Our previous pharmacological screening of a series of s-triazolo [4, 3a] [1, 4] benzodiazepines (1), originally synthesized by Meguro and Kuwada (2), has demonstrated that several compounds are more effective in the central nervous depression than the well-known analogues, diazepam and nitrazepam. In the present study, two of these compounds, 8-chloro-6-phenyl-4H-s-triazolo [4, 3a] [1, 4] benzodiazepine (D-40TA) and its 1-methyl analogue (D-65MT) (Fig. 1) were undergone more detailed pharmacological evaluation of effects on central nervous system, in comparison with other benzodiazepines and chlorpromazine.

**Fig. 1. Chemical structures of D-40TA and D-65MT.**

**MATERIALS AND METHODS**

Unless otherwise stated, D-40TA, D-65MT and other benzodiazepines, synthesized in this Chemical Laboratories, were suspended in 5% arabic gum solution for oral and intraperitoneal administration. All other following agents were dissolved in physiological saline for the administration: chlorpromazine HCl (Contomin®), morphine HCl, mecamine HSO₄, methamphetamine H₂SO₄, apomorphine HCl, methylhexabital Na (Cyclopan natrium®), chlorprothixene (Chlorthixen®), metrazol HCl (Cardiazol®), reserpine (Serpa-sil®), tetrabenazine methanesulfonate (Regu-line®) and oxotremorine.

The experimental animals used were male ICR-JCL mice (18-23 g), male SD-JCL rats (150-300 g), cats of either sex (2.1-3.2 kg), beagle dogs of either sex (5-10 kg), and Japanese and cynomolgus monkeys of either sex (5-10.5 kg).

The ED₅₀'s in all-or-none (quantal) response or LDₕ₀'s and their confidence limits (p = 0.05) were calculated by the method of Litchfield and Wilcoxon (3). The ED₅₀'s in graded
response were determined graphically on the logarithmic dose-response curve.

*Acute toxicity and gross behavior:* Following a single oral or intraperitoneal administration of D-40TA or D-65MT in groups consisting of 6-10 mice each and of 6 rats each, acute toxicity was evaluated on 7th day in association with observation of gross behavior. Four cats and 5 beagle dogs were used once a week for observing the effect of oral dosage of D-40TA on gross behavior.

*Spontaneous locomotor activity in mice:* Groups of 10 mice given orally either saline or various doses of the test agent were placed individually in an activity wheel 30 minutes later, and the revolution counts during the next 1 hour were determined. The ED$_{50}$ dose of the test agent to reduce the mean revolution count to half the value of the control group was determined.

*Morphine-excitation in mice:* Either saline or various doses of the test agent was given orally 45 minutes prior to subcutaneous injection of morphine HCl, 50 mg/kg, in groups of 5 mice. Morphine-treated mice were placed individually for 1 hour in a round transparent cage in order to evaluate the frequency of circle movements and maximal score of tail-erection during 30-second period each every 10 minutes. The degree of tail-erection was rated by the method of Holton (4). From the sum of these 6 determinations, the ED$_{50}$ of the test agent to reduce the mean value of the respective response to half the control value was determined.

*Mescaline-scratch behavior in mice:* Mescaline H$_2$SO$_4$ (100 mg/kg) was given subcutaneously to groups of 5 mice 45 minutes following oral dosage of the test agent. The frequency of scratching episodes in an individual transparent cage was then counted for 1 minute each every 10 minutes for 50 minutes. From the sum of these 5 determinations, the mean value in each group was determined. The ED$_{50}$ of the test agent to reduce the scratching frequency to half the control value was calculated.

*Methamphetamine-excitation in mice:* Following oral dosage of the test agent, groups of 8 mice were treated subcutaneously with 10 mg/kg of methamphetamine H$_2$SO$_4$, 10 minutes later. The intensity of stereotyped gnawing and agitated behaviors was scored according to the method of Quinton and Halliwell (5) by observing the mice for 1 minute each every 30 minutes for 1.5 hours in an individual cage. The mean score in each group was determined from the sum of 3 determinations in each mouse, and the ED$_{50}$ of the test agent to reduce the score to half the saline-treated control value was evaluated.

*Apomorphine-gnawing in rats:* Apomorphine HCl (5 mg/kg) was injected intravenously 30 minutes after intraperitoneal dosage of D-40TA or other benzodiazepines, or 1 hour after oral dosage of chlorpromazine in groups of 5 rats. The intensity of stereotyped gnawing and agitated behaviors provoked by apomorphine was scored by observing the rat for 1 minute each every 5 minutes for 30 minutes in an individual transparent cage, as described in anti-methamphetamine test. The ED$_{50}$ of the test agent was determined from the number of rats failing to gnaw.

*Septal lesioned rats:* Bilateral electrolytic lesion, utilizing anodal DC current of 2 mA for 20 seconds, of the septal area of the rats was performed stereotaxically under
pentobarbital anesthesia. After several days, the attenuating effect of the test agent on hyperirritability to a rod presented and handling was evaluated in groups of 5 rats each. The degree of hyperirritability was scored by the method of King and Meyer (6), before and after intraperitoneal dosage of the test agent. The ED_{50} of the test agent to reduce the score of hyperirritability to half the pre-drug control value at the peak of drug effect was determined.

**Olfactory bulb-ablated rats:** Bilateral olfactory bulbs of the rats were exposed by removing the skull 9 mm anterior to bregma with a dental drill and were ablated by aspiration under pentobarbital anesthesia. After more than 7 days, attack or defensive response of each rat to i) a rod presented visually before the rat’s snout, ii) a light tactile stimulation of the back with a rod, and iii) clipping the tail with forceps was assessed before and at 0.5- or 1-hour intervals after intraperitoneal administration of the test agent. Each of these parameters was scored from 0 for no response to 5 for violent attack behavior such as biting. The rats showing the total score more than 13 were randomly divided to groups of 6 rats each for drug testing. Percent depression of aggressiveness by drug was calculated by comparison of the post-drug score with the pre-drug score.

