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THEORETICAL REVIEW

A Review of Methods to Induce Alcohol Addiction in Animals

NANCY K. MELLO[1]

Laboratory of Alcohol Research, National Institute on Alcohol Abuse and Alcoholism
National Institutes of Mental Health, Washington, D. C. 20032

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MELLO, N. K. *A review of methods to induce alcohol addiction in animals.* PHARMAC. BIOCHEM. BEHAV. 1(1) 89–101, 1973.– Since alcoholism has been shown to be a form of addiction, there has been increasing attention to the development of an animal model of alcoholism. Within the past five years, several techniques have been developed to produce physical dependence upon alcohol in a number of species. This review presents a summary and evaluation of the various approaches used and a discussion of the relative merits of the behavioral (self-administration) and pharmacological (forced administration) models. Several questions are raised concerning the relationship between physical dependence and subsequent self-administration of alcohol. It is concluded that although it has been possible to produce physical dependence upon alcohol in animals, the critical determinants of the addictive process are still unknown.

Alcohol Addiction Physical dependence Alcohol withdrawal Animal models of addiction

ALCOHOLISM has recently been shown to be a form of addiction, as defined in terms of the traditional pharmacological criteria of tolerance and physical dependence [23, 51, 74]. Recognition that alcoholism is an addictive disorder has proceded slowly and at one time it was thought that the alcohol withdrawal syndrome reflected intercurrent illness, vitamin or nutritional deficiencies [74]. Now it has been shown that alcohol withdrawal signs and symptoms occur in healthy, well-nourished alcoholics [51] and in experimental animals, solely as a function of cessation of drinking.

The crucial determinants in the development of alcohol addiction are unknown and the nature of the addictive process remains a matter of conjecture. It has been generally agreed that only the development of an alcohol dependent animal would permit the study of the natural history of the addictive process at a behavioral, biochemical and neurophysiological level. In man, alcohol dependence usually evolves over many years and consequently, its developmental antecedents are obscured by time. A more rapid and systematic induction of alcohol dependence in experimental animals could contribute to clarification of the neural, endocrine and metabolic changes which may be critical for the expression of physical dependence upon alcohol. Many lines of evidence suggest that addiction involves some, as yet, unspecified alteration in the central nervous system [24, 25, 45, 53, 54]. The importance of CNS alterations in alcohol addiction can be inferred from demonstrations of behavioral tolerance for alcohol in alcoholics [45] which cannot be explained by metabolic factors alone. The limitations on experiments that can be performed on human subjects have necessarily restricted our progress in understanding the central nervous system mechanisms involved in the phenomena of alcohol addiction.

For many years, investigators have concentrated upon devising techniques for inducing preference for alcohol in animals. These efforts have failed, primarily because the taste of alcohol is aversive to most animals. There has also been an unfortunate tendency to equate a transitory alcohol preference with addiction, even though no withdrawal signs and symptoms occurred upon cessation of chronic drinking. Removal of the factors which accelerated alcohol preference, e.g. noxious stimuli, is usually accompanied by a decrease in alcohol intake (cf. [32, 35, 43, 57, 84] for reviews).

[1]Send reprint requests to: Dr. N. K. Mello, Laboratory of Alcohol Research, NIAAA, NIMH, William A. White Building, Room 493, Saint Elizabeths Hospital, Washington, D. C. 20032.

Recently, several groups of investigators have succeeded in producing physical dependence upon alcohol in animals using oral, intragastric, intravenous and inhalation routes of administration. The procedures and results of some representative studies are summarized in Table 1. Both forced administration and self-administration procedures have been effective in producing alcohol abstinence signs. The remainder of this review will describe the current status of models of alcohol addiction in animals with emphasis upon the differences between behavioral (self-administration) and pharmacological (forced administration) models. Before describing the details of the various experimental procedures, some general considerations concerning the evaluation of animal models and criteria for evaluating physical dependence will be discussed.

CRITERIA OF ALCOHOL DEPENDENCE

Comparison of Behavioral and Pharmacological Models

Uniform criteria for evaluating the adequacy and potential applicability of the various techniques for inducing physical dependence upon ethanol have not been developed. It is obvious that demonstration of physical dependence per se must be unequivocally attributed to the chronic administration and subsequent removal of alcohol and not to the confounding effects of nutritional deficiency, illness or nonalcohol drug toxicity [60]. Evidence concerning the induction of behavioral and pharmacological tolerance should be obtained whenever possible [25]. It has also been argued that levels of ethanol intake should be sufficient to produce intoxication as indicated both by

TABLE 1

METHODS USED TO INDUCE PHYSICAL DEPENDENCE ON ALCOHOL IN ANIMALS

	PROCEDURES			
Investigator & Year	Technique	Species	Dose	Days of Exposure
Falk *et al.*, 1972 (12)	Polydipsia	rat	13 g/kg/day	90
Lester, 1961 (31)	Polydipsia	rat		2
Ogata *et al.*, 1972 (60)	Polydipsia	mouse	14–24 mg/g/day	7–14
Mello & Mendelson, 1971 (49)	Polydipsia	rhesus monkey	3 g/kg/day	90–195
Woods & Winger, 1971 (84)	Polydipsia	rhesus monkey	4–7 g/kg/day	no data
Freund, 1969 (14)	ETOH-Liquid Diet	mouse	0.51 ml abs. ETOH	4
Ogata *et al.*, 1972 (60)	ETOH-Liquid Diet	mouse	15–18 mg/g	4
Branchey *et al.*, 1971 (2)	ETOH-Liquid Diet	rat	4.26–4.33 ml abs. ETOH	4–21
Pieper *et al.*, 1972 (63)	ETOH-Liquid Diet	chimpanzee	2–8 g/kg/day	42–70
Pieper & Skeen, 1972 (64)	ETOH-Liquid Diet	rhesus monkey	5–7 g/kg/day	40
Mendelson & Mello, 1971 (55)	ETOH-Sole Fluid	infant Rh. monkey	4–10 g/kg/day	730
Majchrowicz, 1972 [Pers. Com.]	Intubation	rat	9–14 g/kg/day	5–7
Ellis & Pick, 1969, 70, 71 (5, 7, 8)	N. G. Intubation	rhesus monkey	4–8 g/kg/day	10–18
Ellis & Pick, 1970 (6)	N. G. Intubation	beagle dog	3–7 g/kg/day	14–56
Essig & Lam, 1968 (9)	Intragas. Infus.	beagle dog	4–4.5 ml/kg/day (40%)	40–54
Yanagita *et al.*, 1969 (87)	Intragas. Self Infus.	rhesus monkey	3.2–7.5 g/kg/day	35
Goldstein & Pal, 1971 (19)	Inhalation ETOH Vapor	mouse	11 mg/liter air	1–3
Goldstein, 1972 (17)				1–3
Mello & Mendelson, 1971 (48)	Drinking to Avoid Shock	rhesus monkey	2.5 g/kg/day	70–700
Deneau *et al.*, 1969 (4)	Intravenous Self Administration	rhesus monkey	8.6 g/kg/day	120+
Woods *et al.*, 1971 (85)	Intravenous Self Administration	rhesus monkey	6–8 g/kg/day	90–360
McQuarrie & Fingl, 1958 (40)	Gavage	mouse	5.4 g/kg/day	14
Gibbins *et al.*, 1971 (15)	Intubation & ETOH-Sole Fluid	rat	3–7 g/kg/day	37
Ratclif, 1972 (65)	ETOH-Sole Fluid	rat	- - no data - -	35–49

