Effects of Prenatal Alcohol Exposure on Pup Development and Vocalization Behavior and on Dam Retrieval Behavior

by

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(ABSTRACT)

An animal model (Rattus norvegicus) was employed to study the effects of chronic prenatal alcohol exposure on pup development and on the functional efficacy of pup vocalizations on the maternal behavior of the dam. Subjects were 72 dams and their litters. Dams were matched by weight and assigned to either an Ethanol (EtOH), a Pair-fed (PF), or an Untreated Control (UC) group. Ethanol dams received 15% ethanol as their sole source of fluid throughout the experiment. Pair-fed dams were fed isocalorically to EtOH dams. Untreated Control dams received food and water ad libitum.

Dam's retrieval behavior was assessed in a runway choice situation when pups were 3, 5, 7, and 9 days old. Developmental measures were taken on pups from ages 0 through 13 days. Blood ethanol concentrations were also analyzed for dams and pups.

The data showed that the BEC of EtOH dams was .1% and that EtOH pups showed a negligible BEC postpartum. Prenatal alcohol exposure was shown to have a direct pharmacological and indirect nutritional effect on pup development. Ethanol dams retrieved a reliably smaller percentage of pups and retrieved reliably more slowly than did controls. Pair-fed pups showed a higher rate of calling than did other pups and tended to be chosen more often by UC and PF dams than were EtOH or UC pups. Ethanol dams tended to chose UC pups more often than other pups.

These findings suggest that chronic prenatal alcohol exposure produces altered behavior and responsiveness in the dam and the pup. This altered behavior and responsiveness may have a synergistic effect on the interaction between the dam and the pup.

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Fetal Alcohol Syndrome

The adverse consequences of maternal alcohol consumption on the fetus have been noted as far back as Aristotle's time (Warner & Rosett, 1975). However, it was Jones and Smith (1973) who first brought world attention to the detrimental effects of maternal alcohol consumption on fetal morphogenesis and called it "fetal alcohol syndrome"(FAS). As many as 8000-10,000 children are born annually in the U.S. with at least a partial expression of FAS (Clarren & Smith, 1978). This statistic, however, may underestimate the true frequency of FAS, since many children who have the syndrome exhibit varying degrees of its symptoms, and thus are often misdiagnosed (Abel, 1980).

Fetal alcohol syndrome refers to a pattern of anomalies observed in children born to women who consumed alcohol during pregnancy. The main characteristics of FAS are pre- and postnatal growth deficiency, craniofacial dimorphism, retardation of psychomotor development, and limb and organ anomalies. Pre- and postnatal growth deficiencies include intrauterine growth retardation and postnatal delays in development. These delays in postnatal development are not due to differences in relative gestational age between FAS and "normal" children. When gestational age is taken into account, FAS children still exhibit delays in morphological development when compared to "normal" children (Abel, 1982). Craniofacial dimorphisms are distinctive and include short palpebral fissures, epicanthic eye folds, small eyes, microcephaly, and occasionally ptosis and strabismus (Majewski & Goecke, 1982). Microcephaly is of particular clinical importance because it is indicative of possible underdevelopment (Abel, 1981). Retardation of psychomotor development is a general category for behavioral symptoms associated with FAS. These symptoms include poor coordination, mental retardation, hypotonia, irritability, and hyperactivity (Van Thiel, 1981). Limb and organ anomalies are not uncommon, the most frequent of which are heart defects, which occur in about 30% of FAS cases (Abel, 1982).

Cinical and animal studies have shown that in the absence of definitive physical symptoms, fetal alcohol exposure may result in a wide range of subtle disruptions which often lead to the misdiagnosis of FAS (Abel, 1980). These effects include delay of physical and behavioral development (Abel, Dintcheff, & Bush, 1981; Diaz & Samson, 1981; Durand & Carlen, 1984) altered responsiveness to external and internal stimuli (Abel, Bush, & Dincheff, 1981; Anandam, Felgi, & Stern, 1980), and impaired performance on various learning tasks (Mukherjee & Hodgen, 1982; Riley, Lochry, & Shapiro, 1979).

Historically, research on FAS has presumed that postnatal deficits in infant behavioral development results directly from prenatal alcohol exposure. Conversely, it is possible that FAS may be mediated by alterations of the postnatal environment, which, in part, may result from the behavior of the infant. The proposed research incorporates a model of early behavioral development which has recently been formulated by developmental psychologists working in the psychobiological tradition (Cairns, 1979; Gottlieb, 1976; Kuo, 1969). This model, called the bidirectional model of development, suggests, that chronic perinatal exposure to alcohol may produce altered behavior and responsiveness in both infant and mother. These modifications may have a synergistic effect on the quality of mother-infant interactions and thereby, influence the infant's behavioral development.

