THE ACUTE AND CHRONIC BIOCHEMICAL AND BEHAVIORAL EFFECTS OF CYCLOTRIMETHYLENETRINITRAMINE

Introduction:

The object of this report is to present the results of a study of the toxic effects of cyclomethylenetrinitramine on the brain after chronic administration to male rats.

In 1973 the Department of Pharmacology and Toxicology of the University of Maryland School of Pharmacy contracted with Office of Naval Research to screen several parameters of central nervous system function for possible effects from three months' exposures to cyclomethylenetrinitramine.

Background:

Acute high doses of cyclotrimethylenetrinitramine had been reported to induce severe signs and symptoms of central nervous system injury, which, although generally transient, occasionally persisted for some time. The effects of lower chronic doses are relatively unstudied.

Trimethylenetrinitramine, a biologically and chemically stable compound, produces confusion, hyperirritability, myoclonic seizures progressing to severe motor seizures with prolonged postictal mental confusion and amnesia in humans (Kaplan, A.S. et al, 1965). Acute and chronic exposures produce a similar toxicity in animals (von Oettingen, W.F., et al, 1949). The acute oral LD_{50} in rats is 200 mg/kg and chronic oral exposures of 25 to 100 mg/kg produces central excitation in both rats and dogs. Chronic oral dosing of rats with 25 and 50 mg/kg produces severe weight loss progressing for 3 and 4 weeks, which then reverses until 12 weeks at which time body weight returns to control levels. Since trimethylenetrinitramine is not metabolized in vivo (von Oettingen, W.F. et al, 1949), these biphasic effects on body weight do not appear to be due to an increase in the rate of its metabolism. After these dosies, cytological or histological changes were not found in the brain or any other tissue.

The absence of cytotoxicity suggests that the mechanism of the central toxicity to trimethylenetrinitramine is biochemical, althougn this has not been investigated. The central effects are dose related (Slanskaya, R.M. and Pozharsky, F.I., 1944) and antoagonized by prior administration of pentobarbital (Sunderman, F.W. et al, 1944). In contrast to von Oettingen, Slanskaya and Pozharski reported patholigical changes in several organs, but none in the brain. They suggest that the mechanism of acute poisoning involves changes in vascular walls and chronic poisoning involves changes in lipid metabolism. The biphasic changes in body weight with chronic dosing suggests that tolerance occurs to some of the effects, but no efidence was found which suggested tolerance to the central toxicity.

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The trimethylenetrinitramine induced convulsions were shown to be abolished in decerebrate animals and initiated by stimulation of higher centers (Kaplan, A.S. et al, 1965), but the effect of acute, subacute and chronic dosing on brain biochemistry or on sensitivity to centrally acting drugs has not been investigated in any species.

OVERALL CONCLUSIONS

1. Cyclomethylenetrinitramine administration to male rats daily for 12 weeks produced transient changes in biochemical and behavioral parpmeters of CNS function at dosage ranges which produced no overt signs of toxicity other than a 25% decrease in growth rate at the highest dose (615 - 12.5 mg/k.).

2. Time and dose ralated changes were found in whole brain monoamine oxidase, cholinesterase and oxygen uptake in chronically treated rats. Although acute administration of CMT increased norepinephrine, 5-HT, and decreased dopamine steady state levels 6 hours after an acute dose, no remarkable changes were found after 24 hours in either acute or chronically dosed rats. Chronic exposure to CMT produces larger alteration in brain enzyme levels than acute exposure. Moreover, the changes are dose-related with an elevation in brain cholinesterase induced by the lowest dose (0.3 mg/k/da) after 12 but not 6 weeks of dosing. The changes possibly reflect only hemostatic modification of enzyme activity in response to serial dosing.

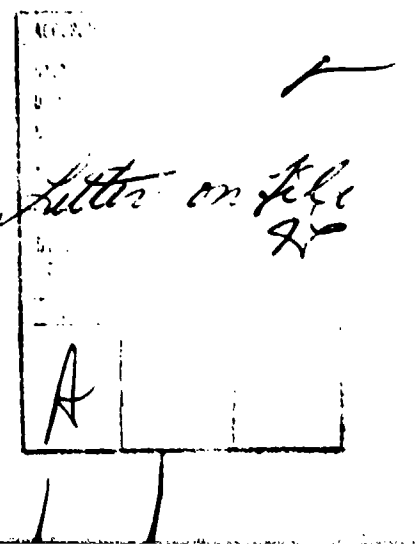
3. CMT inhibited both brain monoamine oxidase and cholinesterase in vitro and stimulated brain O_2 uptake, suggesting that CMT or a contaminate may have a direct effect on these enzyme systems. The blood levels of CMT 24 hours after dosing are related to dose administered and duration of exposure. The alteration in enzyme activities are directly associated with the increase in blood CMT levels. Thus chronic exposure in addition to inducing increases in brain MAO, cholinesterase, and O_2 uptake also increase blood CMT levels. These changes are dose related.

4. None of the effects were found in rats chronically dosed with 0.30 mg CMT/kg/day for 12 weeks, the lowest dosage level used. Thus 0.30 mg/kg/day is a no effect level.

5. Measures of indirect blood pressure in acute and chronically treated rats were measured. No changes were found 24 hours after dosing in either acute or chronically treated rats. However, a hypertensive response was induced after administration of CMT in chronically treated rats but not in acutely treated rats. This elevation was blocked by pretreatment with the ganglionic blocker hexomethionium which suggested that the elevations are due to a central rather than peripheral action of CMT.

6. Several of the studies yeilded negative results after both chronic and acute treatment. These include: Brain weight studies; brain protein levels, DNA-RNA studies; hexabarbital sleep time; spontaneous locomotor activity; and the examination for gross neurological abnormalities which included positional sense, righting reflex, gait and stance, muscle tone and equilibrium. Thus the biochemical parameters (MAO, cholinesterase and 0_2 uptake) appear to be the sensitive measures of chronic effects of CMT.

7. The significance of these changes in evaluating occupational and environmental exposure to CMT is yet to be established. However, the observation that all changes found are dose related show that a safe level of exposure can be established.



TECHNICAL DESCRIPTION

Objective - The overall objective of this project was to determine if trimethylenetrinitramine, a central excitatory agent, induces chenges in behavior, in brain metabolism and in neurotransmitters. This objective was approached through a comparison of doses and time relationships between changes in biochemical parameters and changes in behavioral measures of brain activity.

This report presents the results of studies designed to

1. Establish the effect of trimethylenetrinitramine on oxygen uptake and determine the effect on two specific brain enzyme systems, brain cholinesterase and monoamine oxidase.

2. Establish the effect of trimethylenetrinitramine on steady state levels and turnover rates of brain norepinephrine, dopamine, 5-hydroxytryptamine and possible gama-aminobutyric acid (GABA).

General Research Plan

Dose and time relationships often indicate the significance and mode of toxicological changes. Therefore, in this study changes in brain biochemistry and behavioral correlates were determined in rats administered a range of 4 doses of trimethylene-trinitramine daily and tested at selected intervals over a 12 week dosing period.

The general experimental design will be that of a Latin Square for each biochemical parameter and behavioral correlate.

The study consisted of three phases, some of which were run concurrently. Thase I included measurements of the effect of trimethylenetrinitramine on brain biochemistry in animals receiving four chronic dosage levels sacrificed on 4 selected intervals. Vehicle-treated controls and zero time intervals were included. Phase II included measurements of effects on brain transmitter steady state levels and turnover rates in animals treated according to the same protocol. Phase III included measurements of effects on some *in vivo* behavioral correlates in animals treated according to the same protocol.

