CERTAIN ASPECTS OF PHARMACOLOGICAL PROFILES OF CHLORDIAZEPoxide AND DIAZEPAM*

By

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In recent years, chlordiazepoxide and diazepam, two agents of the benzodiazepine series, have been found to be useful in a variety of psychoemotional and autonomic nervous disturbances (14); in cases of skeletal muscle spasm of varied aetiology (16, 20); in epilepsy (9, 25, 31); in obstetrics (2, 4) and dermatology (10) and in the management of chronic alcoholism (14). Tranquilisedative, muscle relaxant, anticonvulsant and taming effect of chlordiazepoxide was first described by Randall et al., (23) and by Randall (21); later, diazepam was found to be 'generally more potent' in many tests (22). Since then the agents have been the subject of extensive pharmacological and clinical research. Many authors, however, have not been able to share the general enthusiasm (3, 14, 17) in the therapeutic use of these agents and as a result the therapeutic status of these agents remains to be settled. In view of this, certain aspects of pharmacological profiles of both the drugs were examined in different species with particular attention to their relative potency and overt neurotoxicity.

MATERIALS AND METHODS

Experimental seizure techniques

Maximal electroshock seizures (MES) were evoked in mice and rats as described by Swinyard et al. (29) using 'Convulsiometer' (Techno, Lucknow). Abolition of the tonic extensor phase of the seizure pattern was taken as anticonvulsant effect of drugs. Neurotoxicity (NT) of the drugs was determined considering any evidence of minimal neurological deficit as described by Swinyard et al. (29). Groups of 4 to 6 animals were given graded doses of the benzodiazepines intraperitoneally 1 hr before the MES or the NT tests. In some groups subjected to MES test phenobarbitone sodium was given intraperitoneally as a reference drug; the animals were tested 2 hr later.

Antimetrazol activity: Rats in groups of 10 were given graded doses of chlordiazepoxide or diazepam intraperitoneally 1 hr prior to the subcutaneous injection of challenging dose (CD 97) of metrazole (90 mg/kg). Abolition of the tonic extensor phase of the seizure was considered as an anticonvulsant effect (a brief clonic seizure was followed by a tonic component).

Antistrychnine activity

Groups of guineapigs (10 animals each) received chlordiazepoxide or diazepam intraperitoneally in graded doses; one hr later the animals were challenged with strychnine hydro-

*Preliminary experiments performed as described by Swinyard et al. (29) showed that the peak anti-MES effect of chlordiazepoxide and diazepam was fully developed at this time. In the remaining tests also the effect under observation was, hence, assumed to be fully developed 1 hr after the intraperitoneal injection of these agents.
chloride (2 mg/kg, intraperitoneally). Complete protection from convulsion of any type and/or death was considered as antisystrynine activity.

Sleeping time

Male adult guineapigs (not fasted) weighing between 350 and 500 gm were used once only. Two groups of animals (25 each) were given chlordiazepoxide (4 mg/kg) and diazepam (2 mg/kg) as single intraperitoneal injections; 50 control animals received equivalent quantity of saline. One hr later pentobarbitone sodium (30 mg/kg) was injected intraperitoneally as a hypnotic. The time of onset of sleep was taken as time between injection of the hypnotic and the loss of righting reflex; the sleeping time was taken as the interval between the loss and the reappearance of the righting reflex (i.e. 3 successive rightings in a period of 1 min.).

Analgesiometry

Thermal method: Heat was applied to the tail of rats using ‘Analgesiometer’ (Techno, Lucknow). A sudden flick of the tail was taken as the end point, which was always determined in duplicate. Heat intensity was adjusted to give a control reaction time of 4 sec. Different doses of chlordiazepoxide or diazepam were injected intraperitoneally in rats (in groups of 10) and the reaction time was determined again at 15 min. intervals for next 3 hr.