Infant's Effects on its Environment

The infant cry has been shown to play a role in the development of the mother-infant interactive system (Thoman, Acebo, & Becker, 1983). Bell and Ainsworth (1972) reported that the infant can influence its environment through the signaling quality of its behaviors and, initially, the infant's cry is its most effective signal. However, the mother-infant interactive system can go awry if the infant's cry signal is uninterpretable or aversive to the mother (Thoman et al., 1983). This is typically the case when the infant demonstrates obstetric and anthropometric indexes of high risk (Zeskind, 1980). Lounsbury and Bates (1982) showed that infants, who were rated (Infant

Characteristics Questionnaire) by parents as having an average or difficult temperament, had cries which were described as mildly irritating and spoiled' sounding. Zeskind and Lester (1978) demonstrated that men and women rated cries of high-risk infants as more aversive and arousing than cries of low-risk infants. In addition, Frodi, Lamb, Leavitt, Donovan, Neff, and Sherry (1978) reported that adults evinced greater autonomic arousal and higher ratings of aversiveness upon hearing the cries of high-risk as opposed to low-risk infants. Acoustical analyses of cries emitted by high-risk infants reveal short duration, high average fundamental frequency and maximum frequency, and fewer numbers of harmonics in the cry sound (Zeskind, 1983).

In general, children who exhibit various types of congenital defects are typically characterized by high-pitched disagreeable screaming, are difficult to tolerate, and are likely to elicit abusive behavior (Frodi, 1981). Prenatal alcohol exposure also results in a number of congenital defects and is also frequently associated with child abuse (e.g., Kempe & Kempe, 1978; Mayer, Black, & Mc Donald, 1978). In light of this, it is conceivable that prenatal exposure to alcohol may affect the acoustical properties of the infant cry signal. Thus, the infant who is born to a chronically alcoholic mother may experience pathological interactions with the caregiver as well as be at risk for the biological anomalies associated with FAS. Pathological interactions may, then have a synergistic effect on the infant's development (Sameroff & Chandler, 1975).

The Animal Model

The present research employs an animal model to study the effects of chronic alcohol exposure on the functional efficacy of neonatal rat (Rattus norvegicus) vocalizations on rat maternal behavior. Use of the animal model allowed for the experimental control necessary for isolating specific behavioral effects of alcohol while avoiding the numerous confounds associated with human subjects (Stott & Latchford, 1976).

However, the animal model was not used soley because it allows for control. There are two underlying isomorphic processes which give credence to the use of an animal model of FAS. First, in rats as in humans, neonatal vocalizations are an important mediator of maternal behavior (Murray, 1979; Noirot, 1972; Scoville, 1982). Second, the deleterious effects of prenatal alcohol exposure in rats are similar to those in humans on birth weight, growth rate, behavioral reactivity, pharmacodynamics, and neural development (e.g., Abel & Greizerstien, 1982; Riley, Lochry, & Shapiro, 1980; Salaspuro & Lieber, 1980; Samson & Diaz, 1982).

Among mammals, maternal behavior is closely synchronized with the behavior of the developing offspring (Rosenblatt & Lehrman, 1963). This behavioral synchrony is especially true for mammals bearing altricial young, because of the extended period of maternal dependence which antecedes independent function in the offspring (Rosenblatt, 1965). Specifically, Rosenblatt & Lehrman (1963) found that the behavior of a dam and its pups are, in part, the result of synchronous developmental changes in each. Futhermore, mutual and reciprocal elicitations of behavior by the dam and its pups are appropriate to the stimuli each provides to the other.

Ultrasonic cry stimulation by neonatal rat pups is crucial for normal interactions between dams and pups (Bell, 1979). Studies have reported that ultrasonic vocalizations to thermal and tactile cues change with pup age. Pups between the ages of 0 and 3 days are essentially ectothermic (DeGhett, 1979). At nest temperatures (35°-40° centigrade) pups do not vocalize. As nest ambient temperature falls below normal, vocalizations start and continue until nest temperature approaches 0° centigrade. At 0° centigrade pups stop vocalizing and eventually become comatose (Noirot, 1972). Between 5 and 7 days of age, pups achieve endothermy provided that the ambient temperature is above 25° centigrade (DeGhett, 1979). Peak frequency of calls per minute occurs between 5 and 7 days of age (Elwood & Keeling, 1982). When pups reach 9 days of age acoustical call properties begin to change. Calls gradually begin to shift from long call duration (up to 140 msec) and gradual changes in frequency to calls of shorter duration (less than 65 msec) and more marked changes in frequency (Sales & Smith, 1978).

The species-typical neonatal rat vocalization, which stimulates dam maternal behavior, has a fundamental frequency of approximately 45 KHz. These ultrasonic vocalizations initiate and direct the dam's retrieval of pups that are separated from the nest (Allin & Banks, 1972). Further, pup calls serve to establish and maintain the dam's long term pattern of maternal behavior (Bell, 1979; Hennessy et al., 1980). Emission of distress ultrasounds from pups evokes orienting behavior from the dam, departure from the nest, and retrieval of the pup back to the nest (Allin & Banks, 1972; Sewell 1970; Smotherman, Bell, Hershberger, & Coover, 1978). Ultrasonic pup cries also affect prolactin release in lactating dams (Terkel, Damassa, & Sewyer, 1979) and seem to have more long term effects on dams' maternal behavior with subsequent litters. For example, pups which were handled in order to augment their ultrasonic emissions, received increased maternal behavior from the dam (Bell, 1979; Wright, Bell, Schreibe, Villescas, & Conley, 1977). In multiparous dams, this increased responsiveness carried over from first to second litter. Dams whose first litter was handled showed increased responsiveness to their second litter, whether or not it was handled.