Specific Methodology

A. Animals and animal care:

Adult, male, Sprague Dawley rats, 125-150 grams were housed in an air conditioned animal room with a 12 hour light-dark cycle and allowed free access to food and water throughout the study. The animals were housed in community cages of not more than 10 animals each.

B. Dosing Procedure:

For chronic studies, trimethylenetrinitramine was administered IP daily in either an oil or carboxymethycelluose suspension. Controls received the vehicle only. Body weights were recorded and overt toxic signs noted (See also Section C.3). The doses are described in the text.

C. Analytical Procedures:

1. Biochemical studies -

Oxygen uptake was determined using fresh brain slices in a Warbaugh apparatus, using the method described by Umbright, 1957.

Brain cholinesterase was determined by the colorimetric method of Ellman, et al, 1957.

Monoamine oxidase was determined by the radiochemical method of Wurtzman and Axelrod (1963), utilizing C^{14}-L-tyramine as substrate.

2. Neurotransmitter dynamics -

The pulse-labeling technique of Algeri and Costa (1971) was used for the determination of brain steady-state concentration and turn-over rates for norepinephrine, dopamine and 5-hydroxy-tryptamine. This technique utilizes the monoamine precursors H^3-L-tryptophan and C^{14}-L-tyrosine.

3. Behavioral Studies -

Barbiturate sleeptimes were determined using hexabarbital according to the standard technique used in our laboratory, in which rats are placed on their backs only at regular (1 or 5 minute) intervals to avoid respiratory complications.

Pentylenetetrazol (Metrazol) seizure thresholds and patterns were determined by the intravenous infusion procedure described by Orloff, et al, (1969).

Electroshock seizure thresholds and patterns were determined by the method described by Woodbury and Davenport (1952) modified to use ear alectrodes.

Spontaneous motor activity were determined by the procedure described by Tedeschi, et al (1964) modified in our laboratory to use circular photoelectric cages. Amphetamine-induced motor activity will be determined in the same manner after rats have received amphetamine sulfate, 10 mg/kg, IP.

Evidence of neurological abnormalities (toxicity) were assessed by the method described by Swinyard et al (1952). Accordingly, five tests were used for the evaluation of the animals.

a. Positional Sense - When a hind limb of a rat is lowered over the edge of a table, the rat normally quickly lifts it back to the table.

b. Righting Reflex - A rat placed on its back will normally right itself quickly.

c. Gait and Stance - Deficits indicated by a circular or zigzag gait, ataxia, abnormal spread of the legs, abnormal body posture, tremor, hyperactivity, stupor or catalepsy.

d. Muscle tone, - Determined subjectively by handling the animals

e. Equilibrium - Maintenance of equilibrium while walking on a narrow elevated edge.

RESULTS

A. Range finding studies:

A series of acute and sub acute experiments were conducted to establish the range of doses to be tested. The results are shown in tables 1, 2, and 3.

B. Enzyme studies:

The time and dose related effects of CMT on oxygen uptake, Monoamine oxidase and brain cholinesterase are shown in Figures 1, 2, and 3 and Tables 1, 2, and 3, respectively.

1. Oxygen uptake

Oxygen uptake is often taken as an overall index of brain energetics. Therefore in the 6-hour study the O_2 uptake of brain homogenates was determined using succinate, glucose or pyruvate malate as the substrate. The effect of *in vitro* addition of CMT on O_2 uptake by control tissue was also determined. In the chronic experiments succinate was used as the substrate and O_2 uptake was determined within 2 hours of sacrifice. The results are shown in Tables 4, a, b, and c.

No significant change in O_2 uptake by whole brain homogenates prepared from rats sacrificed 6 or 24 hours after administration of the final dose of CMT was found after acute and chronic dosing with CMT in the 6-hour, 24-hour, 48-hour, 2-week or 12-week groups. Figure 1, Table 5. However, there was an increase to about 175% of control in O_2 uptake in the 6-week group dosed with 2.5 or 6.5 mg/kg. The increase was significant at the $P < 0.05$ level.

In a preliminary study changes were found in O_2 uptake which suggested that CMT may have a direct effect on O_2 uptake in homogenates when succinate but not when glucose or pyruvate malate was used as the substrate (Table 4D). No differences were found when either the supernatant or shaken RDX preparation was used suggesting that only the RDX in solution had an effect on O_2 uptake in the *in vitro* study (Table 4 D).

These results show that CMT does not produce an acute effect on O_2uptake of duration over 6 hours. Nor does chronic dosing for 2 days or 2 weeks significantly alter O_2 uptake 24 hours after administration. Therefore, the effect of 6.5 mg CMT/kg IP on O_2 uptake at 1/2, 1 1/2, and 3 hours was determined. The O_2 uptake in brain homogenates increase from 115% at 1/2 hour to 126% at 1 1/2 hours and 145% at 3 hours after dosing with CMT. The O_2 uptake had returned to control levels by six hours. These results suggest that CMT may slightly alter axidative phosphorylation leading to increases in O_2 uptake.

The effect of CMT of O_2 uptake in vitro was also tested and did not differ from control. Thus the effect of CMT on O_2 uptake may be indirect.

The 6 & 12 week values do show that chronic dosing can alter O_2 uptake and that these changes are reversable. Based on these results it is unlikely that CMT uncoupling of oxidative phosphorylation produced long-term alterations in brain energetics.

2. Monoamine oxidase.

Measures of enzyme activity often provide the first indication of toxic damage. Since CMT produced acute convulsions it was considered possible that changes in amine or acetylcholine levels due to changes in MAO of cholinesterase activity may be associated with these convulsive or preconvulsive conditions in the brain. Moreover, measurement of MAO and cholinesterase are established indices of CNS toxicity.

Therefore, monoamine oxidase activity was determined or 24 hours after dosing. The results are shown in Figure 2 and Table 6. In this study ^{3}H-Tyramine was used as the substrate. There was a small but not significant ($P > 0.05$) decrease in MAO at all doses in the 1/2 hour, 1 1/2 hour, 3-hour, 6-hour, 24-hour and 2-week group. In contrast, there was a significant ($P < 0.05$) increase of greater than 150% in monoamine oxidase in the 6-week group treated with 2.5 or 6.5 mg/kg and in all of the treated rats in the 12-week group. These changes were dose related.

The in vitro effects of CMT on MAO activity was tested to determine whether the action was direct due to CMT or indirect due to metabolite or secondary to other changes. A dose related inhibition of MAO activity of 76 and 66% of control was induced in vitro by concentrations of CMT ranging from 0.25 to 1.0 mg/ml ($1.12 - 4.50 \times 10^{-3}$M). This indicates that CMT is a direct inhibiter of MAO activity in brain homogenates at these concentrations.

These results show that CMT does alter monoamine oxidase activity and with chronic dosing the effect is biphasic producing a decrease 6 or 24 hours after short-term exposure and an increase ofter 6 and 12-week exposures.

These results show that subconvulsant doses of CMT can alter MAO activity and suggest that amine levels in the brain may be also changed.

3. Brain Cholinesterase

Brain cholinesterase was determined at 6 and 24 hours after dosing with CMT. The results are shown in Figure 3 and Tables 7 & 8. Acute administration of CMT produced a small but significant decrease in brain cholinesterase at 1 1/2, 3 and 6 hours. The activity had returned to control levels by 24 hours. Activity was inhibited at 24 hours after 2 weeks of chronic dosing. In contrast, an increase of activity was observed in the 2.5 mg/kg and 6.5 mg/kg group after 6 and 12 weeks of chronic dosing. This shows a biphasic effect similar to the O_2 uptake and brain MAO activity. In order to determine whether CMT has a direct effect on brain cholinesterase, brain homogenates were incubated with $1.12 - 4.50 \times 10^{-3}$M concentrations of CMT and brain homogenates. CMT decreased cholinesterase activity to 53% of control at the 4.5×10^{-3}M level.