*Assessed by observation
†Assessed by a threshold measure
NA = not applicable

behavioral measures and blood alcohol levels [84]. Moreover, an adequate behavioral model of addiction should show that alcohol functions as a reinforcer, i.e. the rate of operant responding for alcohol increases and is sustained [84,85]. Addictive drug seeking behavior presumably is maintained by its consequences and it is the identification and analysis of the complex of drug related reinforcers which presents a major challenge to the behavioral pharmacologist.

Implicit in these comments is the notion that voluntary self-selection techniques for inducing alcohol addiction may eventually prove more valuable than pharmacological models achieved through forced administration techniques. Although the rapid induction of physical dependence by intravenous or nasogastric alcohol administration provides an important tool for assessing the end product of addiction, the analysis of developmental correlates of addiction may be obscured by an accelerated induction time. Only a behavioral technique designed to produce alcohol self-administration would permit examination of those factors which contribute to the development of the addictive process as a function of time. Ideally, a behavioral method should permit the identification and subsequent manipulation of the environmental determinants that affect the acquisition and maintenance of addictive drinking. The effects of a variety of pharmacological interventions could then be studied.

The relative value of a behavioral (self-administration) or pharmacological (forced administration) model is of course dependent upon the types of questions that are subsequently asked. However, there is reason to argue for explicit comparison of results obtained with these two techniques. Recent human data suggest that the pattern of alcohol consumption (spontaneous rather than programmed

TABLE 1 (cont.)

	RESULTS			
Other Variables	Physical Depend.	Enhanced Metabolic Rate	Intoxication (Bal Range)	ETOH as a Reinf.
	yes*	yes	100–300 mg/100 ml	yes
	no*	no data	100–200 mg/100 ml	yes
	no*	no data	73–279 mg/100 ml	yes
	no*	no data	50 mg/100 ml	no
	no*	no data	+200 mg/100 ml	yes
35% weight reduction	yes*	no data	100–650 mg/100 ml	yes
35% weight reduction	yes*	no data	178–499 mg/100 ml	yes
33% weight reduction	yes*	no data	- - no data - -	no
	yes*	yes	50–500 mg/100 ml	yes
	yes*	yes	+ 150 mg/100 ml	no
peer & maternal depriv.	no*	no	50–200 mg/100 ml	no
	yes*	yes	+ 600 mg/100 ml	NA
	yes*	yes	100–500 mg/100 ml	NA
	yes*	no data	100–500 mg/100 ml	NA
	yes*	no data	no data	NA
	yes*	no data	no data	yes
Pyrazole (1.0 mmol/kg/day)	yes*	no data	147–186 mg/100 ml	NA
	no*	no data	30–70 mg/100 ml	no
	yes*	no data	- - no data - -	yes
	yes*	no	300–400 mg/100 ml	yes
electro-convulsive seizure threshold	yes†	no data	no data	no
startle threshold to electric shock	yes†	no data	104–200 mg/100 ml	no
audiogenic & drug induced withdrawal seizures.	yes†	no data	- - no data - -	no

dosage) does produce significant differences in biological and behavioral correlates of intoxication and withdrawal [47].

Assessment of Withdrawal Signs

Although there is some interspecies variability in the types of withdrawal signs shown upon removal of alcohol, there has been a reasonable consensus between observations from different laboratories. A comparison of the types of alcohol withdrawal signs observed in man, monkey, chimpanzee, dog, rat and mouse is presented in Table 2. It is important to recognize that, for the most part, these signs have been assessed on the basis of visual observation. The development of quantitative methods for assessing withdrawal severity has received relatively little experimental attention. Determination of the presence or absence of specific withdrawal signs can be done with a reasonable degree of confidence. Reliable examination of the gross behavioral signs of alcohol withdrawal requires frequent observation over an 8–12 hr period. Comprehensive behavioral rating scales for signs of physical dependence in mouse have been developed by Irwin [21]; Goldstein [17] and Freund [14]. Aside from the data provided for opiate dependence by Seevers and Deneau [66] comparable behavioral rating scales have not been developed for other species. A rating scale for primates which has been useful at the NIAAA Laboratory of Alcohol Research is shown in Appendix I.

Determination of the relative severity of withdrawal signs and symptoms, e.g. tremors or convulsions, and permitting meaningful comparisons between laboratories remains an unresolved problem. The general usefulness of the several physiological and movement monitoring devices currently available has not been assessed in the context of quantifying alcohol withdrawal signs in animals [61,68]. Recent texts on behavioral pharmacology [73] and methods of drug evaluation [71] have not discussed the several problems involved in the reliable assessment of physical dependence. There has been considerably more progress in developing quantitative behavioral techniques for the assessment of tolerance and these studies have recently been reviewed by Kalant and associates [25].

Since the grossly observable withdrawal signs presumably reflect central nervous system hyperexcitability, a few investigators have attempted to assess CNS excitability directly by examining the seizure threshold to electroconvulsive shock [1,40] audiogenic stimuli [65] and convulsive drugs [65]. Gibbins and co-workers have recently reported that the threshold of a startle response (flinch and jump) to foot shock provides a sensitive measure of the degree of physical dependence upon alcohol [15]. A similar approach to the measurement of physical dependence in animals addicted to opiate narcotics has recently been reported [72].