The rats, which did not necessarily show the emotional score more than 13 but killed a mouse placed in the rat’s cage, were assigned to groups of 6 rats each for observing the drug effect on muricidal behavior. These rats were presented a mouse for 10 minutes each prior to and at 0.5- or 1-hour intervals after the drug administration, and % depression of the test agent on the muricidal response was calculated. The same rats were used repeatedly but once a week for drug testing.

**Aggressiveness in monkeys:** Three consistently hostile, cynomolgus monkeys were used repeatedly but once a week for drug testing. The behaviors of the animals in an individual cage were rated on a check-list devised by Norton (7), with some modifications, prior to and every 30 minutes for 3 hours after oral dosage of the test agent. Each component of aggressive behaviors such as forward lunge, baring teeth, grabbing, attacking posture, vocalization and defensive behavior was scored as 3 for response to confrontation with the observer without any other stimulus, 2 for response to threat with a glove, 1 for response to presentation of a rod and 0 for indifference to either stimulus. The attenuating activity of the test agent on aggressive behavior was expressed as the ED_{50}, dose reducing the aggressive score to half the pre-drug control score at the peak of drug effect.

**Conflict behavior:** The effect of the test agent on fear and apprehension of rats experimentally produced by a combination of reward and punishment was examined. The principle of the procedure used was based on that described by Geller and Seifter (8). The apparatus used was a small sound-attenuated Skinner box (Grason-Stadler Co.) equipped with a lever, a device for presentation of milk reward, an electrifiable grid floor, and a tone generator. Twenty one rats maintained on limited feeding were trained to press a lever in order to obtain a reward on an 1-minute variable interval schedule (VI-1) or once every 1 minute on the average (9). After establishment of the stable lever-pressing responses, a 2-minute non-aversive tone stimulus was presented once every 20 minutes as the signal
that every lever-pressing response was rewarded with milk but simultaneously punished with a brief (0.5 seconds) foot-shock. The shock intensity was adjusted for the animals to show only 0-4 lever responses during each 2-minute tone (conflict) period. Switching and timing circuits allowed automatic programming and recording of lever responses, rewards and foot-shocks received. Experimental sessions of 2-hour duration were carried out after intraperitoneal administration of either saline or the test agent.

Non-discriminated avoidance behavior: Twelve rats were trained on continuous non-discriminated avoidance schedule developed by Sidman (10) in a Skinner box until stable lever-pressing avoidance response was achieved. The animals received a brief (0.5 seconds) foot-shock once every 20 seconds unless they pressed a lever to postpone the occurrence of the next foot-shock for 40 seconds. The well-trained animals were subjected to this schedule continuously for 1 hour before and for 3 hours after oral administration of the test agent. Programming and recording of lever-pressing responses and foot-shocks received were carried out automatically by switching and timing circuits.

Discriminated avoidance: In the box equipped with a buzzer, a vertical pole and an electrifiable grid floor, the rats were trained to avoid a foot-shock by climbing onto a pole (avoidance response) during an 8-second warning or subsequent 3-second silent period in more than 90% of trials. An 8-second foot-shock resulting from the avoidance failure was terminated by pole-climbing response of the rats (escape response). This trial was repeated every 1 minute automatically. Twelve well-trained rats were subjected to 10 trials each before and at 30-minute intervals after oral administration of the test agent. The same rat was used repeatedly but once a week for drug testing.

Barbiturate or chlorprothixene hypnosis: Non-hypnotic dose of methylhexabital Na (MHB, 60 mg/kg) or chlorprothixene (CPT, 2 mg/kg) was given intraperitoneally to groups of 10 mice 30 minutes or immediately after oral administration of the test agent in various doses. Thereafter, the number of mice which lost the righting reflex for more than 30 seconds with MHB and 3 minutes with CPT was counted in order to determine the ED₅₀ of the test agent, dose producing hypnosis in half the mice.

Sleep induction test in monkeys: Two docile Japanese monkeys semi-restrained chronically in a chair with a neck yoke were placed in a sound-attenuated room for behavioral observation through a TV camera, for 5.5 hours from 9:00 a.m. to 2:30 p.m. Following the adaptation period of 30-minute duration, an adequate volume of orange juice alone or with the test agent suspended was taken orally from a small feeding bottle, and the total time-length spent by sleeping behavior, i.e. head-drop and complete eye-closure, was measured for consecutive 5 hours. On a basis of the total time-length during 3 hours from 30 minutes after the drug administration, % increase of the sleeping time by the test agent was calculated by a simple formula: \[ \frac{A-B}{3} \times 100 \] where A and B were the total sleeping time under the test agent and the mean sleeping time of 5 or 6 control experiments with orange juice alone, respectively. The dose of the test agent to cause 50% increase in the sleeping time was determined from the logarithmic dose-% sleep increase curve in each monkey, and the mean value of these doses obtained in two monkeys was taken as
mean hypnogenic dose (HD₃₃). The animals were used twice a week as a rule; the first session for the control experiment and the second for drug testing.

**Anticonvulsion tests:** Maximal electroshock seizure (MES) was produced essentially by the technique of Swinyard et al. (11). Mice were challenged with a supramaximal electroshock (30 mA, 0.2 seconds) delivered through corneal electrodes 45 minutes after oral dosage of the test agent. The ED₃₀ of the test agent preventing a hind limb extension in 50% of the mice was calculated. Six or 8 mice per dose were used.

Groups of 6 mice similarly pretreated with the test agent were challenged with subcutaneous injection of 150 mg/kg of metrazol HCl. The ED₃₀ of the test agent to prevent convulsion and death in 50% of mice for 1 hour after metrazol challenge was evaluated.