4. Comparison of CNS effects.

The relative changes in the brain enzymes are summarized in table 7. The enzyme levels are not correlated with changes in amine levels.

C. Physiological studies.

1. Chronic feeding of rats with 15, 50, 100 mg/kg per day in the diet produced a decrease in body weight through 2 and 4 weeks at the 50 and 100 mg/kg dose. Therefore, the body weights were plotted weekly. Figure 4 and Table 10 show that 0.03, 2.5 and 6.5 mg/kg also produced a decrease in body weights at 2 weeks but the weight had returned to normal levels by 6 and 12 weeks. Thus rats chronically dosed with CMT appear to develop a tolerance to it over the first 3-4 weeks of dosing.

2. The blood levels of CMT 24 hours after dosing are shown in Table 11. The blood levels are related to dose administered and duration of exposure. The alteration in enzyme activities shown in Table 9 are directly associated with the increase in blood CMT levels. Thus chronic exposure in addition to inducing increases in brain MAO, cholinesterase, and O_2 uptake also increase blood CMT levels. These changes are dose related. None of the effects were found in rats chronically dosed with 0.30 mg/kg/day for 12 weeks, the lowest dosage level used. Thus 0.30 mg/kg/day is a no effect level.

3. Measures of indirect blood pressure in acute and chronically treated rats were measured. (Table 12). No changes were found 24 hours after dosing in either acute or chronically treated rats. However, a hypertensive response was induced after administration of CMT in chronically treated rats but not in acutely treated rats. This elevation was blocked by pretreatment with the ganglionic blocker hexo-

4. Brain weight, brain protein levels, DNA-RNA, hexabarbital sleep time, spontaneous locomotor activity and the examination for gross neurological abnormalities which included positional sense, righting reflex, gait and stance, muscle tone and equilibrium were no different from controls after either chronic or acute treatments.

Thus the biochemical parameters (MAO, cholinesterase and O_2 uptake) appear to be the sensitive measures of chronic effects of CMT.

D. Brain transmitter studies

The brain transmitter determinations are summarized in figures 5, 6, and 7 and in tables 13, 14, and 15.

Time and dose related changes were found in whole brain monoamine oxidase, cholinesterase and oxygen uptake in chronically treated rats. Although acute administration of CMT increased norepinephrine, 5-HT, and decreased dopamine steady state levels 6 hours after an acute dose, no remarkable changes were found after 24 hours in either acute or chronically dosed rats.

These results suggest that the effects of CMT on the brain excitatiry state either are not induced at low doses of CMT or that they are not cummulative on the brain.

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TABLE 1

Range Finding Study RD:

Dose[A]	Number of Rats	Results
200 mg/kg (1 ml/kg)	3	Convulsed 10 minutes; dead 15-20 minutes
100 mg/kg (0.5 ml/kg)	3	Convulsed 10 minutes; dead 15-20 minutes
50 mg/kg (0.25 ml/kg)	3	Convulsed 20-90 minutes; dead 120-200 minutes
25 mg/kg (1 ml/kg)	3	Convulsed 30-90 minutes; 1/3 dead 120 minutes
125 mg/kg	3	No convulsions and no deaths

Summary

Dose mg/kg	Number responding/number treated	
	Convulsions	Death
12.5	0/3	0/3
25.0	3/3	1/3
50.0	3/3	3/3
100.0	3/3	3/3
A. 200.0	3/3	3/3

Effects noted: exothalmus, pyloeriction, facial clonus, alternating unilateral and bilateral clonus, diarrhea, violent convulsions, nose bleeding, eye bleeding, and death.

TABLE 2

Effect of Chronic RDX Dosing on Body Weight

	Body Wt. (, Mean ± S.E.) (6 Rats)			
Treatment[A]	Day 1	Day 5	Day 10	Day 15
1% Methyl Cellulose	180 ± 5.1	201 ± 8.2	230 ± 12.1	250 ± 12.1
0.3 Mg/Kg RDX	178 ± 7.0	205 ± 9.1	241 ± 9.1	253 ± 10.2
2.5 Mg/Kg RDX	172 ± 3.1	202 ± 4.9	237 ± 6.1	243 ± 3.3
25 (c) "	180 ± 7.3 (6)	198 ± 8.3[b](3)	—	—
12.5 "	232 ± 8.7	224 ± 10.8	243 ± 5.4	

a. 1 Ml/Kg volumes administered each day.

b. One of 6 rats gained weight, of the remaining 5, none gained weight and 3 died by the 5th day.

c. All of this group showed convulsions during dosing.

TABLE 3

Time Course of Lethality Study Chronic Dosing (A)

Dose	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	0/6													0/6
0.3 mg/kg	0/6													0/6
2.5 mg/kg	0/6													0/6
12.5 mg/kg[B]	0/6													0/6
25.0 mg/kg	0/6	1/6	2/6	2/6	3/6	4/6	4/6	5/6	5/6	dose lowered				5/6
		17%	33%	33%	50%	67%	67%	83%	83%					

A. 1 ml/kg/da volume

B. 0.5 ml/kg/da volume day 1-10 sacrificed 72 hours after the last dose.

TABLE 4A

Effect of RDX on O_2 Uptake in Rat Brain (in vivo)

% of Control Values

Dose RDX*	Duration of Exposures 6 Hr.	48 Hr.	288 Hr.
Control	100	100	100
0.3 Mg/Kg	111	94	101
2.5 Mg/Kg	110	97	85+
12.5 Mg/Kg	-	-	128**
25.0 Mg/Kg	98	105	-

* 1 Ml/Kg volume each day, sacrificed 24 hours after last dose.

** $P < 0.05$ by T test, sacrificed 72 hours after last dose.

\+ Not different from control $P < 0.20$ two-tailed test, or by rank-sign test.

TABLE 4B

In Vivo Effect of RDX on Oxygen Uptake in Rat Brain (Succinate)

$\mu l\ O_2/30/gm$ brain (% Control)

Dose	Acute 6 hour(3)	2 days 48 hour (4-6)	2 weeks 288 hours (6)
Methylcellulose (1%)	509.8 ± 31.4	328.53 ± 15.5	320.21 ± 14.7
0.3 Mg/Kg	567.0 ± 23.4	309.43 ± 52.5	323.06 ± 13.4
2.5 Mg/Kg	545.0 ± 70.5	321.68 ± 29.9	272.38 ± 24.4
12.5 Mg/Kg	----	----	409.80 ± 29.7 (Control 324.23)
25 Mg/Kg	501.3 ± 14.4	344.80 ± 29.1	----

A. Male rats received i.p. injections either 6 hours before sacrificing or every 24 hours, and sacrificed 24 hours after the last injection.

TABLE 4C

Preliminary Experiments on the In Vitro Effect of RDX on O_2 Uptake Using Various Substrates (μl O_2/30 min./gm brain)

(Pooled brain in each experiment)

Substrate: Succinate (0.1 Molar)

Experiment I. Preincubation for 10 minutes

Concentration RDX mg/ml	μl O_2/30 min./gm brain Values	Average
0	621.0; 588; 701.5	736.5
0.70×10^{-3}	687.0; 743.1	715.1
7.0×10^{-3}	727.0	726.7
70×10^{-3}	696.8; 703.1	699.9
140×10^{-3}	680.2; 706.1	693.1

Experiment II. Preincubation for 45 minutes

Concentration RDX (mg/ml)	μL O_2/30 min./gm brain Values	Average
0	443.6; 421.51; 505.8; 439.4	452.6 ± 18.4
70×10^{-3}	344.9; 384.7	364.8
140×10^{-3}	469.5; 551.6	510.5
210×10^{-3}	564; 598.6	581.8

No effects were found using glucose or pyruvate as the substrate at 140×10^{-3} mg/ml RDX concentrations.