Behavioral interactions between dams and pups are not fixed but malleable. The way this interaction is expressed is not a result of the pup's nor the dam's behavior but a result of their behavioral relationship (Bell, 1979; Wright, Bell, Schreiber, Villescas, & Coonely, 1977). The quality of the behavioral relationship between dams and pups is essential to pup development. Galler and Rosenthal (1979) made this point in their study of the effects of intergenerational malnutrition on dam-pup interactions. Pups reared by malnourished dams weighed significantly less than those reared by well nourished dams. Well nourished dams that reared malnourished pups spent less time nursing and contacted fewer pups than did well nourished dams that reared well nourished pups. Weizenbaum & Hartigan (1982) studied the quality of the behavioral relationship between dams and pups. They employed a cross-fostering technique to separate prenatal from postnatal effects of alcohol on maternal behavior. Maternal behavior was measured by the latency of a dam to retrieve her pups to a nest site, after the pups were randomly placed about the nest box. Weizenbaum & Hartigan (1982) found that maternal behavior of alcoholic dams was differentially effected by the prepartal condition of the subject pups. That is, maternal behavior was less impaired in alcoholic dams who had control pups crossfostered to them, as compared to alcoholic dams who had fetally alcoholic pups crossfosterd to them. These findings suggest that the alcoholic condition of the dam may not be soley responsible for the decline in maternal behavior. Rather, some characteristic of the pup may have contributed to the disruption of the dam's maternal behavior.

One possible characteristic is the pup vocalization as a signal for maternal behavior. Fetally alcoholic pups may call less frequently than control pups, and such differences may alter maternal reponses. Alternatively, if dams consistently prefer control pups over fetally alcoholic pups, but the rate of calling does not differ between alcohol and control pups, then an effect of alcohol on some acoustic quality of the calls would be implicated. Finally, differences in the maternal competency of the dam should be reflected in group differences in dam's responses to the vocalizations of the alcohol and control pups.

Behavior Study

Method

Subjects

Maternal subjects were 72 virgin female Sprague-Dawley rats (Rattus norvegicus), bred in the colony maintained by the department of psychology at VPI & SU. Thirty-six dams were used in the choice test phase and 36 dams were used in the developmental measures phase. Dams were 100-120 days of age at the start of the experiment. They were housed in a 3 m x 4 m x 3 m experimental animal maintenance room, which was isolated from the main colony. Room temperature was maintained at 23 +1°C. Room illumination was on an L-D 14:10 photoperiod with light onset at 1200 hours EST.

Neonatal subjects were 432 rat pups derived from breeding the maternal subjects to outbred Sprague-Dawley males from Dominion Laboratories, Dublin, Virginia. Two hundred-sixteen pups were used in the choice test phase and 216 pups were used in the developmental measures phase.

Apparatus

The choice test apparatus was a 120 cm x 15 cm x 20 cm runway constructed of 1/4 in. plexiglas. A start box was positioned in the center of the runway and was partitioned from the rest of the runway by two guillotine doors. Each door was 7.5 cm from either side of the runway midline. The start box and the two arms of the runway were covered with three separate hinged sections of 1/4 in. plexiglas. Three red incandescent lights above the runway provided illumination. All but the observation side of the runway was painted black.

At each end of the runway were 15 cm x 5 cm x 5 cm wells located 5 cm below the surface of the runway floor. Bat detector microphones were located beneath each end of the runway and were fit into 1/2 in. holes drilled into the center of each respective 15 cm x 5 cm well wall. An 1/8 in. hole was drilled into the center of each of the 15 cm x 5 cm wall opposite to the ones mentioned above. This hole had an aspirator hose connected to it which provided an air flow of 2 liters per minute from the start area into each arm of the runway.

Pup vocalizations were monitored by a QMC mini bat detector and a QMC S100 bat detector each tuned to 45 KHz. Tuning was accomplished by sampling, via a Bruel and Kjear 1/4 in. microphone, one minute of calls emitted by 20 nonexperimental pups and observing the output signal on a Tektronix oscilloscope. A mean frequency was calculated from the sample and a matching frequency was created by a tone generator and played to each of the bat detectors. This tone was used to tune the bat detectors so that both instruments measured the same frequency and relative amplitude. The bat detector outputs were plugged into separate inputs of an Ampex ATR 700 two track reel to reel tape recorder.

The choice test apparatus was located in a 3 m x 4 m x 3 m observation room. The room was isolated from the main colony and the experimental animal maintenance room. The observation room was maintained at 23 +1° C and was illuminated by red incandescent lights.

Procedure

The behavior study used three sets of 12 dams. This study consisted of five consecutive phases; a 7 day entrainment phase, a 7 day weight phase, a 7 day alcohol acclimation phase, a 27 day maternal phase, and a 10 day testing phase. The testing phase appears in the text as two separate subsections: Developmental Measures and Choice Test.

Entrainment Phase. Maternal subjects were removed from the main colony and were housed individually in 20 cm x 20 cm x 30 cm single-hanging wire cages in the experimental animal maintenance room. Subjects were allowed 7 days to acclimate to the light-dark cycle. Food (Wayne Lab Blox) and tap water were available ad libitum.

Weight Phase. Body weights of female rats were recorded at 1300 hours daily over a seven day period. Mean body weight was calculated for each animal over that period. Means were calculated because the weight of a female rat systematically varies in relation to its estrous cycle (Weizenbaum, Kenny, & Alder, 1979).

