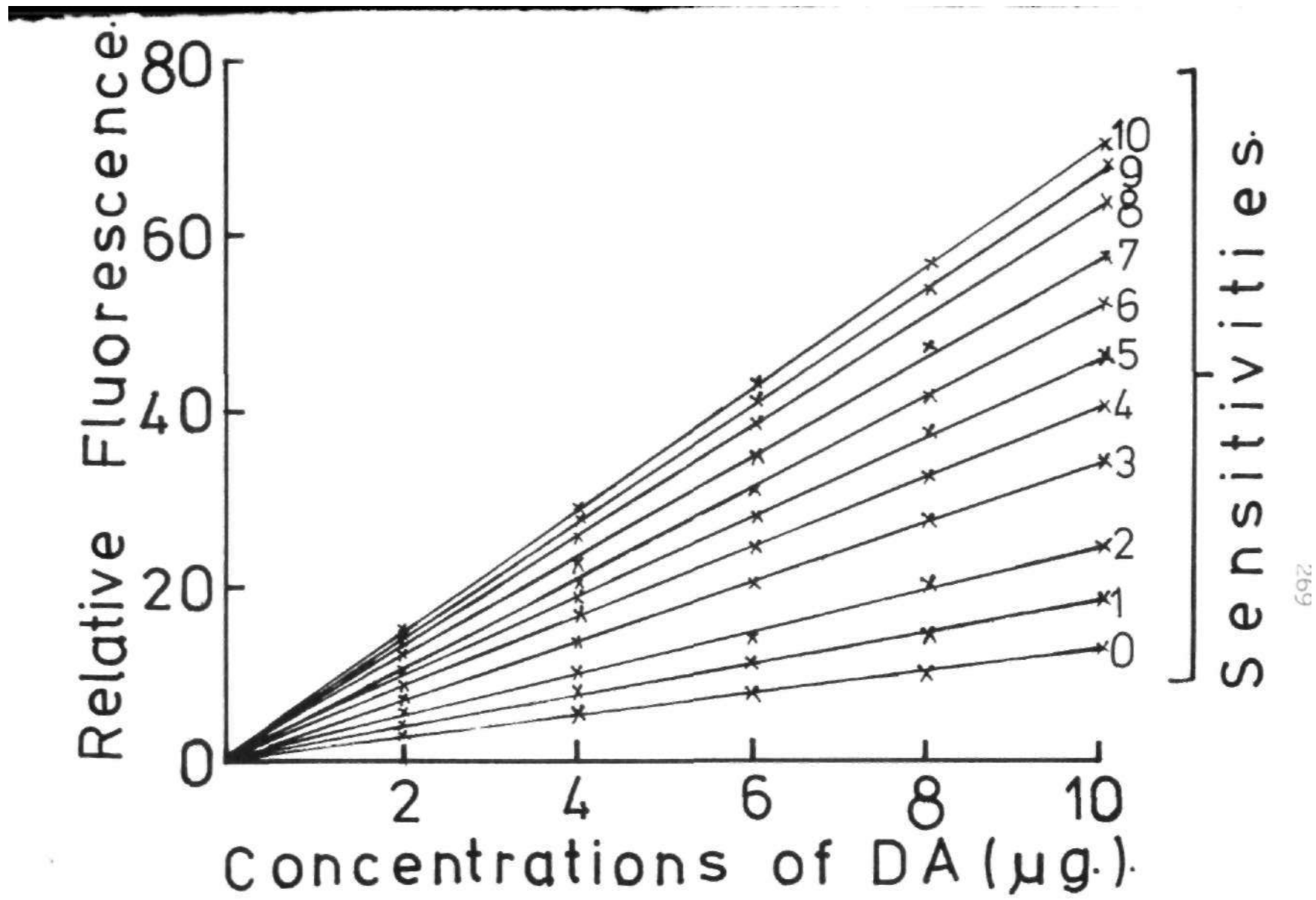



Fig. 11.3. Calibration Curve.
Coarse sensitivity of the amplifier = 3. The fine sensitivities are shown on the right-hand panel of the figure (0,10).