TABLE 4D OXYGEN UPTAKE, EFFECT OF USING CMT-RDX SUSPENSION OR SUPERNATANT

Vol RDX/3cc[A]	mg/ml RDX	μL O_2/30 min./gm brain Values	Average
0.1 cc suspension	70×10^{-3}	105.4; 106.2	105.8
0.2 cc suspension	140×10^{-3}	118.9	118.9
0.1 cc supernatant	?	99.5; 105.4	102.5
0.2 cc supernatant	?	114.5; 117.0	115.7
Control	0	98.6; 80.7; 101.7; 124.5	101.4 ± 9.0

A. 2 mg/ml RDX

Note an increase may be due to soluble fraction.

FIGURE 1

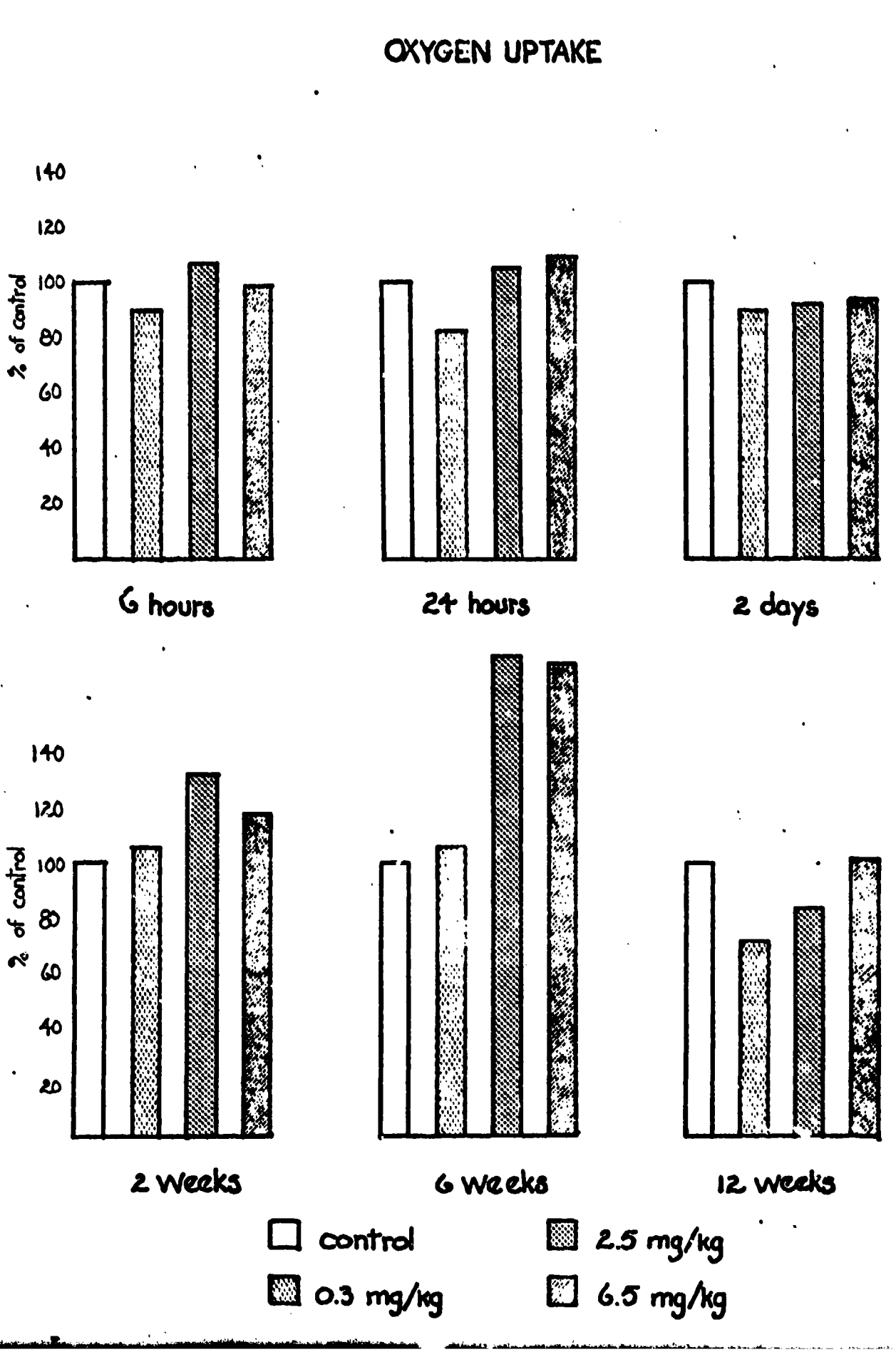
OXYGEN UPTAKE
140
120
100
80
60
40
20
% of control
6 hours
24 hours
2 days
2 weeks
6 weeks
12 weeks
control
2.5 mg/kg
0.3 mg/kg
6.5 mg/kg

TABLE 5

EFFECTS OF RDX ON OXYGEN UPTAKE ($\bar{X}$ ± SE)

ul/60min/gram

Duration of Treatment	Dose (Mg/Kg/Day) Controls	0.3	2.5	6.5 - 12.5
6 hours	509.7±31.4	567.0±23.4	550.0±70.5	501.3±14.4
24 hours	311.3±22.9	257.0±22.6	324.8±26.9	341.9±16.4
2 days	418.7±22.9	377.3±73.5	383.6±20.6	391.8±22.5
2 weeks	278.7±13.7	298.1±27.2	364.9±29.4	326.5±18.6
6 weeks	348.6±14.0	370.2±47.1	620.5±61.3	604.3±14.2
12 weeks	352.8	252.8±27.8	288.3±40.8	-----