Alcohol Acclimation Phase. This phase consisted of assigning the dams to groups, allowing them seven days to acclimate to their respective experimental conditions, and initiating the collection of maintenance data. The dams were assigned to one of three conditions (n=12); an ethanol group (EtOH), a pair-fed group (PF), or an untreated control group (UC). Subjects were assigned to triplets by matching them in threes according to mean body weight. Dams within each triplet were assigned to groups by counterbalancing the dams across triplets according to weight. This procedure was essential for the effectiveness of the PF control group (Abel, 1980).

The EtOH group dams received a 15% ethanol solution as their sole source of fluid throughout gestation and lactation. The solution was prepared by mixing 15 g of 190 proof ethanol (Fisher Brand) for every 80 g of 0.1% sodium saccharin with tap water as the solvent. In order to maximize intake and minimize stress, dams were gradually acclimated to the 15% ethanol solution; two days at 5%, two days at 10%, and three days at 15%. These maternal subjects also received ad libitum food throughout the experiment.

The PF group dams received a 0.1% sodium saccharin solution as their sole source of fluid throughout gestation and lactation. The PF dams received an amount of food equal to the amount of food their ethanol dam counterpart consumed for that day. In addition, the PF dams received a daily ration of sucrose equal to the caloric value of the ethanol consumed by their ethanol group counterpart for that day. The sucrose ration was calculated by the following equation:

$$[(0.15X(7.1\text{Kcals/gm EtOH})) / 3.94 \text{ Kcals/gm Sucrose}],$$

where X is the amount of fluid consumed. Thus, the PF group dams were matched isocalorically to their EtOH group counterparts. The UC group dams received tap water and food ad libitum throughout gestation and lactation.

Fluid intake, food intake, and body weight for each dam was measured at approximately 1300 hours daily from this phase through the completion of the experiment.

<u>Maternal Phase</u>. At the start of the fourth week of the experiment, the dams were bred. Breeding took place in the male's cage daily for six days from 0800 to 1200 hours (food and fluid removed). Copulation was verified by the presence of copulation plugs. Plugs were checked for when the dam was removed from the male's cage. The detection of copulation plugs was used to mark Day one of gestation.

Two weeks postcopulation maternal subjects were removed from their wire cages and were placed in standard 50 cm x 20 cm x 20 cm lab maternity tubs. At day 19 of gestation dams were checked for liters at 1300 and 1700 hours daily. The day on which a litter was first detected was designated as day 0 for that litter. On day 0, the length of gestation, number of pups born per litter, litter birth weight, average pup weight per litter, and number of pups born dead were recorded for each dam. The gestation period was measured as the length of time from the appearance of copulation plugs to parturition. Number of pups per litter was determined by counting the number of live pups per litter. Litter weight was determined to the nearest tenth of a gram (Mettler P1000 balance). Average pup weight per litter was calculated by dividing litter weight by number of pups per litter. Number of pups born dead was determined by counting the number of dead pups per litter.

After the parturition data were collected, litters were culled to six pups, 3 males and 3 females. Culling was accomplished by retaining pups whose weights were nearest to the median weight of the litter. This procedure was used to decrease interlitter variability in body weight and to minimize the confounding of behavioral and body weight effects (Abel, 1982). Pups that were retained were marked with nontoxic ink for identification.

Developmental Measures. Developmental measures were taken on all pups from ages 0 through 13 days at approximately 1500 hours daily. The measures were body weight, body length, age at which ear unfurling occurred, age at which upper incisor eruption occurred, and latency to perform a righting reflex (Martin, Martin, Sigmund, and Radow, 1977).

Individual body weights were measured to the nearest tenth of a gram. Body length was recorded by placing a pup on its ventral side and gently flattening the pup along the straight edge of a metric ruler. Body length was measured, to the nearest millimeter, from the tip of the pup's nose to the base of the pup's tail. Ear unfurling was recorded independently for each ear and was defined as the pinna coming away from the ear canal over which it had been folded. The upper incisor eruption test was conducted by gently dragging a straight edge across the gums of a pup. Upper incisor eruption was measured by recording the day on which the straight edge met resistance from the upper incisors penetrating the pup's gums. The righting reflex test was conducted on a smooth, level, unobstructed surface with surface temperature at 23 $\pm 1^{\circ}$C. Righting reflex was measured (to the nearest second) as the time the pup was placed on its dorsal side to the time the pup rotated 180° to its ventral side with all four paws contacting the surface.

Choice Test. The dam's retrieval behavior was assessed in the choice test apparatus when her pups were 3, 5, 7, and 9 days of age. Habituation trials and choice testing were conducted in the observation room between 0600 and 1200 hours, which was during the dark phase of the L-D cycle. Habituation trials occurred on Days 1 and 2 postparturition. For an habituation trial a dam and a handful of her bedding were placed in the start box of the choice test apparatus. The guillotine doors were raised and the dam was allowed five minutes in the apparatus. Between habituation trials, the apparatus was wiped down with 100% EtOH (Fisher Brand) in order to minimize the influence of olfactory cues on subsequent trials.

For a choice test session the maternity tubs, containing the dams to be tested and their litters, were transported, in the dark, to the observation room. Dams and their litters were acoustically isolated from the choice apparatus. Consequently, the dam in the apparatus was exposed only to vocalizations being emitted by the pups in the apparatus.

