Psychopharmacologia 4, 247—260 (1963)

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Tolerance to and Physical Dependence on Morphine in Rats

By

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With 3 Figures in the Text

(Received December 4, 1962)

In recent years the rat has been used extensively in studies on the basic mechanisms involved in the development of tolerance to, and both "physical" and "psychic" dependence on morphine (Nichols et al. 1956; Beach 1957; Gunne 1960; Wikler et al. 1960; Davis and Nichols 1962; Maynert and Klingman 1962; Sloan et al. 1962). Although tolerance to and physical dependence on morphine have been studied by several investigators in the rat (Joël and Ettinger 1926; Himmelsbach et al. 1935; Fichtenberg 1951; Kaymakcalan and Woods 1956; Hanna 1960) most of these studies have restricted themselves to a description of gross behavioral changes observed during acute and chronic morphine intoxication and abstinence precipitated by either abrupt withdrawal of morphine or by the administration of nalorphine. This has led to an oversimplified characterization of abstinence in the rat, which according to some investigators is excitatory in nature, while others contend that it is predominantly depressant. The present study was undertaken with two specific objectives in view: (1) to obtain additional and essential information on the characteristics of chronic morphine intoxication and abstinence in the rat, and (2) to obtain a quantitative determination of the time course of abstinence precipitated by abrupt withdrawal of morphine.

In this paper, the term "addicted" or "addiction" is used to refer exclusively to a state of physical dependence on morphine. The expression, "experimentally addicted" will be used to refer to the procedure of administering morphine to rats on a specified schedule.

Methods

The experiments herein reported were conducted on male rats of the Wistar strain taken from a group that had a mean weight of 447 g (range 365—543 g) at the beginning of the study, when the animals were about four months old. A portion of these rats were experimentally addicted to morphine over a period of 42 days. Selection of the final daily dose level of morphine to which the rats were to be experi-

mentally addicted was determined by two considerations: a) induction of maximal physical dependence, and b) avoidance of convulsive or otherwise lethal effects. The schedule of experimental addiction employed was one that in a previous study (SLOAN et al. 1962) had been found not to cause loss of body weight in the experimental rats. The initial dose was 5 mg/kg administered intraperitoneally twice daily at 8 a.m. and 3 p.m. The dose was increased biweekly until the rats were receiving 320 mg/kg/day (140 mg/kg at 8 a.m., 180 mg/kg at 3 p.m.) by the 35th day. The rats were stabilized at this dose level for a week and then morphine was withdrawn abruptly. Another group of rats, which served as controls, received normal saline (0.9% aqueous solution of sodium chloride) intraperitoneally at the same time and in the same relative volume as the rats receiving morphine. Throughout the entire study, all rats were allowed food (Purina Chow base) and tap water *ad libitum* in the home cages. At each observation period 6 rats were studied and the following protocol was followed:

0 hours: rats removed from home cages, weighed and placed in individual cylindrical battery jars, 12 inches (30.5 cm) in diameter and 18 inches (45.7 cm) high. In the experiments on acute and chronic intoxication, the experimental and control rats were first weighed, given the injections of morphine or saline respectively, and returned to the home cages where they remained for one hour before being placed in the battery jars. As soon as rats were placed in their respective jars the level of spontaneous motor activity was assessed for 15 consecutive one-minute intervals, using a modification of an activity check list previously described by SLOAN et al. (1962) (see Fig. 3). In addition, a count of the number of "wet dog" shakes (WIKLER et al. 1960) exhibited by the rats during this 15 min. period was made. These "wet dog" phenomena (so-called because they resemble the behavior of a dog shaking water off its back) are very brief episodes of rapid repetitive shaking of the entire trunk, occurring most often after a prolonged grooming period. Their frequency is greater immediately after handling the rat or changing its place of "residence" than after a lapse of time (e.g., one hour) during which the animal has remained undisturbed. In making these observations, 6 rats (generally 4 experimental and 2 control) were studied concurrently by two experimenters, each of whom observed 3 of the rats.

1 hour: another 15-min "activity" and "wet dog" count was made. In the interim between the two "activity" measurements, respiratory rate was determined.