Phenylnquinone: Male mice, 18 to 25 gm were employed in chemical analgesiometry which is based upon antagonism by drugs of the typical ‘writhing syndrome’ (26) produced by an intraperitoneal injection of 0.25 ml of 0.02% solution of phenylquinone in 5% alcohol. Mice in groups of 7 were given chlordiazepoxide or diazepam intraperitoneally and subjected to the analgesiometry 1 hr later. In pilot experiments all the 50 control mice exhibited writhing within 30 min. of the injection of phenylquinone. Absence of writhing within a period of 30 min. was taken as evidence of analgesia.

Mechanical method: The method was similar to that described by Bianchi and Franceschini (5). Male albino mice weighing from 18 to 24 gm were used. An artery clip with branches enclosed in rubber tubing was applied to the root of the tail for 30 sec. Only the mice which made attempt to turn and remove the clip within this time were selected for experiments. Benzodiazepines were administered as in the chemical method; morphine sulphate was used as a reference drug and was injected intraperitoneally. Percentage of the animals failing to react to the obnoxious stimulus within 30 sec was determined from all or none response at various times after the injection of the drugs.

Potentiation of analgesia: Mice in groups of 7 were given an intraperitoneal injection of morphine sulphate (1.3 mg/kg, i.e. ED 20) 40 min. after the intraperitoneal injections of different doses of the benzodiazepines or saline (in control groups). Analgesia was tested by the mechanical method 20 min. after the injection of morphine (i.e. at the time of peak effect) and at various intervals thereafter.

Locomotor activity

Female albino mice weighing between 18 to 24 gm were used. The animals were in the laboratory for at least two weeks before use and they were given standard laboratory diet. The
locomotor activity of individual animals was studied with a revolving activity cage mounted on ball bearing axel and fitted with arrangement to record the rotations. The experiments were always conducted in a quiet room under uniform lighting conditions and at the same time in the day (late afternoon). Each animal was housed separately for 2 hr before the experiment.

Effect of benzodiazepines: Increasing doses of chlordiazepoxide and of diazepam were given intraperitoneally in groups of mice (5 animals each) : control groups received equivalent quantity of normal saline. The locomotion was recorded for 10 min. periods at 1 hr, 2 hr and 3 hr after the injections. The animals were in the activity cage for 10 min. before the record was obtained.

Antagonism of drug induced motor activity by benzodiazepines

Groups of mice (5 animals each) were injected with a fixed dose of chlordiazepoxide (25 mg/kg) or diazepam (7 mg/kg) intraperitoneally: control groups were injected with equivalent quantity of normal saline. 45 min. later, either cocaine (10 mg/kg), dexamphetamine (5 mg/kg) or methylphenidate (10 mg/kg) was injected intraperitoneally. The motor activity was recorded as described above 15 min. after the injection of the stimulants and again at 1 hr and 2 hr thereafter.

Mice. Effect of benzodiazepines on spontaneous locomotor activity and on increase in activity induced by stimulant drugs. The locomotor activity was recorded using a revolving cage for periods of 10 min. at 1 hr, 2 hr and 3 hr after intraperitoneal administration of saline (○——○), chlordiazepoxide (25 mg/kg, ◦——◦) and diazepam (7 mg/kg, ◦——◦) in mice in groups of five. The mean activity is expressed as % of (saline treated) control group, Group A; spontaneous activity (no stimulant was used). Group B, C and D; injected intraperitoneally with cocaine (10 mg/kg), methylphenidate (10 mg/kg) and dexamphetamine (5 mg/kg) respectively. The stimulants were given 15 min. before the first record, i.e., at 1 hr.
The results in all tests were interpreted in terms of activity observed in saline treated control groups.

Synergism with alcohol

The method followed was the one described by Friedman and Ingalls (9) for study of enhancement of alcoholic intoxication by drugs. Unfasted female rats, weighing between 180 and 200 gm were used. The animal was placed precisely on a wooden plane, facing the elevated rim of the plane. The plane was now inclined so as to turn through 90° in a period of 5 sec. The angle at which the animal slides back was precisely recorded by a device involving a 'shutter release' mechanism. The 'critical sliding angle' was determined as a mean of five successive observations.

