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# Orally Delivered Pentobarbital as a Reinforcer for Rhesus Monkeys with Concurrent Access to Water: Effects of Concentration, Fixed-Ratio Size, and Liquid Positions

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DENOBLE, V. J., D. S. SVIKIS AND R. A. MEISCH. *Orally delivered pentobarbital as a reinforcer for rhesus monkeys with concurrent access to water: Effects of concentration, fixed-ratio size, and liquid positions.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 113–117, 1982.—The number of liquid deliveries and pattern of concurrent pentobarbital and water drinking were studied in three food deprived rhesus monkeys during daily 3-hr sessions. During the daily sessions, deliveries of approximately 0.6 ml of each liquid occurred under fixed-ratio (FR) schedules of lip contact responses. Between sessions water was freely available. Session drinking was studied as a function of pentobarbital concentration (1.0, 1.41, 2.0, and 4.0 mg/ml) and FR size (4, 8, 16 and 32 lip contacts per delivery). The number of drug deliveries decreased with increases in drug concentration. Drug intake ranged from 21 to 52 mg/kg of body wt./3-hr session. At all concentrations and FR values tested, the number of pentobarbital deliveries substantially exceeded the number of water deliveries. The positive reinforcing effect of the pentobarbital was indicated by a consistent choice of drug over water irrespective of the side position of pentobarbital and by higher rates of drug responding. Pentobarbital drinking occurred in a negatively accelerated pattern whereas water drinking did not have any consistent pattern. Marked intoxication followed bouts of drug drinking.

Pentobarbital reinforcement  Oral drug self-administration  Concurrent schedules  Fixed ratio schedules  Drug concentration  Rhesus monkeys

DENEAU and co-workers [4] and Yanagita and Takahashi [19] were the first to study intravenous self-administration of pentobarbital (PB) in rhesus monkeys. Under conditions of continuous 24-hr drug access, drug naive monkeys initiated and maintained intravenous infusions of PB. Drug intake increased gradually across several weeks of testing, and self-imposed periods of abstinence did not occur. Occasionally drug intake was high enough to produce anesthesia. At night self-administration was 50 percent of daytime levels. These results have been replicated and extended [14, 16, 18].

Goldberg and co-workers [6] demonstrated that rhesus monkeys would intravenously self-administer PB (0.25 mg/kg) under conditions of drug access limited to 3 hr per day. The time course of intake was characterized by an initial burst of infusions during the first 30 min of the session and by irregularly spaced responding over the remainder of the session. After the initial burst of responding the monkeys often showed ataxia and lethargy. Pentobarbital intake (mg/kg of body wt./3-hr session) increased less than two-fold across an eight-fold increase in injection doses.

The rate and pattern of barbiturate reinforced lever pressing were also studied in rhesus monkeys during daily 3-hr sessions by Winger *et al.* [16]. Responding was increased and maintained by barbital (2.5–10.0 mg/kg), PB (0.25–4.0 mg/kg), thiopental (0.5–4.0 mg/kg), methohexital (0.125–2.0 mg/kg) and amobarbital (0.25–4.0 mg/kg/injection). For each barbiturate, responding for low to intermediate doses occurred in bursts followed by pauses. The largest bursts came at the beginning of the session, and subsequent bursts were distributed in a negatively accelerated pattern. Response rate was inversely related to drug dose whereas drug intake (mg/kg of body wt./3-hr session) was directly related to dose.

While these studies provide valuable information concerning the variables that control intravenous barbiturate self-administration, there is a lack of research involving the oral route, even though with humans the oral route is the most common mode of drug self-administration. Over the last several years procedures have been developed for establishing orally delivered ethanol [9], etonitazene [2], phencyclidine [3], and PB [11] as reinforcers for rhesus monkeys.

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The purpose of the present study was to extend previous work [11] in several ways. Naive instead of ethanol experienced rhesus monkeys were used. Pentobarbital was presented concurrently with water instead of sequentially, and all monkeys were tested under identical rather that different fixed-ratio schedules. In the present study the effects of changes in the drug concentration and fixed-ratio (FR) schedule of reinforcement were also studied.