The assessment of withdrawal severity presents similar dificulties and ambiguities in human addicts. Neurological examination of tremor, nystagmus and hyperreflexia is of course easier, but the degree of severity remains a subjective judgement. Assessment of CNS hyperexcitability by determining threshold for photomyoclonic seizures has recently been explored by Wolfe and Victor and their associates [81,82]. It was found that upon cessation of drinking, alcoholics develop a significant respiratory alkalosis which correlates both with susceptibility to stroboscopically induced seizures and the occurrence of the most severe form of alcohol withdrawal, delirium tremens [82]. These findings are of considerable interest since several other hyperventilation syndromes also associated with a respiratory alkalosis, yield clinical symptoms similar to those of alcohol withdrawal [82]. These data have been interpreted to suggest that following removal of the depressant effect of alcohol, there is an increased sensitivity of the respiratory center to carbon dioxide and hyperventilation [82]. The basis for the CNS hyperexcitability during withdrawal has often been attributed to a rebound effect following drug induced depression of neural activity. However, the biological mechanisms underlying the expression of the abstinence syndromes generally are unknown [53,54].

ANIMAL MODELS OF ALCOHOL DEPENDENCE

The following description of animal models of alcohol addiction will be organized in terms of species and routes of administration of alcohol, i.e. oral and intragastric administration; intravenous administration; and administration via an inhalation technique. The route of administration determines the rapidity with which an effective dose of alcohol is achieved and also influences the duration and stability of drug action.

Inhalation Techniques

In 1971, Goldstein and Pal [19] reported that alcohol dependence could be produced in mice by exposing them to a situation in which they inhaled alcohol vapor at concentrations between 10 and 16 mg/l. Maintenance of blood alcohol levels of 180 mg/100 ml for three days was found sufficient to produce withdrawal signs of tremor, convulsions on handling, tail lift, startle reaction to noise and spontaneous seizures [17,19].

In order to ensure stable high blood alcohol levels, the alcohol inhalation technique was combined with daily doses of pyrazole (1.0 mmol/kg I.P.). Pyrazole reduces the rate of alcohol metabolism [16,33] even more effectively than does starvation, i.e. by an estimated 70% [19]. Since pyrazole has toxic side effects on hepatic function [34], evaluation of the adequacy of this technique for inducing a true model of physical dependence is complicated. Mice exposed to alcohol vapor alone do show withdrawal signs and symptoms but the sometimes lethal fluctuations in blood alcohol concentrations achieved without pyrazole seemed to the investigators to contraindicate its omission [17,19]. All animals exposed to the inhalation technique lost weight rapidly and those mice treated with pyrazole and maintained at moderate blood alcohol levels (140 mg/100 ml) lost as much as 17% in 6 days (17). Moreover, in mice exposed to alcohol only, coma and death occurred under conditions where blood alcohol levels averaged 200–300 mg/100 ml (17); a level which is rarely lethal in mice [14, 60, 70]. Solubilization of ethanol exerts osmolar pressure which leads to a relative dehydration. Control studies to examine the possible role of the dehydrating effect of high levels of alcohol vapor on nasal mucosa with attendant susceptibility to infection have not been reported.

Goldstein [17] has reported that the intensity of withdrawal convulsions (as rated by a special scoring system) increased as a function of the duration of exposure to alcohol vapor and concomitant blood alcohol levels. Moreover, mild withdrawal signs were observed following a single injection of alcohol, with or without pyrazole, while mice were still intoxicated [17]. Barbiturates and benzo-

TABLE 2

COMPARISON OF ALCOHOL WITHDRAWAL SIGNS OBSERVED IN DIFFERENT SPECIES*

	MAN (74,82)	MONKEY (4, 7, 8)	CHIMPANZEE (63,64)	DOG (6, 9, 10)	RAT (12)	MOUSE (14, 17, 60)
Tremor	X	X	X	X	X	X
Muscle Fasciculation	X	X	--	X	--	--
Hyperreflexia	X	X	X	X	--	--
Spasticity	--	X	X	X	X	--
Rigidity	--	X	X	X	--	--
Nystagmus	X	--	--	--	--	--
Tonic-Clonic Seizures	X	X	X	X	X	X
Convulsions on Handling	NA	NA	NA	NA	--	X
Fatal Convulsions	X	X	X	X	X	X
tail beating & arching	NA	NA	NA	NA	--	X
tail lift	NA	NA	NA	NA	--	X
lethargy	--	--	--	--	--	X
hyperactivity	X	--	--	--	X	X
irritability	X	X	X	--	--	
AUTONOMIC HYPERACTIVITY						
mydriasis/photophobia	X	X	X	--	--	--
tachycardia	X	--	X	--	--	--
respiratory rate	X	--	X	--	--	--
temperature	X	--	X	--	--	--
sweating	X	--	X	--	--	--
salivation	X	X	--	--	--	--
GASTROINTESTINAL SIGNS						
retching	X	X	--	--	--	--
vomiting	X	X	--	--	--	--
GROSS BEHAVIORAL CHANGES						
spontaneous fear reactions	X	X	--	X	--	--
startle to noise	X	--	--	--	X	X
decreased response to noise	--	--	X	--	--	--
exaggerated postures	--	X	--	X	--	--
intense scratching	--	X	--	--	--	--
stereotyped movements	--	--	--	--	--	X
sleep disturbances	X	--	--	X	--	--

observed = X
not reported = - -
not applicable = NA

*References are indicated in parenthesis under each species.

diazepines were effective in suppressing alcohol withdrawal reactions after three days of intoxication, whereas the phenothiazines and chlormethiazole were not [18]. Ethanol, paraldehyde and meprobamate each produced a temporary suppression of withdrawal reactions, as is the case in man [18].