FIGURE 2

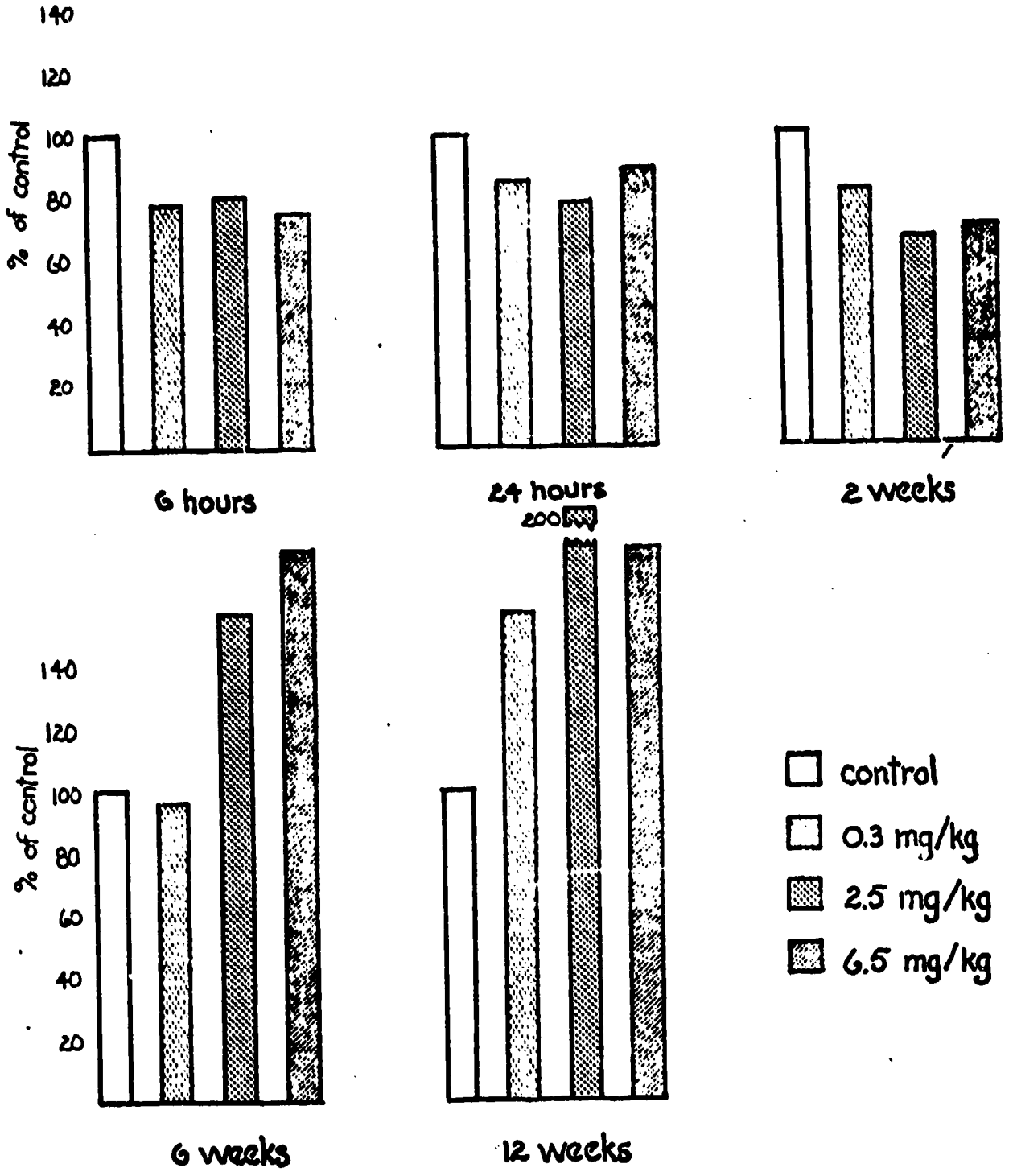
MONOAMINE OXIDASE
% of control
140
120
100
80
60
40
20
6 hours
24 hours
2 weeks
6 weeks
12 weeks
control
0.3 mg/kg
2.5 mg/kg
6.5 mg/kg

TABLE 6A

EFFECTS OF RDX ON MAO

(um/60 min/Mg protein $\bar{X} \pm SE$)

Duration of Treatment	Dose (Mg/Kg/Day) Controls	0.3	2.5	6.5 - 12.5
6 hours	1.49±0.27	1.17±0.04	1.20±0.02	1.12±0.05
24 hours	2.57±0.09	2.22±0.34	2.00±0.21	2.27±0.20
2 days	---	---	---	---
2 weeks	3.50±0.57	2.86±0.27	2.31±0.35	2.45±0.52
6 weeks	2.41±0.69	2.49±0.51	3.78±0.42	4.32±0.85
12 weeks	1.64±0.47	2.68±0.48	3.24±0.52	2.80±0.40

N = 5-6 unless indicated otherwise

TABLE 6B

Effect of an Acute Dose of RDX on Monoamine Oxidase Activity in the Brain of Rats[B]

Dose RDX	Activity CPM/0.01 ml 10% homogenate/20 minute (N) Mean ± SE
Control	8 386.4 ± 20.0
12.5 mg/kg	4 419.4 ± 60.1
25.0 mg/kg	3 254.5 ± 5.8 (P<0.01)

Rats sacrificed 4 hours after dosing.

TABLE 7

Effect of RDX on Brain Cholinesterase Activity % of Control Value

(1 Ml/Kg administered i.p. daily for 2 days or 2 weeks and sacri- 24 hours after last dose)

	Durations of Exposures		
Dose RDX	6 Hr. (3)	48 Hr. (4-6)	288 Hr. (2 wks.) (6)[A]
Control	100	100	100
0.3 mg/kg	87	107	149 $P < 0.05$
2.5 mg/kg	79	107	138 $P < 0.05$
12.5 mg/kg	-	-	113*
25.0 mg/kg	71	105	-

* A separate experiment control 0.93 vs. 1.006 treated sacrificed 72 hours after last dose.

A. There is no overall lap between control and treated values. 0.89 ± 0.01 (C) 1.33 ± 0.01 (0.3 mg/kg) $1.23 (\pm 0.04$ (2.5 mg/kg)

Conclusion: (A) 6 hour inhibition of brain cholinesterase is significant and probably real.

(B) The increase after 288 days is also statistically significant $P < 0.05$. However, in this case brains are removed 24 hours after inhibition vs. 6 hours in the group showing inhibition.