The incoordination was studied in rats (in groups of 5) one hr after intraperitoneal administration of increasing doses of the benzodiazepines or 15 min. after intraperitoneal administration of increasing doses of 50% alcohol. An increment of 7° in the critical sliding angle (as determined from the difference between the control and the test readings) was arbitrarily taken as evidence of incoordination and the results used as quantal data for further analysis.

In studies on synergism between alcohol and the benzodiazepines the groups of rats were injected with chlordiazepoxide (15 mg/kg) or diazepam (5 mg/kg) intraperitoneally; 45 min. later the animals were given 900 mg/kg (i.e. ED 20) of alcohol intraperitoneally as a 50% solution. The incoordination was determined 15 min. after the injection of alcohol.

Drugs: The drugs used were: phenobarbitone sodium, pentobarbitone sodium, strychnine hydrochloride, dexamphetamine sulphate, morphine sulphate, cocaine hydrochloride and methylphenidate hydrochloride. The doses are of the salts. Diazepam (Valium, Roche) was used as 5 mg/ml solution. Chlordiazepoxide (Librium, Roche) was dissolved in the special solvent just prior to the use. Solutions of pentylenetetrazole, alcohol and of phenylquinone were prepared fresh prior to use. Alcohol and phenylquinone were injected as described above. Other intraperitoneal injections were made in a constant volume of 2 ml/kg.

Statistical methods: In tests designed as quantal end point procedures, at least three and often five dose levels were established between the limits of 100% effect and no effect. The data were analysed by semigraphic method of Litchfield and Wilcoxon (18). Conventional methods (6) were used for analysis of other data.

RESULTS

Anticonvulsant activity

Anti-MES activity. (Table 1). Both chlordiazepoxide and diazepam were active in modifying the MES pattern in rats as well as in mice. In each species the regression lines for both agents indicating the anti-MES activity were statistically significant (p<0.05) and did not deviate significantly from parallelism. Diazepam was significantly more potent than chlordiazepoxide [the potency ratios and 19/20 confidence limits: 3.25 (2—5.26) in mice and 3.5 (2.35—5.32) in rats]. However, the deviation from parallelism was significant when these lines
Anti-MES activity of the benzodiazepines was not associated with favourable 'protective indices' in mice or rats, as the doses required to produce anti-MES effect were nearer to those producing overt neurotoxicity. In the NT tests diazepam was found to be significantly more potent than chlordiazepoxide [the potency ratios and 19/20 confidence limits : 3.26 (1.91—5.142) for mice and 4.86 (2.13—11.07) for rats]; however, the protective indices associated with the anti-MES activity of these agents were comparable in both species.

**Antimetrazole activity.** (Table 1). Both chlordiazepoxide and diazepam exhibited considerable antimetrazole activity in rats. The regression lines for this activity were statistically significant (p < 0.05) and deviation from parallelism was not significant. Diazepam was more potent than chlordiazepoxide in this test also [potency ratios and 19/20 confidence limits : 3.5 (1.88—6.51)]. The antimetrazole activity was associated with much more favourable protective indices.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Animal</th>
<th>1 Neurotoxicity</th>
<th>1 Anti-MES activity</th>
<th>2 Antimetrazole activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTD 50</td>
<td>ED 50</td>
<td>P.I.</td>
<td>ED 50</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>Mice</td>
<td>50.7</td>
<td>39.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33.8—76.05)</td>
<td>(30.0—50.7)</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>40.77</td>
<td>27.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(27.18—61.55)</td>
<td>(21.6—33.75)</td>
<td>(2.19—5.6)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Mice</td>
<td>16.8</td>
<td>12.0</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11.2—25.2)</td>
<td>(8.0—18.0)</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>13.01</td>
<td>7.7</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9.28—18.2)</td>
<td>(5.5—10.78)</td>
<td>..</td>
</tr>
<tr>
<td>Phenobarbitone sodium</td>
<td>Mice</td>
<td>..</td>
<td>26.1</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(20.0—33.9)</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>..</td>
<td>12.5</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(10.4—15.0)</td>
<td>..</td>
</tr>
</tbody>
</table>