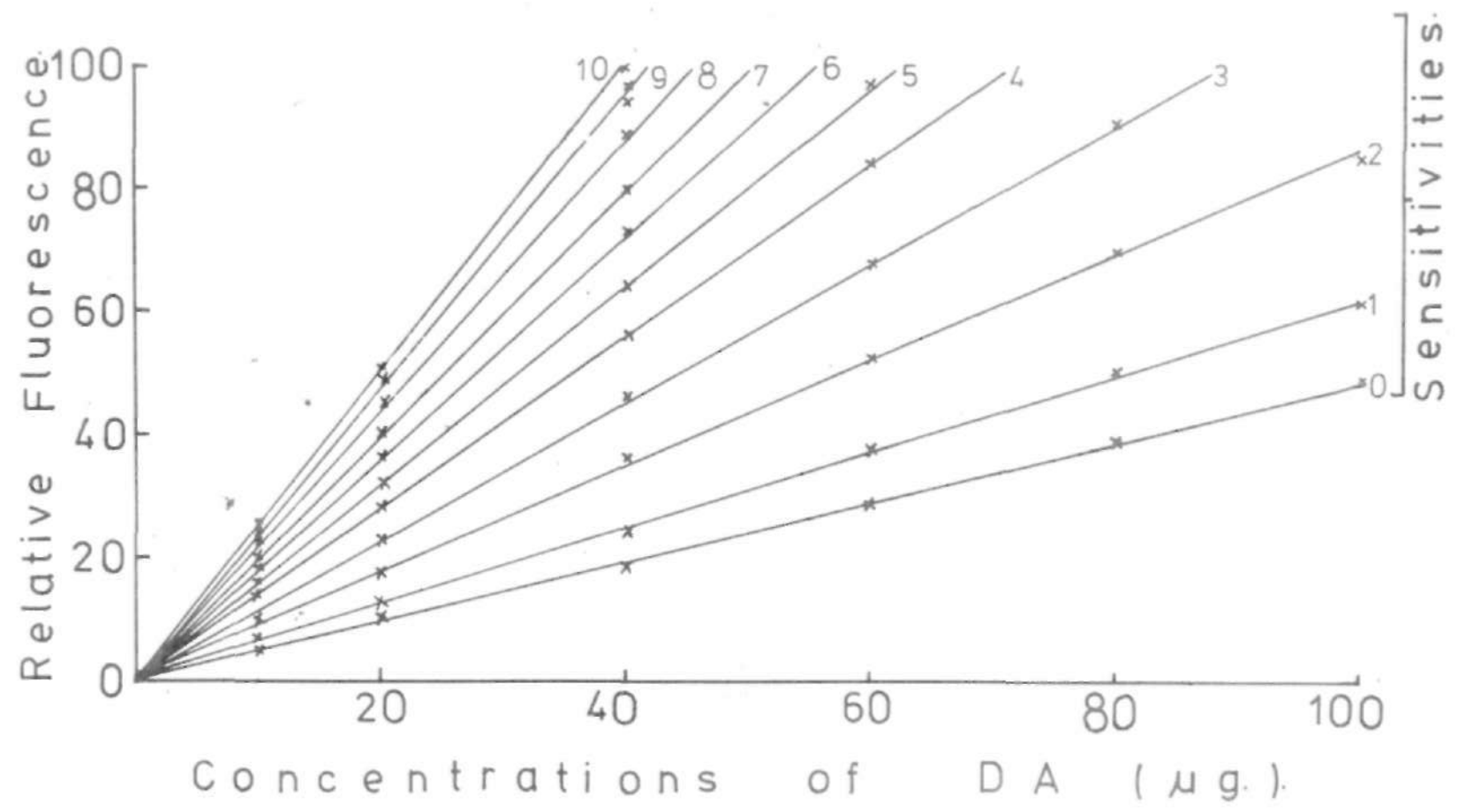



Fig. 11.2. Calibration Curve.
Coarse sensitivity of the amplifier = 1. The fine sensitivities of the amplifier are shown on the right-hand panel of the figure (0.10).