## METHOD

### *Subjects*

Six male adult rhesus monkeys (*Macaca mulatta*) served as the subjects. At the beginning of the study free-feeding weights ranged from 7.8 to 10.2 kg. All six monkeys were experimentally naive. Five monkeys were reduced to 70 to 86 percent of their free-feeding weights by limiting the amount of Purina Monkey Chow they received. A sixth monkey (G2) became ill and lost 44 percent of his weight during the initial acquisition phase. During this time (approximately 3 weeks) the animal received veterinary care. Over the next few months his weight increased to 70 percent of his free-feeding weight. Once each week the monkeys were given 2.0 ml of Poly-Visol multivitamins to drink. They were housed in their experimental chambers in a continuously illuminated room.

### *Apparatus*

The experimental chambers were stainless steel Hoeltge (no. HB-108) primate cages equipped with a work panel on one wall that contained two symmetrically placed drinking spouts, a food receptacle, a response lever, and stimulus lights. Each spout was 2.7 cm long and was constructed of nonconductive material. A small brass plate was recessed in the spout 1.0 cm from its end. Responses occurred when a monkey made lip contact with the brass plate. Each lip contact response activated a drinkometer circuit and for the duration of each contact illuminated one of two pairs of stimulus feedback lights (1.1 W) that were located directly behind a Plexiglas plate supporting the spout. When water was available each response turned on a pair of white lights. When drug was available each response turned on a pair of green lights. In addition to the two pairs of feedback lights, a larger green light (4.76 W) was mounted 11.5 cm above each spout. This light was constantly illuminated when water was present and blinked at a rate of 10 Hz when drug was present. A similar-sized light, red in color, was located 22 cm above the food lever.

Lip contact responses were programmed to operate a solenoid that delivered a maximum of 0.65 ml from the spout in 0.13 sec. If the monkey broke contact before completion of the 0.13-sec interval, then the solenoid operation terminated. Liquids were placed in covered stainless steel reservoirs. There was no detectable evaporation. Liquid intake was not restricted by the amount of solution in the reservoir. Details of the drinking device [7] and work panel [10] have been reported. Experimental events were scheduled and recorded by equipment (Coulbourn Instruments, Inc.) located in an adjacent room.

### *Procedure*

Daily experimental sessions were 3 hrs in duration. Each session was preceded and followed by a 1-hr stimulus blackout during which time data were recorded and solutions changed. Another 1-hr stimulus blackout occurred during the third hr that followed the end of the 3-hr sessions. During the 18-hour inter-session period a constantly illuminated green light signalled water availability under an FR 1 schedule. Orally delivered PB was established as a reinforcer in four phases. First, water drinking was induced by giving the monkeys their daily food ration during the 3-hr session. Second, increasing concentrations of PB were substituted for water during the session. Third, food availability was shifted from within the session to the third hr after the end of the session. Fourth, during the session drug and water were made concurrently available. Sessions were conducted seven days per week at a constant starting time.

*Phase 1. Induced drinking of water.* Two different feeding procedures were used to induce drinking. With one group of monkeys (B2, S2, and G2) the daily ration of Purina Monkey Chow (70 g) was placed in their food receptacles 20 min after the start of the session. With the other group (F2, E2, R2) the daily ration of 1-g Noyes banana-flavored pellets was delivered according to a signalled differential reinforcement of low rates (DRL) 120-sec schedule. Under this schedule each lever press separated by a minimum interresponse interval of 120 sec resulted in the illumination of the red light above the food lever. The first response in the presence of this light both delivered a food pellet and extinguished the light. Responses on the food lever during the 120-sec interval reset a clock and delayed the opportunity for pellet delivery by 120 sec. Onset of the food schedule occurred with the beginning of the second hr of the session. The food schedule remained in effect until all pellets were delivered or the 3-hr session ended. Intermittent schedules of food pellet delivery engender excessive drinking termed schedule-induced polydipsia [5,15].

*Phase 2. Pentobarbital solutions substituted for water.* When large volumes of water were reliably drunk, PB (0.0078 mg/ml) was substituted for water during 3-hr sessions. At each concentration drinking was allowed to stabilize so that there were no increasing or decreasing trends over five consecutive sessions in the volume consumed. After stable performance was obtained at 0.0078 mg/ml, increasing concentrations of PB (0.0156, 0.0312, 0.0625, 0.25, 0.35, 0.5, 0.707, 1.0, 1.41 and 2.0 mg/ml) were presented. Not all monkeys were exposed to the full range of concentrations before beginning phase 3, and some monkeys required increases in the FR size which either limited drug intake or slowed the rate of drug intake (see Table 1). Water was freely available between sessions according to an FR 1 schedule for the same spout that delivered PB during the session.