1.5 hours: metabolic rate measured, using a modification of the apparatus described by HOLTCAMP et al. (1955). After the animals were placed in their respective metabolic chamber they were allowed to

acclimate for approximately 15 min. Following the acclimation period, oxygen consumption was determined for three consecutive 10-min periods. Hourly oxygen consumption rates, corrected for atmospheric pressure and expressed in units of liters/m^2/hr, were calculated from these determinations.

2.5 hours: colonic temperature was determined with a thermistor probe inserted 4 cm.

The following experiments were conducted:

1. Replicate determinations of the measures listed above were made on 12 saline-treated, non-addicted rats.

2. The effects of 100 mg/kg of morphine i.p. were investigated on the same 12 non-addicted rats. Although several preliminary experiments indicated that this dose of morphine was not lethal for the strain of rats employed, 2 of the 12 rats died following this dose and several other rats that were severely depressed were given nalorphine after the observations to assure their survival.

3. Fourteen rats were addicted to 320 mg/kg/day of morphine, and 8 rats that served as controls were injected with saline. During the course of addiction 2 morphine-treated animals and one saline control died. The effects of 100 mg/kg and 140 mg/kg of morphine were studied in 8 rats on the 29th or 30th day as well as on the 45th or 46th day of the addiction period. At these respective times, the rats were addicted to 80 and 320 mg/kg/day. Four nontolerant saline-treated rats were studied concomitantly. Because the metabolic apparatus contained only six chambers, it was necessary to divide the rats into two groups consisting of 4 addicted and 2 nontolerant rats. These groups were studied on alternate days after drug injection at 8 a.m.

Following abrupt withdrawal the two groups were studied at alternate four-hour periods for the first 72 hours of abstinence. Thereafter, observations were started at 8 a.m. for the first group and at 12 p.m. for the second group. Morphine sulfate was employed in all experiments, and the doses stated refer to this salt.

Results

Tolerance to large doses of morphine. The nontolerant rats, following a 100-mg/kg dose of morphine, exhibited signs of general motor depression consisting of cyanosis and the almost total absence of spontaneous activity (Fig. 1 and Table 1). Under the conditions of this experiment body temperature and metabolic rate were slightly depressed. Despite the presence of cyanosis in many of the animals, respiratory rate was not decreased. In contrast, large doses of morphine (100 or 140 mg/kg) produced in the addicted rat an increase in activity, body temperature and metabolic rate (Fig. 1 and Table 2). Although

Table 1. *Effects of 100 mg/kg of morphine in nontolerant rats*

Each mean ± one standard error was calculated for the control group on the basis of replicate determinations in 12 rats receiving a saline injection, and on the basis of a single determination in the same 12 rats for the 100 mg./kg. dose level of morphine. The "activity" scores were obtained after the rats had remained in the observation jars one hour.

	Control ($n = 12$)	Morphine ($n = 12$) (100 mg/kg i.p.)
Body weight (grams)	443 ± 15	446 ± 15
Respiratory rate (breaths/min)	69 ± 5	72 ± 5
Temperature (° C)	37.5 ± 0.1	37.2 ± 0.5
Metabolic rate (l/m²/hr)	7.77 ± 0.25	6.81 ± 0.58
Activity (15 min score)	2 ± 2	−36 ± 3*

* $p < 0.05$.

Table 2. *Effects of 100 mg/kg and 140 mg/kg of morphine in 8 rats addicted to 80 and 320 mg/kg/day*

The control group received equal volumes on a weight basis of saline during the addiction cycle and at the time of administration of morphine to the addicted rats.

Addiction level: Morphine, 80 mg/kg/day	Control ($n = 4$)	Morphine ($n = 8$) (100 mg/kg i.p.)
Body weight (grams)	485 ± 16	393 ± 15*
Respiratory rate (breaths/min)	80 ± 5	90 ± 9 *
Temperature (° C)	37.9 ± 0.2	39.8 ± 0.2*
Metabolic rate (l/m²/hr)	7.47 ± 0.28	11.39 ± 0.44*
Activity (15 min score)	16 ± 13	34 ± 6*
Addiction level: Morphine, 320 mg/kg/day	**Control ($n = 4$)**	**Morphine ($n = 8$) (140 mg/kg i.p.)**
Body weight (grams)	517 ± 27	395 ± 20*
Respiratory rate (breaths/min)	77 ± 5	91 ± 5
Temperature (° C)	37.4 ± 0.4	39.6 ± 0.2*
Metabolic rate (l/m²/hr) . . .	7.16 ± 0.33	11.92 ± 0.59*
Activity (15 min score)	16 ± 5	46 ± 7*