Oral and Intragastric Administration

(1) Rodents. Falk has recently reported the successful application of a behavioral technique, schedule-induced polydipsia, in producing physical dependence in the rat [12]. Schedule-induced polydipsia refers to a situation in which rats will consume as much as 1/2 of their body weight in water within a few hours when dry food is presented intermittently. The polydipsia phenomenon was first reported by Falk in 1961 [11]. Rats reduced to 80% of their free feeding weight were exposed to a schedule in which a pellet was delivered automatically every two minutes during one-hour sessions. There were six one-hr sessions in each 24-hr day. Following establishment of polydipsia with water, an ethanol concentration of 1% was introduced and increased in 1% increments every 6–8 days

until 5 or 6% solutions were reached. This procedure resulted in an intake of between 11 and 15 g of ETOH/kg/day, which yielded blood alcohol levels maintained above 100 mg/100 ml and usually ranging between 150 and 300 mg/100 ml. Following three months exposure to this polydipsia regime, alcohol was abruptly withdrawn and animals consistently showed evidence of physical dependence including hyperactivity, tremor, spasticity and audiogenically induced tonic-clonic seizures [12]. Lester [31] was the first to show that polydipsia was an effective procedure for inducing intoxication in rats; however, he did not report evidence of physical dependence. High levels of alcohol intake in rats have been reported by several investigators using variations on a polydipsia technique, but none has produced physical dependence [20, 41, 42].

The polydipsia paradigm for inducing physical dependence upon alcohol in the rat as reported by Falk [12], meets the evaluative criteria previously described insofar as animals ingested more alcohol through time, showed evidence of intoxication, and evidence of physical dependence upon alcohol withdrawal. The polydipsia technique has the particular advantage of maintaining high levels of alcohol ingestion in the presence of adequate food intake, since animals can be maintained between 80 and 85% ad lib weight.

The potentially confounding effects of severe weight reduction in the model of physical dependence upon alcohol in mouse, developed by Freund in 1969 [14] have introduced some serious questions about the adequacy of that model [60]. By restricting food depleted mice to a liquid diet, in which 35% of the calories came from ethanol, gross intoxication and evidence of physical dependence were produced within 4 days [14]. The drastic reduction in weight to 65% of free feeding weight induced a higher effective dosage of alcohol because of the concomitant reduction in the rate of alcohol metabolism. It has been previously shown, both in man and in animals, that the rate of alcohol metabolism is reduced by as much as 50% in fasted, as contrasted to well fed organisms [13, 29, 30, 53, 62, 70]. Freund's basic observations have been replicated [60]. However, interpretation of the findings is unclear. The sucrose-alcohol diet was shown to produce a consistently greater percentage of severe withdrawal signs than the Metrecal-alcohol diet. However, the Metrecal-alcohol animals had a consistently higher blood alcohol level than the sucrose-alcohol animals [14,60]. Since a specific toxic factor cannot be attributed to the sucrose-alcohol diet, it appears that the nutritional deficiency associated with both diets may contribute to the withdrawal syndrome. Both diets produced a high level of mortality and liver pathology was comparable in the replication by Ogata and co-workers [60]. It is important to note that during the four days of alcohol administration, the mice already reduced to 65% of their free feeding weight, lost an additional 10% of their weight.

In order to determine if equivalent alcohol consumption with an adequate diet would be effective in producing physical dependence, a naive group of mice were exposed to a variation [60] of the polydipsia procedure first described by Falk [11]. Dry food pellets were automatically delivered at 180 sec intervals in four daily sessions. Following establishment of polydipsic drinking with water, a 3, 6 or 10% alcohol solution was introduced as the only fluid. This procedure was effective in inducing the consumption of large quantities of fluid each day which approached 40% of total body weight. Alcohol intake in the polydipsia paradigm was between 0.58 and 0.71 ml of absolute alcohol or 14–24 mg/gm. This volume exceeded that observed in the replication of the Freund procedure, i.e. 0.45–0.46 ml of absolute alcohol or 15–18 mg/gm. Average blood alcohol levels in the polydipsia procedure ranged between 73 and 279 mg/100 ml, a level significantly lower than that observed in the nutritionally deprived Freund replication animals (178–499 mg/100 ml). Despite sustained high alcohol intake for periods of 7–14 days, no polydipsia mouse showed evidence of physical dependence during a 10-hr observation period after the substitution of water for alcohol. Consequently, consumption of alcohol in amounts exceeding 15 mg/g or 0.50 ml of absolute alcohol per day for prolonged periods of time is not a sufficient condition to produce withdrawal signs in mice upon removal of alcohol [60].

The difference between the polydipsia and the liquid diet procedure cannot easily be interpreted in terms of liver histopathology and cannot be attributed to differences in strain, age, amount and duration of alcohol intake between the two groups. A severe nutritional deficiency in combination with the profound weight loss in the Freund replication mice clearly resulted in a higher effective dosage of alcohol as reflected by higher blood alcohol levels. There is also the possibility that differences in the pattern of drinking between the two groups contributed to the absence of withdrawal signs and symptoms in the polydipsia mice [60]. The differences between the polydipsia procedure which produced physical dependence in rats [12] and the polydipsia procedure which was unsuccessful in mice [60] is probably related more to the duration of exposure to alcohol, i.e. 14 days versus 3 months than to species differences. Further studies attempting to clarify this point are currently in progress and preliminary findings suggest that a 3-month exposure to the polydipsia procedure does result in physical dependence in mice (Mello, unpublished observations).

The Freund liquid diet plus severe weight reduction procedure has also been applied to the rat by Branchey and co-workers [2]. In contrast to the Freund procedure, rats were maintained at 66% of their ad lib weight during an alcohol administration period of 3 weeks. Upon withdrawal from alcohol, rats exhibited tremulousness and hyperactivity, muscle spasticity and occasionally, generalized convulsions. After two weeks of abstinence, alcohol was administered for 4 days to the 8 physically dependent animals and to naive controls. During the second alcohol withdrawal procedure, no control animal showed signs of physical dependence and only half of the previously addicted rats showed withdrawal signs [2].

Another technique which has proven effective in producing oral ingestion of intoxicating amounts of alcohol involves electrical stimulation of the lateral hypothalamus in rat [75]. During stimulation, rats will ingest aversive fluids and this phenomenon is referred to as "stimulus bound drinking". Thirty stimulation events over a period of 45 min produced consumption of alcohol concentrations as high as 20% at dose levels comparable to those observed using polydipsia procedures (16–21 mg/g). Rapid, high alcohol intake produced signs of gross intoxication. However, no animals showed evidence of physical dependence after as long as 25 days exposure to LH stimulation-alcohol sessions. Presumably the absence of physical dependence was a function of the single dose pattern of consumption

which did not yield the sustained high blood alcohol levels produced by multiple daily polydipsia sessions. No data are reported on the effects of multiple LH stimulation sessions. No animal showed enhanced preference for lower concentrations of alcohol in the home cage situation as a function of alcohol exposure [75].