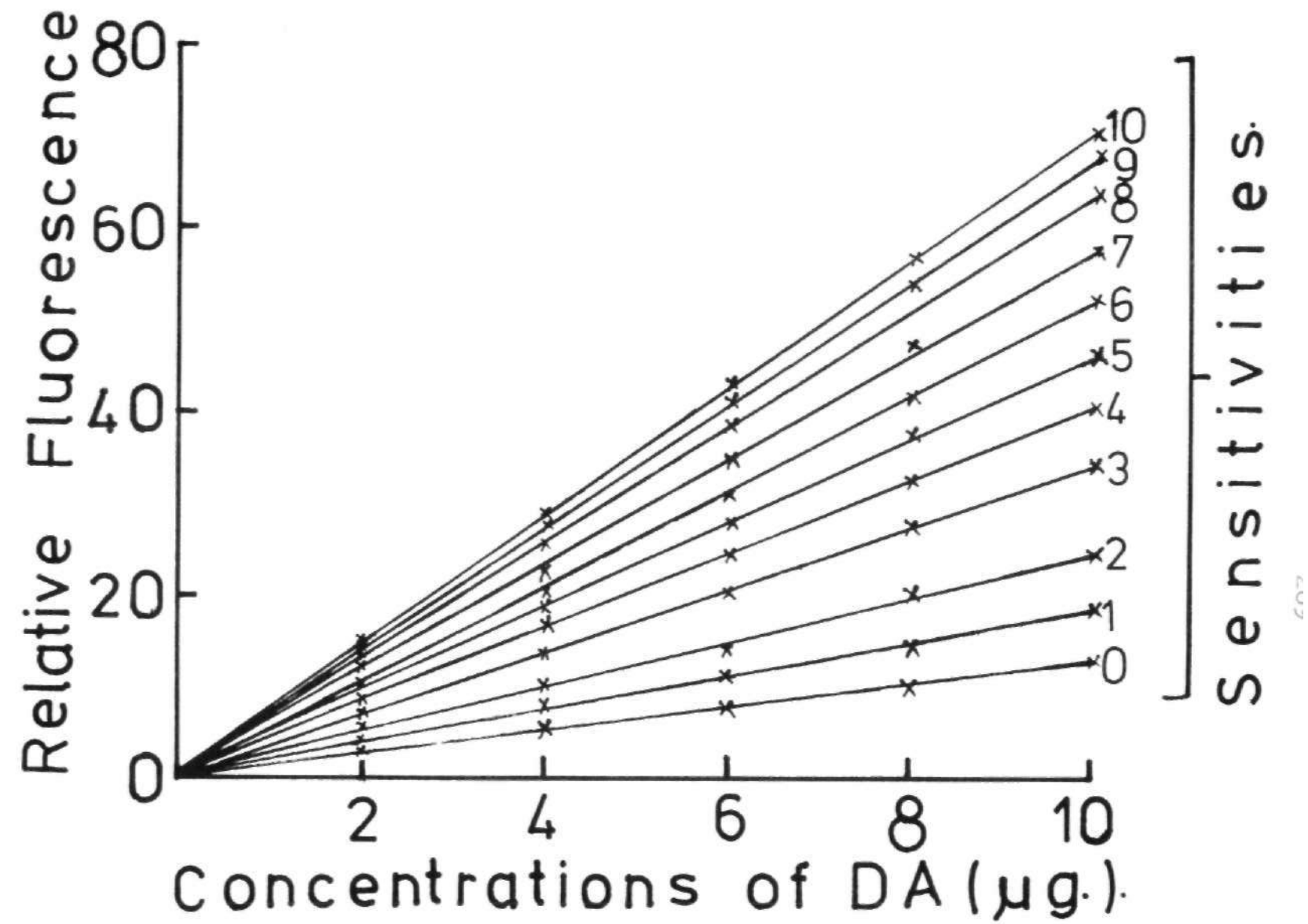



Fig. 11.3. Calibration Curve.
Coarse sensitivity of the amplifier = 3. The fine sensitivities are shown on the right-hand panel of the figure (0,10).