**Muscle relaxant tests:** Three kinds of tests were used as measures of muscular atony and motor inco-ordination. In inclined screen test, mouse was placed on a 60° inclined wire screen at 10-minute intervals after oral administration of the test agent. The ED₃₀ of the test agent to cause 50% of the mice to slide off a screen within 1 minute was determined. Six or 8 mice per dose were used.

In rota rod test, the rats trained previously to remain on a rota rod of 3.5 cm in diameter (8 revolutions/minute) for more than 1 minute were used. The animals were subjected to this test at 15- or 30-minute intervals after intraperitoneal dosage of the test agent, and the ED₃₀ of the test agent to cause 50% of the rats falling off a rota rod within 20 seconds was determined. Six rats per dose were used.

In rearing test, six cats which could rear up skillfully to catch food presented from a hole in the ceiling of an observation cage, were used once a week for drug testing. When muscle relaxation occurred by a drug, the animal was incapable of supporting the body upon hind limbs when reared and thus fell down backwards. The oral dose of the test agent to produce falling-down response in one of 3 cats was determined as the minimum muscle relaxant dose (MMD).

**Anti-reserpine and anti-tetrabenazine tests:** The effect of D-40TA on reserpine-induced hypothermia and blepharoptosis, which has been known to be antagonized by tricyclic antidepressants, was examined in groups of 8 mice each. Each mouse received 2 mg/kg of reserpine intraperitoneally 1 hour after oral administration of either D-40TA or saline. The degree of reserpine-induced hypothermia was determined by measuring the rectal temperature with an electronic thermometer before and 2, 4 and 6 hours after administration of reserpine. The 6-hour integral hypothermia calculated by the method of Brittain and Spencer (12) was compared in the groups treated with saline alone, reserpine alone and reserpine combined with D-40TA. The degree of blepharoptosis was also scored from 0 for complete opened-eye to 4 for complete closed-eye.

As another test detecting tricyclic antidepressant-like action, antagonism of D-40TA against tetrabenazine-induced catalepsy and blepharoptosis was examined. Groups of 5 rats received 30 mg/kg of tetrabenazine subcutaneously immediately after intraperitoneal injection of either D-40TA or saline. Maintenance of the imposed posture for more than 30 seconds when one of forepaws of the rat was placed on a rubber stopper 7 cm high,
was regarded as catalepsy. The degree of blepharoptosis was also rated in the same procedure as that in anti-reserpine test. Both tests were carried out at 30-minute intervals after administration of tetrabenazine.

**Anti-oxotremorine test:** One mg kg of oxotremorine was administered subcutaneously to groups of 8 mice 30 minutes after intraperitoneal injection of the test agent. The intensities of oxotremorine-induced tremor and salivation were scored on a rating scale devised by Rathbun and Slater (13) every 5 minutes for 30 minutes, and the total value of 6 scorings was compared in the drug-treated and control groups.

**Anti-apomorphine emesis test:** D-40TA was given orally to 4 beagle dogs 1 hour before subcutaneous injection of a standard dose (0.1 mg/kg) of apomorphine HCl, which induced emesis in all control dogs. The onset and frequency of emesis were measured for 30 minutes after apomorphine challenge. The animals were used once a week for test.

**RESULTS**

1. **Acute toxicity and gross behaviors**

   **Mice and rats:** A single oral dosage of D-40TA produced the following behavioral changes in mice and rats; one mg/kg produced some restless symptoms consisting of rearing, grooming and sniffing for the initial 5-10 minutes, and subsequently sign of drowsiness for 30 minutes. After dosage of 5 or 10 mg/kg, the animals gradually became sedated in crouched or prone position in association with frequent eye-closure and decrease in flight behavior to tactile stimulation, these sedative symptoms persisting for about 3 hours. A dosage of 50 or 100 mg/kg caused moderate or marked ataxia and sedation with recumbent posture in association with partial or complete eye-closure for 6-8 hours after the administration. The righting reflex, however, was still intact. After a dosage of 500 mg/kg or more in mice and 1500 mg/kg or more in rats, reduction or loss of the righting reflex, opisthotonus when touched, lowered body temperature were noted. Although some of these mice and all rats recovered 72 hours later, other mice died from respiratory arrest following the intoxicated symptoms consisting of profound salivation, lacrimation and gradual emaciation. Maximal mortality was observed on 3rd or 4th day. Lethal symptoms observed after intraperitoneal dosage of D-40TA in rats were also similar to those after oral dosage in mice. D-65MT was about twice as potent as D-40TA in inducing sedation in mice and rats. Acute toxicities of both compounds are shown in Table 1.

### Table 1. Acute toxicity.

<table>
<thead>
<tr>
<th>Species of animals</th>
<th>Route of admin.</th>
<th>LD$_{50}$, mg/kg (confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D-40TA</td>
</tr>
<tr>
<td>Mice (♀)</td>
<td>p.o.</td>
<td>678 (611–753)</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>438 (393–485)</td>
</tr>
<tr>
<td>Rats (♂)</td>
<td>p.o.</td>
<td>&gt;2000</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>885 (750–1044)</td>
</tr>
</tbody>
</table>
**CENTRAL DEPRESSANT ACTIVITY OF D-40TA**

**Cats:** D-40TA was given orally as a powder in gelatine capsule. The animals given the dose ranging from 0.25 to 2 mg/kg showed ataxic hypermotility for 1–2 hours from 30 minutes after the administration, and became drowsy in crouched position intermittently for the next 3–5 hours. Twenty mg/kg produced the above-mentioned symptoms more remarkably and persistently but no other symptom.