1 Determined by the method of Swinyard et al. (29).
2 Challenging dose—90 mg/kg, s.c.

**Antistrychnine activity**

The benzodiazepines could abolish the strychnine toxicity in guineapigs only after comparatively large doses. The regression lines indicating this activity were statistically significant.
the ED 50s (with 19/20 confidence limits), in mg/kg, were 200 (66.6 - 600) and 10 (40.82 - 245) for chlordiazepoxide and diazepam, respectively. The deviation of the lines from parallelism was not significant; however, the lines were comparatively flat and the agents did not differ significantly in potency. Though we did not study the neurotoxicity in guinea-pigs after doses of these drugs effective against strychnine the animals were heavily sedated and ataxic indicating rather unfavourable protective indices.

**Sleeping time**

Chlordiazepoxide (4 mg/kg) or diazepam (2 mg/kg) had very little effect on central nervous system in guinea-pigs prior to the administration of pentobarbitone. However the agents significantly reduced the time of onset of sleep and increased the duration of sleep due to pentobarbitone (Table II).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Mean time for onset of 'sleep' (min.±s.e. of mean)</th>
<th>Mean duration of 'sleep' (min.±s.e. of mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (i.p., 1 hr before) (Control group)</td>
<td>50</td>
<td>34.08±17.4</td>
<td>48.85±20.8</td>
</tr>
<tr>
<td>Chlordiazepoxide (4 mg/kg, i.p., 1 hr before)</td>
<td>25</td>
<td>11.6±7.95</td>
<td>82.06±31.3</td>
</tr>
<tr>
<td><strong>(p &lt; .01)</strong></td>
<td></td>
<td><strong>(p &lt; .01)</strong></td>
<td></td>
</tr>
<tr>
<td>Diazepam (2 mg/kg, i.p., 1 hr before)</td>
<td>25</td>
<td>7.64±1.8</td>
<td>150±60</td>
</tr>
<tr>
<td><strong>(p &lt; .01)</strong></td>
<td></td>
<td><strong>(p &lt; .01)</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Pentobarbitone sodium (30 mg/kg) was administered intraperitoneally in all groups.

**Values in parenthesis indicate the probability for the difference between the test and the control (saline treated) groups.

**Analgesic activity**

Chlordiazepoxide (up to 30 mg/kg) or diazepam (up to 7 mg/kg) did not show any analgesic activity when tested by thermal method in rats or by mechanical method in mice.

In control mice the phenylquinone writhing syndrome was characterized by intermittent contractions of the abdomen, twisting and turning of the trunk, opisthotonous and extension of hindlegs. After chlordiazepoxide (30 mg/kg) or diazepam (7 mg/kg) the writhing syndrome was somewhat modified in most of the animals. Thus the extension of hindlegs and the torsion movements of the body were abolished while the animals still exhibited opisthotonous and contraction of the abdomen, so that the belly touched the ground. As complete abolition of all features of writhing syndrome was considered as evidence of analgesic activity it was concluded that in the doses employed, the benzodiazepines had no analgesic activity when tested with the phenylquinone method. However the method was not employed in studies of potentiation of morphine analgesia in view of the aforesaid modification of writhing by the benzodiazepines.
Potentiation of analgesia: ED 50 of morphine sulphate in mice when determined at 20 min. after the intraperitoneal injection by the mechanical method was 4 mg/kg (95/20 confidence limits, 2 and 8). ED 20 was 1.3 mg/kg. Chlordiazepoxide or diazepam, injected intraperitoneally 40 min. before the injection of morphine increased the proportion of mice showing analgesia (Table III). The duration of morphine analgesia also was apparently increased. None of the animals injected with morphine alone or with a sequential combination of a benzodiazepine and morphine exhibited the Straub-Herrman tail reaction.

### Table III

<table>
<thead>
<tr>
<th>Agent</th>
<th>Percentage showing analgesia (%)</th>
<th>min. after intraperitoneal injection of morphine sulphate (1.3 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Normal saline</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>15 mg/kg</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>10</td>
</tr>
<tr>
<td>Diazepam</td>
<td>3.5 mg/kg</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7.0 mg/kg</td>
<td>10</td>
</tr>
</tbody>
</table>

*Injected intraperitoneally 40 min. before.
*Estimated by the mechanical method (details in text).
*Value differs significantly ($p < 0.05$) from saline treated (control) group.