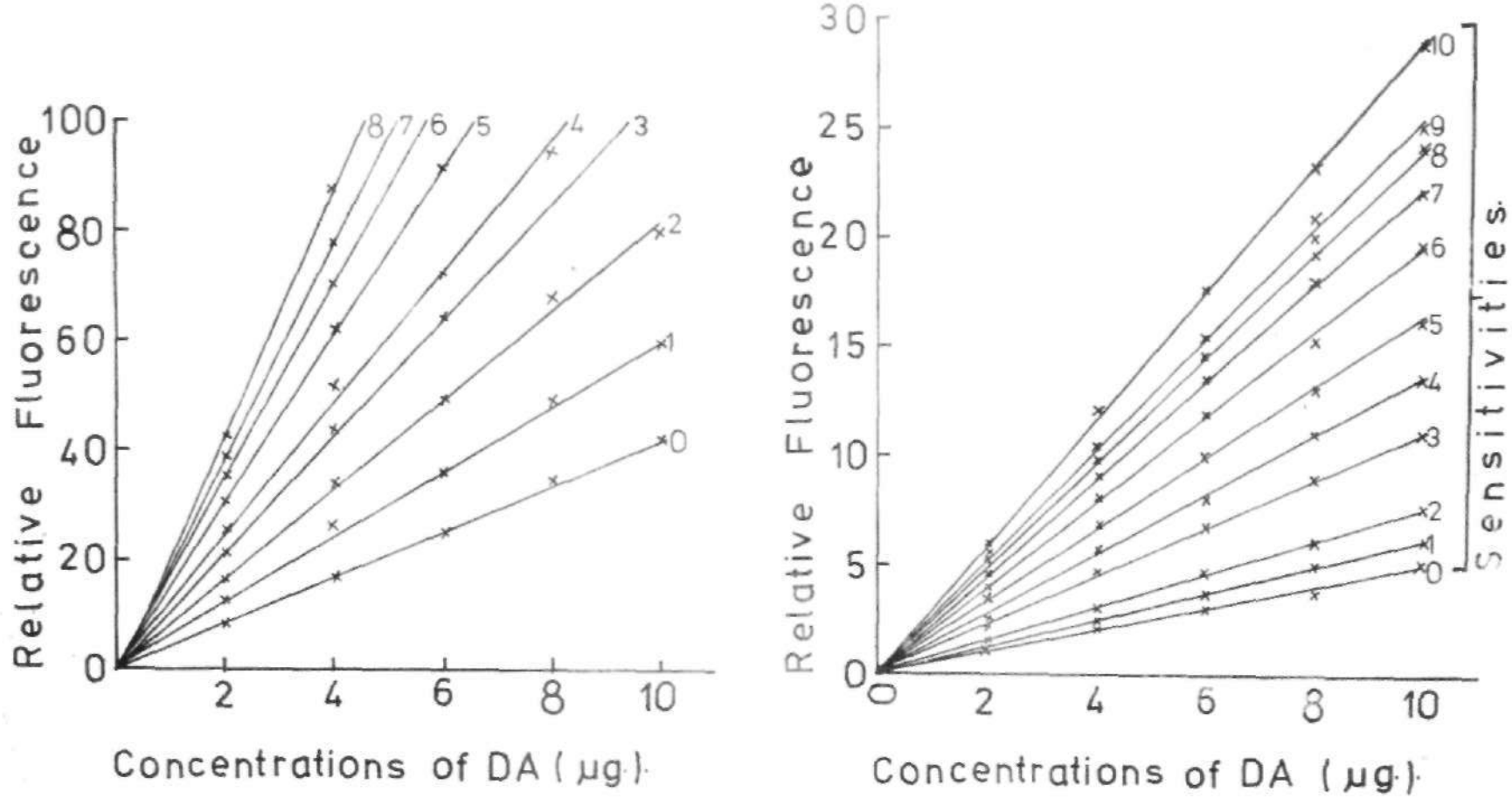



Fig. 11.4. Calibration Curve.
Coarse sensitivities of the amplifier are 10 and 1 for left-hand and right-hand figures respectively. The fine sensitivities are shown on the right-hand panel of the figures (0.10).

| Drug | Dose | Estimated DA (Mg) | | | |
|---|---|---|---|---|---|
| | (mg/kg.) | Cerebellum | Hyperstriatum | Optic tectum | Pons and medullary oblongata. |
| 0 | 0 | 1.1 ± 0.1 | 3.6 ± 0.3 | 1.5 ± 0.4 | 1.3 ± 0.3 |
| DA | 100 | 2.9 ± 0.7 | 6.8 ± 6 | 4.5 ± 0.7 | 3.5 ± 0.7 |
| DA | 400 | 9.6 ± 0.7 | 26.5 ± 1.0 | 16.7 ± 1.1 | 9.7 ± 0.9 |
| L-dopa | 25 | 13.2 ± 1.4 | 33.6 ± 1.6 | 22.3 ± 2.0 | 18.2 ± 0.8 |
| L-dopa | 100 | 48.6 ± 1.6 | 138.2 ± 16.2 | 67.2 ± 7.7 | 59.0 ± 5.7 |

Table 11.1. Estimated brain DA after an intraperitoneal injection of DA and L-dopa into 5 day old chicks.

n = 3, the values in the table are arithmetic means ± s.d.

11.6. Discussion.

Both the radioassay and the fluorimetric assay clearly show that DA accumulated in the brain after an intraperitoneally injected DA. Such a finding is compatible with the hypothesis of Waelsch (1955) that in the chick, the blood-brain barrier to catecholamines is not well developed at birth until five weeks after hatching thus, injected DA could penetrate the brain of young chicks. The results obtained using labelled DA, suggest that DA does not accumulate in muscles (skeletal and cardiac) after an intraperitoneal injection. This finding implies that most of the pharmacological actions observed after an intraperitoneal injection of DA might not be due to a direct effect on neither the skeletal muscle nor the myocardium. The percentage of $^{3}H$ - DA which was detected in the brain after an intraperitoneal injection was very high

(i.e. 90). However, this result does not show how much of the radioactivity was due to $^{3}H$ - DA per se. The total radioactivity recorded could be due to $^{3}H$ - DA, the metabolites of $^{3}H$ - DA, $^{3}H$ - NA (i.e. $^{3}H$ - DA was converted into $^{3}H$ - NA in vivo). It is equally possible that some of the metabolites of $^{3}H$ - DA resulting from its peripheral metaboliamwere transported into the brain thereby contributing to the total radioactivity.