**Dogs:** Among 5 dogs used, two were friendly to the observer, but the other three were very timid and always retreated in a corner of cage with fearful eye when the observer approached. D-40TA was administered orally to these dogs by the same procedure as in cats. For 3–4 hours after a dosage of 2 mg/kg, all dogs became restless in a characteristic of frequent rearing and pawing a front door of the cage, and wagged their tails violently as if to ask for contact with the observer. In fact, even timid dogs were fearless so markedly as to lick or bite lightly the observer’s hand like a well-domesticated puppy. Eight or 10 mg/kg caused the above-mentioned tamed behavior for more than 6 hours in all animals but slight staggering of the lower body trunk at 1 hour only in one animal. Slight or moderate ataxic gait was clearly observed for about 4 hours from 15 minutes after a dosage of 30 mg/kg in all animals. When given 50 mg/kg, the tamed behavior accompanied with ataxic gait for the initial 3.5–4 hours and the frequent sleep in crouched or recumbent position for the next 5 hours were noted in all animals. These sleeping dogs were relatively easily arousable by an ordinary acoustic or tactile stimulation and could stand up with staggering after arousal. After 20–24 hours, all these animals recovered completely via the tamed behavior with or without ataxia. Other noticeable symptoms were marked palpebral ptosis and retching at 1–2 hours after a dosage of 50 mg/kg only in one dogs.

II. Effects on spontaneous locomotor activity and drug-induced excitation

1. Spontaneous locomotor activity

D-40TA, D-65MT and chlorpromazine decreased the spontaneous locomotor activity of the mice in dose-related way, and the \(ED_{50}\) values were 10.5, 6.2 and 4.9 mg/kg, respectively. On the other hand, the locomotor depression by diazepam and nitrazepam in 5–30 mg/kg was variable from 27 to 50%, decrease.

2. Antagonism against morphine-induced excitation

All benzodiazepines including D-40TA and D-65MT produced more inhibition of tail-eversion than of circle movement, while chlorpromazine was more effective against

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Circle</td>
<td>Tail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-40TA</td>
<td>12.4</td>
<td>2.8</td>
<td>7.6</td>
<td>4.1</td>
</tr>
<tr>
<td>D-65MT</td>
<td>3.1</td>
<td>0.95</td>
<td>1.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>14.8</td>
<td>14.2</td>
<td>–</td>
<td>17.2</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1.6</td>
<td>0.8</td>
<td>3.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>3.8</td>
<td>1.3</td>
<td>2.8</td>
<td>9.5</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>6.8</td>
<td>12.5</td>
<td>1.2</td>
<td>9.1</td>
</tr>
</tbody>
</table>
circle movement, as shown in Table 2. D-40TA was more active than chlordiazeoxide in inhibition of the tail response but less active than D-65MT, diazepam and nitrazepam.

3. Antagonism against mescaline-induced scratching behavior

As shown in Table 2, D-65MT was very highly active in inhibition of mescaline scratching response, while D-40TA was weakest in the test agent except chlordiazepoxide.

4. Effects on methamphetamine-induced excitation

Chlorpromazine suppressed remarkably the methamphetamine-induced stereotyped gnawing and agitation as indicated by the ED₅₀ value of 13.1 mg/kg. Neither D-40TA, D-65MT nor other benzodiazepines in 4-100 mg/kg affected the agitation significantly but appeared to rather augment the gnawing behavior.

5. Effects on apomorphine gnawing

No significant effect of D-40TA and D-65MT on the apomorphine-induced stereotyped gnawing was observed at the dose levels of 5, 10 and 20 mg/kg in rats. Likewise, diazepam and nitrazepam were also ineffective. Chlorpromazine rather potentiated the apomorphine gnawing behavior during the initial 10 minutes no matter how the interval between the administration of chlorpromazine and apomorphine challenge was altered. This drug, however, suppressed markedly the gnawing behavior thereafter, and its ED₅₀ value (confidence limit p = 0.05) was 16.2 (10.6-24.8) mg/kg.

III. Anti-aggressive effects

1. Calming effect on hyperirritability in septal lesioned rats

D-40TA and D-65MT were most potent in attenuation of hyperirritability in septal

---

**FIG. 2. Anti-aggressive and anti-muricidal activities of D-40TA and diazepam.**

- , 1.25 mg/kg; - , 2.5 mg/kg, ○, 5 mg/kg; ●, 10 mg/kg and ○, 20 mg/kg.

Shown are the mean values in 6 rats except in 4 rats for anti-muricidal activity of diazepam (1.25 mg/kg).
lesioned rats and were approximately 2 or 3 times as potent as diazepam or nitrazepam (Table 2).

2. Anti-aggressive and anti-muricidal effects in olfactory bulbectomized rats

Both D-40TA and diazepam reduced aggressiveness and abolished remarkably the muricidal behavior in olfactory bulbectomized rats (Fig. 2). It was difficult to demonstrate the comparative potency of both agents, since there was no clear-cut dose-response relationship. However, when the minimum effective doses of both agents were compared, D-40TA was roughly 2 times as potent as diazepam in both anti-aggressive and anti-muricidal activities. As far as compared at dose level of 20 mg/kg, diazepam was effective more persistently than was D-40TA in anti-muricidal activity. The maximal effects of both agents were obtained within 0.5–1 hour.

3. Taming effects in monkeys

D-65MT was most potent, and D-40TA was approximately equipotent to nitrazepam and was 2.3 times as potent as diazepam in taming the ordinarily hostile cynomolgus monkeys (Table 2). The monkeys receiving the dose of D-40TA above 0.5 mg/kg approached without hostility to the observer, and parried off only gently a rod presented and hooted frequently as if they relaxed. Furthermore, these animals were tamed so markedly as to be touched easily without a protective glove outside a cage. The monkeys with 2 mg/kg of D-40TA yawned frequently and became drowsy unless disturbed. Five mg/kg of this agent produced only slight decrease in the strength of forepaws to grip an object, but higher dose (10 mg/kg) caused slight or moderate staggering of the body trunk and slippy gait.