Gastric intubation of alcohol in a range of 9–14 g/kg over a 5–7 day period has also been shown to produce physical dependence in rats. Alcohol was administered every 6 hr to insure consistent high blood alcohol levels. The reliability of the procedure in terms of the total number of animals showing positive signs of physical dependence is greatest when alcohol administration is maintained for at least 7 days. Refinement of this technique is still in progress, (Majchrowicz, personal communication).

(2) Primates and dogs. Efforts to develop behavioral methods to induce addictive drinking in rhesus monkey have been, thus far, unsuccessful. The major impediment appears to be the aversive taste of alcohol which is difficult to mask effectively. A second factor is the inevitable delay between alcohol ingestion and intoxication. It requires about 2 hr for a 25% alcohol solution, administered via a nasogastric tube, to reach peak absorption as indicated by enzymatic blood alcohol determinations [44]. The rate of ethanol metabolism in rhesus monkey is similar to the metabolic rate in man, i.e. about 25–30 mg/100 ml per hr [7, 8, 52, 76, 85]. Consequently, if alcohol consumption is only associated with an aversive taste, and never with intoxication, monkeys might never discover the potential reinforcing properties of oral alcohol ingestion. In contrast, the immediacy of the effects obtained from intravenous alcohol administration is undoubtedly a critical factor in maintaining self-administration.

The rationale for applying behavioral techniques to induce addictive drinking derives from the argument that a situation in which an animal drinks alcohol as a form of motivated behavior in order to avoid pain or to obtain a reward, is most analogous to the human condition. Operant conditioning techniques permit evaluation of the efficacy of positive and aversive stimulus control in maintaining alcohol consumption. Moreover, operant techniques can require drinking as the contingent response.

In a paradigm which involved using a consummatory (lick) response as the operant response, rhesus monkeys were trained to drink in order to avoid a noxious shock [48]. Both bourbon and ethanol solutions were presented in concentrations ranging from 5–25%. A 6-hr Sidman avoidance period [67] was alternated with a 6-hr rest period during which no shocks occurred. Experiments were run 24 hr a day, 7 days a week. Each monkey learned to drink to avoid shock at a rate sufficient to avoid virtually all possible shocks. However, the amount of fluid consumed did not remain stable across concentrations but decreased linearly as a function of increasing alcohol concentrations even though the rate of response was the same. It was found that monkeys had learned a dual avoidance response in which it was possible to postpone the occurrence of a noxious shock by making a lick response, and to avoid consuming an aversive fluid by modulating the duration of the lick response. As the ethanol concentration was increased, the mode of the lick duration distributions was shifted towards shorter lick durations which presumably resulted in smaller amounts of fluid dispensed per lick [48].

The apparatus was then modified so that only discrete licks of a specified duration were effective in postponing shock [48]. Monkeys were run for 60 days on a 10% alcohol solution and lick duration requirements were increased in 50 msec increments. Each monkey's lick duration shifted towards longer durations in accordance with the programmed lick duration requirement. However, despite the increase in lick durations, the volume of alcohol consumed did not increase. Monkeys drank about 2.5 g/kg per day and blood alcohol levels ranged between 30 and 70 mg/100 ml. No monkey showed evidence of intoxication or of physical dependence upon removal of alcohol. These data testify to the monkey's aversion to alcohol and suggest that monkeys had learned to control the amount of fluid dispensed by manipulating the displacement of the ball valve [48]. Studies are currently underway in which both lick duration and lick displacement are specified as part of the avoidance schedule.

In a reward association paradigm, rhesus monkeys were required to make a consummatory (lick) response to earn banana pellets on an intermittent schedule of reinforcement (Mult VI 1-DRL-20) [49]. Monkeys were run in single daily 3-hr sessions. During the water baseline, it was found that the amount of fluid consumed from a second bottle (adjunctive drinking) was far greater than fluid consumed from the response contingent bottle. Subsequently, alcohol concentrations in the response contingent bottle were gradually increased from 5–15% in 5% steps and fluid in the noncontingent bottle was increased at a slower rate in an effort to manipulate alcohol intake through contrast effects. Although monkeys ingested volumes of 5% ethanol equal to or exceeding baseline water consumption, (200 ml within 3 hr) a sustained intake of higher concentrations of alcohol was not observed. This consumption level yielded an alcohol dose which averaged about 3 g/kg. Blood alcohol levels averaged about 50 mg/100 ml [49].

Despite daily consumption of alcohol in doses of 2.5–5 g/kg, no monkey showed signs of gross intoxication. There were no signs of physical dependence following approximately 10 months of continuous access to alcohol under conditions of schedule-induced polydipsia. Moreover, 10 months of exposure to alcohol under these conditions did not increase preference for bourbon in the home chair or result in an increased intake of alcohol in the polydipsia situation [49].

One serious limitation of the polydipsia paradigm used in this study was that maximal levels of alcohol intake were induced only for 3 hr each day, and ingestion of bourbon during the remaining 21 hr was optional. It has recently been reported that induction of blood alcohol levels of 400 mg/100 ml for only 3 hr each day is not sufficient to produce physical dependence in an intravenous self-selection paradigm [80]. It appears that the maintenance of a consistent high blood alcohol level over successive days is important for the induction of physical dependence upon alcohol. Consequently, the potential utility of a single session polydipsia paradigm is greatly limited. Also, it is somewhat difficult to achieve a frequent and perhaps aperiodic high dose of alcohol during consecutive 24-hr intervals with a behavioral technique involving food reinforcement, since satiation for banana pellets occurs quite rapidly.

The use of relatively low alcohol concentrations (2.5 w/v) in a polydipsia paradigm has been more effective in inducing severe intoxication in the monkey [84]. Total

volumes of alcohol consumption exceeding 1000 ml over a 24-hr period were occasionally observed in monkeys with a prior history of intravenous alcohol administration and alcohol doses as high as 7.1 g/kg were self administered [84,85]. It was found that monkeys with an intravenous alcohol administration history drank more alcohol in a 2.5 to 4-hr polydipsia paradigm than naive controls during the initial 3–4 weeks of exposure to this schedule. Subsequently, the naive controls reached about the same level of intake as the alcohol experienced animals. About 5 out of 6 animals showed signs of intoxication and blood alcohol levels ranged between 150 and 200 mg/100 ml (Woods, personal communication). Signs of physical dependence were not observed in these monkeys under these conditions.