* $p < 0.05$.

the early effects of morphine in the tolerant rat were not systematically studied (since observations were not begun until one hour after administration of drug and not completed until 2.5 hours later), there seemed to be a transient phase during which the rats were inactive. Following this phase the rats' activity increased and they consumed food and water voraciously, if allowed.

Abstinence from morphine in the tolerant rat. The changes observed during abstinence are summarized in Fig 1—3 and in Tables 3 and 4. The abstinence syndrome in the rat can be divided into two phases

which will be designated as primary and secondary abstinence. After the last dose of morphine the excitatory effects became manifest and then subsided from the 4th to the 16th hour. During this time the rats showed a small gain in body weight; however the first signs of abstinence, an

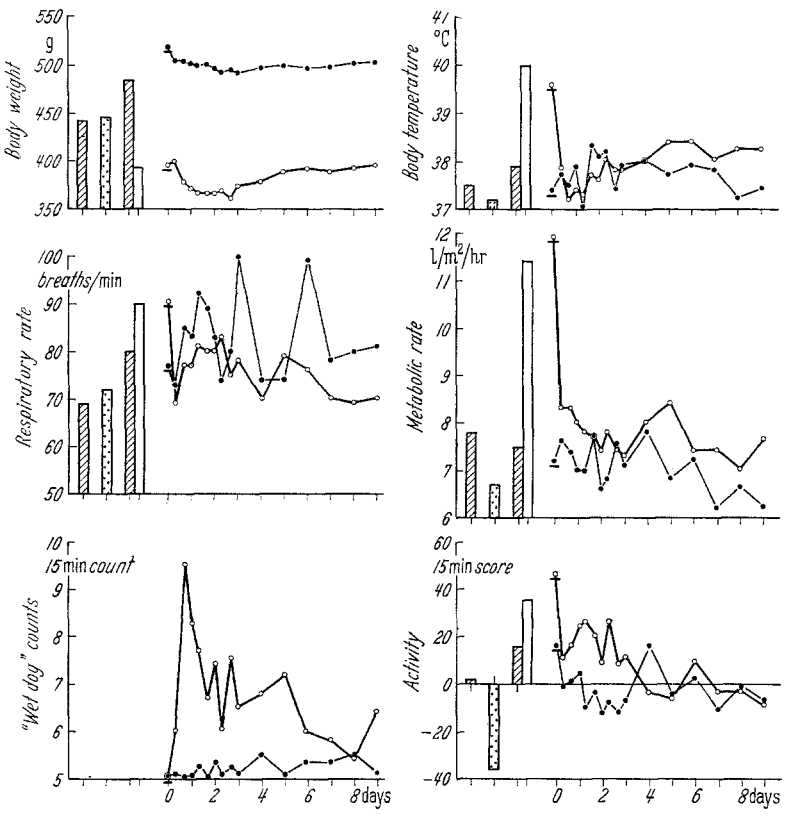

Fig. 1. Time course of primary and early secondary abstinence signs. For comparison, values presented in Tables 1 and 2 are illustrated by bars. Cross hatched bar, indicates saline control; stippled bar, 100 mg/kg of morphine in nontolerant rats; open bar, effects of 100 mg/kg in in rats addicted to 80 mg/kg/day at about the 29th day of experimental addiction. Open circles (o) indicate mean values for abstinent rats ($n = 7$); closed circles (•), mean values for saline controls ($n = 4$). The first point (underlined) on each graph summarizes determinations made during the first four hours following an injection of 140 mg/kg of morphine in rats tolerant to 320 mg/kg/day

increase in frequency of "wet dog" shakes, became manifest. From the 16th to the 24th hour, the primary abstinence syndrome became almost fully developed. The addicted animals lost weight precipitously, body temperature and metabolic rate fell to subnormal or normal levels and the floors of observation jars housing addicted rats showed more soft stools and urine than did those of nonaddicted control animals. After decreasing to almost control levels, spontaneous activity increased and