IV. Effects on conditioned behaviors in rats

1. Conflict behavior

A. Acute effects: In a series of acute experiments, rats were used repeatedly but once a week for drug testings. When given an effective dose of the test agent, the number of foot-shocks received during the tone period increased ("conflict" attenuation). Fig. 3 shows the data averaged from 3–12 rats at each dose level of the agents. The numbers of the lever-pressing responses and rewards during the periods of 1-minute variable interval schedule (VI) in the post-drug sessions were expressed as percentage of the saline-treated control values. The number of shocks received during the tone periods was calculated as the ratio to the saline-treated control value. However, if the rat never pressed a lever during the tone periods in the control sessions, the control value was regarded tentatively as 1 in order to make the calculation of the ratio possible.

Following the injection of 0.25 mg/kg of D-40TA, moderate conflict attenuation was observed without significant depression of the lever response rate during the VI period. The conflict attenuation appeared to be greater after dosage of 0.5 mg/kg, although considerable depression of the VI lever responses was accompanied. Higher dose (2 and 3 mg/kg), however, caused only profound depression of the VI lever responses without further increment of the conflict attenuating activity. On the other hand, diazepam and nitrazepam produced remarkable alleviation of conflict relative to depression of the VI
lever response rate as the dose was increased. Especially, chlordiazepoxide in the wide dose range from 2.5 to 20 mg/kg alleviated conflict markedly without concomitant reduction of the VI lever responses.

**B. Chronic effect of D-40TA:** Six rats exhibiting a stable VI lever response rate and conflict situation in the saline-treated control sessions were used for chronic experiments. Three mg/kg of D-40TA, the dose causing a clear-cut suppression of the VI lever response rate under single dosage, was administered intraperitoneally once every other day, and the rats were subjected to the experimental sessions of 2-hour duration after each dosage. On 2 or 3 successive days after termination of the chronic dosage of D-40TA, saline-treated control experiments were resumed. The % lever response during the VI periods and the shock ratio after the beginning of the chronic dosage schedule were calculated on a basis of the mean values obtained from at least 3 control experiments which were carried out prior to the chronic dosage schedule.

The representative cumulative records in the rat CD-10 and the data averaged from 6 rats are shown in Figs. 4 and 5, respectively. Following 3 or 4 repeated administra-
tions of D-40TA, the remarkable conflict attenuation indicated by marked increase in shock ratio was disclosed in association with gradual disappearance of the suppressed VI lever responses. Seventh dosing did not produce any longer the depression of the VI lever responses and concomitantly did alleviate the conflict situation markedly. Saline experiment on the 1st day after cessation of chronic dosage of D-40TA demonstrated similar pattern to those in the pre-drug control experiments.

2. Non-discriminated avoidance

A. Acute effects: In a series of acute experiments, rats were used repeatedly but once a week for drug testing. The numbers of lever-pressing responses and shocks received during each of three successive post-drug 1-hour periods were expressed as percentage and ratio to those during pre-drug 1-hour period, respectively. The average data ob-

![Figure 4](image-url)  
**Fig. 4.** Cumulative records representing the effect of chronic administration of D-40TA on "conflict" situation in rat CD-10. Pen offsets in baselines indicate conflict (tone) period. The slope of curves indicates the lever-pressing response rate; downward strokes of the pen superimposed on the curves during VI period show the receipt of milk reward alone, and those during tone period are the receipt of reward coupled with foot-shock.
FIG. 5. Histograms representing the summarized data of the effects of chronic D-40TA dosage on conflict behavior in 6 rats. Mean % lever responses (±S.E. as vertical bars) during VI periods (□) and mean shock ratio (±S.E. as vertical bars) during tone periods (□) on various drug days and on post-drug day are shown.

Fig. 6. Histogram illustrating the effects of single administration of D-40TA and other benzodiazepines on non-discriminated avoidance in rats. Mean % lever-pressing responses (±S.E. as vertical bars) and mean shock ratio (±S.E. as vertical bars) during 1st (□), 2nd (■) and 3rd (□) 1-hr periods after the drug administration. N: number of rats used.

D-40TA in 1-5 mg/kg caused slight or mild depression of the lever responses and increased the shock ratio to 4-6. At highest dose, 10 mg/kg, the lever responses decreased to 40-60% of the pre-drug control level, and as a consequence, the shock ratio increased to 15-20. The effect of D-40TA appeared gradually from 15-20 minutes after the ad-
ministration, persisting for more than 3 hours. Although the comparative potency of D-40TA with other reference benzodiazepines was unable to be stated precisely because of a few data available for the latter agents in the present experiments, anti-avoidance activity of D-40TA appeared approximately equivalent to nitrazepam and 3 or 4 times that of diazepam or chlordiazepoxide.

B. Chronic effect of D-40TA: In 4 well-trained rats, 10 mg/kg of D-40TA was administered daily for 4-13 days except Sunday. The representative data obtained from the rat F-4 are shown in Fig. 7. In this rat, the comparatively steady rate of 170-190 lever-pressing responses/hour and the receipt of only a few shocks/hour were observed in 3 control session carried out before the beginning of chronic dosage of D-40TA. The number of lever responses did not alter significantly for the initial 4 days of chronic dosing of D-40TA, while subsequent dosings gradually reduced the lever responses to 20-40% of the control level on 12-14th day. The number of foot-shocks received increased gradually from the initial dosing, and at final stage of chronic dosage the animal received a large number of shocks. However, almost complete restoration to the normal avoidance level was noted before each of daily dosages of D-40TA and 24 hours after cessation of chronic dosage as well. The almost same pattern of results was obtained in the other 3 chronically treated rats.