### Locomotor activity

Chlordiazepoxide (up to 25 mg/kg) or diazepam (7 mg/kg) had no appreciable effect on spontaneous locomotor activity of mice (Fig. 1). Doses higher than this were not studied as they tended to produce ataxia in a variable proportion of the animals.

The stimulant effect of dexamphetamine, methylphenidate or cocaine was fully developed 15 min. after the injection of stimulants. In doses mentioned above the benzodiazepines (injected 45 min. earlier) did not counteract the stimulant effect of any of the three drugs. On the other hand there was some augmentation of the stimulation due to methylphenidate though the effect was not statistically significant. Moreover some of the animals treated with the benzodiazepines showed evidence of ataxia 15 min. after the injection of cocaine; none of the animals pretreated with saline exhibited this feature. This might explain the apparent reduction in motor activity of drug treated mice at 15 min. after the injection of cocaine.

### Synergism with alcohol

The critical sliding angle varied between 38° and 42° in different animals in control period, though it was remarkably constant for the same animal at different times.
The sliding angle was little affected one hr subsequent to the intraperitoneal administration of saline, chlordiazepoxide (up to 30 \( \text{mg/kg} \)) or diazepam (10 \( \text{mg/kg} \)). However, a dose related intoxication was seen 15 min. after intraperitoneal injection of 50% alcohol, as evidenced by an increment in the sliding angle. The ED 50 of alcohol (\( \text{g/kg} \)) was 1.7 (with 1.42 and 2.04 as 19/20 confidence limits). ED 20 was 0.9 \( \text{g/kg} \).

Both chlordiazepoxide and diazepam (administered intraperitoneally 45 min. before) significantly enhanced the intoxicating effect of ED 20 of alcohol (Table IV).

**TABLE IV**

*Effect of benzodiazepines on the intoxicating effect of alcohol in rats*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Number of animals</th>
<th>Percentage showing intoxication 15 min. after intraperitoneal injection of alcohol (900 ( \text{mg/kg} ), as 50% solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (control group)</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Chlordiazepoxide (30 ( \text{mg/kg} ))</td>
<td>10</td>
<td>*60</td>
</tr>
<tr>
<td>Diazepam (10 ( \text{mg/kg} ))</td>
<td>10</td>
<td>*70</td>
</tr>
</tbody>
</table>

*Value significantly different (\( p < 0.05 \)) from the saline pretreated (control) group.

'Intoxication' was determined by the inclined plane test as described by Friedman and Ingalls (9). An increment of 7° in the critical sliding angle was considered as evidence of intoxication.

**DISCUSSION**

Anticonvulsant effect of chlordiazepoxide or diazepam has been repeatedly confirmed in past against electroshock seizures (1, 7, 22) and against convulsions induced by anoxia (28), metrazole (1, 23) or bemegride (15, 27). Diazepam has also been reported to prevent the effects of stimulation of various areas of cat's brain (11). On the strength of these and other observations the agents have also been investigated against human epilepsy. We found that the protective indices of the benzodiazepines in relation with anti-MES activity were not very favourable though they were favourable in relation with antimetrazol activity. Nevertheless, these pharmacological findings in themselves do not appear to indicate the status of these agents as antiepileptic drugs. Firstly, the single dose-toxicity data, as encountered in the present study does not clearly indicate the margin of safety involved in the clinical use of the drugs. Secondly, the antagonism of metrazole by drugs below the hypnotic level is thought to be a measure of sedative activity and may not necessarily indicate suppression of epileptogenic focus (30). Useful supplementary data could perhaps be obtained by using the drugs in human studies on convulsions associated with electroshock therapy. In any case it appears probable that undesirable sedation may accompany the full antiepileptic doses of benzodiazepines in clinical practice.
In the present study the benzodiazepines exhibited considerable myorelaxant effect in comparatively small doses, while the strychnine toxicity could be abolished only after high doses of these agents. The agents had very little effect on neuromuscular transmission (authors' unpublished observations). This agrees with the view that both these agents are centrally acting muscle relaxants (14) with very little action on spinal cord.