For a choice test trial, a dam and a handful of her bedding were placed in the start box. One pup of a preselected pair was placed in each of the runway wells. After the dam was in the start box for 30 seconds, the guillotine doors were raised, and a stop watch, event recorder, and tape recorder were simultaneously activated. The trial was terminated either when the dam retrieved both of the pups from the wells or after five minutes had elapsed. After a test trial the choice test apparatus was wiped down with 100% EtOH (Fisher Brand).

Each dam was placed into the apparatus with a pair of same sexed pups from unfamiliar litters. The pups were randomly selected with replacement from the litters of the dams being tested that day. Placement of the pups in the apparatus was counterbalanced according to the treatment condition of the pup and the dam. The pup pair conditions tested with each of the three dam conditions were EtOH vs PF, EtOH vs UC, and PF vs UC. Each dam was tested once each session on each pup pair condition. Pup pair condition and pup sex were counterbalanced across dam conditions.

Maternal behavior was recorded on a 20 channel Esterline Angus event recorder (1 mm/s) by depressing a key for the duration of a particular behavior. The following behaviors were recorded: the latency of initial choice of a pup (Pre-Choice), the duration of time in the vicinity of a pup (Choice), the latency to initial contact with a pup (Pre-Contact), the duration of contact with a pup (Contact), and the latency to retrieve a pup (Retrieval). Pre-Choice and Choice behaviors were defined with the aid of a criterion area demarked by a line 10 cm from the edge of each well. Pre-Choice was measured as the time from the beginning of a trial to the time the dam's front paws first crossed a criterion line. Choice was measured as the amount of time which the dam spent in the criterion area. When the dam's front paws crossed the criterion line Choice was defined as initiated and when the dam's front paws were withdrawn back over the criterion line Choice was defined as terminated. Pre-Contact was defined as the time which elapsed from the beginning of the trial to the dam's first physical contact with a pup. Contact was defined as the duration of physical contact between the dam and a pup. Retrieval was defined as the time which elapsed from the beginning of a trial to the dam's removing the pup from the well.

Pup calls were measured during each test trial by recording the audible outputs from the bat detectors onto separate tracks of a tape recorder. After the experimental session was over, the tapes were played back and the calls were counted for each pup. The number of calls was then divided by the retrieval latency for that pup and multiplied by 100 to yield a percent calling score.

Blood Ethanol Study

Method

Subjects

The subjects were 32 female rats and their pups which had the same characteristics and received the same treatment conditions as those in the previous study.

Procedure

The entrainment, weight, and maternal phases of this study were identical to those in the behavior study. The only difference in the alcohol acclimation phase was that only EtOH and PF groups were used with 16 dams assigned to each group.

Blood Ethanol. Blood was collected from the maternal subjects at approximately 0800 hours when their pups were either 3, 5, 7, or 9 days of age. Blood was collected at this time because these dams were used as a parallel control group to provide the best estimate of the blood ethanol concentration (BEC) of the dams in the behavior study (Sturtevant & Garber, 1981).

To obtain a blood sample a dam was terminated by exposure to CO_2. Blood was drawn from the dorsal artery with a 10 cc syringe. When the Syringe was filled (10 cc), the blood was immediately transferred to a tube containing heparinized beads. The tube was capped and stored in an ice bath.

Pup blood was taken from the carotid artery through an incision in the pup's throat. Blood was drawn up through a heparinized 20 microliter micropipet. It was immediately diluted in 3.4% w/v (water per volume) perchloric acid, and was stored in a sealed tube in an ice bath. After all samples for a day were collected, the blood was chemically analyzed with the Calbiochem Ethyl Alcohol Stat Pack (sensitivity of 0.01 g EtOH/dl) according to the technique described by Jones, Gerber, & Drell (1970).

Principle 10 in the "Ethical Principles of Psychologists", which adresses the humane care and treatment of animals, was strictly adhered to at all times.

Maintenance, BEC, developmental, and vocalization data were analyzed using a randomized block design. Choice test data were analyzed using a mixed design with repeated measures on dams across days of testing. In all cases the data were interpreted from type three sums of squares generated by the general linear model (GLM) option of the Statistical Analysis System (SAS) software package. A 0.05 alpha level was chosen a priori as the level of significance at which the null hypothesis was rejected.

Results

Dam Maintenance

Food Intake. Figure 1 shows mean food intake (g) by Dams as a function of Test Weeks. For all Dam Conditions food intake increased to a peak at week 4 and then decreased through week 7. Dams in the Untreated Control Condition ate more food than did EtOH Dams which ate slightly more than did PF Dams. An Analysis of Variance (ANOVA) yielded reliable effects for Dam Condition ($p<.0001$) and Week ($p<.0001$) with no reliable effect for Dam Condition x Week (see Table 1). A Duncan's Multiple Range Test (DMRT) yielded reliable differences for all comparisons of Dam Conditions (see Table 1a).

Fluid Intake. Figure 2 shows mean fluid intake (ml) by Dams as a function of Test Week. Intake increased for all Dam Conditions. The UC and PF Dams drank similar amounts, EtOH Dams drank less than these groups. An ANOVA yielded reliable effects for Dam Condition ($p<.0001$) and Week ($p<.0001$) with no significant effect for Dam Condition x Week (see Table 2). A DMRT showed that PF and UC Dams did not differ reliably but each drank more than did EtOH Dams (see Table 2a).