The amount of DA which accumulated in the brain after an intraperitoneal administration of unlabelled DA was very low (less than 1%). Some of the factors which may account for this result include problems with methodology, rapid metabolism of DA by enzymes, biosynthetic conversion of injected DA to NA and pharmacokinetics. It can also be speculated that the immature blood-brain barrier partially restricted the penetration of DA into the brain thereby exposing the exogenously administered DA to enzymatic destruction. This proposal is made with the understanding that

the immaturity of the blood-brain barrier to catecholamines in the chick is not absolute. However, it should be noted that after an intraperitoneal injection of 100 mg/kg. of DA, the brain content of DA rose by 235%. It is interesting to note that the distribution of injected DA in various brain regions follows the same pattern as that of the native DA. Thus, the lowest and highest DA contents were found in the cerebellum and the hyperstriatum respectively in both the controls as well as DA-pretreated chicks. Such a data may suggest that the pharmacological actions of injected DA might be mainly exagerations of its physiological effects. From these results, the endogenous brain content of DA was apparently high (i.e. 7.6 µg) suggesting that DA may subserve a physiological role in the chick. However, more electrophysiological, biochemical and pharmacological data are required to justify the proposal that DA may be a transmitter substance in the brain of chicks.

It is generally believed that the pharmacological actions of injected L-dopa are due to DA formed from it *in vivo*. The data presented show that brain level of DA increased 11 times after a subcutaneous injection of 25 mg/kg. of L-dopa indicating that DA may play a significant role in the pharmacological actions of injected L-dopa. The results also show that the distribution of DA in different brain regions of chicks pretreated with L-dopa follow the same pattern as that of the endogenous DA. This observation indicates that there may be a close correlation between the pharmacological actions of L-dopa and the physiological roles of endogenous DA.

# CHAPTER XII

## GENERAL DISCUSSION.

The age of chicks seems to play a significant role in determining their susceptibility to both electroshock and strychnine-induced seizures. The seizure threshold to electroshock and strychnine decreased and increased respectively with age of chicks-while leptazol-induced seizure was not significantly affected by the age of chicks. The results obtained with strychnine are similar to those obtained with some convulsant agents by Osuide (1968; 1972). It has been suggested by Vernadakis & Bulkhalter (1965) that as the chick grew older, the myelination process improves both the rate of conduction and capacity for conduction of impulses along neurons. This explanation is compatible with the increased susceptibility of chicks to electroshock seizure with age. In the case of strychnine-induced seizures both neuronal and enzymatic factors may contribute to the increase in their seizure threshold to strychnine.

The seizure-potentiating effects of high doses of DA, L-dopa and apomorphine in 1 - 7 day old chicks might be due to activation of DA receptors- especially as this activity was antagonised by specific DA receptor antagonists (pimozide and haloperidol). In addition, the seizure-enhancement was demonstrated in FLA-63-pretreated chicks. If the effect is due to a genuine activation of DA receptors, why did it disappear with age? Is it that the receptors have developed/matured such that their functions have been altered? Undoubtedly, this finding needs further investigation both electrophysiologically and neurochemically. It is, however, possible that there are two types of DA receptors in chick's brain subserving two distinct physiological roles as suggested for the rat, cat and snail by Cools & Van Rossum in 1976 - i.e. DA - excitatory (DE) and DA - inhibitory (D1) mediating receptors. In such a case, the seizure-enhancement by high doses of DA, L-dopa

and apomorphine might be due to activation of DE receptors while their anticonvulsant effect might be mediated via DI receptors. According to Cools & Van Rossum (1976), in the rat, the DE receptors are more developed than the DI receptors at birth. If a similar situation exists in the chick, it can be speculated that the overall response observed after the injection of these DA agonists would be an algebraic sum of the opposing influences produced by activation of DI and DE receptors. The results indicate that DI receptors may be more sensitive to low doses of DA agonists than high doses. Thus at birth, the DI mechanisms might be poorly developed resulting in preponderance in the activity of DE receptors when high doses of DA agonists were used. On the other hand, as the chick grew older, the inhibitory mechanisms become more developed thereby leading to a preponderance in the activity of DI receptors when high doses of DA agonists were used.

