AN EVALUATION OF MORPHINE DEPENDENCE IN THE RAT

A Thesis

Presented To

the Faculty of the College of Pharmacy

The University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Pharmacy

by

Yogendra Pragatrai Kharode

December 1974

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ABSTRACT

A semiquantitative method for assessing the degree of physical dependence upon morphine in rats has been examined. A time-course measure of dependence due to repetitive and increasing daily doses of morphine was obtained between one and eleven days of treatment utilizing a narcotic antagonist to precipitate withdrawal and scoring six specific withdrawal symptoms. High starting doses of morphine produced a mixiмal withdrawal score after two days which remained constant until the eleventh day. However, low starting doses of morphine produced a more typical dose-effect relationship from day one through day seven.

The scoring system utilized in this study was analyzed and found to be acceptable, but by no means optimal to assess the degree of morphine physical dependence.

The effects upon the narcotic antagonist precipitated withdrawal score, and therefore, the degree of physical dependence upon morphine, of concomitantly administered drugs was investigated. Dextroamphetamine, apomorphine and atropine were shown to decrease the total withdrawal score whereas levoamphetamine, haloperidol and atropine methyl nitrate failed to reduce significantly the total withdrawal score.

Based upon these data a hypothetical model for the development of morphine physical dependence was proposed. Although this model appeared to hold for total withdrawal scores certain discrepancies became apparent when drug-induced effects upon individual withdrawal symptoms were analyzed.

Irrespective of mechanism, the research shows a clear differential effect between the two isomers of amphetamine, which could be due to differences on central dopaminergic mechanisms.

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Chapter 1

INTRODUCTION

Morphine, the principal alkaloid of opium, was first isolated by Serturner in 1808 and has long been used as a potent analgesic. The chemical structure of morphine was elucidated by Gulland and Robinson in 1925 and confirmed by Gates and Tschudi, who synthesised the compound in 1952.

The literature is abundant with studies on the pharmacology of morphine, its therapeutic effects and side effects, including tolerance and physical dependence (Jaffe, 1971; Murphree, 1971).

TOLERANCE AND PHYSICAL DEPENDENCE

With narcotic analgesics like morphine, tolerance develops after repeated administration and more of the drug must be given to produce the same effect as that obtained with the initial dose. Physical dependence may also develop quite rapidly, i.e., a characteristic set of symptoms appears on withdrawal of the drug. These symptoms are called the "Withdrawal-Syndrome" (Shuster, 1971). The withdrawal syndrome can be precipitated by either terminating the drug administration or by treating with a narcotic antagonist, such as nalorphine, levallorphan or naloxone. The severity of the withdrawal-syndrome is believed to be directly proportional to the degree of physical dependence. According to Akera and Brody (1968), the loss of body weight, measured over a period of 48 hours following withdrawal, is the most reliable index of the severity of physical dependence in rats chronically treated with morphine. Alternatively, Buckett (1964) has reported a semiquantitative scoring method to measure morphine-induced physical dependence in rats.

His method consists of scoring various clearly defined symptoms of withdrawal which occur within 30 minutes following the administration of a narcotic antagonist. Thus, as described, the technique provides a clear, quick and reproducible means of assessing the severity of narcotic-induced physical dependence. The observed symptoms and their respective scores are shown below:

SYMPTOM	SCORE
Writhing	3
Squealing	2
Diarrhea	2
Teeth Chatter	1
Ptosis	1
Wet Dog	1
TOTAL:	10

According to the method, the responses are recorded following the precipitation of withdrawal from rats that have received daily doses of morphine for a given period.

The degree of tolerance to narcotic analgesics can be measured by comparing the response of a dose after tolerance has developed with that of a standard dose before tolerance development. Parameters(Cox et al., 1968) that are commonly measured include analgesia, respiratory rate and body temperature changes. In experimental animals the criteria of tolerance is usually assessed by measuring the decreased analgesic response in animals treated repeatedly with a narcotic analgesic. The

degree of tolerance may be expressed as a percentage of the response elicited in a non-tolerant, control animal. On the other hand, the response measured before the start of narcotic treatment may be utilized as the control measure (Martin et al., 1961; Way et al., 1969; and Shuster et al., 1963).

THEORIES OF TOLERANCE AND PHYSICAL DEPENDENCE

A complete biochemical basis of narcotic physical dependence and tolerance has yet to be elucidated. General conceptual theories have been proposed for these phenomena, but they are necessarily vague in nature since so much remains unknown with regard to the physiological functions of the central nervous system. Goldstein and Goldstein (1961) made the first attempt to explain physical dependence and tolerance based upon the Jacob-Monod (1961) concepts of repression and depression of protein synthesis as fundamental mechanisms of biochemical regulation. Shuster (1961) has advanced the same idea independently. Collier (1965, 1972) proposed a somewhat different but related theory and finally Goldstein and Goldstein (1968, 1973) further refined and generalized their hypothesis, which was presented under the name of the "enzyme (receptor) expansion theory."

The essence of the theories pertaining to tolerance and physical dependence is as follows: the narcotic analgesic is believed to elicit effects by interfering with synaptic transmission in the central nervous system. The drug may act by modifying the receptor and/or the release of the transmitter. Irrespective of the actual site, the normal steady-state

level of synaptic transmission is disturbed, resulting in excitation or inhibition depending upon the physiological function of the affected synapses. The phenomenon of tolerance is explained by proposing that synaptic transmission adapts to the continued presence of the drug such that a new functional steady-state is achieved which opposes the pharmacological action of the compound. A probable explanation for adaptive change is that "feed-back" neurogenic pathways control the functional level of the pre-synaptic neurons. For instance, if post-synaptic receptors are inhibited, the release of the transmitter from pre-synaptic neurons would be predicted to increase. Increased release might overcome the drug-induced receptor inhibition, which would then become manifest as overt tolerance. On the other hand, the receptor itself may change ("receptor expansion theory") so as to allow normal function despite the continued presence of the drug. Other mechanisms are equally feasible but all are assumed to call for enhanced protein synthesis so as to aquire the new steady-state level. Upon withdrawal of the drug, the "supra-normal" events at affected synapses continue to function and become manifest as the withdrawal syndrome. This phenomenon accounts for physical dependence and will only fully disappear when synaptic events re-adjust to normal physiological limits.

CONSEQUENCES OF THE THEORIES

The general concept relating to tolerance and physical dependence as outlined above fits fairly well with certain experimental observations concerning these phenomena. For example, it was predictable that the states of tolerance and physical dependence would be prevented by inhibitors

of nucleic acid and protein synthesis, and this has been found to be true (Cox et al., 1968; Way et al., 1968). Also, the theory predicts that the signs of withdrawal should be opposite to those of narcotic intoxication, a prediction which is known to be valid (Jaffe, 1970). The faster appearance of the pharmacological effects of narcotics and associated withdrawal symptoms compared with the development of tolerance and physical dependence is inherent in the theory and is a well known fact with regard to narcotic analgesics. Finally, as described below, the generalized concept fully predicts changes in the synthesis, release, effectiveness, and metabolism of endogenous neurotransmitters.

The phenomena of tolerance and physical dependence have been investigated intensively with particular emphasis on the interaction and effects of morphine and certain morphine-like agents on brain neurotransmitters. The following discussion will outline the more significant observations concerning changes in the steady-state levels of 5-hydroxytryptamine (serotonin), norepinephrine and dopamine.

The possibility of serotonergic involvement in the development of tolerance and physical dependence to morphine has been intensively studied over the past decade particularly in relation to the endogenous synthesis rate of this indoleamine. However, conflicting data has been obtained. Way et al., (1968); Loh et al., (1969); and Shen et al., (1970) reported that the rate of brain serotonin synthesis increased in morphine tolerant-dependent animals. They also pointed out that para-chlorophenylalanine (pCPA), an inhibitor of tryptophan hydroxylase (320 mg/kg i.p., 24 hours prior to a morphine pellet-implantation), reduced the development of

tolerance and dependence. Ho et al., (1972) confirmed this work, again reporting that pCPA pretreatment inhibited morphine dependence and tolerance. This was demonstrated by a decreased amount of morphine necessary to produce analgesia and an increase in the amount of naloxone required to induce withdrawal jumping. The pCPA-induced inhibition of morphine physical dependence was further evidenced by the fact that pretreatment with the inhibitor decreased the loss in body weight, following abrupt withdrawal of morphine. Loss in body weight following withdrawal is claimed to be an excellent criteria of dependence (Akara and Brody, 1968). Chronically morphine-dependent rats undergoing abrupt withdrawal, lose more weight than control rats deprived of food over a period of 48 hours.

The findings of Shen et al., (1970) were immediately opposed by Marshall and Grahamme-Smith (1970) who failed to find any change in the rate of brain serotonin synthesis during chronic morphine treatment. These authors also reported that pCPA failed to attenuate the withdrawal-syndrome in mice. Chenny and Goldstein (1971) found no correlation between narcotic tolerance-dependence and serotonin turnover rate in the mouse brain. These workers studied the conversion of radiolabelled tryptophan to serotonin and found no difference in highly morphine tolerant and dependent mice compared with control animals.

Knapp and Mandall (1972) suggested that two different enzymes regulate serotonin levels, namely particulate and soluble tryptophan hydroxylase. Morphine administration produced an immediate decrease and a long term increase in the activity of the nerve ending (particulate) enzyme but did not change the activity of the cell body (soluble) enzyme.

The presence of two measurable forms of tryptophan hydroxylase in brain, their varying regional distribution, differing modes of regulation, and differential response to pharmacological agents may explain some of the conflicts seen in the literature relating the action and effects of morphine on serotonin biosynthesis. Thus, before definite statements can be forthcoming with regard to the effects of morphine on serotonin synthesis experimental designs must be so constructed as to minimize the above variables. In relation to this latter comment, Tilson and Rech (1974) studied the effects of pCPA on morphine analgesia, tolerance development and physical dependence, using two different strains of albino rats. Pretreatment with pCPA in morphine tolerant and dependent Sprague-Dawley rats was shown to attenuate morphine analgesia and significantly decreased the withdrawal symptoms. However, they failed to find any change in analgesia of withdrawal effects in Fischer rats also pretreated with pCPA. Therefore, their results emphasised the importance of strain differences with respect to the involvement of serotonin in morphine analgesia, tolerance development, and physical dependence.

The role of brain catecholamines in the various pharmacological effects of morphine have been a center of attention for many investigators during the last twenty years. Vogt (1954), a pioneer in these studies, noticed that morphine caused a reduction of brain catecholamines. These results were consistent with those of the other workers (Gunne, 1963; Takagi and Nakama, 1966; Reis et al., 1969) who used fluorimetric assays in contrast to the bio-assay technique of Vogt (1954). It has now been established that morphine analgesia (Ayhan, 1972), excitation (Holdinger 1969; Rethy et al., 1971; Zeigler et al., 1972), tolerance production

and physical dependence are also associated with changes (i.e. decrease) in brain catecholamine levels (Gunne, 1963; Sloan et al., 1963; Takagi and Kuriki, 1969). Ayhan and Randrup (1970) suggested that brain norepinephrine may play an important role in morphine-induced sterotyped behavior which consists of contineous sniffing, licking, and biting. Ayhan and Randrup (1973) further showed that inhibition of catecholamine biosynthesis by alphamethylparatyrosine (α-MT), an inhibitor of tyrosine hydroxylase, and more importantly FLA-63, an inhibitor of dopamine beta-hydroxylase, could depress the stimulatory effects of small doses of morphine on behavior. The findings of Glick et al., (1973) that pretreatment with α-MT could depress the oral intake of morphine, in addition to ameliorating withdrawal-induced weight loss, again implicated an important role of the catecholamines in morphine-induced physical dependence. Villarreal et al., (1973) independently demonstrated that pretreatment of mice with reserpine (5 mg/kg, i.p., 24 hours) or α-MT (200 mg/kg, i.p., 3 hours) markedly reduced the hyperkinetic effects of morphine. However, the same drug pretreatment schedule failed to decrease the locomotor activity due to dextroamphetamine, thereby indicating the different mechanisms of action for these two agents. These findings agreed well with those of Ayhan and Randrup (1973) who failed to notice any effect of FLA-63 on amphetamine-induced hyperactivity. In contrast to these findings, several other workers (Fog et al., 1967; Randrup and Munkvad, 1970; Iverson et al., 1971) have proposed that the sterotyped behavior of rats following the chronic administration of morphine is closely related to that seen following amphetamine. The latter syndrome is believed to be mediated by an action on central dopaminergic pathways

and/or receptors, probably at the level of corpus striatum.

Recently, Puri and Lal (1973), using morphine dependent rats, demonstrated that dopaminergic stimulation by pretreatment with L-dihydroxyphenylalanine (150 mg/kg), D-L-dihydroxylphenylalanine (200 mg/kg), dextroamphetamine (2 mg/kg) or apomorphine (1.25 mg/kg) enhanced withdrawal-induced aggression characterized by rearing, vocalization, and attack-bites, several fold. Dopaminergic blockade by haloperidol pretreatment (0.63-2.5 mg/kg) abolished the withdrawal-aggression due to mere abstinence of morphine or supersensitized withdrawal using dextroamphetamine. Similarly, methadone (5-20 mg/kg) blocked morphine-withdrawal aggression supersensitized by treatment with apomorphine. These results suggested a dopaminergic basis for morphine withdrawal aggression and thus, the possibility of the involvement of dopaminergic neurogenic pathways during morphine-induced physical dependence.

The synthetic narcotic analgesic, methadone, shares with morphine several common pharmacological properties. Methadone is reported to accelerate the synthesis of dopamine in the rat brain. Sasame and Perez-Cruet (1972) revealed a dopamine-receptor blocking action of methadone, evidenced by a marked elevation of urinary homovanillic acid excretion accompanied by a marked increase in dopamine synthesis. As mentioned earlier, this latter effect might be a compensatory feedback response consequential to dopaminergic receptor blockade. The work of Gassa et al., (1973), lends support to this concept and further proposes that methadone may possess an amphetamine-like action at dopamine nerve terminals. Furthermore, the stimulant effects of methadone, similar to

those of dextroamphetamine, could be antagonized by α-MT or haloperidol.