An ANOVA to check for possible differences in caloric intake among Dams Conditions yielded a reliable effect for Dam Condition ($p<.001$) (see Table 3) . A DMRT revealed that UC Dams differed reliably from EtOH and PF Dams which did not differ reliably from each other. Ethanol Dams derived 30% of their total caloric intake from the ethanol consumed.

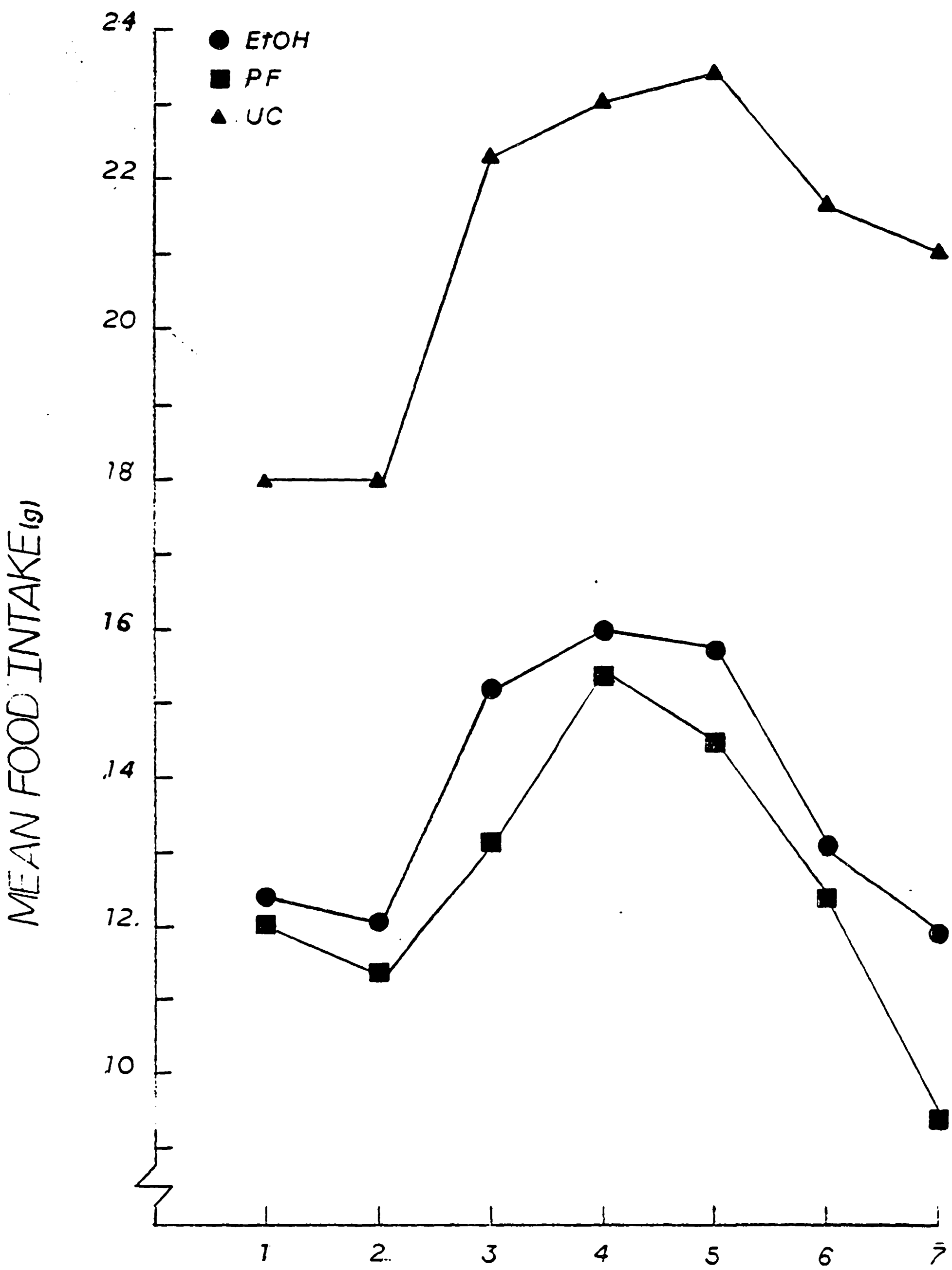

EtOH
PF
UC
24
22
20
18
16
14
12
10
MEAN FOOD INTAKE (g)
1
2
3
4
5
6
7
TEST WEEK

FIGURE 1

TABLE 1

Analysis of Variance of Food Intake for

EtOH, PF, and UC Dam Conditions Over Test Weeks

Source	df	SS	F
Dam Condition	2	220090.27	155.01 *
Week	6	95386.45	22.39 *
Dam Cond. x Week	11	12200.90	1.56

* p<.0001

TABLE 1A

Duncan's Multiple Range Test for Dam Condition and Week

Alpha Level=.05   df=302   MSE=709.91

Dam	N	Mean	
UC	49	21.0	A
EtOH	137	14.3	B
PF	136	13.3	C

Week	N	Mean	
4	62	17.2	A
5	55	16.3	A D
3	37	15.4	A D
6	51	14.5	B D
1	60	13.2	B
2	54	13.0	B
7	3	8.3	C