The optimal parameters of schedule-induced ethanol consumption to produce self-intoxication remain to be determined. These data suggest that a procedure involving multiple polydipsia sessions in which large volumes of low alcohol concentrations are consumed might prove most effective. However, it could also be argued that monkeys would have to consume very large volumes of a low concentration alcohol solution before intoxication levels would be reached.

A successful model of alcohol addiction in young chimpanzees (*Pan troglodytes*) has recently been reported [63]. One to 7-month old chimpanzees were given a liquid diet with 45% of the calories from ethanol, 4–5 times daily at standard feeding times. Chimpanzees maintained normal weight gain and consumed alcohol in doses from 2 g/kg to 8 g/kg. Blood alcohol levels ranged between 50 and 300 mg/100 ml with peaks as high as 500 mg/100 ml depending on the concentration of alcohol in the liquid diet. During the alcohol administration period, mild tremulousness, hyperreflexia and irritability were observed prior to the morning feeding (a 9-hr abstinent interval) when blood alcohol levels fell below 100-150 mg/100 ml. After 6–10 weeks on this regimen, alcohol was abruptly withdrawn and withdrawal symptoms observed included hyperreflexia, irritability, photophobia, rapid respiration, sweaty palms and feet, spastic rigidity and, in some instances, tonic and clonic convulsions resulting in death in one animal. An induced increase in the rate of ethanol metabolism comparable to that observed in man [56] was also found in chimpanzees. Liver biopsies indicated reversible fatty infiltration comparable to changes observed in man following ethanol ingestion [63].

This liquid diet procedure has recently been extended to adult rhesus monkeys with comparable results [64]. Monkeys maintained at 75–85% of ad lib weight developed withdrawal signs after 155 days of consumption of 2.5–7 g/kg of alcohol presented in gradually increasing doses. Alcohol was administered twice daily and withdrawal signs were frequently seen before the morning dose. Significant increases in the rate of ethanol metabolism were consistently observed.

In contrast to these findings on alcohol addiction in young chimpanzees [63], infant rhesus monkeys did not develop physical dependence after prolonged exposure to ethanol as the only fluid in an otherwise normal diet [55]. In order to determine the addiction potential of early experience with alcohol, four infant monkeys were provided with alcohol continuously from within two hr of birth. Newborn monkeys were separated from their mothers immediately and subsequently maintained under conditions of maternal and peer deprivation. Four monkeys were given increasing concentrations of alcohol as their only fluid source and four control monkeys were maintained under identical conditions and given a free choice between comparable concentrations of alcohol and water. During the first three months of life, the experimental infant monkeys consumed between 2 and 5 g/kg of ethanol with blood alcohol levels ranging between 50 and 80 mg/100 ml. During the next six months, alcohol intake increased and varied between 4 and 10 g/kg and blood alcohol levels of 200 mg/100 ml were occasionally observed. This pattern was sustained for the remainder of the first year and stabilized during the second year with intakes between 5 and 8 g/kg. No monkey showed evidence of physical dependence when withdrawn from alcohol. Subsequent assessment of blood alcohol disappearance curves after acute nasogastric intubation of 2 g/kg of 25% ethanol showed no significant differences from control monkeys, raised under identical conditions with access to water. Examination of alcohol selection in comparison to water showed that no monkey had developed a preference for alcohol [55].

Forced alcohol administration procedures have proved to be consistently effective in producing alcohol addiction in monkeys [5, 7, 8] and in dogs [6, 9, 10]. Ellis and Pick [5] were first to report that nasogastric intubation of alcohol (25%) in 2 or 3 divided doses of 4–8 g/kg is effective in producing physical dependence upon alcohol in rhesus monkey within 10–18 days. These monkeys showed tremor, spasticity, hyperreflexia, mydriasis and clonic-tonic convulsions which could be suppressed with ethanol. Monkeys also exhibited an alcohol-induced increase in metabolic rate [5, 7, 8].

Essig and Lam [9] were the first to report alcohol dependence in the dog following prolonged administration via a surgically implanted gastric cannula. The 8 out of 12 dogs that survived the alcohol administration regimen did show definite signs of physical dependence including tremulousness, tonic extension of the extremities, and seizures. Some animals who did not exhibit convulsions appeared to be attending to nonexistent visual stimuli, described by the authors as hallucinations, and they reported a disruption of sleep during the withdrawal period [9,10]. These animals lost an average of 1.6 kg during the total period of intoxication.

The effect of intragastric administration of alcohol in monkeys has recently been examined by Yanagita and colleagues [87]. An intragastric catheter was implanted via a nasogastric route in contrast to the technique of Essig and Lam which involved surgical implantation of a gastric cannula in the upper quadrant of the stomach [9]. All monkeys were maintained for 5 or 6 weeks using either voluntary or forced ingestion of alcohol. The reinforcing properties of alcohol were apparently dependent upon the prior history of reinforcement, as has also been reported with intravenous alcohol administration [85]. Naive monkeys did not initiate self-administration and programmed alcohol was presented at 1.0 or 2.0 g/kg/infusion every 3 hr or every 6 hr for 5 or 6 weeks. After only 5 days of intragastric administration of 2.8 g/kg of alcohol every 3 hr, mild abstinence signs were observed. However, a withdrawal probe did not result in increases in lever pressing behavior for alcohol.

Three monkeys that were familiar with intravenous self-administration procedures, administered intragastric

doses of alcohol ranging from 2.8–7.5 g/kg. These monkeys showed severe abstinence syndromes. It appears that the critical distinguishing variable is experience with drug self-administration rather than experience with alcohol since two naive monkeys failed to initiate self-administration even after programmed administration of alcohol at a dose level of 8.0 g/kg/day for periods of 2 months [87]. Comparable data have been reported for initiation of intravenous self-selection [4]. These data strongly suggest that alcohol self-administration is controlled by the reinforcing properties of alcohol rather than by pharmacological exposure per se.

Intravenous Administration.

Yanagita, Deneau and Seevers [86] were the first to report the successful addiction of monkeys to alcohol, using a paradigm in which a monkey could press a lever to activate an automatic intravenous injection apparatus. Their original observations have been subsequently confirmed and extended [4, 80, 84, 85]. Consistent delivery of 6–8 g/kg/day for 10 weeks or less appears to be sufficient to produce signs of physical dependence. Upon discontinuation of alcohol, an abstinence syndrome appeared within 6 hr characterized by tremor, vomiting, hallucinatory behavior and convulsions [4].