*Phase 3. Food shifted from within session to after session.* When the monkeys consistently became intoxicated (see [11]) at intakes of approximately 60 mg/kg/3-hr session and above, food access was shifted to the third hour after the session. This shift occurred at different concentrations and FR values for individual monkeys. Three monkeys did not persist in responding for drug with food removed. The other three monkeys went on to phase 4.

*Phase 4. Concurrent availability of pentobarbital and water.* Once performance was stable in the absence of food, the side location from which PB was delivered was alternated from session to session. During this phase the water location during the 18-hour intersession period was also alternated between the right and left spouts. Within a particular session, the spout chosen for PB delivery was the one which had not delivered water during the preceding inter-

TABLE 1

MEAN (n=5) PENTOBARBITAL INTAKE (mg/kg/3-hr SESSION) DURING THE INDUCED DRINKING CONDITION

| | | Pentobarbital (mg/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| Monkey | Schedule | 0.35 | 0.50 | 0.707 | 1.0 | 1.41 | 2.0 |
| M-G2 | FR1 | 25.5 | 39.7 | 61.4 | | | |
| M-B2 | FR 1 | 44.7 | | | | | |
| | FR 2 | 44.7 | 55.5 | 66.1 | | | |
| | FR 4 | | | 57.9 | 67.7 | | |
| M-S2 | FR 1 | 33.4 | 53.9 | | | | |
| | FR 2 | | 45.5 | 58.3 | 71.4 | | |
| | FR 4 | | | | 73.7 | | |
| M-E2 | FR 1 | 22.2 | 27.1 | 39.4 | 65.3 | 76.2 | |
| | FR 2 | | | | | 68.3 | 78.0 |
| M-R2 | FR 1 | 30.1 | 41.4 | 48.6 | 71.7 | | |
| | FR 2 | | | | 57.1 | | |
| M-F2 | FR 1 | 46.6 | | | | | |
| | FR 2 | 45.4 | 51.4 | 58.4 | | | |
| | FR 4 | | | 61.2 | 68.4 | | |

Reading the table from left to right and top to bottom gives the sequence of conditions. Note that the last concentration and fixed ratio at which each monkey was tested with food in session was also the concentration and fixed ratio at which food was subsequently removed from within the session.

session period. After 10 stable sessions were obtained with PB alone, water was made concurrently available. For monkey G2 the PB concentration was increased to 1.0 mg/ml and the fixed ratio was increased from 1, to 2 and then to 4.

*Effects of FR Size and Pentobarbital Concentration.*

The FR size was gradually increased for both drug and water from 4 to 8. At FR 8 with water concurrently available increasing concentrations (1.0, 1.41, 2.0 mg/ml) of PB were presented. Each concentration was present for at least 10 sessions and until performance was stable. At 2.0 mg/ml the FR was increased from FR 8 to FR 16, and additional concentrations (2.0, 2.8, and 4.0 mg/ml) were tested. The FR was again increased for both drug and water when drug concentration was 4.0 mg/ml; the increase was from 16 to 32. Increases in the FR size were made in small increments; for example in going from FR 8 to 16 several sessions were conducted at the intermediate values of 10, 12, and 14.

*Pentobarbital*

Drug solutions were prepared two hrs before use and were at room temperature when used. Concentrations are in terms of the sodium salt.

## RESULTS

*Induced Drinking*

When the monkeys received their food during the session, they reliably drank from 400 to 800 ml of water. There was no disruption of drinking when PB was introduced at a concentration of 0.0078 mg/ml. As the concentration was increased, the total drug intake (mg/kg of body wt./3-hr session) also increased. Table 1 lists intakes for each monkey at concentrations of 0.35 mg/ml and above. At intakes of approximately 40 mg/kg/session, intoxication was noted. The severity of intoxication increased in proportion to the amount of drug consumed, and at intakes of 60 mg/kg/session and above, periods of anesthesia were observed. When severe intoxication was reliably obtained, food availability was shifted to the 3 hrs after the session. This occurred at different concentrations and FR values. Table 1 indicates the specific conditions for each monkey.

For three monkeys (F2, E2, and R2) removal of food resulted in a cessation in drug intake; by the fourth session these monkeys were obtaining less that 50 liquid deliveries. These three monkeys were the ones who obtained their food according to the signalled DRL schedule. In contrast the three monkeys who obtained their food in a single feeding persisted in drinking PB solutions after food was shifted to between the sessions. However, their drug intake was also decreased by the elimination of food, and the three monkeys, B2, S2, and G2, showed decreases of 11, 61 and 63 percent, respectively. The introduction of concurrent water availability had little effect on PB intake.