![Fig. 7. The effect of the chronic D40TA administration on non-discriminated avoidance in rat F-4. Stipple indicates the control days before and after the chronic dosage schedule of D-40TA. Number of lever-pressing response (○) and number of shocks (●) during 1-hr period prior to the administration of either saline or D-40TA. Mean number of lever-pressing responses per hour (●) and mean number of shocks per hour (●) during 3 successive 1-hr period after the administration of either saline or D-40TA.](image-url)
3. Discriminated avoidance

Table 3 shows the comparative data in the effects of D-40TA and other reference agents on avoidance and escape responses in pole-climbing procedure. Percent inhibition of avoidance responses was determined by comparing the post-drug avoidance rate with the pre-drug rate. Percent escape failure was calculated from the number of escape failure in unconditioned trials resulting from avoidance failure. D-40TA caused remarkable avoidance failure without escape failure in the doses ranging from 5 to 40 mg/kg. On the other hand, other benzodiazepines and even chlorpromazine disrupted escape behavior as well in a little higher doses than those producing avoidance failure. The effect of D-40TA on avoidance behavior was attained to the maximum 0.5 or 1 hour after the oral administration and gradually disappeared thereafter, as seen in other benzodiazepines. Whereas, the effect of chlorpromazine was at the maximum 2-4 hours later.

<table>
<thead>
<tr>
<th>Test agents</th>
<th>Dose mg/kg p.o.</th>
<th>N</th>
<th>Mean % inhibition at peak of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Avoidance</td>
</tr>
<tr>
<td>D-40TA</td>
<td>2.5</td>
<td>4</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4</td>
<td>67.5</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>4</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>3</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>4</td>
<td>72.5</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10.0</td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>3</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>3</td>
<td>90.0</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>20.0</td>
<td>3</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>2</td>
<td>85.0</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>8.0</td>
<td>3</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>3</td>
<td>56.7</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>3</td>
<td>96.7</td>
</tr>
</tbody>
</table>

V. Sleep potentiation and hypnogenic effects

1. Potentiation on isobutylnitrate (MHB)- or chlorpromazine (CPT)-hypnosis in mice

As shown in Table 4, D-655MT was most potent in potentiation of MHB and CPT hypnosis. D-40TA was also very effective in MHB test but appeared to be a little less active in CPT test than nitrazepam.

2. Hypnogenic effects in monkeys

Fig. 8 is histogram representing the % sleeping time during each 30-minute period of the consecutive 5 hours after the oral administration of D-40TA in two monkeys. The doses above 0.23 mg/kg in the monkey M-1 and above 0.45 mg/kg in the monkey M-2 distinctly increased the sleeping time dose-related way. Hypnogenic activity of D-40TA appeared 30 minutes later in low dose and more rapidly in high dose, and persisted in dose-
Fig. 8. Histograms of % sleeping time representing the hypnogenic effect of D-40TA in two monkeys. ■, percentage of the total sleeping time during each 0.5-hr period. Vertical bars are standard error of the mean value in 5 control experiments with M-1. Monkey M-2 never slept in 6 control sessions.

### TABLE 4. Sleep potentiating and hypnogenic effects.

<table>
<thead>
<tr>
<th>Test agents</th>
<th>Potentiation of hypnosis, mice $ED_{50}$, mg/kg, p.o.</th>
<th>Hypnogenic activity, monkeys $HE_{50}$, mg/kg p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHB</td>
<td>CPT</td>
</tr>
<tr>
<td>D-40TA</td>
<td>0.35 (0.18 - 0.69)</td>
<td>1.0 (0.55 - 1.95)</td>
</tr>
<tr>
<td>D-65MT</td>
<td>0.13 (0.09 - 0.21)</td>
<td>0.52 (0.27 - 1.01)</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>7.2 (4.9 - 10.5)</td>
<td>6.2 (3.9 - 9.9)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1.7 (1.2 - 2.4)</td>
<td>1.5 (0.9 - 2.6)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>1.5 (1.1 - 2.4)</td>
<td>0.5 (0.3 - 0.8)</td>
</tr>
</tbody>
</table>

related way. The animals given D-40TA slept so deeply as to awake barely by intensive acoustic stimulation or by swaying the body but sometimes awakened intermittently without external stimulation. Hypnogenic activities of D-40TA and D-65TA were most potent in benzodiazepines tested here, and was about 1.6 times as potent as nitrazepam (Table 4).

### VI. Anticonvulsant effects in mice

In anti-electroshock activity, D-65MT and D-40TA were about 5.2 and 1.8 times, respectively, as potent as nitrazepam, the most active of the reference benzodiazepines tested here. Anti-metrazol activity of D-65MT was 3 times that of nitrazepam, while D-40TA was 3 times less than the latter agent (Table 5).

### VII. Muscle relaxant effects in mice, rats and cats

The muscle relaxant effect of D-40TA was found to be roughly equipotent to nitrazepam in the inclined screen test of mice but about 1.7 times more potent in the rota
VIII. Other central nervous effects

1. Anti-reserpine and anti-tetrabenazine effects in mice and rats

Neither reserpine-induced ptosis in mice nor tetrabenazine-induced ptosis in rats was significantly antagonized by D-40TA in 2.5-30 mg/kg. The oral dosage of D-40TA in 5 mg/kg, the dose causing respiratory depression, rather potentiated reserpine-induced hypothermia in mice. The hypothermic effect of D-40TA alone in higher doses interrupted the assessment of antagonistic activity against reserpine-induced hypothermia. Furthermore, D-40TA in higher doses than 5 mg/kg caused muscular atony by itself so that the rats could not maintain the imposed posture on a rubber stopper in catalepsy test, thereby abolishing the occurrence of catalepsy by tetrabenazine.