Costa and co-workers (1973) compared the effects of morphine and dextroamphetamine with respect to their augmenting action on the turnover rate of striatal dopamine and cyclic-AMP concentrations. They found that morphine (52 μ moles/kg, i.p.) increased the turnover rate of dopamine, but larger doses of the drug (104 μ moles/kg, i.p.) were required to increase the concentration of cyclic-AMP. The increase in dopamine turnover rate was accompanied by a corresponding increase on tyrosine hydroxylase activity. On the other hand, dextroamphetamine increased concomitantly the turnover rate of dopamine and cyclic-AMP concentrations. Thus, differences with regard to the pharmacological effects of morphine and dextroamphetamine were quantitative rather than qualitative with regard to the particular systems investigated. Another report by Iwamota et al., (1973) relates to an observed elevation of brain dopamine during naloxone-induced withdrawal from morphine-dependent mice and rats; again implicating the involvement of dopaminergic pathways in the morphine withdrawal syndrome. These workers also showed the antagonizing effects of physostigmine on morphine withdrawal, and on the subsequent increase in dopamine levels, thus pointing to the possibility of cholinergic involvement in physical dependence to morphine. This observation fits with the known interrelationship of central dopaminergic neurons and cholinergic pathways.

The involvement of cholinergic mechanisms in morphine withdrawal was previously proposed by Martin and Eades (1967), Crossland (1970), and Jahmandas et al., (1970). This view was further supported by Jahmandas, Sutak and Ball (1973). They employed cholinergic drugs (e.g. atropine,

physostigmine, etc.) and adrenergic receptor blocking agents (e.g. phentolamine, propranolol) in rats undergoing morphine withdrawal, with naloxone. The drugs were administered in three doses (10 mg/kg, 20 mg/kg, and 30 mg/kg), 15 to 20 minutes prior to precipitation of abstinence. The effects of cholinergic and adrenergic drugs were assessed on the development of the autonomic, non-autonomic, and total signs of the morphine withdrawal syndrome. Their results suggested that autonomic signs were suppressed by atropine but increased by physostigmine. The total signs of withdrawal were intensified by atropine and were not effectively suppressed by any drug. The adrenergic drugs failed to effect the severity of any of withdrawal symptoms. Thus, their study confirmed that drugs affecting cholinergic mechanisms could modify certain aspects of the morphine withdrawal syndrome but could not completely suppress it. Jahmandas et al., (1973) also demonstrated that atropine sulfate and the ganglionic blocking agent mecamylamine could suppress the development of the precipitated withdrawal syndrome after morphine or methadone.

Likewise, Martin and Eades (1967) showed the partial blocking effects of atropine pretreatment on morphine withdrawal. However, in contrast to this observation, Grumbach (1969) reported that atropine pretreatment intensified the development of the behavioral signs of withdrawal in rats. Collier et al., (1972) demonstrated that atropine could suppress or intensify the behavioural signs of the precipitated withdrawal in the rat depending upon the time of drug administration during the developmental course of dependence. However, this work demands further attention since it may allow a greater insight into the mechanisms involved during the

development of morphine-induced physical dependence.

In conclusion, the reports on the role of serotonergic and cholinergic mechanisms in physical dependence induced by morphine, are conflicting. Dopaminergic mechanisms, however, seem to be more conclusively involved since methadone has been shown to increase the synthesis of dopamine, to block morphine dependence, and also both morphine and methadone share certain common pharmacological properties with dextroamphetamine. Furthermore, considerable evidence has recently accumulated that morphine can, like methadone, inhibit dopaminergic receptors and enhance dopamine synthesis (Smith et al., 1972; Kukui et al., 1972). If dopaminergic involvement in morphine physical dependence is a major factor, then cholinergic mechanisms need to be re-examined more closely because of the known interrelationship of central dopaminergic neurones and cholinergic pathways in certain areas of the brain (Horneykiawicz, 1966; Bartholini et al., 1973).

STATEMENT OF PROBLEM AND INITIAL RATIONALE

The objective of the present research was to gain a further insight into the mechanism or mechanisms whereby repeated administration of morphine produces physical dependence. In view of the possible involvement of central dopaminergic pathways in this phenomenon, and the known dopamine releasing properties of dextroamphetamine, it was decided to study the effect of this sympathomimetic amine on the severity of certain drug-precipitated withdrawal symptoms elicited from morphine-dependent rats. Although drug-induced modifications of withdrawal symptoms is not a unique approach in the investigation of physical dependence, it must be emphasized that the present experimental design differed from that normally encountered in such studies. In virtually all of the present experiments the dextroamphetamine was administered concomitantly with the morphine, rather than adopting the more usual procedure of employing a single administration of the potential modifying agent, at or close to the time of withdrawal. It was argued that this approach might reveal more dramatic changes in the withdrawal syndrome since the continued presence of antagonizing or potentiating influences would presumably be more effective than a single brief challenge to a system or systems which might have already undergone considerable functional modification.

In the design of this work it was realized that an attempt must be made to divorce the pharmacological effects of dextroamphetamine on the central nervous system from those elicited in the periphery. Therefore, experiments were proposed in which levoamphetamine would be utilized in place of the dextro-isomer. Furthermore, it was envisaged that if the

above studies revealed encouraging findings concerning central dopaminergic neuronal involvement, that further experiments would be made employing a direct dopamine receptor agonist (apomorphine) and antagonist (haloperidol). Such experiments would serve to further control and confirm the findings obtained utilizing the amphetamines.

The method of assessing withdrawal symptoms was based on that described by Buckett (1964). Although this method appeared to combine simplicity with a means of semi-quantitative analysis, certain inherent disadvantages in the technique were not apparent at the onset of this work. Therefore, experiments were undertaken to further clarify and validify this particular technique.

Chapter 2

MATERIALS* AND METHODS

A. Maintenance of Animals:

Male, albino, Sprague-Dawley [a] rats weighing approximately 100 g (± 10 g) were used. Upon arrival from the supplier the animals were housed in standard plastic cages (46 cm x 26 cm x 20 cm) in groups of five or six. Room temperature was maintained at 22° ± 2° C under a light and dark cycle of 12 hours light and 12 hours dark (6:00 AM/6:00 PM). Purina lab chow[b] and tap-water were provided ad libitum.

B. Preparation of Drugs:

The drugs used in the experiments are listed below. All drugs were made up in saline 0.9 % w/v with the exception of levallorphan which was used directly from the vial.

TABLE 1: DRUG CONCENTRATIONS AND SOURCES

#	Drug	Concentration Used	Supplier
1	Apomorphine	1.5 mg/ml	Mallinckrodt[c]
2	Dextroamphetamine sulfate	0.25 mg/ml	Elkin Sinn Inc.[d]
3	Levallorphan tartarate	1 mg/kg	Roche[e]
4	Morphine sulfate	1-30 mg/ml	Merck[f]
5	Atropine sulfate	2 mg/ml	Mallinckrodt[c]
6	Atropine Methyl nitrate	2 mg/ml	Regis[g]
7	Haloperidol	0.1 mg/ml	McNeil[h]
8	Levoamphetamine	0.25 mg/ml	Aldrich Chem.Co.[i]
9	Naloxone	0.4 mg/ml	Endo Lab.[j]

* Letter in the superscript denotes the source (Appendix I)

C. Administration of the Drugs:

All drugs were injected intraperitoneally using a sterile 1 ml disposable plastic syringe and 25 gauge needle.

Morphine was administered in three different dose schedules. Starting doses of 20, 10, and 1 mg/kg are referred to as dose schedule one, dose schedule two, and dose schedule three, respectively (Table 2, 3, and 4).

The doses of other drugs were kept constant regardless of whether they were administered alone or concomitantly with morphine.

Table 2 DAILY DOSES AND TOTAL CUMULATIVE DOSES FOR MORPHINE SULFATE; STARTING DOSE 20 mg/kg

(Dose Schedule: 1)

DAY	NO. OF DOSES	DAILY DOSES* MORPHINE SO_4 (mg/kg)I.P.	TOTAL CUMULATIVE DOSE (mg/kg)
1	1-3	3 x 20	60
2	4-6	3 x 60	240
3	7-9	3 x 100	540
4	10-12	3 x 140	960
5	13-15	3 x 180	1500
6	16-18	3 x 220	2160
7	19-21	3 x 260	2940
8	22-24	3 x 300	3840
9	25-27	3 x 340	4860
10	28-30	3 x 380	6000
11	31-33	3 x 420	7260

* 1) Dose injected every 8 hours
  2) Initial individual dose 20 mg/kg
  3) Individual doses were increased daily by 40 mg/kg

Table 3 DAILY DOSES AND TOTAL CUMULATIVE DOSES FOR MORPHINE SULFATE; STARTING DOSE 10 mg/kg

(Dose Schedule: 2)

DAY	NO. OF DOSES	DAILY DOSES* MORPHINE SO_4 (mg/kg)I.P.	TOTAL CUMULATIVE DOSE (mg/kg)
1	1-3	3 x 10	30
2	4-6	3 x 30	120
3	7-9	3 x 50	270
4	10-12	3 x 70	480
5	13-15	3 x 90	750
6	16-18	3 x 110	1080
7	19-21	3 x 130	1470
8	22-24	3 x 150	1920
9	25-27	3 x 170	2430
10	28-30	3 x 190	3000
11	31-33	3 x 210	3630

* 1) Dose injected every 8 hours
  2) Initial individual dose 10 mg/kg
  3) Individual doses were increased daily by 20 mg/kg

Table 4 DAILY DOSES AND TOTAL CUMULATIVE DOSES FOR MORPHINE SULFATE; STARTING DOSE 1 mg/kg

(Dose Schedule: 3)

DAY	NO. OF DOSES	DAILY DOSES* MORPHINE SO_4 (mg/kg)I.P.	TOTAL CUMULATIVE DOSE (mg/kg)
1	1-3	3 x 1	3
2	4-6	3 x 3	12
3	7-9	3 x 5	27
4	10-12	3 x 7	48
5	13-15	3 x 9	75
6	16-18	3 x 11	108
7	19-21	3 x 13	147

* 1) Dose injected every 8 hours
  2) Initial individual dose 1 mg/kg
  3) Individual doses were increased daily by 2 mg/kg

D. Assessment of Withdrawal Symptoms:

The development of physical dependence was measured according to the method of Buckett (1964). The narcotic antagonists levallorphan or naloxone, 1 mg/kg, were administered four hours after the last dose of morphine in order to precipitate withdrawal symptoms. In some experiments the various physical symptoms listed below (Table 5) were recorded in a double blind manner by a person who did not have any prior knowledge of the experimental treatment. The results obtained from a typical experiment are illustrated in Table 6.

TABLE 5 MORPHINE WITHDRAWAL SYMPTOMS, DEFINITIONS AND SCORES

Symptom	Definition	Score
Writhing	Dragging the abdomen along the floor of the cage with inward rotation of one or both of the hind feet and concurrent drawing in of the abdominal wall.	3
Squealing	Spontaneous or provoked audible response.	2
Diarrhea	Expulsion of soft wet feces which does not possess or retain a pellet shape.	2
Teeth Chatter	Distinctly spontaneous audible response	1
Ptosis	Tightly closed eyelids which remain closed for at least one minute	1
"Wet Dog"	Characteristic shaking of the animal in a manner described by the title	1
	Total Possible Score	10

TABLE 6 SAMPLE DATA SHEET FOR WITHDRAWAL SCORE AFTER TREATMENT WITH SIX DOSES OF MORPHINE (DOSE SCHEDULE: 2)

Date: 03-14-74

Group	Rat No.	Body Wt. (Gms)	Writhing 3	Squealing 2	Diarrhea 2	Teeth Chatter 1	Ptosis 1	"Wet Dog" 1	Total Out of 10	Avg.
Morphine	1	85	-	-	✓	✓	✓	✓	5	5.6
	2	95	-	✓	✓	✓	✓	✓	7	
	3	102	-	-	✓	✓	-	✓	4	
	4	105	-	-	✓	✓	✓	✓	5	
	5	96	-	✓	✓	✓	✓	✓	7	
Normal Saline	1	98	-	-	-	-	-	-	0	0
	2	101	-	-	-	-	-	-	0	
	3	94	-	-	-	-	-	-	0	
	4	89	-	-	-	-	-	-	0	
	5	106	-	-	-	-	-	-	0	

E. Statistical Methods:

Differences between the mean values of drug-treated and control groups were examined for significance using the following formula:

a) Student's"t" test

$$e = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n(n - 1)}}$$

where $\bar{x}$ = mean of all observations, x = individual observations, n = number of observations and e = standard error.

b) Values for "t" were found using

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{e_1^2 + e_2^2}}$$

where $\bar{x}_1$ = the highest mean value and e_1 is the standard error of that mean.

Note: Vertical Lines in figures at different points denote the standard error of mean ($\pm$ SEM).

Chapter 3

RESULTS

(1) Time-Course Effect of Morphine Withdrawal

Object and Rationale:

The study was made in order to evaluate the dose-effect relationship between the dose of morphine and the recorded score. This experiment was not carried out by Buckett (1964) and thus, no information with regard to this important consideration has been reported.

Morphine withdrawal was made with levallorphan as described previously, using the three different dose schedules of morphine.

The results of these experiments are shown in Tables 7, 8, and 9. Tables 7 and 8 show that no dose-related effect exists beyond the 6th dose of morphine, irrespective of whether a starting dose of 20 or 10 mg/kg morphine was used. However, in both instances, a clear dose-related effect is evident over the first two days of morphine treatment (i.e. up to the 6th dose). From Table 9 it can be seen that dose schedule 3 (1 mg/kg starting dose of morphine) did produce dose-related effects over a period of seven days of treatment (1-21 doses). All of the above data are represented graphically in Figure 1. Figure 1 also shows that levallorphan or saline, when given alone, is without effect. Thus, the withdrawal scores are solely due to morphine administration and the attainment of the maximum score of 10 is not even approached by any of the dose schedules of morphine used.

Comparisons of the cumulative dose versus the recorded score are given in Figures 2 and 3. It may be seen again that a deviation from

a direct relationship between these two variables is present, especially with dose schedules 1 and 2 (Figure 2).