FIGURE 3

CHOLINESTERASE

μ moles/minute/gram of brain

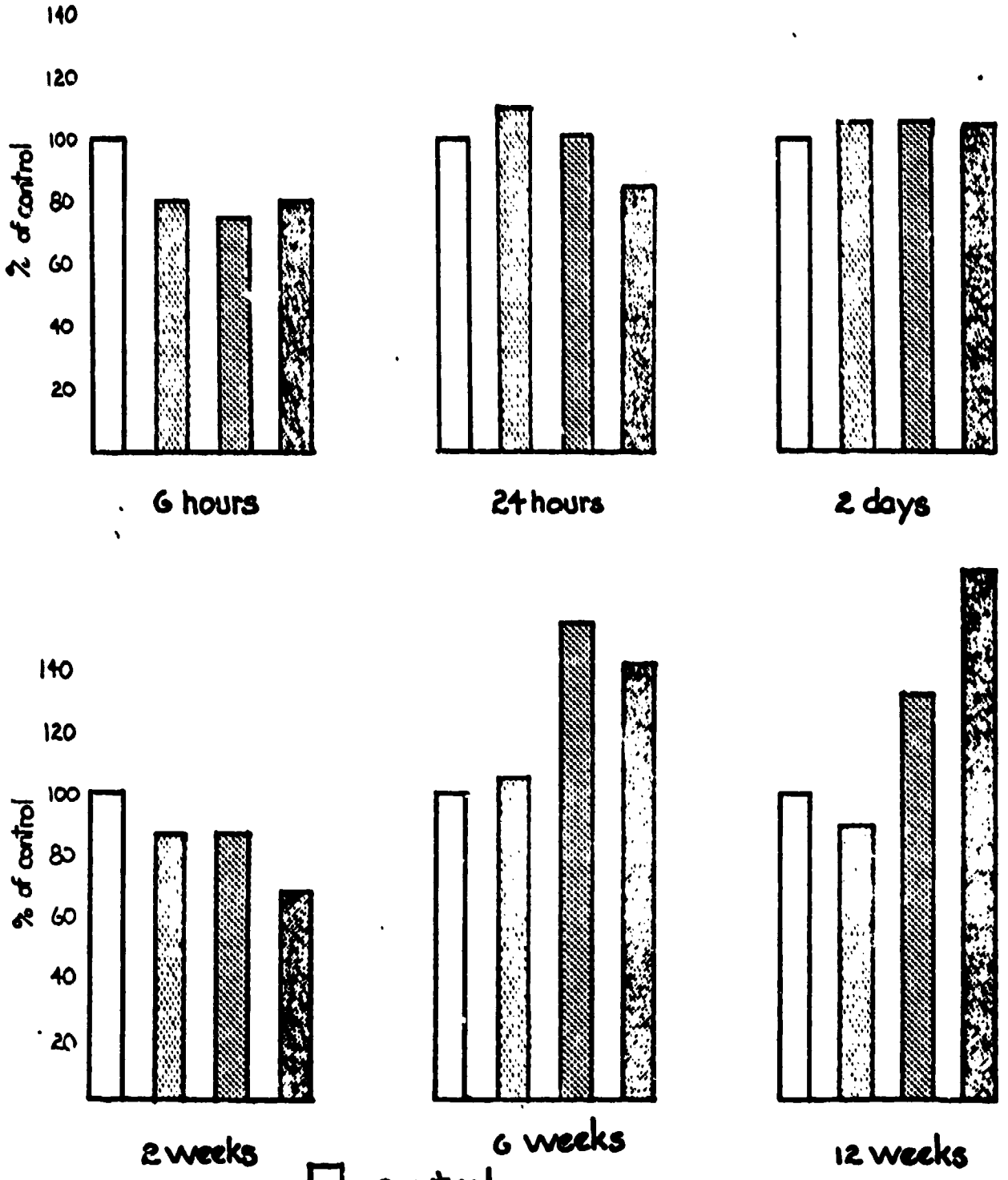

TABLE 8

EFFECTS OF RDX IN CHOLINESTERASE

(umoles/minute/gram of brain)
X 10^6

Duration of Treatment	Dose (Mg/Kg/Day) Controls	0.3	2.5	6.5 - 12.5
6 hours	14.42±0.16	11.62±0.09	10.71±0.11	11.40±0.31
24 hours	15.97±0.71	17.65±0.54	16.15±0.91	13.45±0.41
2 days	19.03±0.92	20.33±0.43	20.28±0.64	20.05±0.20
2 weeks	18.52±0.51	16.06±0.71	15.40±0.75	12.80±0.74
6 weeks	7.895±0.21	8.363±0.32	12.375±0.71	11.236±0.52
12 weeks	5.846±0.40	5.266±0.29	7.727±0.20	9.985±0.18

TABLE 9

Enzyme and Monoamine levels 24 hours after dosing with (6.5 mg/k/da) CMT.

	% Control enzyme activity		% Control Monoamine levels			
Time on Study	Monoamine oxidase	Cholinesterase	O_2Uptake	Norepinephine	Dopamine	5-HT
24 hour	85	85	110	95	110	90
2 week	70	70	95	135	65	110
6 week	180	140	175	90	90	80
12 week	200	180	100	100	75	100
6 hours	75	80	98	72	136	221

FIGURE 4

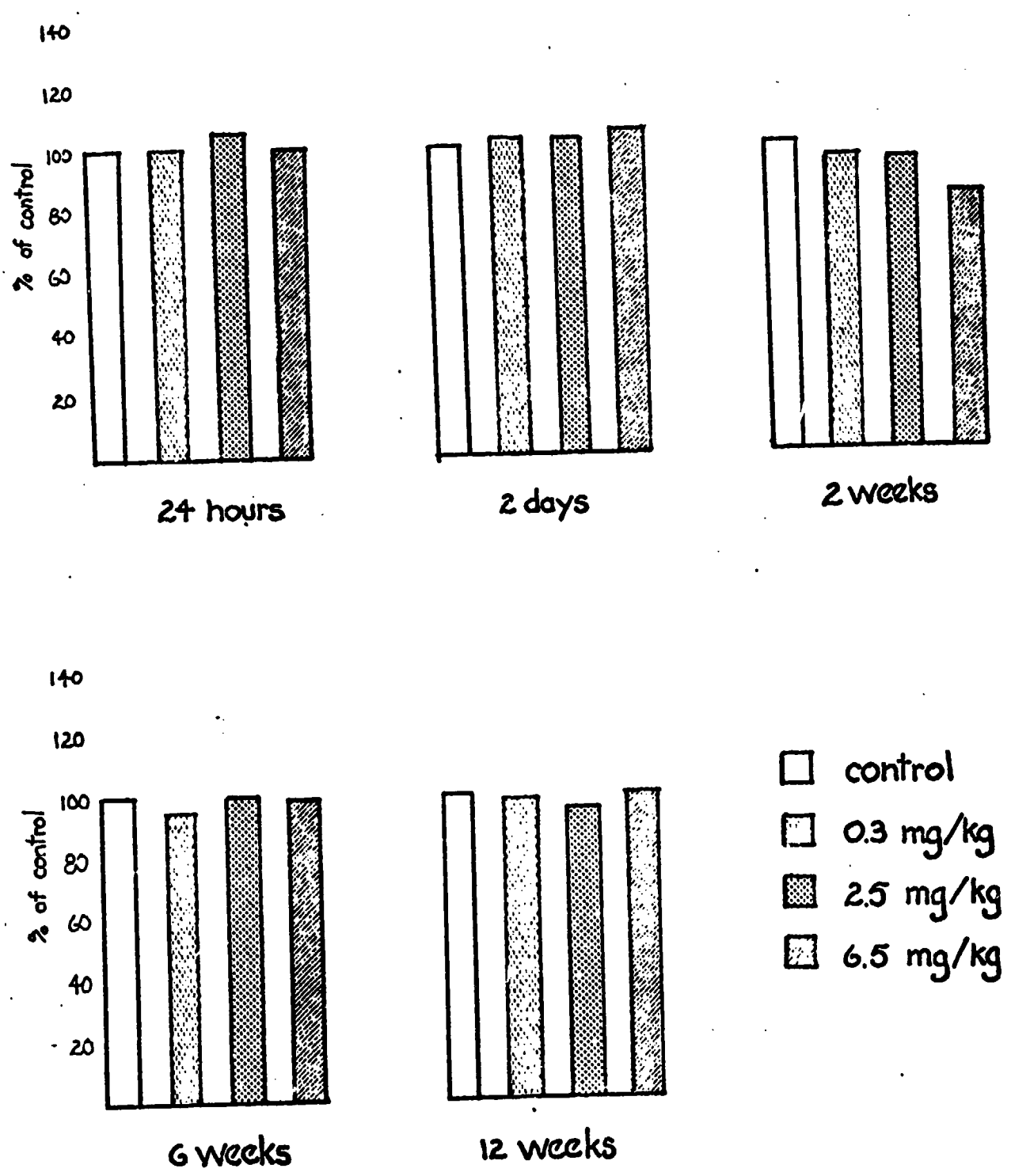
BODY WEIGHTS
140
120
100
80
60
40
20
% of control
24 hours
2 days
2 weeks
6 weeks
12 weeks
control
0.3 mg/kg
2.5 mg/kg
6.5 mg/kg

TABLE 10

BODY WEIGHTS
(Grams)

	Dose (Mg/Kg/Day)			
	Controls	0.3	2.5	6.5 - 12.5
6 hours	273	280	193	216
24 hours	191.8±5.55	189.7±3.63	198.8±5.65	191.3±6.94
2 days	178.3±3.18	181.3±4.10	182.7±4.33	185.7±1.20
2 weeks	344.1±6.39	326.8±5.25	321.2±4.55	282.0±4.66
6 weeks.	276.9±9.61	295.3±9.92	276.0±11.25	280.0±8.82
12 weeks	---	---	---	358.9±6.47

TABLE 11

	Mean CMT in whole blood mg/ml (A)			
Dosing period	0.	0.3mg/k/da	2.5mg/k/da	6.5 mg/k/da
1 day	0	0.03	0.065	0.111
2 weeks	0	0.000	0.000	0.098
6 weeks	0	0.015	0.103	0.134
12 weeks	0	0.009	0.108	0.222

(A)
Means of 5-6 determinations

TABLE 12

Effect of CMT on blood pressure in rats after acute and chronic treatment. (Means ±SE of 8-15 rats)

Treatment	Bloodpressure (MMHg)	Change after CMT	Significance
Acute CMT	155.56 ± 4.98	1.33 ± 5.129	--
Chronic CMT	150.50 ± 5.40	10.33 ± 2.250	$P < 0.05$ (A)
Acute Vehicle	167.25 ± 10.67	0.88 ± 4.250	--
Chronic Vehicle	163.00 ± 4.10	3.23 ± 2.240	--