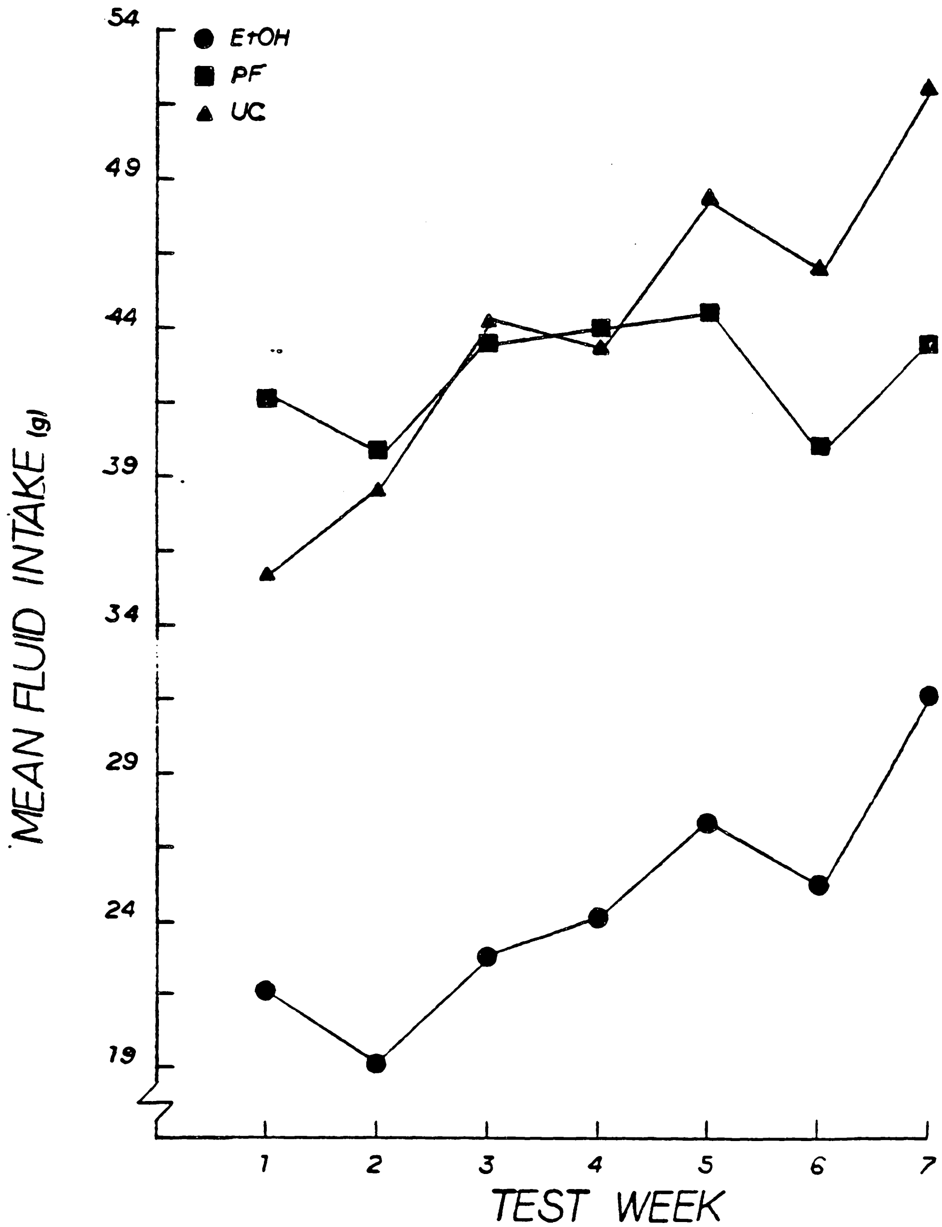

EtOH
PF
UC
54
49
44
39
34
29
24
19
MEAN FLUID INTAKE (g)
1
2
3
4
5
6
7
TEST WEEK

FIGURE 2

TABLE 2

Analysis of Variance of Fluid Intake for

EtOH, PF, and UC Dam Conditions and Test Weeks

Source	df	SS	F
Dam Condition	2	2231704.77	125.21 *
Week	6	342470.55	6.40 *
Dam Cond. x Week	12	95536.99	0.89

* p<.0001

Table 2A

Duncan's Multiple Range Test for Dam Condition and Week

Alpha Level=.05 df=323 MSE=8911.55

Dam	N	Mean
UC	63	42.1 A
PF	135	41.8 A
EtOH	146	24.2 B

Week	N	Mean
7	20	43.0 A
5	57	38.1 B
3	40	35.4 B
6	53	34.8 B C
4	47	34.2 B C
1	68	31.0 C
2	59	30.9 C

Table 3

Analysis of Variance of Mean Caloric Intake for Dam Condition

Source	df	SS	F
Dam Cond.	2	9137.54	7.51**

**p<.001

Table 3A

Duncan's Multiple Range Test for Dam Condition

Alpha Level=.05 df=96 MSE=608.68

Dam	N	Mean
UC	33	91.2 Kcals A
EtOH	33	85.6 Kcals B
PF	33	80.8 Kcals B

Body Weight. Figure 3 shows mean body weight for Dams as a function of Test Week. Body weights for all Dam Conditions increased to week six (parturition) and decreased from week 6 to 7 (lactation). Weights of the UC Dams were higher than those of the PF Dams, which were higher than those of EtOH Dams. An ANOVA yielded a reliable effect for Dam Condition ($p<.0001$) and Week ($p<.0001$) with no reliable interaction, Dam Condition x Week (see Table 4). A DMRT revealed that all Dam Conditions differed reliably (see Table 4a).