TABLE 7

THE TIME COURSE EFFECT OF MORPHINE WITHDRAWAL IN RATS TREATED WITH AN INITIAL DOSE OF MORPHINE 20 mg/kg (DOSE SCHEDULE 1, METHODS AND MATERIALS)

DAY	TOTAL NO. OF DOSES	TOTAL CUMULATIVE DOSE (mg/kg)	MEAN WITHDRAWAL* SCORE ± S.E.	
			MORPHINE TREATED	SALINE CONTROL
1	3	60	3.4 ± 0.4 (5)	0.2±0.14 (5)
2	6	240	5.5 ± 0.6 (6)	0.0 (5)
11	33	7260	5.8 ± 0.7 (6)	0.0 (8)

* (1) Number in brackets indicate number of animals used in each experiment

(2) Significance of Differences:
(i) All morphine treated vs. Saline Control ($P<0.001$)
(ii) Morphine treated, Dose number 3 vs 6 ($P<0.01$) and 6 vs 33 (N.S.)

TABLE 8

THE TIME COURSE EFFECT OF MORPHINE WITHDRAWAL IN RATS TREATED WITH AN INITIAL DOSE OF MORPHINE, 10 mg/kg, I.P. (DOSE SCHEDULE 2, METHODS AND MATERIALS).

DAY	TOTAL NO. OF DOSES	TOTAL CUMULATIVE DOSE (mg/kg)	MEAN WITHDRAWAL* SCORE ± S.E.M. MORPHINE TREATED	SALINE CONTROL
	1	10	1.28 ± 0.42 (5)	0 (5)
1	2	20	2.14 ± 0.45 (5)	-
	3	30	3.00 ± 0.44 (5)	0.20±0.14 (5)
	4	60	3.83 ± 0.47 (5)	-
2	5	90	4.80 ± 0.80 (5)	0 (5)
	6	120	5.60 ± 0.34 (5)	-
5	15	750	5.75 ± 0.75 (5)	0 (4)
8	24	1920	5.75 ± 0.62 (5)	-
11	33	3630	5.8 ± 0.58 (5)	0 (8)

(1) Numbers in Brackets indicate number of animals used in each experiment

(2) Significance of differences:
(i) All morphine treated are significantly from saline control ($P<0.001$)
(ii) Morphine treated dose number 1 vs 3 ($P<0.01$)
dose number 3 vs 6 ($P<0.01$)
dose number 6 vs 33 (NS.).

TABLE 9

THE TIME COURSE EFFECT OF MORPHINE WITHDRAWAL IN RATS TREATED WITH AN INITIAL DOSE OF MORPHINE 1 mg/kg, I.P. (DOSE SCHEDULE 3, METHODS AND MATERIALS)

DAY	TOTAL NO. OF DOSES	TOTAL CUMULATIVE DOSE (mg/kg)	MEAN WITHDRAWAL* SCORE ± S.E.	
			MORPHINE TREATED	SALINE CONTROL
1	3	3	0.16 ± 0.16 (6)	0.2±0.14 (5)
2	6	9	1.33 ± 0.33 (6)	0 (5)
5	15	75	3.40 ± 0.50 (6)	0 (5)
7	21	147	4.20 ± 0.20 (5)	

(1) Numbers in brackets indicate number of animals used in the experiment

(2) Significance of Differences:

(i) All morphine treated (Except Day 1) are significantly different from saline control ($P<0.001$)

(ii) Morphine treated:
Dose number 3 vs 6 ($P<0.02$)
15 vs 6 ($P<0.02$)
21 vs 6 ($P<0.001$)

Figure 1

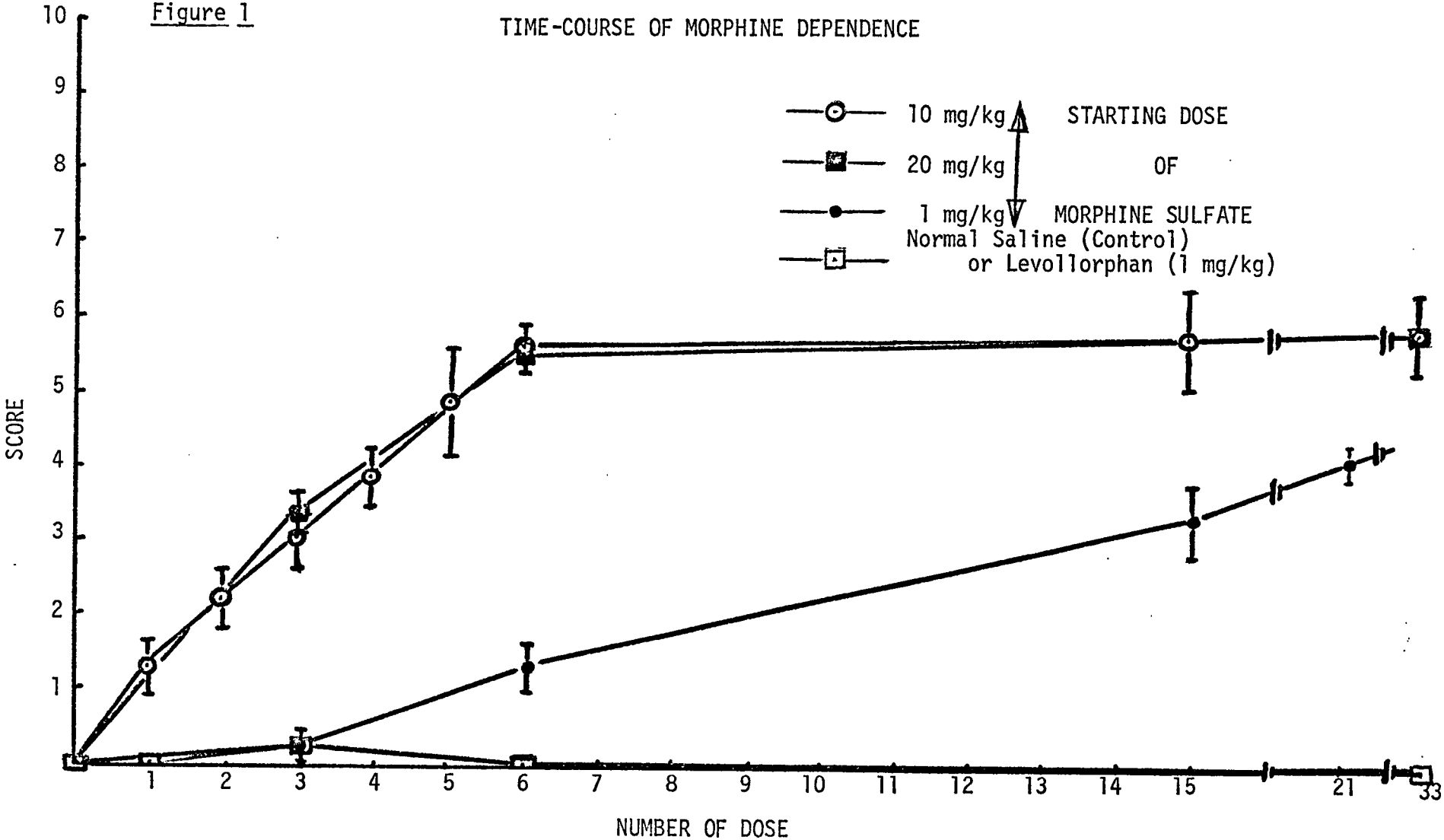

TIME-COURSE OF MORPHINE DEPENDENCE
10 mg/kg
20 mg/kg
1 mg/kg
STARTING DOSE
OF
MORPHINE SULFATE
Normal Saline (Control)
or Levollorphan (1 mg/kg)
SCORE
NUMBER OF DOSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
21
33

Figure 2

CUMULATIVE DOSE - EFFECT PLOT FOR MORPHINE SULFATE

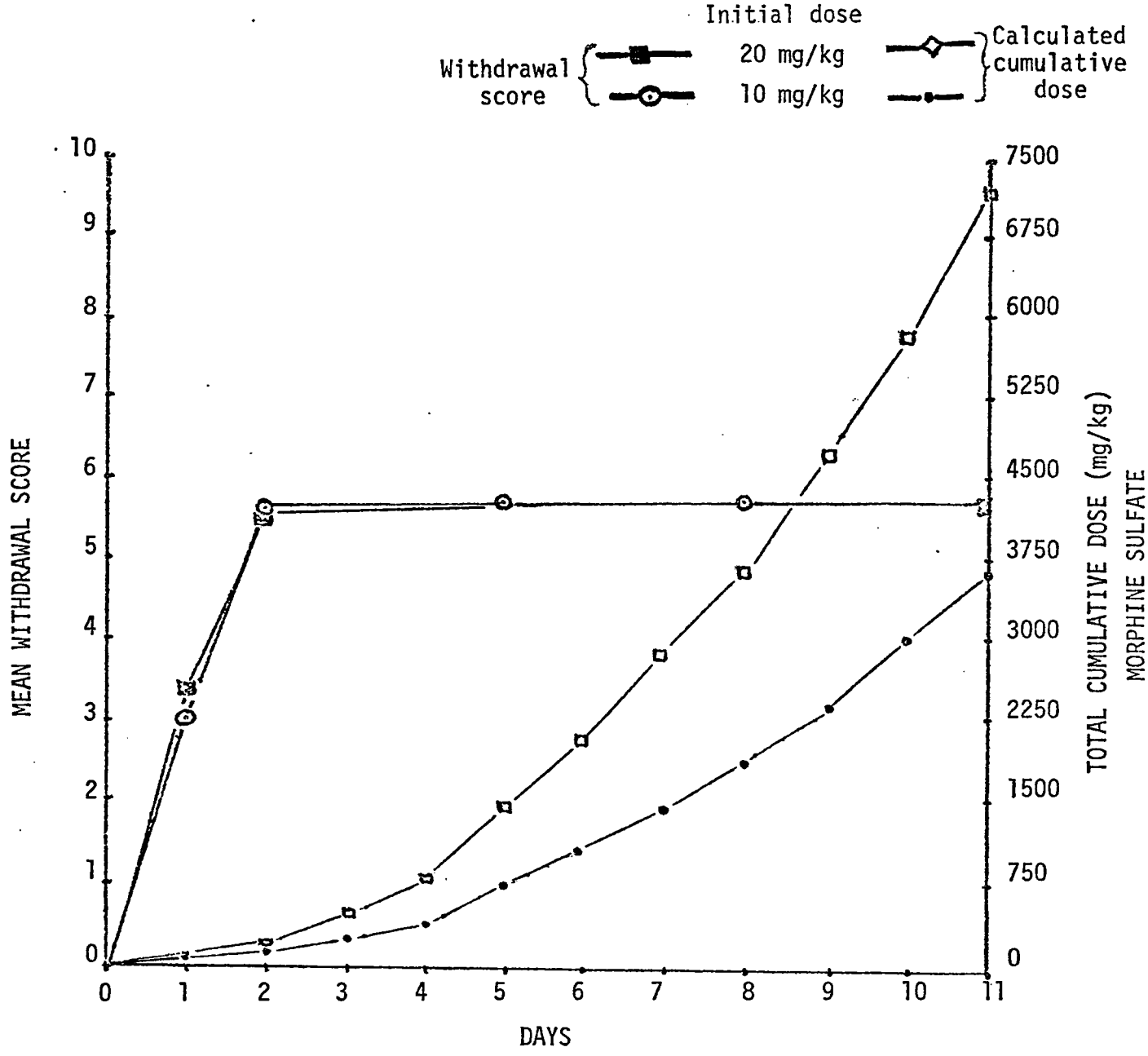
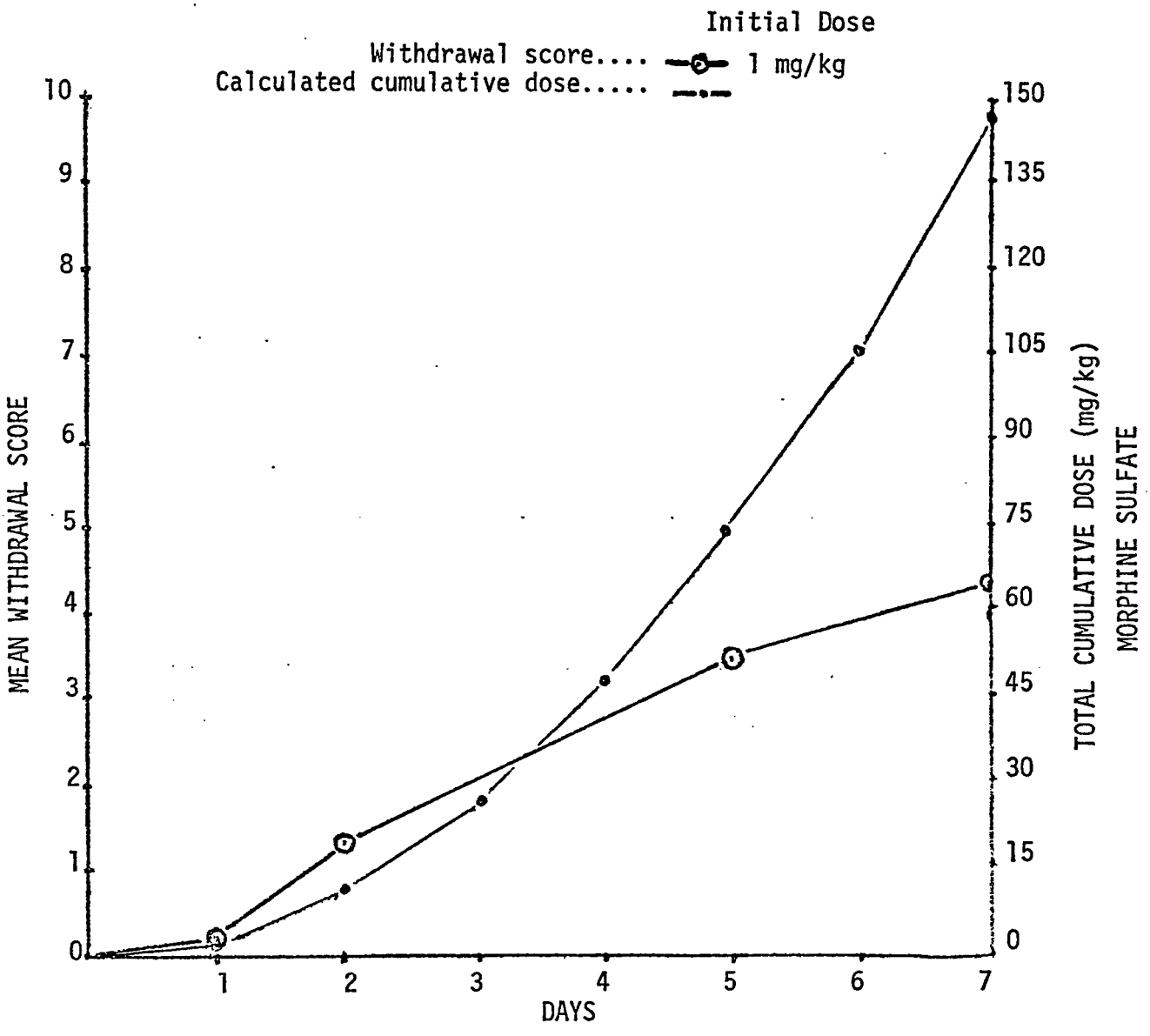


Figure 3

CUMULATIVE DOSE EFFECT PLOT FOR MORPHINE SULFATE



(2) Effect of Dextro and Levoamphetamine on Morphine Withdrawal Symptoms

Object and Rationale:

Both morphine and methadone have been implicated with certain actions on central monaminergic pathways (see Introduction). Dextroamphetamine is known to release central catecholamines, particularly dopamine, and to possess direct dopaminergic receptor agonist properties. Thus, an investigation concerning the interaction between morphine and dextro-amphetamine might well aid in the understanding of the mechanisms involved in morphine-induced physical dependence. In an attempt to activate central catecholamine pathways continuously, the dextroamphetamine was administered concomitantly with every dose of morphine. In another experiment, levo-amphetamine was employed in exactly the same manner. This compound is generally considered to be less effective centrally than the dextro-isomer, while retaining marked peripheral sympathomimetic properties.