<p>| TABLE 5. Anticonvulsant activity (ED₅₀, mg/kg, p.o.). |</p>
<table>
<thead>
<tr>
<th>Test agents</th>
<th>Anti-MES, mice</th>
<th>Anti-metrazol, mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>D40TA</td>
<td>4.6 (2.3-7.1)</td>
<td>2.2 (1.5-3.2)</td>
</tr>
<tr>
<td>D-65MT</td>
<td>1.6 (1.2-2.2)</td>
<td>0.25 (0.17-0.37)</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>28.6 (19.7-41.5)</td>
<td>7.5 (5.3-10.8)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>14.0 (9.3-21.1)</td>
<td>3.0 (1.8-4.5)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>8.4 (5.6-12.7)</td>
<td>0.75 (0.5-1.1)</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Test agents</th>
<th>Inclined screen mice ED₅₀, mg/kg, p.o.</th>
<th>Rota rod, rats ED₅₀, mg/kg i.p.</th>
<th>Rearing cats MMD, mg/kg p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-40TA</td>
<td>6.1 (3.4-11.0)</td>
<td>0.7 (0.5-0.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>D-65MT</td>
<td>3.2 (2.0-5.1)</td>
<td>0.9 (0.5-1.7)</td>
<td>0.125</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>220.0 (150.7-321.2)</td>
<td>11.9 (7.2-19.6)</td>
<td>-</td>
</tr>
<tr>
<td>Diazepam</td>
<td>44.0 (28.4-68.2)</td>
<td>2.5 (1.8-3.5)</td>
<td>-</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>5.8 (3.9-8.5)</td>
<td>1.5 (0.9-2.4)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

rod test of rats, as shown in Table 6. This compound was also very effective in impairment of rearing in cats. In other tests than rota rod, D-65MT was most active.

VIII. Other central nervous effects

1. Anti-reserpine and anti-tetrabenazine effects in mice and rats

Neither reserpine-induced ptosis in mice nor tetrabenazine-induced ptosis in rats was significantly antagonized by D-40TA in 2.5-30 mg/kg. The oral dosage of D-40TA in 5 mg/kg, the dose causing respiratory depression, rather potentiated reserpine-induced hypothermia in mice. Hypothermic effect of D-40TA itself in higher doses interrupted the assessment of antagonistic activity against reserpine-induced hypothermia. Furthermore, D-40TA in higher doses than 5 mg/kg caused muscular atony by itself so that the rats could not maintain the imposed posture on a rubber stopper in catalepsy test, thereby abolishing the occurrence of catalepsy by tetrabenazine.

2. Anti-oxotremorine effects in mice

D-40TA in 1.25, 2.5, 5, 10 and 20 mg/kg suppressed the degree of oxotremorine-induced tremor by 29.2, 32.1, 43.5, 45.3 and 29.8%, respectively. On the other hand, no sig-

<table>
<thead>
<tr>
<th>Dose mg/kg p.o.</th>
<th>N</th>
<th>Apomorphine emesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency (mean ± S.E.)</td>
</tr>
<tr>
<td>0 (control)</td>
<td>4</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>1.25</td>
<td>4</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>2.5</td>
<td>4</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>5.0</td>
<td>4</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>10.0</td>
<td>4</td>
<td>0.9 ± 0.4</td>
</tr>
</tbody>
</table>

* No. of dogs showing emesis / No. of dogs used × 100
significant inhibition of oxotremorine-salivation by D-40TA was observed in any of the above doses.

3. *Anti-apomorphine emesis in dogs*

The oral administration of D-40TA decreased in dose-related way the frequency of emesis induced by apomorphine, as shown in Table 7. Five and 10 mg/kg suppressed completely the occurrence of emesis in two of 4 dogs.

**DISCUSSION**

The experimental evidences presented here indicate that pharmacological profiles of D-40TA and D-65MT, though a tricyclic ring in their chemical structures, were more closely related to the well-known benzodiazepines than a neuroleptic drug, chlorpromazine. It is worth notice that both triazolo compounds were apparently more potent than diazepam or nitrazepam in calming the hyperirritable rats with septal lesion and in taming the naturally vicious monkeys. D-40TA was also found to be more effective than diazepam in anti-aggressive and anti-muricidal actions in olfactory bulbectomized rats, and to increase "sociality" of the timid dogs probably due to antianxiety effect.

In the experimentally induced conflict situation of the rats, conflict attenuating effect of D-40TA, when given acutely, tended to be masked by marked depression of the VI lever-pressing response rate. The depression of the VI responses may be attributable to muscle relaxation, which probably reflected upon the physical ability of the rat to manipulate a lever, or other factor, e.g. sedation or reduced appetite. After 3 or more repeated dosages of D-40TA, however, conflict attenuation was gradually evident in association with gradual disappearance of the depressed VI responses. Similar effect of chronic dosage of oxazepam, one of 1, 4-benzodiazepines, has been reported by Margules and Stein (14). As they suggested as to oxazepam, the chronic-dose pattern of D-40TA effect may be due either to increased conversion into conflict attenuating and non-depressant metabolite or some change of neuronal responsiveness to D-40TA in the central nervous system or both. It is possible that conflict attenuating activity which the first dose of D-40TA originally possesses is masked by its depressant effect of the lever responses but is disclosed in chronic dosage as the result of the freedom from depression by adaptation of the neuronal system which is responsible for depression. Concomitantly, the neuronal system responsible for conflict attenuation may be sensitized by chronic administration of D-40TA. An explanation based on neuronal sensitization to drug is also favourable to explain augmentation of anti-avoidance activity by chronic dosage of D-40TA in non-discriminated avoidance behavior of the rats. Judging from gradual disappearance of depression of the VI lever responses by chronic dosage of D-40TA in conflict experiment, augmented depression of the lever responses in non-discriminated avoidance is suggested to be not due to impairment of the physical ability to manipulate a lever by side effect such as muscle relaxation but probably due to alleviation of the psychologically tonic situation, i.e. psychorelaxation, and/or sedation.