*Effects of FR Size and Pentobarbital Concentration*

Figure 1 shows liquid deliveries of pentobarbital and water as a function of FR size and PB concentration. Results from the left and right sides were similar. Therefore, data from the two sides are combined in Fig. 1. The principal finding was that for all three monekys at all concentrations and FR values tested, the number of drug deliveries sub-

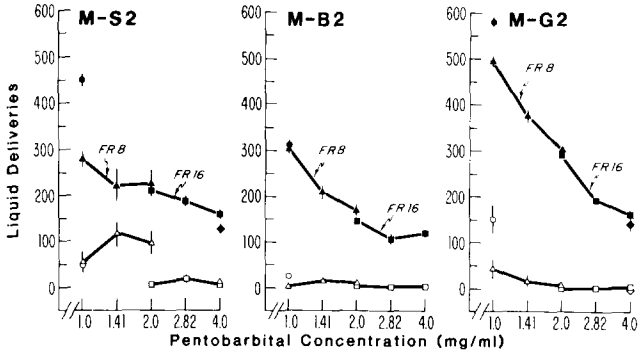


FIG. 1. Liquid deliveries per 3-hr session as a function of pentobarbital concentration and FR size (ascending series) for three monkeys; (circles), liquid deliveries under FR 4; (triangles), liquid deliveries under FR 8; (squares), under FR 16; and (diamonds), under FR 32. Closed symbols show pentobarbital deliveries; open symbols show water deliveries. Each symbol for each monkey is the mean of ten consecutive sessions (five from the right spout and five from the left spout). Vertical lines show the standard errors. Note that monkey M-B2 did not maintain responding under FR 32.

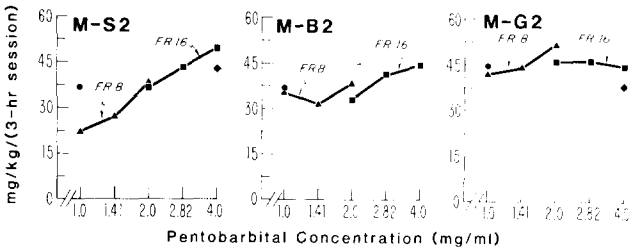


FIG. 2. Pentobarbital intake (mg/kg/3-hr session) as a function of pentobarbital concentration and FR size (ascending series). The symbols representing the FR size are the same as those in Fig. 1. Each point for each monkey is the mean of ten consecutive sessions (five from the right spout and five from the left spout).

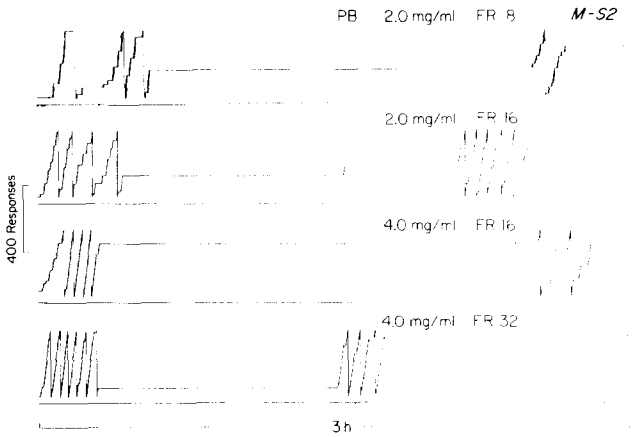


FIG. 3. Cumulative records are presented for monkey M-S2 for four 3-hr sessions. The pentobarbital (PB) concentrations were available under FR values of 8, 16 or 32. The stepping pen recorded lip-contact responses, and each downward deflection of the stepping pen indicated a liquid delivery. The stepping pen reset automatically after 400 responses. The event pen at the bottom of each record indicated water deliveries. Note the occurrence of water deliveries at the beginning of the session when 2 mg/ml pentobarbital and water were concurrently available under an FR 8 schedule.

stantially exceeded the number of water deliveries. Thus, pentobarbital was functioning as a positive reinforcer.

Increases in the FR value generally produced decreases in the number of PB deliveries. These decreases were usually small; however, for both monkeys S2 and G2 at 1.0 mg/ml there was a relatively large decrease when the FR had been increased from 4 to 8. Also, for monkey B2 at 4.0 mg/ml responding was not maintained when the ratio was increased from 16 to 32. Water deliveries were generally low in number and not affected by changes in the FR size. There were two exceptions to this finding. When water deliveries were greater than 75 per session (e.g., M-S2 at a concurrent drug concentration of 2.0 mg/ml, and M-G2 at a concurrent drug concentration of 1.0 mg/ml), increases in the FR produced relatively large decreases in the number of water deliveries (Fig. 1).