The initiation of intravenous self-administration of alcohol does not occur as consistently as with cocaine and opiate narcotics. In one study, only 11 of 27 monkeys initiated responding for alcohol when exposed for 10 or more days. Consequently, it was necessary to elicit high response rates with the use of another consistently reinforcing pharmacologic agent, i.e. cocaine [85]. This procedure proved to be effective in establishing a high response rate which then persisted once ethanol was substituted for the initial drug. Both cocaine and pentobarbitol have been used to establish ethanol reinforced self-administration. The factors which make intravenous ethanol a less potent reinforcer than these other psychotropic agents are unknown.

It has been clearly shown that intravenous alcohol is a reinforcer and that monkeys will self administer to the point of intoxication. The degree of intoxication produced with this technique results in severe motor incoordination, stupor and occasionally light anesthesia [4]. During the course of ethanol self-administration, monkeys show loss of weight, minimal food intake and general ill health associated with malnutrition [4]. Once monkeys begin alcohol self-administration, their usual daily rate of intake remains quite constant. Within a dose range of 0.05–0.20 g/kg/injection, the overall intake within a 3-hr period appears to be independent of the dose delivered with each injection. Moreover, if a presession infusion of alcohol is introduced and increased from one to 3 g/kg, response frequency for ethanol reinforcement is reduced correspondingly [85]. Over a period of six months, pharmacological tolerance as reflected in changed elimination rates of alcohol was not observed [85].

There is a striking similarity between patterns of intravenous self-selection of alcohol by the rhesus monkey and spontaneous drinking patterns observed in human alcohol addicts [4, 50, 58, 59, 85]. Human alcohol addicts, given an opportunity to work for alcohol at a simple operant task, frequently alternate drinking episodes of 3–6 days with relatively abstinent work periods of 2–3 days. These abstinent periods are usually associated with partial withdrawal signs and symptoms [50, 58, 59]. Alcohol self-administration in monkeys is also punctuated by periods of spontaneous abstinence which are associated with withdrawal signs [4,85].

The factors which determine these episodes of self imposed abstinence are difficult to specify. Self imposed abstinence occurs only when monkeys are given 24-hr access to alcohol and never when ethanol access is limited to 3 hr per day [85]. However, only 3 hr a day of intoxication (400 mg/100 ml) is not sufficient to produce physical dependence [80]. Spontaneous termination of self-administration has not been consistently associated with any particular number of days or amount of alcohol taken by monkeys on a 24-hr access paradigm.

DRUG SELF-ADMINISTRATION AND PHYSICAL DEPENDENCE

The ambiguous relationship of physical dependence to subsequent drug self-administration has been repeatedly acknowledged by behavioral pharmacologists, but as yet, there has been no empirical resolution of these issues [24, 45, 50, 78, 79, 83, 84, 85]. The cyclicity of self-administration of alcohol, both in animal and human addicts, raises some basic questions concerning the extent to which physical dependence controls subsequent drug selection; i.e. (1) What is the role of physical dependence in maintaining drug self-administration? (2) What is the role of physical dependence in the reinitiation of a drug self-administration sequence following a period of abstinence? (3) Following the induction of physical dependence, what is the persistence and duration of this condition?

Maintenance of Drug Self-Administration

It is usually assumed that once an addictive drug consumption pattern is in effect, the avoidance of withdrawal signs and symptoms is a critical factor in maintaining drug use. However, if the avoidance of withdrawal signs is the factor which motivates an alcoholic to continue drinking, it would be expected that he should drink consistently enough during a drinking episode to avoid withdrawal signs and symptoms. Both human alcoholics [47,50] and addict monkeys [84,85] frequently exhibit partial withdrawal signs during a period of alcohol administration. Despite the attendant discomfort, the human alcoholic does not invariably respond to these partial withdrawal signs by increased drinking [47,50]. A comparable cyclicity, involving several days of abstinence has not been reported for monkeys addicted to morphine [4]. Data on patterns of heroin self-administration in man are not available for comparison.

Clinical data on narcotics addicts have suggested that incipient withdrawal signs may add to both the gratification and perpetuation of drug use [77]. Wikler has suggested that although "the intensity of the initial 'euphoria' is not regained as long as the uninterrupted schedule of drug administration is maintained . . ." drug use may come to produce another sort of pleasure, ". . . namely, that arising from the relief of such abstinence related discomfort as developed towards the end of the intervals between injections." [77].

Whatever the interpretation of cyclicity of drug administration in a physically dependent individual, these data argue against any simplistic formulations in which drug

effects are automatically defined as "positive" and abstinence effects as "negative." In the literature on the behavioral effects of drugs, there are considerable data which indicate that "a schedule-controlled pattern of responding is itself a fundamental determinant of behavior; that is, the schedule conditions engender what was formerly thought of as motivation." ([27]; p. 27). This idea is illustrated by the perplexing finding that monkeys trained on a continuous shock avoidance schedule, continued to maintain response rates in the presence of unavoidable shocks [28]. These observations have been reconfirmed and extended to show that self-administration of shock can maintain responding [26, 38, 39]. These findings indicate that it is the schedule of shock presentation, rather than the quality of the shock itself, which determines whether responding is suppressed or maintained [26]. Moreover, the effect of response contingent shock is also influenced by the history of reinforcement and the rate of ongoing responding when the initial response-contingent shock is presented [26]. Kelleher and Morse have made the point that "A reinforcer or punisher should not be conceptualized independently of the way it controls behavior." They caution that "When an event following some behavior increases the subsequent occurrence of that behavior, it does not mean that the same event consequent on some other behavior or scheduled in a different way would necessarily have the same effect." ([26]; p. 837).

There has been relatively little attention to the schedules of reinforcement which produce alcohol self-administration and no effects to reanalyze spontaneous self-administration patterns to allow programmed simulation of naturalistic patterns. However, the analysis of alcohol self-administration patterns does provide a potentially powerful tool for better clarifying the functional relationships between physical dependence, intoxication and alcohol self-administration. Animal models of addiction are ideal for such an approach since the confusions introduced by concepts of "psychic" and "social" dependence can be avoided.

Concerning Psychological Dependence

Definitions of drug dependence are usually subdivided into physical and psychological dependence [79]. Psychological dependence is imprecisely defined and often is used to refer both to the motivational factors which underlie drug seeking behavior and the psychological effects of drug use. This semantic confusion between motivational and drug effect aspects of dependence reflects our current limited understanding of the factors which initiate and maintain an excessive drinking episode.