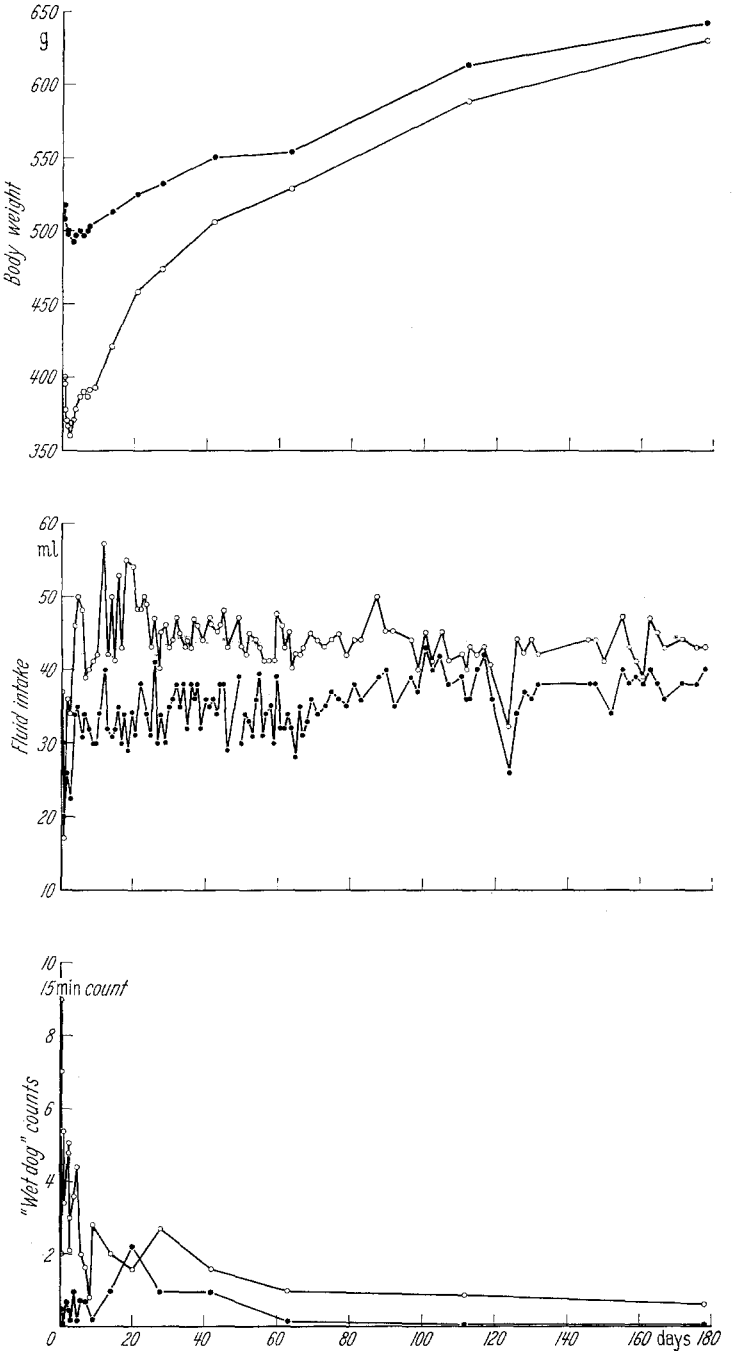
650
g
600
550
500
450
400
350
Body weight
60
ml
50
40
30
20
10
Fluid intake
10
15 min count
8
6
4
2
"Wet dog" counts
0
20
40
60
80
100
120
140
160 days 180