In doses not affecting the locomotor system, the benzodiazepines were found to have no effect on spontaneous locomotor activity of mice. Though the significance of motor activity of small animals is not clear many tranquilizers reduce such activity. On the other hand chlordiazepoxide increased the level of exploratory activity of inexperienced rats (19); a central stimulation following the clinical use of this agent is also reported (13). The benzodiazepines thus appear to differ from typical tranquilizers in this respect as well as in possessing anticonvulsant activity, though like this class of agents, they prolonged the barbiturate sleeping time. The benzodiazepines were also ineffective in counteracting the hyperactivity induced by stimulants which could have been largely, though not exclusively, mediated through the cortical stimulation. This is in agreement with the view that the benzodiazepines exert little cortical inhibitory effect. On the other hand hyperactivity induced by methylphenidate appeared to be somewhat augmented by benzodiazepines, though this effect could not be satisfactorily demonstrated. Moreover, these agents appeared to synergize with cocaine in some animals to produce transient ataxia. In this connection it is of interest to note that hyperactivity induced by amphetamine in rats is increased by amylbarbitone and some of the tranquilizers (24).

We found that the benzodiazepines which are devoid of any analgesic activity enhanced the analgesia induced by morphine. Absence of Straub-Herrman tail reaction in mice showing the peak analgesic effect suggests that all effects of morphine are not probably augmented and that the augmentation is central in origin. In view of restricted number of experiments, this potentially significant phenomenon requires further elucidation.

Present experiments provide evidence suggestive of synergism between alcohol and the benzodiazepines. A synergism between diazepam and alcohol in affecting the attentive motor performance (but not the mental performance) has been demonstrated in a human study (12). These observations appear to be significant in view that the benzodiazepines are clinically used in the management of chronic alcoholism (14).

**SUMMARY**

Diazepam was found to be more effective than chlordiazepoxide as anticonvulsant in MES test in mice and rats. However the protective indices for both these agents were comparable due to greater neurotoxicity of diazepam. Both agents were considerably active in metrazole tests; the protective indices were also much more favourable in this test. Only in high doses the drugs exhibited antistrychnine activity in guineapigs.

The benzodiazepines exhibited no analgesic activity when tested with thermal method in rats, or by mechanical or chemical methods in mice. In doses not affecting the locomotor system the agents did not affect the spontaneous or drug induced locomotor activity of mice. However, both drugs enhanced the barbiturate sleeping time in guineapigs, enhanced the intol-
eating effect of alcohol in rats and also enhanced the morphine induced analgesia in mice. The data agree with the current view that these drugs affect only selected discrete area of brain and are not general depressants of central nervous system.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. E.M. Best, Dean, B.J. Medical College, Ahmedabad for providing facilities to carry out this work. Chlordiazepoxide (Librium) and diazepam (Valium) were generously supplied by Roche Products, Bombay. We are thankful to Dr. O.D. Gunja, Professor of Pharmacology, Medical College, Baroda for providing a sample of phenylquinone.

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EFFECTS OF MORPHINE AS AN ANAESTHETIC AGENT

Morphine as an anaesthetic required for surgical procedures has long been the standard. It is a potent analgesic, but reports in the literature have denied this to be a respiratory depressant. For this reason, nalorphine has been used as a respiratory stimulant in patients who require anaesthesia. It has been claimed that nalorphine causes the respiratory depression caused by morphine.

The purpose of the present study was to evaluate the effects of morphine and nalorphine on respiratory depression induced by anaesthesia in mice.

The volume of loss of righting reflex was measured by the method of Ghosh et al. (1968). A total of 120 mice were used. Each group of 10 mice was given chloroform and halothane i.p. 30 min. before the experiment. The respiratory depression was measured by the percentage reduction in the righting reflex using the method described by Snedecor (1965).

The S.I. of ether is significantly lowered by morphine alone or in combination with nalorphine. The S.I. of ether remains unaltered with nalorphine alone.

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