Rats were treated with morphine (dose schedules 2 and 3, Materials and Methods) in conjunction with dextroamphetamine (0.5 mg/kg I.P.) or levoamphetamine (0.5 mg/kg I.P.).

Another experiment was performed in which the amines were withheld during the development of morphine dependence (dose schedule 2, Materials and Methods), and were administered only once, along with the final dose of morphine, in order to test whether the repetitive

administration of dextroamphetamine was an important factor.

Pilot experiments were made prior to the above work so as to determine a suitable dose of dextroamphetamine. (Appendix 2)

Figure 4 shows that dextroamphetamine administration significantly depressed the withdrawal score at and after the 6th dose of morphine, compared with that obtained with either morphine alone or morphine in combination with levoamphetamine. These latter two conditions were not significantly different at any dose. It is pertinent to note that dextroamphetamine appears to have moved the morphine curve in a manner resembling that of a "non-competitive" antagonist, since the maximal response to morphine was reduced.

Similar results were obtained using a lower initial dose level of morphine (1 mg/kg) (Figure 5). Levoamphetamine failed to alter the withdrawal score but dextroamphetamine again showed evidence of a "non-competitive type" antagonism. However, in this experiment, using the lower dose level of morphine the maximal withdrawal score to morphine alone was not attained.

Figure 6 presents data obtained in some of the above experiments and in addition that obtained using only a single administration of dextro or levoamphetamine. All columns refer to the withdrawal score obtained after the administration of the 6th dose of morphine. The single administration of either dextro or levoamphetamine failed to alter significantly the score obtained with morphine alone. Column B (dextroamphetamine plus morphine, continuous administration) is significantly different ($P < 0.05$) from all other columns.

Figure 4

EFFECT OF AMPHETAMINES ON MORPHINE WITHDRAWAL

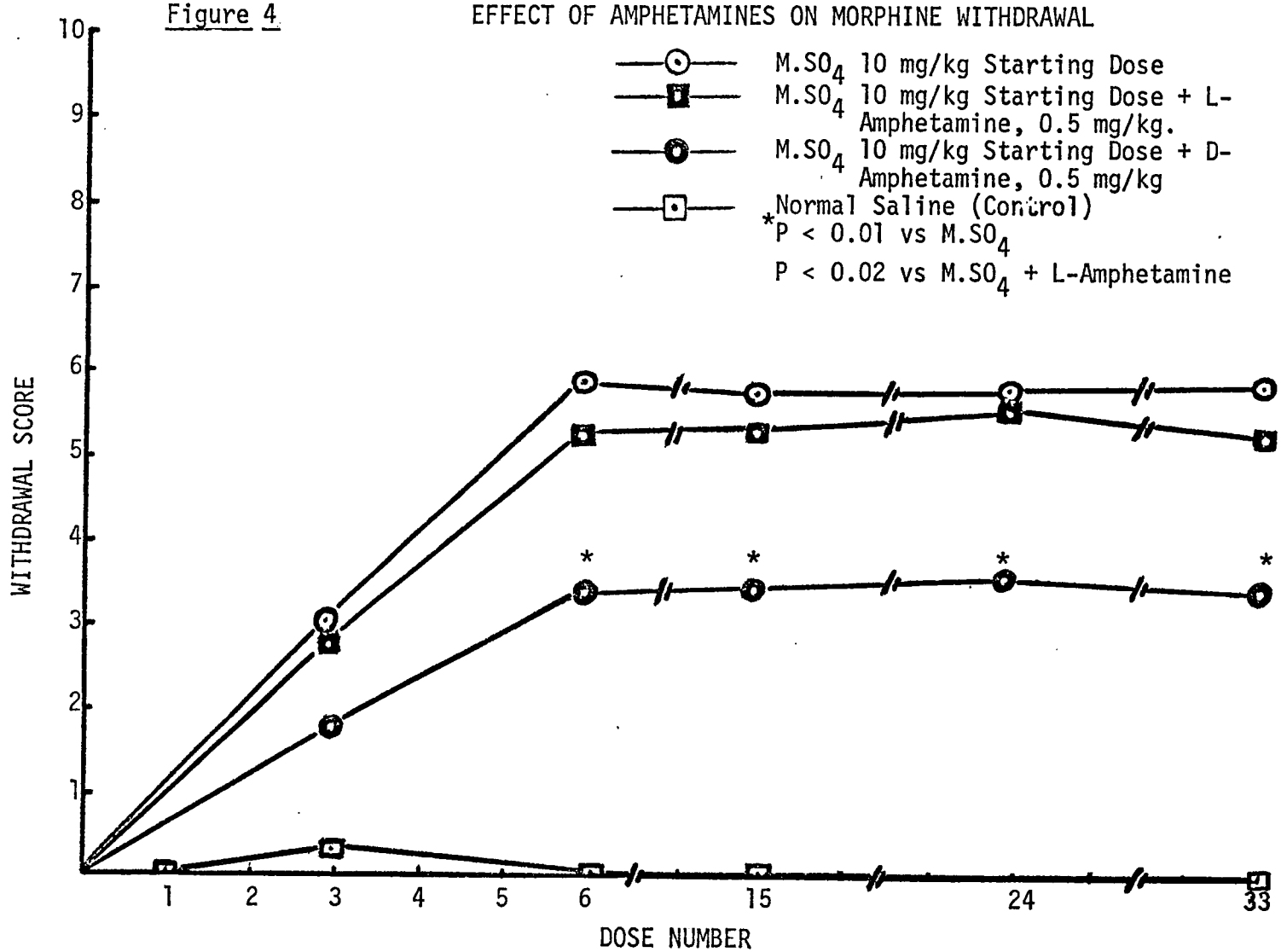


Figure 5

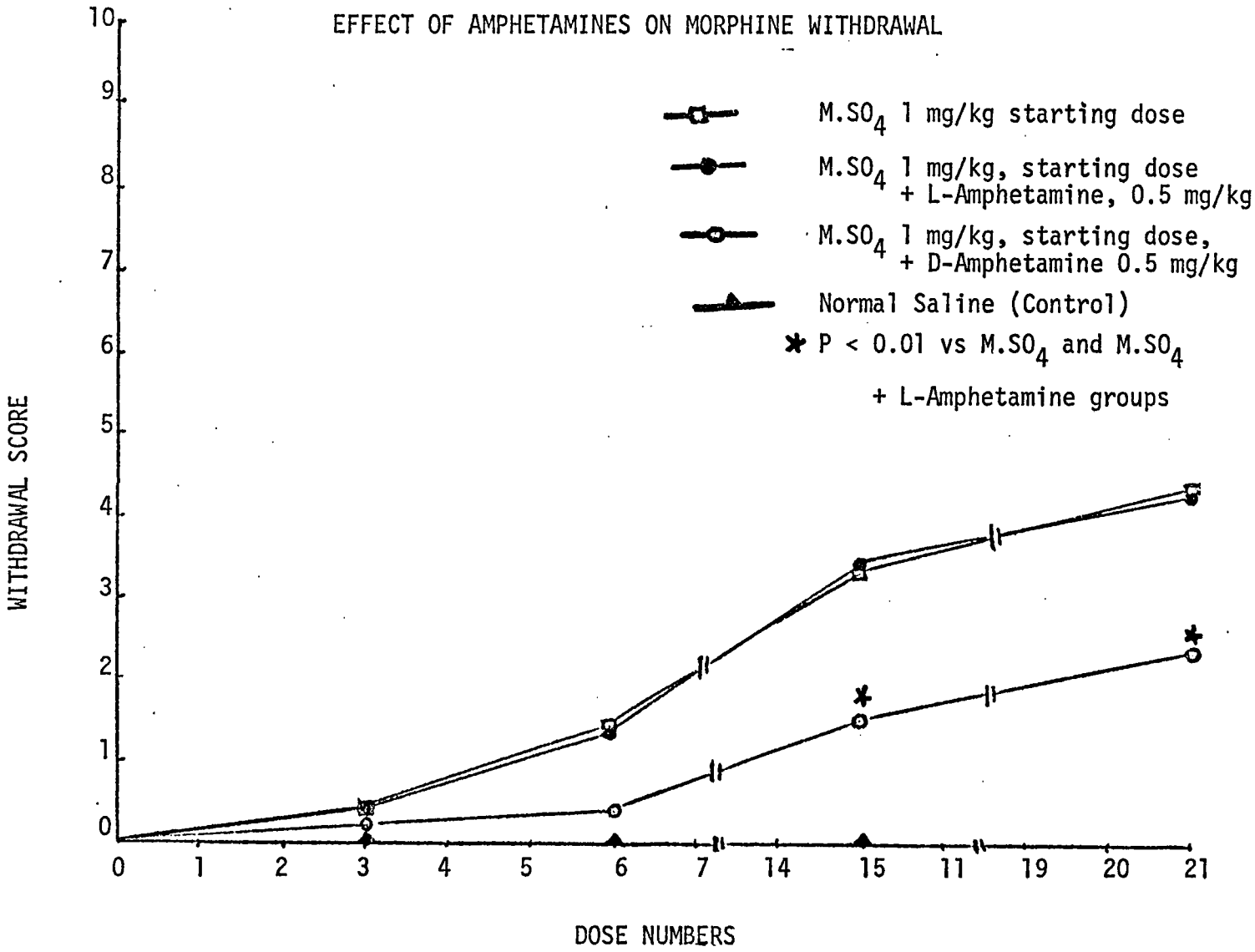
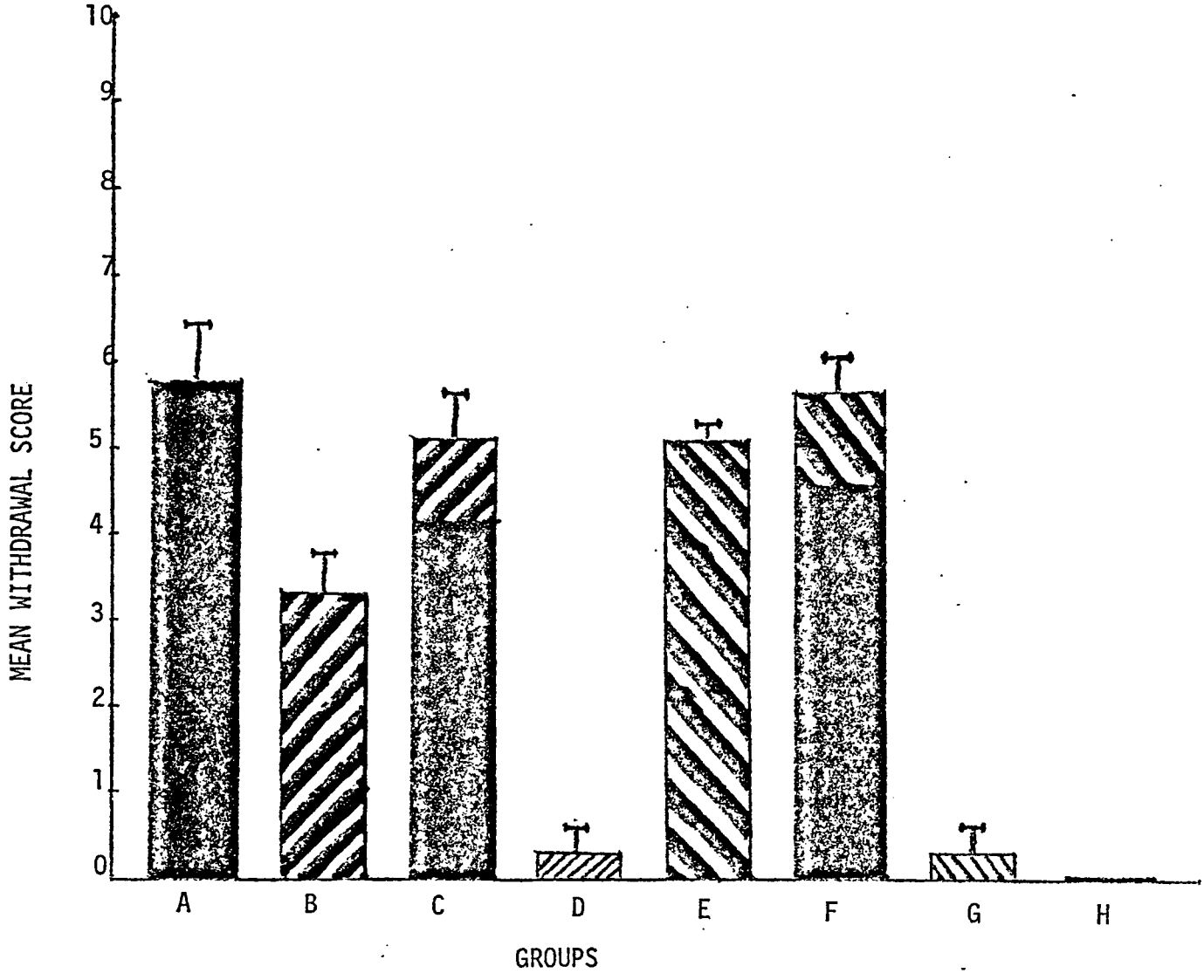

EFFECT OF AMPHETAMINES ON MORPHINE WITHDRAWAL
M.SO4 1 mg/kg starting dose
M.SO4 1 mg/kg, starting dose + L-Amphetamine, 0.5 mg/kg
M.SO4 1 mg/kg, starting dose, + D-Amphetamine 0.5 mg/kg
Normal Saline (Control)
* P < 0.01 vs M.SO4 and M.SO4 + L-Amphetamine groups
WITHDRAWAL SCORE
0
1
2
3
4
5
6
7
8
9
10
0
1
2
3
4
5
6
7
14
15
11
19
20
21
DOSE NUMBERS

Figure 6

THE EFFECTS OF AMPHETAMINES ON MORPHINE PHYSICAL DEPENDENCE, AFTER 6 DOSES



A: Morphine Sulfate, starting dose 10 mg/kg (dose schedule: 2)
B: A + Dextroamphetamine sulfate 0.5 mg/kg
C: A + Dextroamphetamine sulfate 0.5 mg/kg, one dose
D: Dextroamphetamine sulfate 0.5 mg/kg
E: A + Levoamphetamine 0.5 mg/kg
F: A + Levoamphetamine, 0.5 mg/kg, one dose
G: Levoamphetamine, 0.5 mg/kg
H: Control, normal saline

(3) A Comparison of the Nature of the Antagonism by Naloxone with that of Dextroamphetamine on the Morphine Withdrawal Score.