Fig. 2a

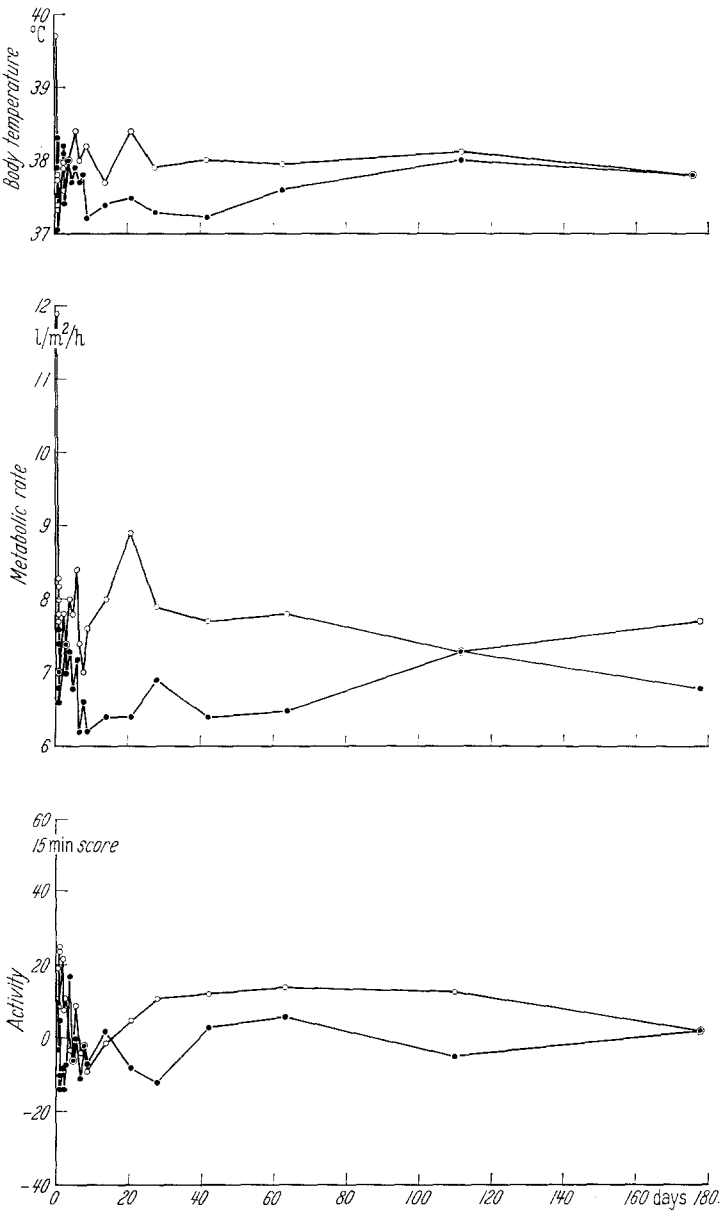

Fig. 2 b

Fig. 2 a and b. Time course of the secondary abstinence signs. Open circles (o) indicate mean value for abstinent rats; closed circles (•), mean values for saline controls

remained significantly above control levels for three days. Two features of the increase in activity seen during the primary abstinence syndrome should be pointed out (Fig. 1 and 3): (1) Although the level of activity

Table 3. *Primary abstinence signs*

Effect of abrupt withdrawal of morphine on rats addicted to 320 mg/kg/day. Each value used in the calculation of these means ± one standard error is the average of nine determinations made from the 8th through the 80th hour after termination of an injection of saline or morphine. "Activity" scores and second (No. 2) "wet dog" counts were made after the rats had been in their observation jar one hour. The first (No. 1) "wet dog" count was made immediately after the rats were placed in the observation jars. One addicted rat died during the primary abstinence phase of withdrawal.

	Control ($n = 4$)	Abstinent rats ($n = 7$)
Respiratory rate (breaths/min) . .	84 ±4	77 ±5
Fluid intake (ml/day)	23 ±2	29 ±5
Temperature (° C)	37.8 ±0.1	37.6 ±0.1
Metabolic rate (l/m²/hr)	7.20±0.13	7.78±0.26
Activity (15 min score)	−5 ±3	17 ±5*
Wet dog count No. 1 (counts/15 min)	0.2 ±0.1	3.7 ±0.9*
Wet dog count No. 2 (counts/15 min)	0 ±0	1.9 ±0.5*

* $P < 0.05$.

Table 4. *Secondary abstinence signs*

Effect of abrupt withdrawal of morphine on rats addicted to 320 mg/kg/day. Each value used in the calculation of these means ± one standard error is the average of eleven determinations made from the 4th through the 63rd day after termination of saline or morphine injections. Determinations are the same as in Table 3.