In response to the dualism inherent in the usual distinctions between psychic and physical dependence, Wikler [79] has recently offered some operational definitions of drug dependence based on the concepts of primary and secondary reinforcement. Primary pharmacological reinforcement is subdivided into two categories of direct and indirect reinforcement. These are differentiated in terms of the extent to which the effects have or have not been generated by the drug itself. According to Wikler's formulation, the positive drug effects usually associated with psychic dependence are direct, drug organism interactions not generated by the drug itself. Whereas, indirect drug organism interactions involve aspects of physical dependence, i.e. suppression of abstinence signs, which have been generated by the drug itself. Secondary reinforcement refers to drug seeking behavior in the presence of stimuli which were previously coincident with primary reinforcement. Wikler suggests that these proposed definitions may provide a common conceptual framework for human and animal research on drug dependence [79]. Presumably, the term "due to experience with" could be substituted for "generated by the drug itself" and thereby avoid ascribing an undefined potency to the drug which cannot be specified in observable terms such as frequency of drug use. Aside from such semantic details, definitions of drug dependence based on observable behaviors seem to offer a decided advantage over the mentalistic vagueness of psychic versus physical dependence.

For example, discussions of drinking patterns in man are frequently confused by introduction of the notion of "craving" which is usually defined to imply that "every time the subject starts drinking, he is compelled to continue until he reaches a state of severe intoxication." ([37]; p. 146). The "craving" concept is often advanced to explain initiation of drinking after abstinence as well as drinking to postpone or diminish withdrawal signs and symptoms. Yet, there is no evidence that the resumption of drinking can be explained by an acquired "craving," any more than can the initial excessive drinking which must precede the development of physical dependence [22]. The conditions which prompt an individual to begin drinking heavily at a certain time and then perpetuate his drinking behavior are unknown. Thus far, there has been virtually no empirical support for the construct of craving [45] as a factor in maintaining drinking behavior.

Reinitiation of Drug Self-Administration

Reinitiation of addictive drinking after a period of abstinence is undoubtedly the complex resultant of a number of psychogenic and stress response factors in man. It has not been established whether the reinforcing properties of a drug are enhanced following development of physical dependence, drug withdrawal and subsequent abstinence. Existing data do not support the notion that the presence or absence of physical dependence influences the rate of response for drug in the same way that the presence or absence of food deprivation affects the rate of response for food. It has been shown that responding for intravenous alcohol under conditions in which physical dependence cannot be produced (i.e. a 3-hr access paradigm) is more consistent than in the 24-hr access paradigm [80]. It has also been shown that monkeys will administer intravenous doses of opiates at levels below that required to produce physical dependence [83]. These data underscore the importance of the momentary reinforcing effects of the drug in maintaining responding. In narcotics addicted monkeys, in contrast to alcohol addicted monkeys, it does appear that responding is maintained to avoid withdrawal signs [4,83].

The contribution of the condition of physical dependence to the reinforcing properties of alcohol is unclear. The extent to which forced administration techniques for producing physical dependence upon alcohol result in subsequent motivated drinking behavior have not been examined. It is not known whether an animal thus addicted will elect to consume large quantities of alcohol once the initial induction of physical dependence and recovery from an abstinence period is past. There have been no studies of

oral alcohol consumption in monkeys during alcohol withdrawal. In fact, since alcohol withdrawal may be associated with considerable gastrointestinal distress, consumption measures during withdrawal may be equivocal.

Duration of Physical Dependence upon Alcohol

Since the relation of physical dependence to subsequent drinking has not been clearly established, it is difficult to examine the duration of physical dependence behaviorally. Branchey and co-workers [2] examined the frequency of abstinence signs in a group of rats which had shown evidence of physical dependence, and were then given alcohol for 4 days after a two-week interval. Half of the animals which had previously been physically dependent on ethanol, became dependent again after 4 days reexposure to alcohol. No control animal showed evidence of physical dependence within that time period. In human morphine addicts, it has been reported that 6–9 weeks following the acute abstinence syndrome, physiological changes (myosis, lower blood pressure, respiratory and cardiac rate and temperature) were observed which persisted for six months or more [37]. Comparable studies have not been reported on alcohol addicts. Prior to the 1966 rulings in the case of Driver vs. Hinnant and Easter vs. The District of Columbia [3], 30–90 day commitments for the crime of public drunkenness were a frequent occurrence. Individuals so sentenced, were often resentenced after an interval of perhaps five days intoxication. Consequently, the skid-row alcoholic might have considerably less opportunity to drink alcohol during a year than many nonaddicted drinkers. Yet, withdrawal signs often occurred following cessation of drinking. There is insufficient evidence available to determine whether this represents persistence of physical dependence or reinduction of physical dependence.

CONCLUSIONS

It has been apparent throughout this review, that although techniques now exist to induce pharmacological dependence upon alcohol; our understanding of the behavioral aspects of alcohol self-administration is severely limited. Although a number of theories have been developed concerning the biological bases of addiction (cf. [53,54]) none have yet been sufficient to account for these phenomena. The many unresolved questions about the biological and behavioral bases of physical dependence upon alcohol and other addictive agents present a continuing challenge to biochemists, pharmacologists and behavioral scientists.

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APPENDIX I (from 66)

Time Drug Removed: _____ Monkey # _____

Total Drug Days: _____ Date: _____

BEHAVIOR	INVOLUNTARY SOMATIC RESPONSES	SYMPATHETIC	PARASYMPATHETIC
Apprehension	Labored breathing	Yawning	Runny Nose
Restlessness	Temperature change	Perspiration	Lacrimation
Resistance to handling	Inflamed eyelids	Piloerection	Cough
Vocalization	Strabismus	Pallor	Miosis
Peculiar Posture (Describe)	Nystagmus		Anorexia
Unusual Behavior (Describe)	Intention tremor		Salivation
Increased Sex Activity (Describe)	Arm tremor		Retching
Sleep/lethargy	Hand tremor		Vomiting
Heart Rate	Leg tremor		
Respiratory Rate	Tongue tremor		Diarrhea
	Generalized tremor		
	Muscle gasaculations		
	Muscle rigidity		
	Elicited hyperreflexia		
	Convulsions: clonic-tonic		
	Hiccough		

Time of Observation _____ Hr since alcohol removed _____ Film Record _____