Object and Rationale:

Since dextroamphetamine appeared to inhibit the morphine withdrawal score in a non-competitive fashion (Experiment 2) a comparison with the effects of naloxone, a known competitive antagonist of morphine, was undertaken.

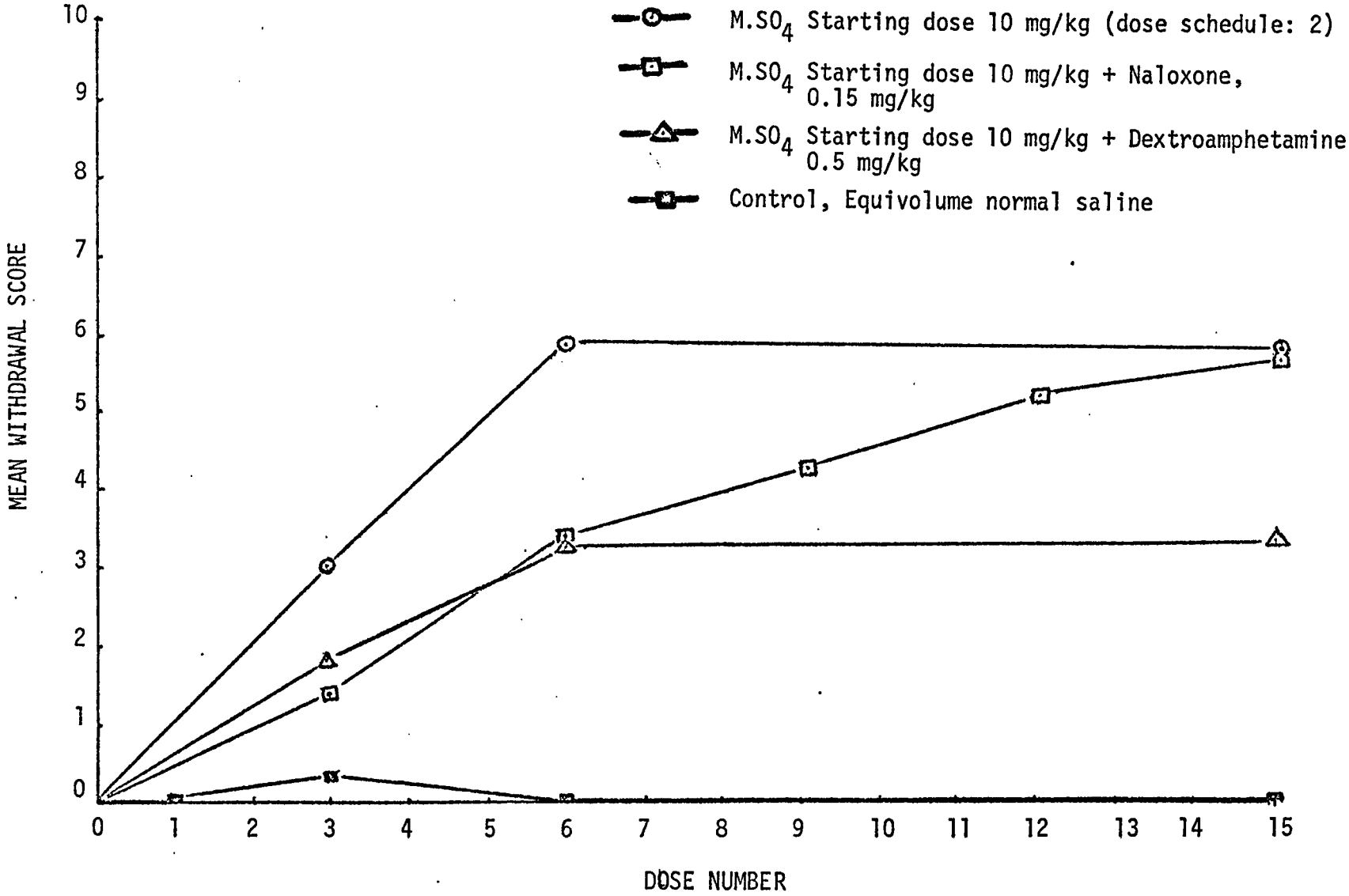
Groups of rats (5-9/group) were treated with morphine (initial dose, 10 mg/kg) alone, or in conjunction with either naloxone (0.15 mg/kg) or dextroamphetamine (0.5 mg/kg) for five days (15 doses).

The combination treatment of naloxone and morphine resulted in a "competitive-type" shift in the curve relating withdrawal score to dose number (Figure 7). The curve shown for dextroamphetamine was obtained from the previous experiment (Experiment 2) and is represented for purposes of comparison.

An important point to observe is that there is no significant difference between morphine alone and morphine plus naloxone after dose number 15. ($P < 0.05$).

Figure 7

ANTAGONISM OF DEXTROAMPHETAMINE ON THE MORPHINE WITHDRAWAL SCORE



(4) The Effects of Haloperidol and Apomorphine on the Development of the Morphine Withdrawal Syndrome.

Object and Rationale:

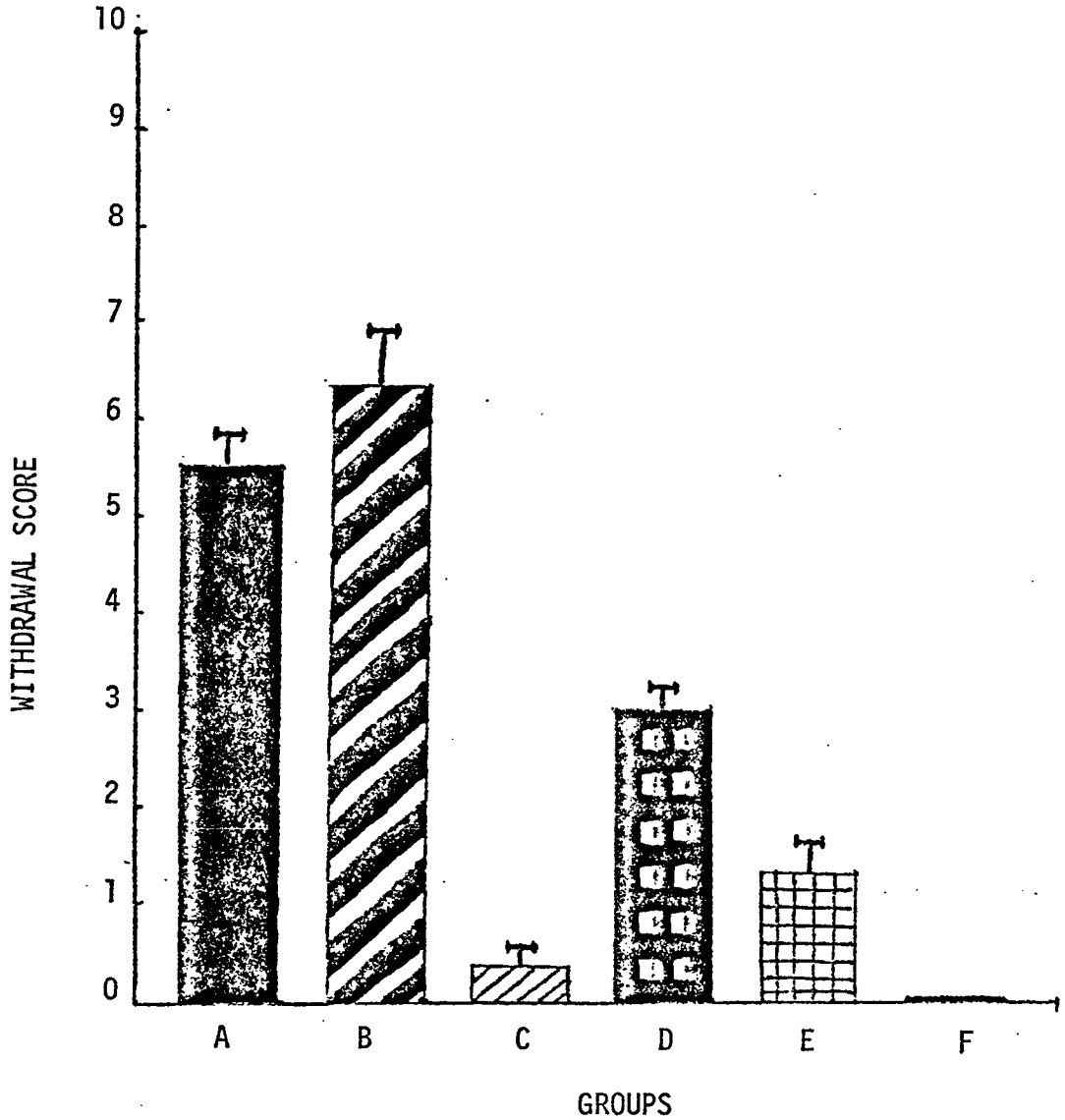
The dopaminergic involvement in morphine induced physical dependence has been indicated directly and indirectly by several workers. The present study on the effects of dextroamphetamine on the withdrawal score also suggests the possibility of the involvement of dopaminergic mechanisms. Therefore, experiments were designed to further investigate the role of dopamine in morphine dependent rats by using a direct dopamine-receptor agonist and antagonist.

Haloperidol (0.1 mg/kg I.P.) and apomorphine (1.5 mg/kg I.P.) were administered along with morphine (starting dose 10 mg/kg - dose schedule 2, Materials and Methods), in two separate groups of rats. Other groups of rats were treated with morphine, haloperidol, apomorphine and normal saline alone and served as the control groups. The withdrawal syndrome was precipitated with levallorphan (1 mg/kg) following the 6th dose of morphine.

Figure 8 clearly shows that apomorphine (Column D) significantly ($P < 0.01$) reduced the withdrawal score of morphine (Column A) whereas haloperidol exhibited a slight increase in the morphine withdrawal score (Column B). However, this increase was not significantly different from morphine alone. Haloperidol itself (Column C) and normal saline (Column F) failed to elicit any marked effect but apomorphine (Column E) gave rise to a small but clear cut withdrawal score.

Figure 8

THE EFFECTS OF HALOPERIDOL AND APOMORPHINE ON THE DEVELOPMENT OF MORPHINE PHYSICAL DEPENDENCE, AFTER 6 DOSES



A: Morphine sulfate, starting dose 10 mg/kg (dose schedule: 2)
B: A + Haloperidol 0.1 mg/kg
C: Haloperidol, 0.1 mg/kg
D: A + Apomorphine, 1.5 mg/kg
E: Apomorphine, 1.5 mg/kg
F: Control, Normal saline

(5) Effects of Atropine and Atropine Methyl Nitrate on the Morphine Withdrawal Syndrome.