	Control ($n = 4$)	Abstinent rats ($n = 7$)
Respiratory rate (breaths/min) . .	80 ±4	72 ±2*
Fluid intake (ml/day)	34 ±2	45 ±2*
Temperature (° C)	37.6 ±0.0	38.0 ±0.1*
Metabolic rate (l/m²/hr)	6.60±0.16	7.85±0.19*
Activity (15 min scores)	−1 ±4	2 ±3
Wet dog count No. 1 (counts/15 min)	0.9 ±0.6	2.3 ±0.6
Wet dog count No. 2 (counts/15 min)	0.2 ±0.1	0.6 ±0.3

* $P < 0.05$.

seen during the primary abstinence syndrome was greater than that seen in control animals, it was less than that seen following morphine in the tolerant rat, and (2) the activity of abstinent rats was qualitatively different from that seen following morphine in tolerant rats. Tolerant rats rarely scratched themselves and their preening movements consisted almost entirely of gnawing of the paw pads. The abstinent rats, on the

other hand, showed a more normal grooming pattern consisting of scratching, preening of fur and licking or gnawing of the nails and tail; whereas the morphine-treated, tolerant rats showed a marked increase in such activity as exploring, walking, standing and circling even in the presence of ataxia, the abstinent rats showed a small increase in this type of activity. The most striking change in the activity of the abstinent

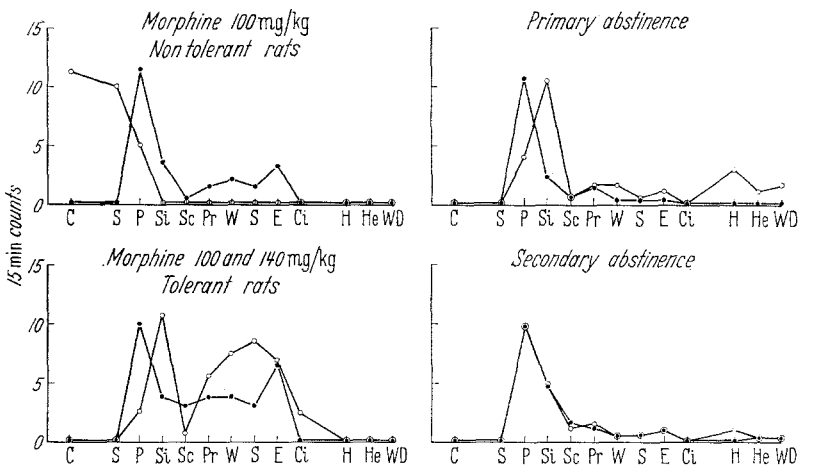

Fig. 3. Activity spectra of morphine-treated nontolerant and tolerant rats, and of abstinent rats (all indicated by open circles) and saline-treated controls (solid circles). The designation of the various signs and the value given them for calculating "activity" scores is as follows.

Cyanosis (C) — 1 × 15 =	—15	Standing (St)	1
On side or supine (S)	—2	Exploring (E)	1
Prone (P)	—1	Circling (Ci)	1
Sitting (Si)	1	Hostile (H) 1 × 15 =	15
Scratching (Sc)	1	Head shake (He)	1
Preening (Pr)	1	Wet dogs (WD)	
Walking (W)	1		

"Wet dog" counts were treated separately and not incorporated in "activity" scores. If animals were either "cyanotic" or "hostile" when placed in their observation jars, these items were checked for each 15 min period

rat was a decreased tendency to sleep. Whereas control rats, following an acclimation period, would commonly assume one of the several sleep postures of the rat, the abstinent rats would become quiet but would remain in a sitting posture with their heads up, exhibiting occasional preening movements. In addition, the abstinent rats developed three signs that were never or rarely seen under other experimental conditions — namely "head" and "wet dog" shakes and the emergence of hostile behavior when handled.

Approximately 72 hours following withdrawal of morphine the primary signs of abstinence subsided and a secondary syndrome emerged (Fig. 2). Abstinent rats began to gain weight rapidly, body temperature

and metabolic rate, which had been at normal or subnormal levels, became elevated, and water consumption of abstinent rats was approximately 30% above that of the control animals. "Activity" and "wet dog" shakes, after subsiding to the level of the nonaddicted control animals at the end of the first week of abstinence, rose again slightly, but not significantly, above control level and remained elevated for four to six months. Other signs of the secondary abstinence syndrome also diminished in magnitude, but even six months following withdrawal minor differences between the control and abstinent rats were still present.