with age. According to Kuno & Weakly (1972), strychnine is a competitive antagonist of the natural inhibitory transmitter (most likely glycine). It has been reported by Vernadakis & Woodbury (1965) that, in the rat, the excitatory neuronal mechanisms are more developed than the inhibitory neuronal mechanisms at birth. It is, therefore, conceivable that as the inhibitory processes develop with age, more molecules of the inhibitory transmitter would be available at the receptive sites consequently, higher concentration of strychnine would be required to effectively antagonise the inhibitory transmitter. Thus, the threshold of the chicks to strychnine seizure would increase with age of chicks. On the other hand, it can be argued that as the inhibitory mechanisms develop, the functional consequence of the antagonistic activity of strychnine against the natural inhibitory transmitter should become more prominent. However, the data do not agree with such a proposal. It is also

speculated that the enzymatic metabolism of strychnine increases with age as its metabolic enzymes become fully functional. It is also possible that the development of the blood-brain barrier with age may reduce the amount of strychnine reaching the central nervous system which may consequently reduce the seizure activity of strychnine. Some of these hypotheses can be tested by the use of specific microsomal enzyme inhibitors and potentiators. Also, fluorimetric assays of monoamines and Ach of young chicks (various ages) may also throw more light on these intriguing observations. The results obtained with leptazol appear anomalous and may need further investigation.

The biphasic effects of DA, L-dopa and apomorphine on electroshock seizure threshold was dose-dependent and age-dependent. Thus, low to moderate doses of these agents protected chicks against seizure while higher doses potentiated seizure activity in 1 to 7 day old chicks.

On the other hand, using 14 day old chicks, low to high doses of DA, L-dopa and apomorphine protected chicks against electroshock seizure. The anticonvulsant activity of DA and L-dopa was potentiated by pargyline and FLA-63 (DA-beta-hydroxylase inhibitor) while both pimozide and haloperidol (DA receptor antagonists) antagonised this effect. These results suggest that the protective action of these dopaminoceptor agents against electroshock and strychnine-induced seizures might be mediated via central DA receptors. Also, the results obtained when FLA-63-pretreated chicks were used suggest that the anticonvulsant effect of DA and L-dopa might be due mainly to increased DA neurotransmission rather than NA formed in vivo from the injected DA or L-dopa. To support this hypothesis further, NA injected to chicks intraperitoneally enhanced electroshock seizure activity. The observation that the anticonvulsant effects of DA, L-dopa and apomorphine increase with age of chicks might be

related to neuronal maturation. If it is assumed that the anticonvulsant activity of these agents is partly due to enhancement of inhibitory processes vis-a-vis seizure mechanisms, then the development of the inhibitory processes with age may apparently increase the seizure-protection offered to chicks by these agents. This explanation becomes more relevant when it is remembered that the inhibitory mechanism in the rat are primitive at birth when compared to the excitatory mechanisms (Woodbury & Vernadakis (1965). It is possible that a similar situation exists in the chick. These results agree with those of Mckenzie & Soroko (1972). These workers studied the effects of L-dopa, apomorphine and d-amphetamine on electroshock seizure using rats and mice. Such a similarity in the results might suggest that the mechanisms underlying seizure protection by these dopaminoceptor agonists in chicks, rats and mice might be very similar.

The results obtained with some miscellaneous agents which interfere with DA neurotransmission support the findings obtained with DA agonists that activation of DA receptors leads to a profound anticonvulsant action in chicks. The dose-dependent anticonvulsant activity of d-amphetamine might be due to enhancement of central DA neurotransmission either directly or indirectly or both. This proposal is hased on the antagonism of d-amphetamine's action by pimozide and haloperidol. In 1972, Mckenzie & Soroko reported a similar protective action of d-amphetamine in rats and mice-suggesting a close similarity between rats, mice and chicks via-a-vis d-amphetamine's anticonvulsant action. Both pargyline and nialamid significantly protected chicks against electroshock seizure in a dose-dependent manner while reserpine potentiated electroshock seizure activity. These findings are compatible with the general