Object and Rationale:

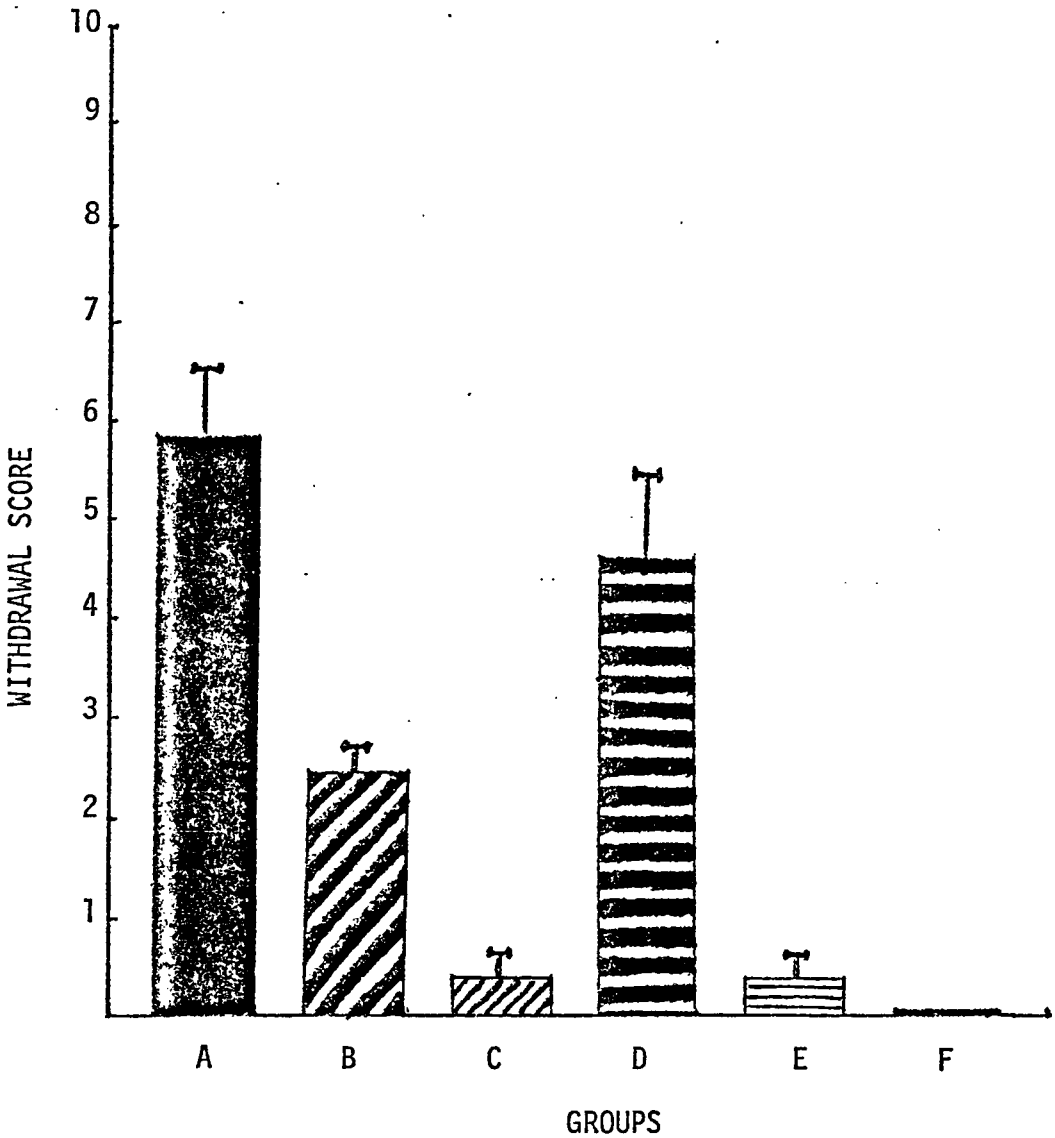
The effects of cholinergic drugs on the development of morphine-induced physical dependence and precipitated withdrawal syndrome have been studied by many workers and implicates the involvement of cholinergic mechanisms. The following study was undertaken therefore to examine the contribution of cholinergic participation under the present experimental conditions.

A group of rats received atropine sulfate (2 mg/kg) concomitantly with morphine (Starting dose 10 mg/kg, dose schedule 2, Materials and Methods). Similarly a group of rats received the centrally inactive muscarinic blocking agent, atropine methyl nitrate (2 mg/kg) along with morphine. Four other groups of rats served as controls and received morphine, atropine, atropine methyl nitrate, or normal saline. The withdrawal syndrome was precipitated with levallorphan (1 mg/kg) after the 6th dose.

As seen in Figure 9, atropine sulfate (Column B) significantly ($P < 0.01$) reduced the withdrawal score whereas atropine methyl nitrate (Column D) failed to produce a significant inhibition. Both drugs failed to induce any marked withdrawal score when administered without morphine (Columns C and E).

Figure 9

THE EFFECTS OF ATROPINE AND ATROPINE METHYL NITRATE ON THE DEVELOPMENT OF MORPHINE PHYSICAL DEPENDENCE, AFTER 6 DOSES.



A: Morphine Sulfate, starting dose 10 mg/kg (dose schedule: 2)
B: A + Atropine Sulfate, 2 mg/kg
C: Atropine Sulfate, 2 mg/kg
D: A + Atropine methyl nitrate, 2 mg/kg
E: Atropine methyl nitrate, 2 mg/kg
F: Control, Normal saline

(6) Frequency-Distribution Study of Withdrawal Symptoms

Object and Rationale:

The previous study on the time-course of morphine withdrawal clearly showed that with the 10 mg/kg starting dose (dose schedule 2, materials and methods) the withdrawal score increased up to 6th dose and then remained unchanged up to, and including, the 33rd dose. The following analysis deals primarily with each individual set of symptoms that comprise the total withdrawal score. The principal objectives of the frequency-distribution analysis are listed below:

1) To examine the relative contribution of each individual symptom over the first dix doses of morphine to the total withdrawal score.

2) To determine whether the maximal withdrawal score from morphine-dependent rats represented a constant occurrence of the same symptoms.

3) To analyze the frequency of occurrence of the scored symptoms for each individual score group. Logically, the order of occurrence for each score group should be, score 1 > score 2 > score 3.

4) To determine how the amphetamines and other drugs used affected the frequency-distribution of the symptoms.

The raw score data from all experiments was computed as a percentage of the maximum possible score (maximum = 100%) and is represented graphically. By this device, the diagrams provide a visual as well as calculated representation of the symptom distribution frequency at the various dosage points illustrated.

Figure 10 (a) represents the frequency of distribution of symptoms found over the first six doses of morphine (10 mg/kg, starting dose). The occurrence of measured symptoms increased progressively with the number of doses. For instance, the primary symptom after the first dose of morphine is wet-dog (60% of maximum), whereas after the 6th dose, wet dog, ptosis, teeth chatter, and diarrhea are all 100%. In addition, there is about a 20% incidence of squealing and a 40% incidence of writhing.

The total score group analysis for Part A of Figure 10 is represented in Part B. The frequency with which the symptoms are distributed approximates to the weighted score system. Thus, there is a 70% occurrence of score 1 symptoms, a 33% occurrence of score 2 symptoms and about a 6% occurrence of the 3 point symptom.

Figure 11 analyzes the maximal response obtained in morphine-dependent rats (10 mg/kg, starting dose). Since this maximal response remained constant from dose 6 to dose 33 (2 to 11 days), the symptoms measured from doses 6 and 15 have been pooled and compared with the combined data derived from doses 24 and 33. By this means, Figure 11 compares the first half of maximal response (days 2 to 5) with the second half (days 8 to 11). The results suggest no great overall difference between these two pooled computations. The decrease in ptosis (more marked) and teeth chatter (less marked) in the 24 and 33 dose group is somewhat compensated for by the slight increase in squealing and writhing.

Figure 10

FREQUENCY - DISTRIBUTION OF INDIVIDUAL (A) AND GROUPED (B) WITHDRAWAL SYMPTOMS WITH MORPHINE SULFATE, STARTING DOSE 10 mg/kg

In A, each square represents the maximum possible response (100%) and the filled in portion shows the percentage response obtained.

B shows the percentage distribution for each score group as indicated by the brackets.

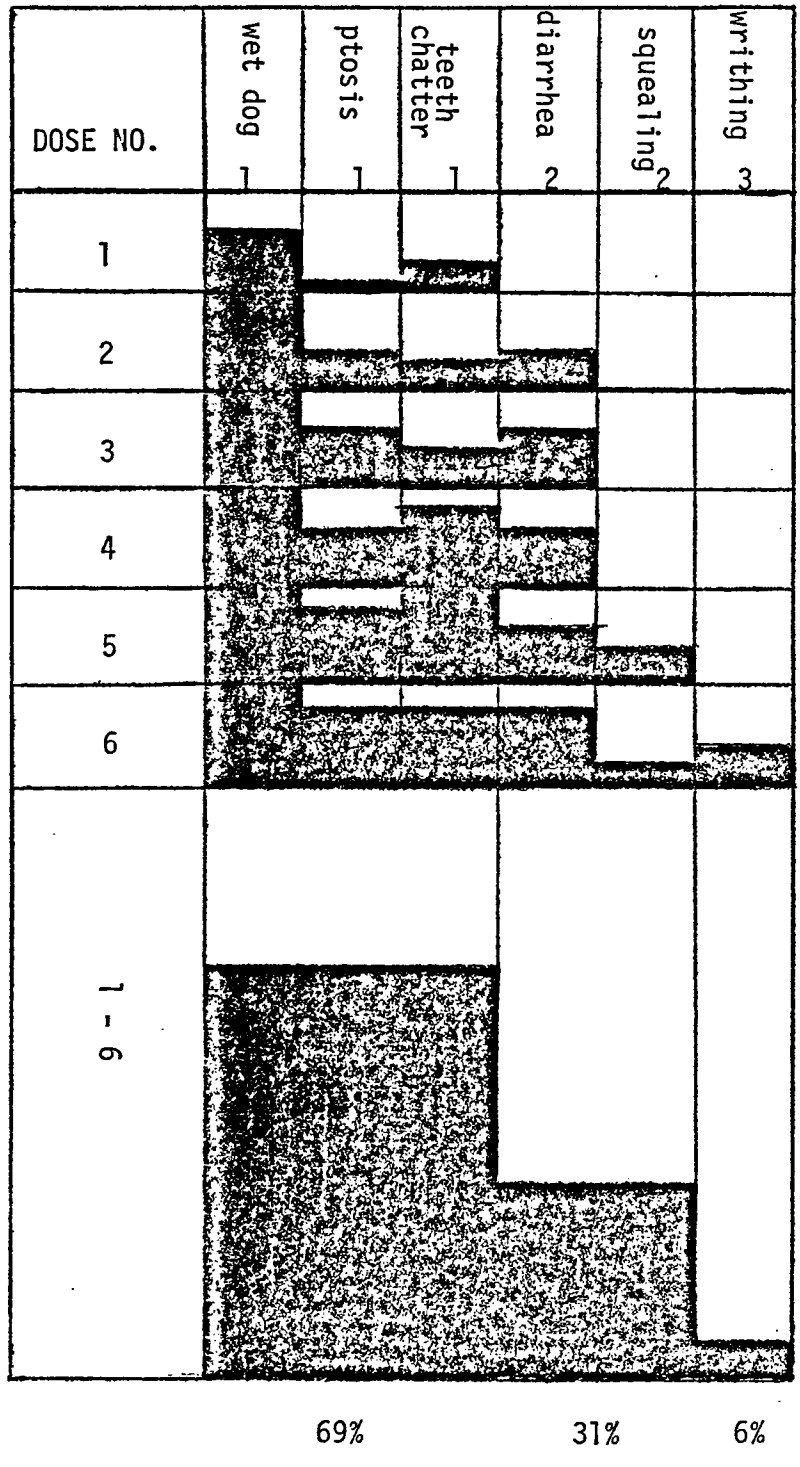
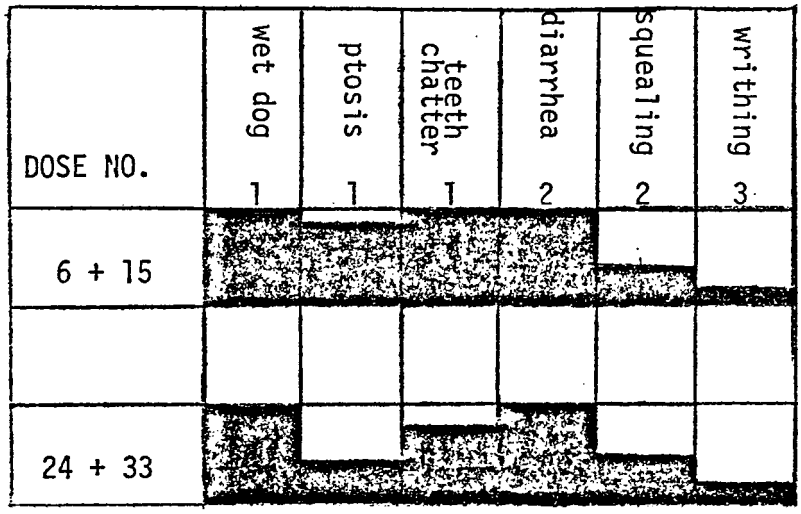


Figure 11

FREQUENCY - DISTRIBUTION OF THE WITHDRAWAL SYMPTOMS FROM DOSE 6 TO 33 USING MORPHINE SULFATE, 10 mg/kg*



* Initial dose, refers to Dose Schedule: 2

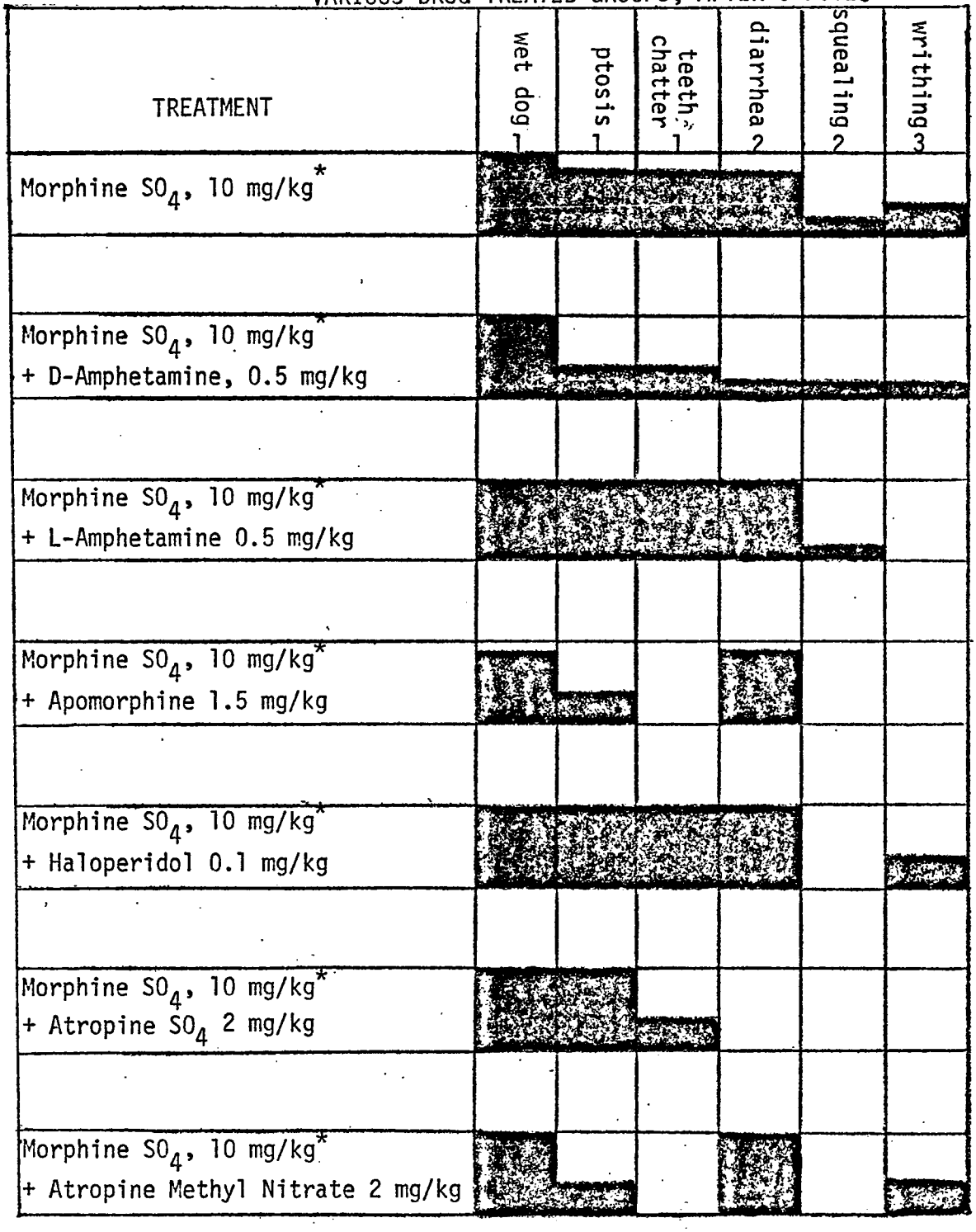
Figure 12 illustrates the effects of combined treatment with morphine plus dextroamphetamine and morphine plus levoamphetamine with that of morphine alone. The data refer to symptoms scored after six injections. Dextroamphetamine decreased all symptoms except wet dog and squealing, whereas levoamphetamine decreased only writhing which, because of its relatively low occurrence in the control morphine group, failed to significantly decrease the total score from that of morphine alone.