Discussion

The effects of morphine on physiological responses in the rat are complicated and depend upon a variety of factors such as dose, length of time after administration, development of tolerance and pretreatment with other drugs. On the basis of a large number of studies of morphine actions in the rat, the following generalizations are either suggested or well documented. Morphine in nontolerant rats has three different types of action which may be designated as excitant, depressant and convulsant[1]. Many of the excitant actions of morphine are seen with doses of 15—30 mg/kg and include an increase in spontaneous activity (JOËL and ETTINGER 1926; SLOAN et al. 1962), an elevation of body temperature (HERRMANN 1942; GUNNE 1960; SLOAN et al. 1962), possibly an increase in release of ACTH (GEORGE and WAY 1955) and an elevation of metabolic rate. Predominantly depressant effects are seen with doses of 60 to 100 mg/kg and consist of depression of body temperature, spontaneous activity and respiration. Dose levels of over 200 mg/kg commonly produce seizures. The following relationship between excitant and depressant effects seem to obtain: (1) The depressant effects become manifest before and are subsequently supplanted by excitant effects (JOËL and ETTINGER 1926; GUNNE 1960; SLOAN et al. 1962); (2) generally the excitant effects predominate at lower doses (below 30 mg/kg) and depressant effects predominate at higher dose levels (60—100 mg/kg); (3) marked tolerance develops to such depressant effects as depression of spontaneous motor activity (JOËL and ETTINGER 1926), analgesia (LEWIS 1949) and depression of body temperature

[1] These terms are used to designate behavioral syndromes, not to infer basic modes of action of morphine. The convulsant action, which is usually included as one of the excitant actions of morphine, has been separately designated in the rat for the following reasons: (1) very large doses of morphine (> 200 mg/kg) are required to produce seizures, whereas moderate dose levels (< 40 mg/kg) produce initial depression which is followed by excitation while large doses (60—100 mg/kg) in the nontolerant rat produce depression; (2) thebaine, when given in convulsant or subconvulsant dose levels in the rat, does not produce other signs of excitation.

(GUNNE 1960). Large doses of morphine in the tolerant rat produce primarily excitant actions such as increased motor activity (JOËL and ETTINGER 1926; FICHTENBERG 1951; KAYMAKCALAN and WOODS 1956), increased body temperature (GUNNE 1960) and an increase in metabolic rate. In addition, large doses of morphine in the tolerant rat probably cause an increased release of ACTH (TANABE and CAFRUNY 1958).

The mechanism whereby morphine produces hyperthermia in nontolerant rats, and hyperthermia and increased metabolic rate in tolerant rats is unknown. On the basis of a limited number of determinations in nontolerant rats, HERRMANN (1942) concluded that the increase in metabolism (CO_2 production) observed following morphine was less than would be expected for acceleration of enzymatic activity due to elevated temperatures and concluded that the increase in body temperature was not due to increased heat production, and consequently must be due to increased heat conservation. In the tolerant rat the increased utilization of oxygen following morphine was considerably more than would be expected on the basis of the rise in body temperature and therefore seems to indicate stimulation of metabolic processes. It is possible that the increase in oxygen consumption may be a consequence of increased activity, although the apparent difference between the motor activity of the addicted and control animals in the metabolic chambers was small. This finding may be related to the observation of SHIDEMAN and SEEVERS (1941) that morphine stimulates metabolism of minces of rat skeletal muscle.