concept that monoamines are involved in the modulation of seizure mechanisms (Jobe et al, 1974; Mckenzie & Soroko, 1972; Schaepdryver et al, 1962). It should be noted that these workers used mammals for their studies. It is, therefore, imperative to conduct biochemical assays of brain monoamines in chicks using reserpine, pargyline and nialamid. FLA-63 produced a time-dependent biphasic effect on seizure threshold - an initial seizure-enhancement followed by a profound seizure-protection. The conventional pretreatment time for effective enzyme inhibition is 4 hours (Corrodi et al, 1970). Thus, the relatively rapid onset (30 min.) of the seizure-enhancing effect suggests that FLA-63 may be mediating this activity independent of its inhibitory influence on DA-beta-hydroxylase enzyme. It is possible that a direct effect of FLA-63 on some neuronal systems, other than DA, may be responsible for this action. On the other hand, the anticonvulsant effect of FLA-63 might

be a consequence of its inhibitory activity on DA-beta-hydroxylase enzyme leading to an accumulation of endogenous DA in the brain. This proposal is compatible with the results obtained with DA agonists. Like FLA-63, DOPS produced a time-dependent biphasic effect on electroshock seizure; an initial non-significant enhancement of seizure followed by a profound anticonvulsant activity. The rapid onset (1 hour compared to the conventional pretreament time of 21 hours) of the seizure-enhancing effect may suggest that such an action is non-specific compared to the conventional influence of DOPS on NA synthesis (i.e. a direct conversion of L-dopa to NA in vivo; Thorn & Ludwig, 1962). The profound anticonvulsant activity of DOPS in chicks might be attributed to elevated brain concentrations of NA. This proposal is incompatible with the results obtained with injected NA (i.e. potentiation of electroshock seizure).

In addition, DOPS hypnotised chicks within 20 min. of injection. The rapid onset of sleep may suggest a non-specific mechanism of action. Therefore, a more intensive investigation involving neurochemical analyses is indicated. Pimozide initially enhanced seizure activity but latter protected chicks against electroshock seizure. The initial influence might be due to the antagonism of the modulatory role of endogenous DA on seizure activity while the seizure-protection might be attributed to a feedback increase in DA transmission. Alternatively, the protective effect of pimozide at 4 hours post-injection might be due to blockade of presynaptic inhibitory DA receptors or autoreceptors which might lead to an increase in the concentration of available DA at postsynaptic receptive sites. In the case of the anticonvulsant effect of haloperidol, the same reasons as given for pimozide might apply.

In addition, however, a possible involvement of other neuronal systems (e.g. NA) might contribute to this action in view of its non-specificity at high doses (Carlsson & Linqvist, 1963).

6-Hydroxydopamine, injected intraperitoneally to chicks produced a time-dependent and dose-dependent effect on electroshock seizure; an initial seizure protection followed by a profound seizure-enhancing effect. The anticonvulsant effect might be due to an initial release of catecholamines by 6-OHDA prior to chemical sympathectomy (Laverty, Sharman & Vogt, 1965). There was no initial anticonvulsant effect when high doses were used. It is possible that the rate of neuronal degeneration was either too rapid or that, with such doses, there was no initial release of catecholamines. Evidently, biochemical estimations of catecholamines in chicks' brains after injection of 6-OHDA may throw more light on the possible mechanisms underlying these observations.

When rats and chicks injected intraperitoneally with 6-OHDA within the first 3 days post-paturition were latter tested at 10 weeks of age, their susceptibility to electroshock seizure was significantly increased. This observation might be due to 6-OHDA - induced neuronal degeneration leading to excessive loss of catecholamines-thus implicating catecholamines in seizure modulation in both the chick and the rat.

The behavioural effects of DA, L-dopa, amantadine, 6-OHDA, DDC and RO4-4602 were studied in chicks. These agents differentially produced various abnormal behaviours including pecking, fanned wings, headshaking and jumping while some normal activities (like chirping, locomotion and water intake) were also affected. It is generally believed that drug-induced stereotyped behaviour is due to activation (directly or indirectly) of DA receptors in the brain (Munkvad & Randrup, 1966; Fog et al, 1966;

Traylor & Snyder, 1971). Therefore, it is suggested that the stereotyped activities induced by these agents might involved increased DA neurotransmission while the other effects might be attributed to some non-specific components of the action of these agents. The specific involvement of DA in the mediation of these behavioural effects would be better understood when neurochemical studies are performed.

DA, L-dopa and apomorphine produced behavioural alerting in chicks, increased the EMG activity and desynchronized the EEG of the HS, OT and RF. These agents antagonised sleep and EEG synchronization induced by pentobarbitone in chicks. Similarly, FLA-63 (which accumulates DA in the brain) desynchronized the EEG and increased EMG activity of chicks. These results suggest that endogenous DA might be involved in the maintenance of wakefuless in the chick.