Figure 12 also shows the effects of a dopaminergic receptor agonist and antagonist on morphine withdrawal after 6 administrations, Both apomorphine and haloperidol were administered along with the morphine. Apomorphine reduced all symptoms except diarrhea in which there was a slight increase; teeth chatter, squealing and writhing were inhibited completely. On the other hand, haloperidol slightly increased ptosis, teeth chatter and diarrhea and showed no effect on writhing; whereas, squealing was abolished.

The effects of concomitant administration of atropine with morphine and that of atropine methyl nitrate with morphine on morphine withdrawal after 6 doses are illustrated in Figure 12. Atropine slightly increased ptosis, decreased teeth chatter and abolished diarrhea, squealing, and writhing. Atropine methyl nitrate decreased ptosis, abolished teeth chatter and squealing, and showed no effects on either wet dog or writhing. The incidence of diarrhea was increased and atropine methyl nitrate failed to alter the symptom of wet dog.

Figure 12

FREQUENCY- DISTRIBUTION OF THE WITHDRAWAL SYMPTOMS IN VARIOUS DRUG TREATED GROUPS, AFTER 6 DOSES



* Refers to dose schedule: 2

Chapter 4

DISCUSSION

The present study attempted to assess the degree of morphine-induced physical dependence by recording certain specific symptoms which occur upon withdrawal of the drug. Many similar methods have been utilized to analyze and quantify narcotic physical dependence (Himmelsbach et al., 1935; Hosoya, 1959; Henna, 1960; Martin et al., 1963; Buckett, 1964; Akere and Brody, 1968; Wei, 1973), however, all of the above methods suffer from being subjective and deal with complex responses which are difficult to interpret from a physiological and pharmacological standpoint. Never-the-less, these responses can be modified by drugs, and thereby produce a broad clue as to the possible influence of morphine upon certain physiological systems.

Studies on the Method Used:

The following discussion will deal with the use, validity and application of the presently used method for assessing morphine dependence. The procedure was based upon that originally reported by Buckett (1964), but certain modifications were made. First, the morphine was administered three times a day (every eight hours) instead of twice daily (every twelve hours). This change in methodology was made to avoid the possibility of spontaneous withdrawal in animals due to infrequent morphine injections, since withdrawal symptoms have been reported to occur within 8 to 12 hours following injections of morphine (Grumbach, 1974). Secondly, other dose schedules of morphine were employed (10 and 1 mg/kg starting dose). These dose schedules were not examined by Buckett (1964).

Using the 20 mg/kg starting dose of morphine (dose schedule 1) the withdrawal score obtained after eleven days of treatment was very comparable to that reported by Buckett (1964). He obtained a mean value ± SEM of 5.8 ± 1.3, and 5.8 ± 0.7 was obtained in the present study. It seems, therefore, that the modification of the number of daily injections fails to influence the score at this time period. However, it remains unknown as to whether the two scores relate to the occurrence of indentical symptoms. Also, the present study showed that virtually the same score (5.6 ± 0.6) is obtained after eleven days of treatment using a lower initial starting dose of morphine (dose schedule, 2). Thus, after eleven days of treatment no dose effect relationship was evident between these two dose schedules. This finding raised questions about the validity of the method.

Clearly, the method would be meaningless if no dose-related effect could be obtained. With regard to this point, Buckett (1964) had not investigated this aspect over the first eleven days, although he did show that greater withdrawal scores could be obtained at later time points (day 17, 8.4 ± 0.6; day 29, 8.8 ± 0.8).

The time-course studies using three different initial doses of morphine showed that there was no difference between the withdrawal scores using 20 and 10 mg/kg starting doses. However, the 1 mg/kg dose was clearly less effective and exhibited a good dose effect relationship.

It is clear from figure 1 and Tables 7 and 8 that the withdrawal score gradually increased up to the sixth dose with the administration of 20 and 10 mg/kg starting doses of morphine, but remained constant from dose 6 to 33. These data show that there is a relationship

between dose and effect up to the sixth dose but beyond this point a maximal score pertained. If Buckett's method computes true physical dependence, then it can be interpreted from the present results that there is no difference in the degree of physical dependence between two and eleven days of morphine treatment. Furthermore, it was extremely surprising to find that the development of maximum physical dependence occurred rapidly, within the first two days. Figure 1 and 2 also show that there is no significant difference in the scores of morphine withdrawal between the two dose schedules, indicating that the administration of morphine in an initial dose greater than 10 mg/kg would not increase the score value.

On the other hand, lower doses of morphine (1 mg/kg, initial dose) appeared to show a distinct dose-effect relationship as seen in Figure 1 and 3 and Table 9. However, the physical dependence was slower to develop in this case than was found under the other experimental conditions. These findings may have some relevance to the development of physical dependence in man. In the human, mild symptoms of spontaneous withdrawal are reported upon the termination of morphine after 2-3 weeks of continuous administration. However, if a narcotic antagonist is used to induce withdrawal, immediate withdrawal symptoms can be seen after only two or three days of continuous treatment (Wikler et al., 1953). Likewise Martin and Eades (1961) showed that they could precipitate withdrawal symptoms in the dog after an 8-hour infusion of morphine (acute tolerance). Therefore, the present finding that physical dependence developed rapidly in rats with higher initial doses of morphine

resemble the above reports.

As discussed above the withdrawal score remained constant from dose 6 to dose 33 (10 and 20 mg/kg, initial dose). There are two possible explanations for this result. First, the result represents a true and constant maximal response for each of the six parameters measured. Secondly, an alternative explaination is that some symptoms increased in occurrence while others decreased compensatorily. If this latter possibility is true, using six different parameters to assess the withdrawal score, one can have ten possible combinations to obtain a score of six. In view of this, it was necessary to analyze the occurrence of individual symptoms at each of the dose levels. A frequency-distribution analysis was made to examine the validity of the time-course of morphine withdrawal and the scoring system suggested by Buckett (1964). The frequency-distribution diagram was constructed in a way that all the symptoms could be visualized individually according to their percentile occurrence. These calculations show a reasonably good correlation to the weighted point system described by Buckett (1964). Thus, the most frequently occurring symptoms (69%) were those assigned the score of 1; the least frequent (6%) were those assigned the score of 3 and the two point symptoms occurred between these two extremes (31%). Therefore, the scoring system appears to carry a fair degree of validity, especially with regard to the 1 and 2 point symptoms. However, it is obvious that alternative scoring methods could be devised which perhaps better fit the obtained frequency-distribution data.

A comparison of the pooled scores observed at dose 6 plus 15 with those obtained at dose 24 plus 33 showed no marked alterations in the

frequency of occurrence of the six measured responses. Thus, the maximal score (flat part of the curve, Figure 1) appears to represent the constant occurrence of the same symptoms, rather than an increase in the incidence of some with a compensatory decrease in others. This consistency of effect serves to further validify the method as tested in the present study. More importantly, however, it also strongly suggests that tolerance is not a complicating factor under the present experimental conditions. Apparently, by utilizing an increasing daily dose of morphine, tolerance is suppressed or virtually eliminated. The above observations would seem to permit direct comparisions between the acute and chronic effects of drugs between the sixth and thirty-third dose. However, since the score was found to be maximal for dose schedules 1 and 2, only drugs with antagonistic effects toward morphine can be studied under these treatments. Furthermore, because of the semi-quantitative nature of assigning the presence or absence of symptoms, only gross changes can be recorded with confidence. As mentioned earlier, this latter point is an inherent disadvantage of the entire procedure.

The fact that the maximum possible score of 10 was not achieved might be used as an argument against the scoring system. Taking the present experiments in isolation, this conclusion would certainly be justified. However, according to Buckett (1964), further treatment with morphine will result in higher withdrawal scores. The significance of a seemingly step-wise relationship between withdrawal score and dose number is impossible to interpret but such relationships often reflect new or additional mechanisms and/or sites of action.

Effect of Drugs: Part I.

The following discussion will deal with the drug effects observed on the total withdrawal score. This approach has been taken since this is presumably how the method was intended for use. Part II of the discussion will re-evaluate the data and conclusions in the light of individual symptom analysis.

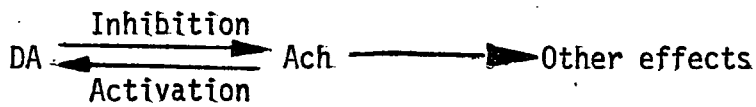
Dextroamphetamine consistently decreased the withdrawal score whereas levoamphetamine failed to do so. The reason for this differential effect might be related to the less marked central action of the levo-isomer on dopaminergic neurons (Cooper et al., 1974). Both isomers are capable of releasing brain norepinephrine (Taylor, K. M. and Snyder, S. H; Sluser, F. and Sanders Bush, E; 1971) but it is also known that the dextro-isomer is a direct dopamine receptor agonist (Coyle, J. T., and Snyder, S. H.; 1969, and Aceto et al., 1970). Since dextroamphetamine is potent at reversing reserpine-induced catalepsy in rats, great emphasis has been placed upon this latter direct agonistic action on dopamine receptors (Hanson, 1966, 1967, Dominic and Moore, 1969, Hollister et al., 1974). In view of the similar effects obtained with the dopamine receptor agonist apomorphine, and the lack of any inhibition with the dopamine-receptor antagonist haloperidol, dopamine release and/or receptor stimulation might be the more important action of dextroamphetamine in the present study. If so, it would appear that persistent activation of central dopaminergic mechanisms decreases dependence upon morphine. It is important to stress that continuous activation of dopaminergic receptors seems to be the important factor, since a single injection of dextroamphetamine to morphine dependent rats failed to reduce the withdrawal score (Figure 6).

There is abundant evidence that neurolepitc drugs, including haloperidol, inhibit central dopaminergic receptors (Koe, 1974). Receptor inhibition leads to an increase fixing and synthesis rate in dopaminergic neurons due to the activation of a neuronal feed back loop (Cooper et al., 1974; Koe, 1974). A similar situation applies to morphine. Morphine elevates brain homovanillic acid and dihydroxyphenylacetic acid levels (Fukui and Takagi, 1972; Kuschinsky and Hornykiawicz, 1972), increases the synthesis of dopamine from labeled tyrosine (Smith et al., 1970, Rosenman and Smith, 1972; Kukui et al., 1972), accelerates the disappearance of brain dopamine following a-methyl-para-tyrosine treatment (Gunne et al., 1969; Puri et al., 1973; Puri and Lal, 1973) and increases the activity of striatal tyrosine hydroxylase (Menon et al., 1967; Davis et al., 1972; Zigler et al., 1972; Pozuelo and Kerr, 1972; Glick et al., 1973). Similar effects are seen after methadone treatment (Sesame et al., 1972). All of the above effects have been attributed to blockade of dopamine receptors (Fuxe and Ungerstedt, 1970; Sesame et al., 1972; Puri et al., 1973; Puri and Lal, 1973). Indeed, morphine-induced analgesia is dependent upon dopaminergic activity as suggested by studies showing analgesic antagonism with apomorphine and amantadine, whereas potentiation followed with certain neuroleptic drugs (Vanerwende and Spoerlein, 1973). In a recent study, a lesion in the nigra-striatal pathway abolished craving and withdrawal symptoms in morphine addicted monkeys (Pozuelo and Kerr, 1972). Moreover, haloperidol apparently abolishes or suppresses the craving in heroin addicts (Karkalas and Lal, 1973). It is noteworthy that the acute effects of morphine on dopamine

synthesis and turnover have been reported to disappear after chronic treatment. For instance, tolerance has been reported to develop to the acceleration of tyrosine hydroxylase activity in the brain (Smith et al., 1970) and to the faster disappearance of dopamine after a-methylparatyrosine (Gunne et al., 1969; Koe, 1974). Also, homovanillic acid and dihydroxyphenylacetic acid levels of morphine-treated mice do not differ from the controls (Fukui and Takagi, 1972). These effects may be related to the development of receptor spread and/or supersensitivity (Goldstein, 1973) with the subsequent loss of feed-back induced neuronal activation. Alternatively, a decrease in the sensitivity of central neurons to excitation by morphine has been recently reported (Bradley and Dray, 1974). Thus, the central pharmacological effects of morphine cannot be solely explained upon inhibition of dopamine-receptors. The maintenance of full dependence in the face of tolerant dopaminergic mechanisms suggests important actions of morphine at other sites. For instance, morphine has been shown to inhibit the firing of noradrenergic neurons in the locus coeruleus (Cooper et al., 1974). However, it may be speculated that an important extra-dopaminergic action of morphine may be exerted at a site which is closely linked with the dopaminergic receptor, such that the active release of dopamine prevents the development of full dependence. The "non-competitive" type antagonism to morphine withdrawal with dextroamphetamine (Figure 7) may be interpreted to support this hypothesis.