It should be also noticed that D-40TA acutely given showed more selective avoidance
depression than diazepam or nitrazepam in pole-climbing test. The rats given D-40TA in the dose as high as 40 mg/kg still retained completely the ability to climb up or pull down a pole for turning off a foot-shock resulting from avoidance failure, while the same dose of diazepam or nitrazepam impaired considerably the escape response. This fact suggests that muscle relaxation by D-40TA is not so severe although the rota rod performance of the rats was impaired by considerably low doses of D-40TA. Anti-apomorphine emetic activity of D-40TA in dogs seemed considerably potent, as compared with diazepam or chlordiazepoxide from the results obtained not by us but by Randall et al. (15). Selective avoidance depression in discriminated conditioned avoidance-unconditioned escape behavior of the rat and anti-apomorphine emesis in dogs have been well known as regards neuroleptics. In these respects, D-40TA resembles neuroleptics. Antagonistic effects against apomorphine gnawing and methamphetamine-gnawing in rats, both of which have been known to be characteristics of neuroleptics, were not shown in D-40TA. Apomorphine- and amphetamine-induced stereotypies in rodents have been shown to require the presence of the corpus striatum and to be probably related to dopamine (16-18). Thus, D-40TA does not seem to act directly on the extra-pyramidal system.

One of prominent actions of D-40TA and D-65MT were potent sleep inducing or hypnogenic activity in monkeys, which was 1.6-1.8 times that of nitrazepam. There seems to be good correlation between hypnogenic activities in monkeys and clinical efficacies as sleep-inducing agents in nitrazepam, diazepam and chlordiazepoxide. Sleeping monkeys with D-40TA or D-65MT were never in anesthetic state but were arousable spontaneously and by external stimulation. In fact, the righting reflex in mice and rats was left intact until almost lethal doses of D-40TA or D-65MT were administered. In these respects, D-40TA and D-65MT must be distinguished from classical hypnotics, barbiturates. The evidence that the midbrain reticular activating system involving in a phasic component of EEG arousal was little affected by D-40TA will be presented elsewhere. The ratios, hypnogenic ED₉₀/taming ED₉₀ in monkeys were 1.12 for D-40TA, 2.1 for D-65MT, 1.7 for nitrazepam, 2.27 for diazepam and more than 11.8 for chlordiazepoxide, respectively. Schallek et al. (19) ascribed the sleep-inducing effect of nitrazepam to the reduction of the emotional activity, based on their observation of its depressive effect on the pressor response to hypothalamic stimulation and on EEG afterdischarge to amygdala stimulation in cats. As far as concerned with monkeys, no clear-cut correlation between taming and sleep-inducing effects exists as seen especially in chlordiazepoxide. Thus, the hypnogenic action might be attributable not only to the emotional depression but also some other mechanism.

The present study also revealed potent anticonvulsive and muscle relaxing effects of D-40TA and D-65MT. From such a pharmacological versatility of D-40TA and D-65MT in animal experiments, there would be a variety of possible clinical utilities, but these compounds would seem to be most promising as a sleep-inducing agent for insomnia and a psychotherapeutic agent for anxiety and psychomotor agitation, etc. in neurotic and psychiatric patients. In clinical use as a psychotherapeutic or sleep-inducing drug, less mus-
cle relaxation of a drug is favourable. Although the doses of D-40TA and D-65MT to
impair the rota rod performance of rats were considerably low, the ratios, rota rod ED₅₀/
anti-"septal" ED₅₀, were 0.17 for D-40TA, 0.31 for D-65MT, 0.16 for nitrazepam, 0.31 for
diazepam and 0.7 for chlordiazepoxide, respectively. Thus, the liability of neurological
side-effect by D-40TA and D-65MT is expected to be a little lower than that of nitra-
pezam clinically. Some comparative neuroanatomical evidences have presented that
subhuman primates are more similar to human than are mouse, rat, cat and dog, in
abundance of the large myelinated fibers and of the direct corticospinal component of
the pyramidal tract (20, 21). Therefore, the results obtained in monkeys seem more
useful for prediction of neurological side-effect in clinical use, and the presence of the
considerably wide spread between taming dose and muscle relaxant dose of D-40TA in
monkeys suggests low incidence of side-effects such as ataxia or muscle weakness in
clinical therapeutic dose.

**SUMMARY**

Triazolobenzodiazepines, D-40TA and D-65MT were found to have potent tranquiliz-
ing, anti-convulsive and muscle relaxant effects in various species of experimental animals.
These properties resemble quantitatively those of well-known 1, 4-benzodiazepines. Both
triazolo compounds were also more effective than nitrazepam in sleep-inducing in mon-
keys. Although attenuating effect of acutely administered D-40TA on the experimentally
induced conflict of the rats was apt to be masked by its remarkable depressant action on
the lever-pressing responses in the periods reinforced with reward alone, chronic adminis-
tration disclosed the powerful conflict attenuation in association with development of toler-
ance to the depressant effect. On the contrary, avoidance failure resulting from the de-
creased lever-pressing responses by D-40TA in non-discriminated avoidance behavior of
the rats were clearly augmented by chronic administration. Selective depression of dis-
criminated avoidance behavior in rats and anti-apomorphine emesis in dogs, which have
been known to be properties of neuroleptics, were also shown by D-40TA. This com-
pound, however, was differentiated from neuroleptics in the absence of anti-apomorphine
and anti-methamphetamine stereotypies in rats. The acute toxicities of D-40TA and
D-65MT in mice and rats were very low.

_Acknowledgement_: The expert technical assistances of Mr. T. Mikoda and Miss
C. Hattori are gratefully acknowledged.

**REFERENCES**

8) Geller, I. and Suffer, J.: *Psychopharmac.* 1, 482 (1960)