Such a proposal agrees with the data of Spooner & Winters (1965). However, these results disagree with the hypothesis that DA is an inhibitory neurotransmitter in the brain (Bloom et al, 1965; Herz & Zieglgansberger, 1966; McLennan & York, 1967; York, 1967). It is however possible that injected DA might depress inhibitory mechanisms to produce behavioural and EEG excitation. Interestingly, high doses of injected DA sedated chicks and potentiated pentobarbitone-induced sleep. These observations might be due to NA formed in vivo from the injected DA, especially as these effects were delayed and an initial EEG activation was observed. It is however, possible that there are DI and DE receptors vis-a-vis sleep mechanisms (as discussed earlier under seizures). A better understanding of the involvement of DA in the maintenance of consciousness may be possible when further studies are undertaken utilising both electrophysiological and neurochemical approaches.

The dose-dependent hypothermia induced by L-dopa and DA in chicks was effectively antagonised by haloperidol suggesting the specific involvement of DA receptors in the mediation of this effect DA did not induce any hypothermia in adult fowls while L-dopa still produced a profound hypothermia. These results suggest that DA-induced hypothermia in chicks may be mediated centrally rather than peripherally. The potentiation of DA-induced hypothermia by FLA-63 conforms with the general concept that this effect might be due to DA rather than NA formed from it in vivo. These results agree with the suggestion of Lal et al (1971) that withdrawal hypothermia in morphine-dependent rats might be due to an increased DA neurotransmission and that of Barnett et al (1973) that apomorphine-induced hypothermia in rats might be due to activation of DA receptors. On the other hand, these data disagree with the observation of Marley & Stephenson (1969) that DA produced hyperthermia when infused into the hypothalamous

of chicks. However, the passibility of the existence of DI and DE receptors vis-a-vis temperature regulation may explain the observed disparity.

The results obtained with both radio-assay and spectrofluorimetric assay support the general concept that injected DA and L-dopa accumulate preferentially in the brain (Scheel-Kruger & Randrup, 1967; Everett & Borcherding, 1970; Friedman & Gershon, 1971).

The data accumulated in this project suggest that DA may play a prominent role in seizure modulation, maintenance of consciousness, temperature regulation and behavioural activities in the chick. DA injected into young chicks penetrates the blood-brain barrier and accumulates in the brain. Most of the actions of injected DA in chicks are similar to the effects it produces in mammals when injected directly into the brain or L-dopa given parenterally. The agents influencing DA neurotransmission produced significant effects on electroshock seizure and

gross behaviour. In addition, these agents apparently produced effects which may not be related to their "primary" actions on DA neurotransmission. The work has been quite exciting and challenging. The basic objectives were achieved and possible courses of future research activities have been recommended in the fields of electrophysiology and biochemical pharmacology.

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# APPENDIX.

## A. STATISTICS.

A.1. Hp 25 calculator was used for the statistical calculations using standard programmes for student-t-test and Chi-Squared test with continuity correction.

A.2. Theory for student-t-test.

Assuming that

$X_{1i}\ X_{2i} \ldots\ldots X_1\ n_1$ and

$X_{21}\ X_{22} \ldots\ldots X_2\ n_2$

are random samples from two normal populations with the same variance -

$$\bar{X}_1 = \frac{1}{n_1} \sum_{i=1}^{n_1} X_{1i}$$

$$\bar{X}_2 = \frac{1}{n_2} \sum_{i=1}^{n_2} X_{2i}$$

The combined estimator of the population is given by

$$s^2 = \frac{\sum_{i=1}^{n_1} (X_{1i} - \bar{X}_1)^2 + \sum_{i=1}^{n_2} (X_{2i} - \bar{X}_2)^2}{n_1 + n_2 - 2}.$$

In evaluating $S^2$, it is more conveniently written as $\Sigma s^2 = \sum_{i=1}^{n_1} X_{1i}^2 + \bar{X}_1 \sum_{i=1}^{n_1} X_{1i} + \sum_{i=1}^{n_2} X_{2i}^2 + \bar{X}_2 \sum_{i=1}^{n_2} X_{2i}$

Then the standard error of $\bar{X}_1 - \bar{X}_2$ is $S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$

The t statistic to test the equality of the two population means is given by:- $t = \frac{\bar{X}_1 - \bar{X}_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$

A.3. Formula for Calculating Chi-Squared Test with Yete's correction for continuity.

| Groups | Results | |
|---|---|---|
| A | a | b |
| B | c | d |

$$X^2 = (a+b+c+d)\ \frac{\left[\,|ad - bc| - \tfrac{1}{2}(a + b + c + d)\,\right]^2}{(a + b)\ (a + c)\ (c + d)\ (b + d)}$$