Increases in the PB concentration led to decreases in the number of PB deliveries (Fig. 1). The effects of increases in the PB concentration on drug intake depended on the level of the monkey's intake at lower PB concentrations (Fig. 2). Monkey S2 had the lowest level of intake at 1.0 mg/ml, and this monkey's intake increased with increases in the concentration. In contrast, monkey G2 had the highest level of intake at 1.0 mg/ml, and his intake did not vary systematically with the drug concentration. At intakes of more than 40 mg per kg, periods of anesthesia were frequently observed.

Figure 3 contains cumulative records that show the pattern of responding and the time course of drinking for monkey S2. All three monkeys generally showed similar patterns of intake with the highest rate of drinking occurring at the beginning of the session. This inital bout was followed by a pause and often by signs of intoxication. When the level of intoxication diminished, a second bout of drinking ensued. Responding, when it occurred, resembled FR responding maintained by other reinforcers (Fig. 3). Water drinking did not show any regular pattern.

## DISCUSSION

Orally-delivered PB maintained substantially higher response rates under FR schedules than did water when both liquids were presented concurrently. Thus, PB functioned as a positive reinforcer. An inverse relationship between concentration and number of drug deliveries was observed. The time course for PB and water drinking was distinctly different. At the beginning of each session pentobarbital drinking occurred at high rates and often resulted in gross intoxication. This initial burst of drinking was followed by a pause and then by more drinking at somewhat lower rates. Water drinking occurred at low rates and was almost nonexistent at higher PB concentrations. These findings extend previous results [11] by showing that PB can be established as a rein-

forcer for drug naive monkeys and that PB can serve as a reinforcer when water is concurrently available. Concurrent presentation of drug and water is superior to sequential presentation in that the additional dependent variable of choice is introduced. When access to food within the 3-hr sessions was terminated three monkeys continued to drink PB solutions whereas the other three monkeys stopped drinking. The principal difference between the two groups of monkeys was that they had received food according to different procedures: the monkeys that persisted in drinking obtained their food in a single feeding while the other monkeys received pellets under a signaled DRL schedule. Why the one procedure was more effective is not clear since both procedures engendered substantial drinking. However, the pattern of drinking induced by receiving food in a single feeding was characterized by a large burst of drinking followed by intoxication. For monkeys receiving pellets under the signaled DRL schedule drinking occurred in small bursts throughout the session.

As the drug concentration was increased form 1.0 to 4.0 mg/ml, the number of liquid deliveries decreased and the total drug intake (mg/kg/3-hr session) either increased or remained high. Similar relationships have been found when other orally delivered drugs functioned as reinforcers for rhesus monkeys [2, 3, 8]. Decreases in the number of liquid deliveries with increases in concentrations may be due to the rate decreasing or intoxicating effects of the large amounts of PB ingested. In studies of intravenous drug intake, an inverse relation between drug dose and number of infusions has often been observed, and this inverse relation has been attributed to the rate decreasing effects that may occur when certain drugs are taken in large amounts [1, 13, 17].

In the present study drug intakes ranged from 21 to 52 mg/kg/3-hr session. These intakes are similar to those obtained with rhesus monkeys and rats in studies of PB self-administration [6, 12, 16]. The temporal pattern of drug intake in the present study was also similar to that seen in the studies of intravenous PB self-administration with rhesus monkeys [6,16]. In both the intravenous and oral studies the highest rate of responding occurred at the beginning of the session, and this initial burst was followed by smaller bursts toward the end of the session. Access to PB was limited to 3 hr/day and no evidence of physiological (physical) dependence, such as that reported by Yanagita and Takahashi [19] was observed.

Responding reinforced by orally delivered PB was well maintained even when the FR was doubled from 4 to 8 and from 8 to 16. In contrast Goldberg and co-workers [6] reported that responding reinforced by the intravenous delivery of PB was not well maintained when the FR size was increased from FR 1 to FR 10. One factor that may partly account or these different findings is that when the oral route is used the taste of the drug solution may come to serve as both a conditioned reinforcer and as a discriminative stimulus, and thus the taste of the drug solution may facilitate performance under intermittent schedules.

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