The observations herein reported on the primary abstinence syndrome are confirmatory in many respects of the works of a sumber of investigators; thus FICHTENBERG (1951) and HOSOYA (1961) have previously reported on the loss of body weight following abrupt withdrawal of morphine in experimentally addicted rats. WIKLER et al. (1960) have previously described "wet dog shakes" as an abstinence sign, and MAYNERT and KLINGMAN (1962) have observed a fall in body temperature following injection of nalorphine in rats experimentally addicted to morphine. KAYMAKCALAN and WOODS (1956) and MAYNERT and KLINGMAN (1962) have observed frequent and loose stools following nalorphine in such morphine-addicted rats. There is however an apparent difference in the literature with regard to behavioral changes observed during the primary abstinence syndrome. KAYMAKCALAN and WOODS (1956) and MAYNERT and KLINGMAN (1962) have characterized the behavioral signs of abstinence precipitated with nalorphine as "sedative". In this regard it should be pointed out that MAYNERT and KLINGMAN (1962) administered nalorphine to some rats that were 24 hours abstinent. On the other hand HIMMELSBACH et al. (1935) and FICHTENBERG (1951) have reported that abstinence following abrupt

withdrawal of morphine from tolerant rats is characterized by hyperirritability, agitation and hostility. HANNA (1960) has reported that morphine-addicted rats treated with nalorphine exhibit hypermotility and hyperexcitability. With the exception of the study of HIMMELSBACH et al. (1935), the above cited studies have characterized behavior on the basis of unquantified observations under environmental conditions that were not rigorously defined. The findings of the present study would indicate that rats exhibiting primary abstinence are more active and less likely to sleep than saline-treated control rats, but less active than morphine-treated tolerant rats, and that the activity seen during abstinence is qualitatively different from the activity seen during chronic morphine intoxication.

The fall in body temperature and metabolic rate to subnormal and normal levels was an unexpected finding, especially in view of the finding that after approximately 72 hours of abstinence both body temperature and metabolic rate again rose to above normal levels. It is possible that by the recruitment of contraadaptive mechanisms (HIMMELSBACH 1943) to morphine-induced hyperthermia and increased metabolism, partial tolerance develops to these effects and that upon withdrawal the contraadaptive mechanisms temporarily prevail. Regardless of the explanation of these findings, it seems reasonable to conclude that abstinence is probably the result of several pathophysiological processes.

The secondary abstinence syndrome in the rat does not appear to have been preciously described, although FICHTENBERG has stated that addicted rats do not lose tolerance for at least 25 days after withdrawal of morphine. The prolonged duration of "wet dog" shakes is confirmatory of unpublished observations of WIKLER. Prolonged polydipsia has been observed by WIKLER et al. (in preparation) after withdrawal of an opioid (etonitazene) following prolonged addiction to that drug. Thus several signs of the secondary abstinence have been confirmed. The significance of this syndrome remains to be demonstrated. In man, both on the basis of HIMMELSBACH'S (1942) fundamental study and clinical impression, six to nine months are required before recovery from abstinence can be considered complete. Because the protracted signs of abstinence in man appear to be a continuation of the early signs of abstinence in progressively diminishing intensity, there has neither been a need nor a basis for differentiating early and late signs of abstinence. It is possible that the secondary abstinence syndrome in the rat, which in many respects is quite similar to the protracted abstinence syndrome of man, may serve as a model for the study of the basic pathophysiological abnormalities of a process known to occur in abstinent human addicts.

Summary

The effects of large doses of morphine in nontolerant and tolerant rats as well as the effects of abruptly withdrawing morphine in rats experimentally addicted to large doses of morphine have been studied on body weight, temperature, metabolic rate, respiratory rate, water consumption and various forms of motor activity and behavior. In confirmation of many early reports, tolerance develops to certain depressant actions of large doses of morphine and the effects of morphine in the tolerant rat are primarily excitatory, consisting of an increase in body temperature, metabolic rate and motor activity. The abstinence syndrome of rats addicted to large doses of morphine seems to have two phases: (1) An early phase, which has been called "primary abstinence" consists of weight loss, an increased number of "wet dog" shakes, increased activity, and a fall in body temperature and metabolic rate. The primary abstinence syndrome becomes clearly manifest within 8 to 16 hours following the last dose of morphine and persists for approximately 72 hours. (2) The secondary abstinence syndrome emerges thereafter and consists of a rapid gain in body weight, elevated body temperature and metabolic rate and an increase in water consumption. The secondary abstinence syndrome is protracted and small differences have been seen between addicted and control animals as long as four to six months after withdrawal of morphine.

Acknowledgment. The authors wish to express their thanks and indebtedness to Mr. WESLEY W. PROCOP for not only constructing the metabolism apparatus but for incorporating many ingenious innovations of his own design into it.

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