A strong case for the close interrelationship between central dopaminergic-cholinergic pathways has been established at least for the

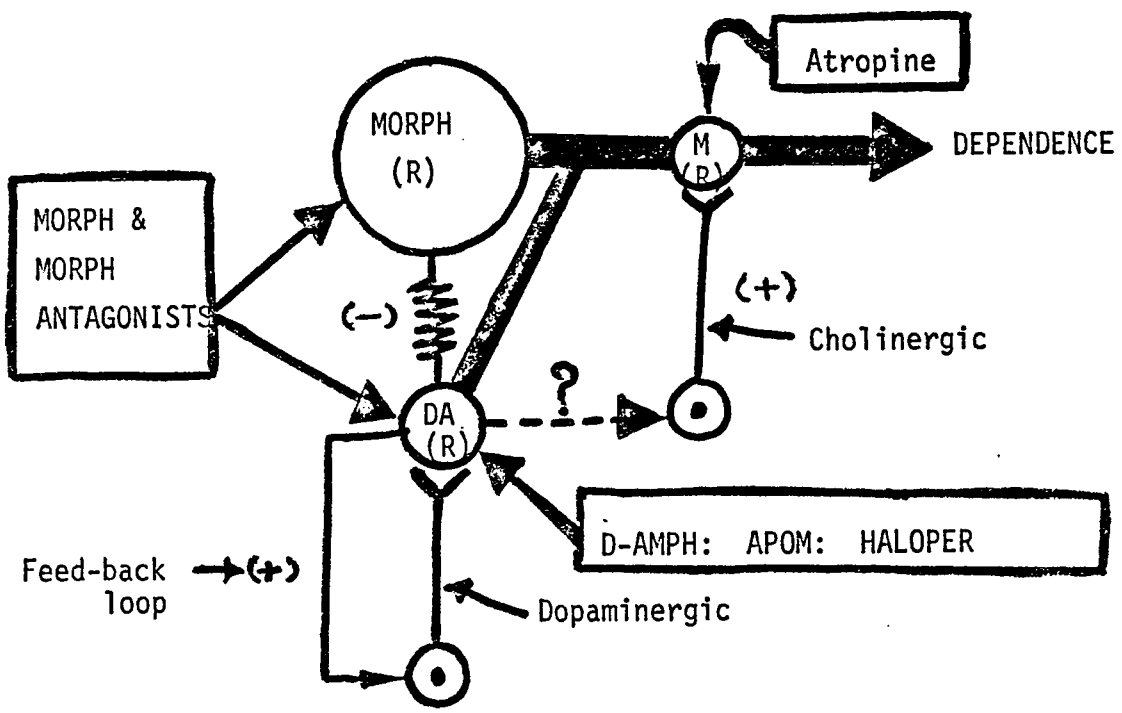
striatum (Horneykiawicz, 1966; Randrup and Munkvad, 1968; Bartholini et al., 1973), where dopamine is an inhibitory transmitter. In this brain region, dopamine release inhibits cholinergic firing and cholinergic activity increases dopaminergic firing (Bartholini et al., 1973);



However, no dopaminergic-cholinergic relationship appears to be present in the limbic structures or the neo-cortex since dopamine does not reduce acetylcholine output (Anden, 1972; Lloyd et al., 1973). Thus, the similarity of effect between atropine and dextroamphetamine is hard to integrate into a composite model. Never-the-less, the greater inhibitory action of atropine than methyl atropine upon morphine withdrawal is conducive with the idea that excessive release of brain acetylcholine accompanies the morphine abstinence syndrome (Crossland, 1970). On the basis of the above assumptions and speculations, the model depicted in Figure 13 may be presented.

Figure 13

" A HYPOTHETICAL MODEL FOR NEUROGENIC AND DRUG INFLUENCES ON MORPHINE-INDUCED DEPENDENCE"



DA(R) = Dopamine receptor; M(R) = Muscarinic receptor; MORPH (R) = receptor to Morphine, other than DA (R); (-) = inhibitory; (+) = excitatory.

For explanation - See text.

Morphine and morphine like drugs (e.g. heroin, methadon) interact with "neuronal receptors" (MORPH (R)) and inhibit dopamine receptors (DA (R)). The dopamine receptor is postulated to be linked to the morphine receptor, either through an undetermined neuronal pathway (-WWW-) or through the cholinergic input (less likely). The receptor interactions of morphine (both MORPH and DA) leads to dependence. The development of full dependence necessitates both receptor interactions whereas the maintenance is perhaps more associated with the MORPH-receptor. Both receptors are acted upon by narcotic antagonists, so as to competitively antagonize the effects of morphine. Blockade of DA receptors by morphine leads to feed-back activation of dopaminergic neurons. Dextroamphetamine releases dopamine and apomorphine stimulates DA receptors directly. If given continuously with morphine, this effect will result in a non-competitive antagonism of morphine-induced dependence. Haloperidol (HALOPER.) blokcs DA receptors and when administered with morphine tends to augment the development of dependence. The extent of such an effect will depend upon the relative affinities of haloperidol and morphine for the DA receptors and the doses used. Atropine simulates the effect of dextroamphetamine by blocking muscarinic receptors (M) and the involvement of acetylcholine in the withdrawal syndrome. If DA receptor activation gave rise to cholinergic depression (--?---), the similarity between dextroamphetamine and atropine would be readily explained.

Effect of Drugs; Part II

The present study attempted to elucidate whether the lowering of morphine-withdrawal scores by dextroamphetamine was surmountable with time. Dextroamphetamine (0.5 mg/kg,I.P.) was given continuously with morphine for up to eleven days. The time-course withdrawal curve exhibited the characteristics of a non-competitive antagonist, in that the curve was displaced to the right and showed a depressed maximal response (Figure 4). This type of antagonism appeared to hold using a lower initial dose of morphine (Figure 5); however, the experiment is not conclusive since the maximal withdrawal score to morphine was not obtained. Levoamphetamine failed to alter significantly the time-course withdrawal curve, irrespective of the initial starting dose of morphine. Thus, over an eleven day period, the present study shows a marked and clear-cut difference between the two isomers of amphetamine which must in turn reflect a difference in their pharmacological action or interaction with morphine.

In order to be sure that the shift in the time-course effect curve with dextroamphetamine was indeed a valid observation, an experiment was undertaken to study the effect of naloxone. Naloxone is a competitive antagonist of morphine (Seevers and Woods, 1953) and Figure 7 shows that, within the limit of experimental error, a competitive type shift was obtained. These data are surprisingly good, considering the semiquantitative and subjective measures used. In addition, such clear cut differences are usually very difficult to obtain *in vivo* since the complexity of the biological environment often leads to drug non-specificity and

diverse responses which serves to cloud interpretations as to the nature of the drug-induced antagonism. One factor which may have aided the clarity of the experiments is the relative low dose of the antagonist used. This in itself points to a marked selectivity and specificity of action with regard to the antagonism of morphine. It should be pointed out here that higher doses of the amphetamines may well produce different effects. In fact, it has been claimed that the mechanism of action of amphetamine on dopaminergic neurons can involve at least five different events, two accelerating dopamine synthesis (low dose) and three decelerating dopamine synthesis (higher dose) (Koe, 1974). Furthermore, dextroamphetamine can accelerate serotonin synthesis possibly by increasing brain tryptophan levels (Schubert et al., 1970; Tagliomonte et al., 1971; Giowinski et al., 1973). In addition, effects on noradrenergic function (Cooper et al., 1974) have been mentioned previously. Thus, it must be emphasized that it is extremely difficult to ascribe one particular action of dextroamphetamine as full explanation for a particular pharmacological effect. Indeed, in the present study, a marked toxicity resulted when experiments were attempted using a higher dose (1 mg/kg, three times per day) of dextroamphetamine along with morphine (10 and 20 mg/kg starting dose), (Appendix II). Thus, drug users taking both morphine and dextroamphetamine may experience less physical dependence upon morphine but risk death due to enhanced toxicity.

So far the interpretation of the results has been based upon changes in the total score and it was upon this basis that the model depicted in Figure 13 was constructed. However, obvious misinterpretations may arise utilizing this procedure since it is possible that the same total

score may be composed of a different set of symptoms. In order to investigate this potential source of error, the frequency-distribution of the withdrawal symptoms was analyzed. Drug induced changes were compared with those seen after morphine alone (Figure 12).

No drug influenced the occurrence of wet dog, suggesting the lack of central monoaminergic mechanisms and peripheral autonomic influences in this response. Dextroamphetamine markedly depressed ptosis, teeth-chatter, and diarrhea, whereas levoamphetamine failed to alter these responses. Thus, a real difference exists between the effect of these two closely related agents with regard to individual symptoms analysis. Apomorphine was postulated to act like dextroamphetamine but, Figure 12 shows that a clear difference exists with regard to diarrhea. Apomorphine was practically ineffective at attenuating this response whereas dextroamphetamine exhibited a marked effect. Furthermore, apomorphine abolished the incidence of squealing and writhing, whereas the symptoms were still evident with dextroamphetamine. Although similarities of effect between apomorphine and dextroamphetamine do exist, it now becomes much more difficult to speculate that both agents antagonize morphine-induced dependence through activating common pathways, especially since apomorphine itself induced some symptoms of morphine withdrawal (Figure 12). If the suppression of diarrhea is speculated to be a peripheral action of dextroamphetamine then a closer similarity is obtained between this agent and apomorphine. However, it is then necessary to postulate differences between the peripheral activity of the dextro and levo isomers. Even though such differences are known to exist (e.g. dextroamphetamine is

20 times more potent at inhibiting the neuronal uptake of norepinephrine in the rat heart than levoamphetamine, (Iversen, 1967), such discussions appear too highly speculative to carry much weight. Haloperidol tended to enhance ptosis, teeth chatter, and diarrhea but no squealing was recorded. Except for the latter symptom, such a pattern is in reasonable agreement with the concept of central dopaminergic receptor inhibition. However, haloperidol, in the same dose, failed to show any greater enhancement of morphine withdrawal symptoms when elicited from rats which were sub-maximally dependent upon morphine (Appendix III). Thus, it is again difficult to reconcile these data with the unified model depicted in Figure 13. The complex nature of drug effects upon the morphine-withdrawal syndrome is also apparent from the studies with atropine and its methyl derivative. Marked peripheral actions were obtained with this latter agent which were not matched by atropine itself. One marked feature was the complete suppression of diarrhea with atropine and complete lack of suppression with the methylated derivative. N-methylation of atropine increases the affinity of the compound for nicotinic receptors (Cullumbine, 1971) and this may be a factor in the present experiments. Although the individual dose of atropine and methylatropine was 2 mg/kg, the total cumulative dose administered was 12 mg/kg. This aspect, along with the long duration of action of atropine and methylatropine, may reflect a large degree of non-specificity of effect with these agents.

Thus, the analysis of drug effects upon individual symptoms reveals a much more complex interaction on morphine withdrawal than is obvious from a consideration of the total score. As seen from the present study, models based upon this latter data can be misleading to the point of being

fundamentally incorrect. This study therefore emphasizes that extreme caution is required before such data can be accepted as being meaningful. Furthermore, studies employing only single dose-levels of a particular drug are also liable to give rise to spurious interpretations. No drug is specific in its pharmacological effects and conclusions based upon a known or even "accepted mode of action" should not be readily accepted. On the other hand, under the limited conditions of the present study, clear evidence was obtained that dextroamphetamine is far more effective than levoamphetamine at antagonizing certain specific withdrawal symptoms from morphine dependent rats. Certain symptoms are also inhibited by apomorphine but not by haloperidol. In relation to current knowledge of the central receptor effects of morphine, the total picture does lend support to the concept that dopaminergic receptors play some role in the development of dependence and/or withdrawal from morphine-dependent states, but the exact mechanism involved is highly speculative and requires further detailed investigation.

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APPENDIX I LIST OF SUPPLIERS

a. Zivic-Miller Labs., Inc.
3848 Hieber
Allison, Pennsylvania 15101

b. Ralston Purina Company
St. Louis, Missouri

c. Mallinckrodt Chemical Works
3600 N. Second Street
Box 5439
St. Louis, Missouri 63160

d. Elkins-Sinn, Inc.
2, Eastbrook Lane
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Division of Hoffmann-LaRoche, Inc.
Roche Park
Huttey, New Jersey 07110

f. Merck Sharp & Dome
Division of Merck & Company, Inc.
West Point, Pennsylvania 19486

g. Regis Chemical Company
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h. McNeil Labs., Inc.
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1000 Stewart Avenue
Garden City, Long Island, New York 11530

APPENDIX II PRELIMINARY STUDIES: DETERMINATION OF DOSES FOR MORPHINE-D-AMPHETAMINE COMBINATION

TREATMENT

3 x Day

Group	No. of Animals	Initial Dose of Morphine	Dose of d-Amphetamine	Withdrawal Score ± SEM After 11 days	Mortality %
I	6	5 mg/kg	0.25 mg/kg	5.4 ± 0.6	16%
II	10	10 mg/kg	0.5 mg/kg	3.4 ± 0.4	10%
III	4	20 mg/kg	0.5 mg/kg	3.0	25%
IV	17	20 mg/kg	1.0 mg/kg	3.0	83%
V	4	10 mg/kg	1.0 mg/kg	3.33 ± 0.6	33%
VI	10	10 mg/kg	None	5.6 ± 0.6	10%

APPENDIX III THE EFFECTS OF HALOPERIDOL AND APOMORPHINE ON MORPHINE WITHDRAWAL SCORE, USING 1 mg/kg STARTING DOSE

	Withdrawal Score ± SEM After:			
Treatment	Dose 3	Dose 6	Dose 15	Dose 21
Morphine Sulfate (initial dose 1 mg/kg)	0.16 ± 0.16	1.33 ± 0.33	3.40 ± 0.4	4.20 ± 0.4
Morphine Sulfate 1 mg/kg, starting dose + Apomorphine 1.5 mg/kg	0.4 ± 0.24	0.4 ± 0.24	1.40 ± 0.4	2.40 ± 0.24
Morphine Sulfate 1 mg/kg, starting dose + Haloperidol 0.1 mg/kg	0.2 ± 0.2	1.50 ± 0.54	3.33 ± 0.40	4.40 ± 0.6
Apomorphine 1.5 mg/kg	0.2 ± 0.2	1.33 ± 0.33	-	1.00
Haloperidol 0.1 mg/kg	0.0	0.33 ± 0.21	-	-
Normal Saline	0.0	0.2 ± 0.2	-	0.0