FIGURE 5

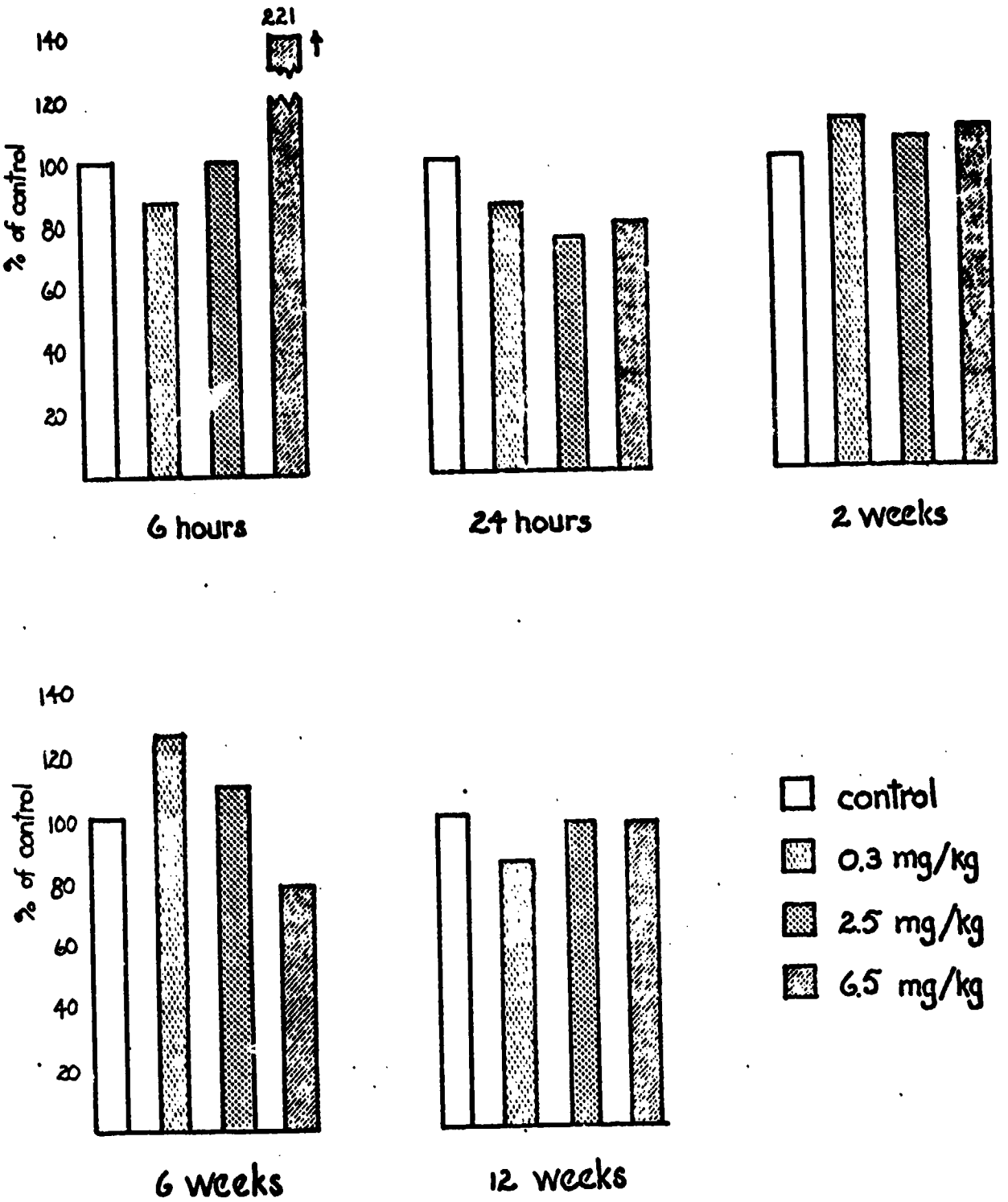
5 HYDROXY TRYPTAMINE
221
140
120
100
80
60
40
20
% of control
6 hours
24 hours
2 weeks
6 weeks
12 weeks
control
0.3 mg/kg
2.5 mg/kg
6.5 mg/kg

TABLE 13

WHOLE BRAIN 5-HYDROXY-TRYPTAMINE

Duration of Treatment	Dose (Mg/Kg/Day) Control	0.3	2.5	12.5
6 hours	0.59[A] (0.56-0.61)	0.51 (0.48-0.54)	0.59 (0.52-0.69)	1.31 (0.91-1.81)
24 hours	0.96 (0.80-1.17)	0.82 (0.65-0.93)	0.72 (0.65-0.77)	0.74 (0.65-0.83)
2 weeks	0.93 (0.82-1.02)	1.05 (0.87-1.14)	0.98 (0.89-1.04)	1.00 (0.95-1.04)
6 weeks	0.93 (0.80-1.18)	1.20 (1.15-1.29)	1.02 (0.91-1.15)	0.72 (0.66-0.81)
12 weeks	0.57 (0.44-0.70)	0.44 (0.37-0.66)	0.56 (0.53-0.61)	0.55 (0.39-0.82)

A Mean and range of 3 brains, duplicate determinations

FIGURE 6

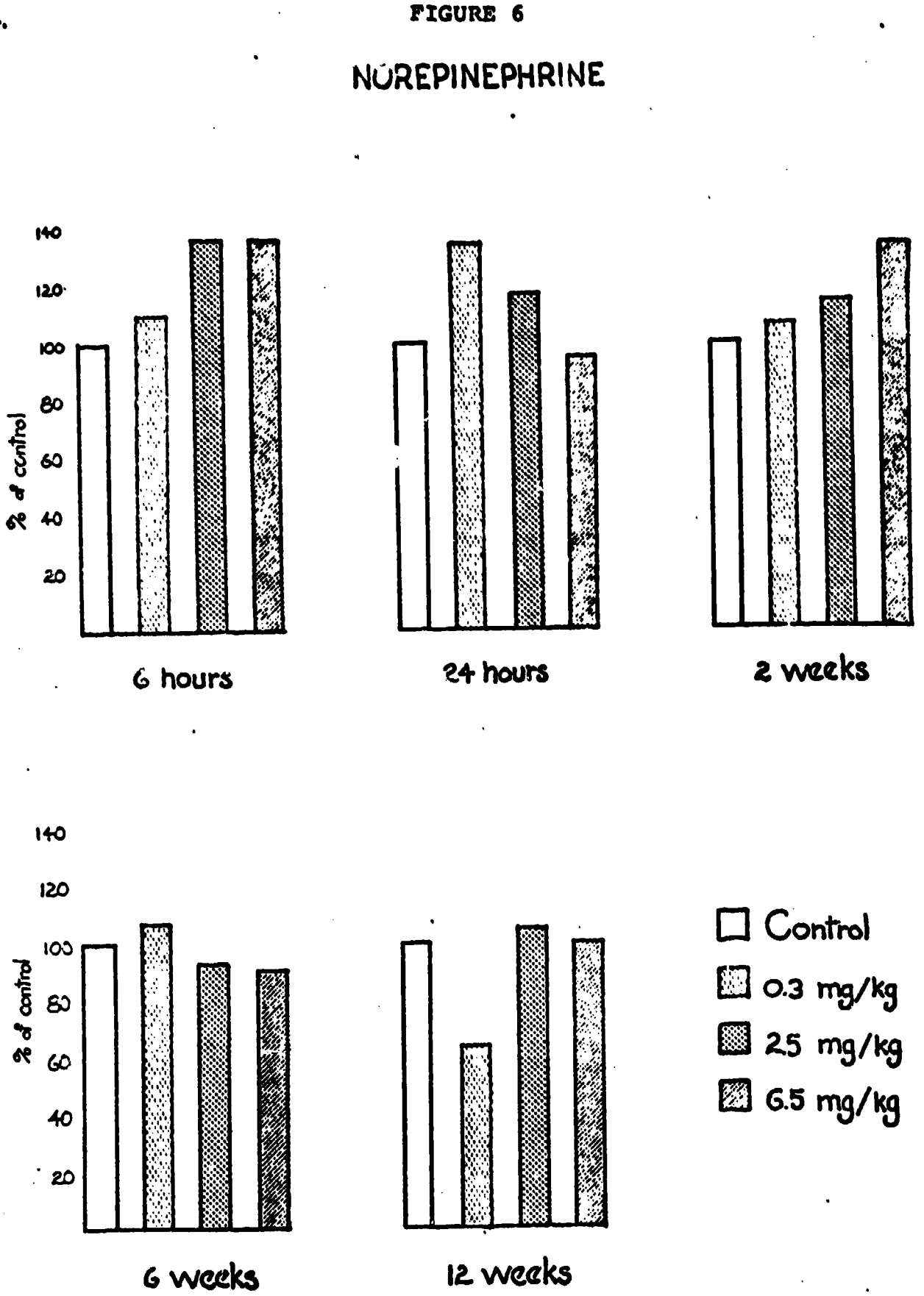
NOREPINEPHRINE
% of control
140
120
100
80
60
40
20
6 hours
24 hours
2 weeks
6 weeks
12 weeks
Control
0.3 mg/kg
2.5 mg/kg
6.5 mg/kg

TABLE 14

WHOLE BRAIN NOREPINEPHRINE

Duration of Treatment	Dose (Mg/Kg/Day) Control	0.3	2.5	12.5
6 hours	0.25[A] (0.22-0.29)	0.26 (0.22-0.32)	0.35 (0.30-0.41)	0.35 (0.28-0.40)
24 hours	0.25 (0.19-0.31)	0.34 (0.29-0.42)	0.29 (0.26-0.31)	0.24 (0.22-0.25)
2 weeks	0.14 (0.11-0.18)	0.15 (0.10-0.18)	0.16 (0.15-0.17)	0.19 (0.14-0.23)
6 weeks	0.30 (0.21-0.46)	0.32 (0.27-0.36)	0.27 (0.23-0.32)	0.27 (0.26-0.28)
12 weeks	0.33 (0.21-0.46)	0.27 (0.25-0.29)	0.35 (0.31-0.38)	0.33 (0.32-0.36)

A Mean and range of 3 brains, duplicate determinations

FIGURE 7

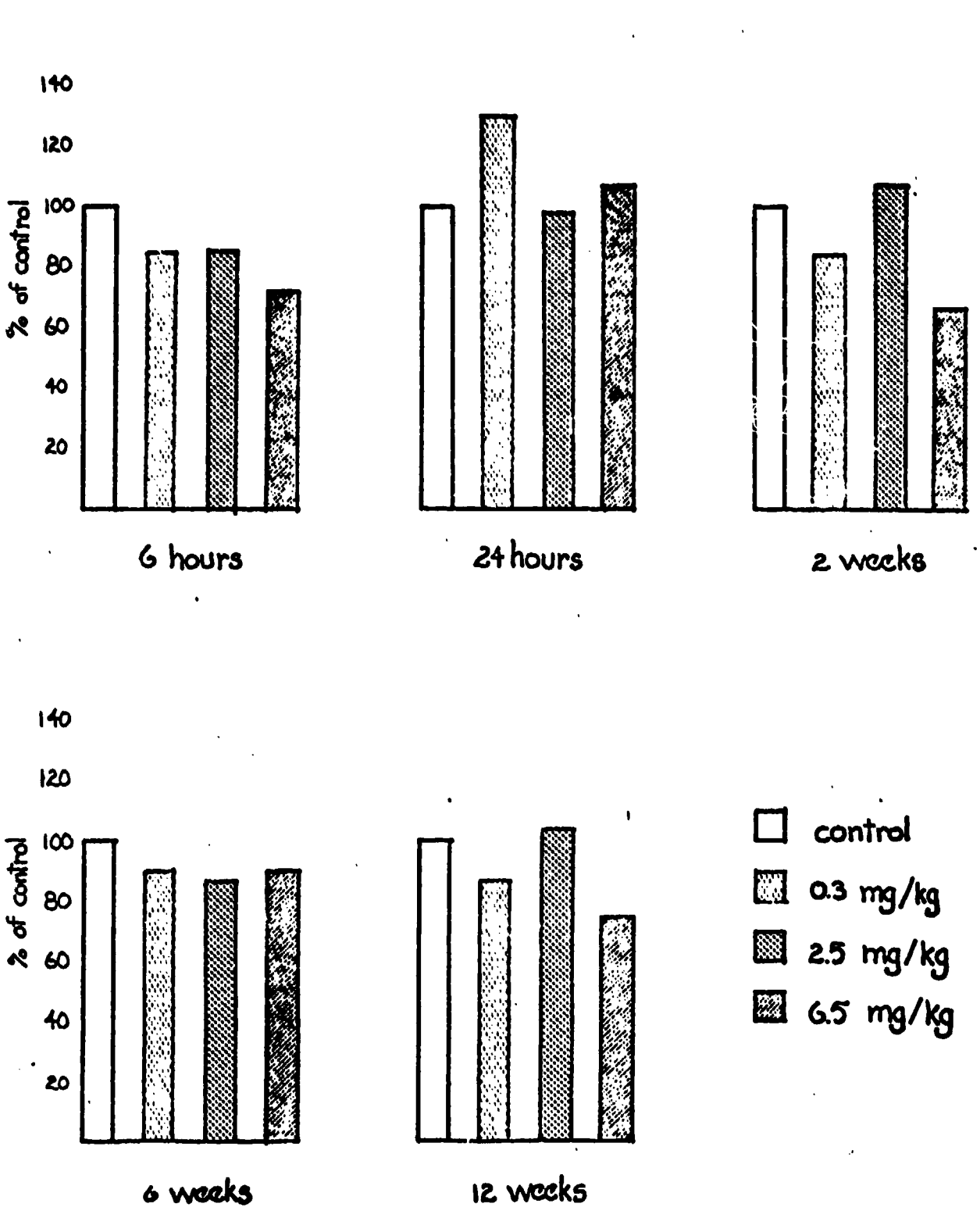
DOPAMINE
% of control
140
120
100
80
60
40
20
6 hours
24 hours
2 weeks
6 weeks
12 weeks
control
0.3 mg/kg
2.5 mg/kg
6.5 mg/kg

TABLE 15

WHOLE BRAIN DOPAMINE

Duration of Treatment	Dose (Mg/Kg/Day) Control	0.3	2.5	12.5
6 hours	0.58[A] (0.52-0.66)	0.48 (0.44-0.50)	0.44 (0.42-0.55)	0.42 (0.36-0.48)
24 hours	0.20 (0.17-0.24)	0.26 (0.20-0.34)	0.19 (0.14-0.26)	0.22 (0.20-0.24)
2 weeks	0.29 (0.24-0.32)	0.24 (0.17-0.26)	0.31 (0.22-0.36)	0.19 (0.12-0.28)
6 weeks	0.47 (0.36-0.64)	0.44 (0.34-0.52)	0.42 (0.38-0.48)	0.44 (0.34-0.50)
12 weeks	0.95 (0.77-1.10)	0.82 (0 72-1.00)	0.99 (0.93-1.05)	0.73 (0.50-0.97)

A Mean and range of 3 brains, duplicate determinations