Litter Characteristics

Gestation. An ANOVA for length of gestation revealed a difference for Dam Conditions ($p<.01$) (see Table 5). A DMRT showed that EtOH Dams had a longer period of gestation than did PF and UC Dams, which did not differ reliably from each other (see Table 5a).

Number of Pups. An ANOVA for number of pups born per litter revealed a difference for Dam Condition ($p<.0001$) (see Table 5). A DMRT showed that EtOH Dams had fewer pups per litter than PF and UC Dams, which did not differ reliably from each other (see Table 5a).

Litter Weight. An ANOVA for litter weight at birth revealed a reliable difference for Dam Condition ($p<.0001$) (see Table 5) . A DMRT reliably showed that the litter weights of EtOH Dams were less than those of PF Dams, which were less than those of UC Dams (see Table 5a).

Average Pup Weight. An ANOVA for average pup weight at birth and prior to culling yielded no reliable differences among Dam Conditions (see Table 5).

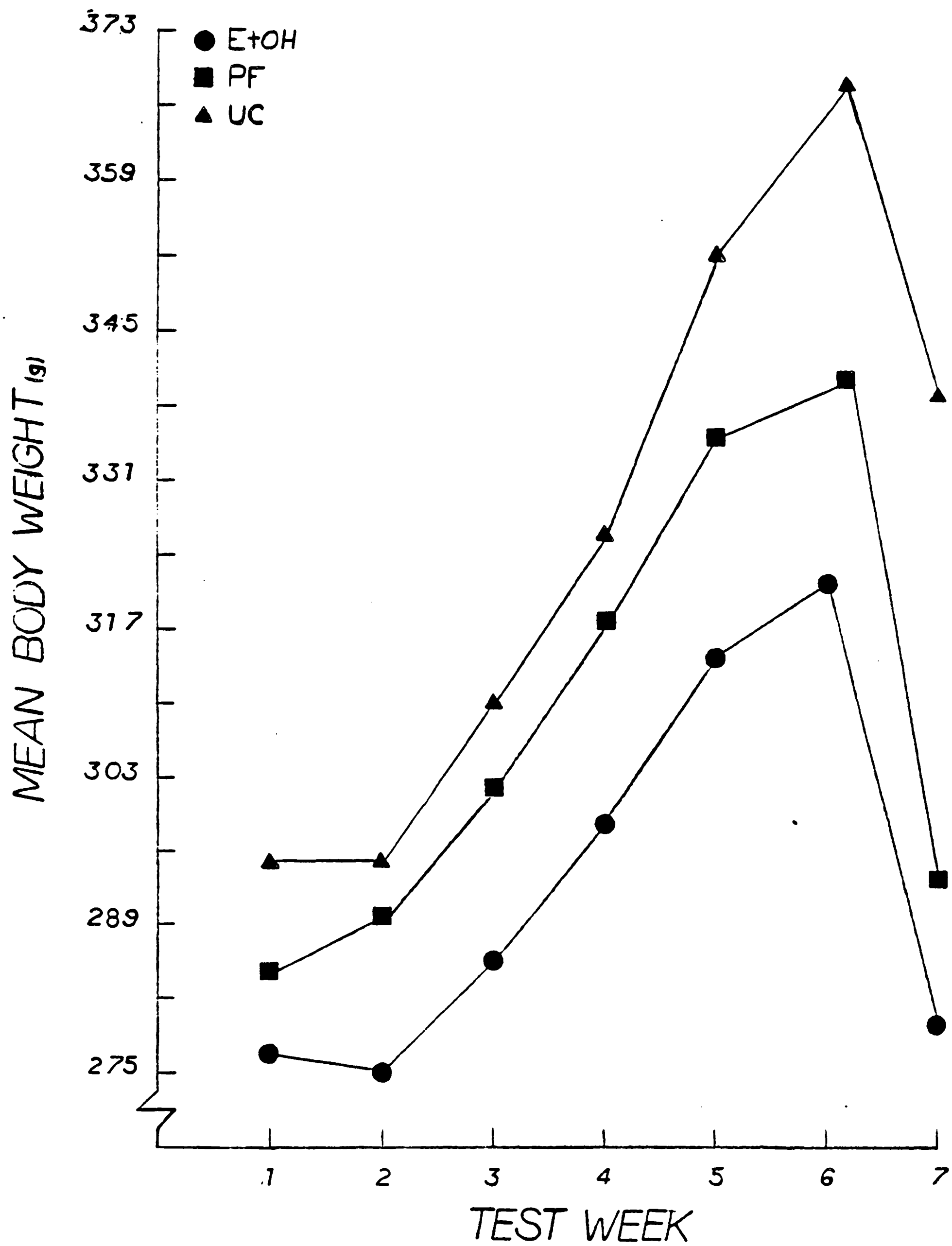

373
359
345
331
317
303
289
275
E+OH
PF
UC
MEAN BODY WEIGHT (g)
1
2
3
4
5
6
7
TEST WEEK

FIGURE 3

Table 4

Analysis of Variance for Dam Body Weight Over Test Week

Source	df	SS	F
Dam Cond.	2	35924.10	24.71*
Week	6	109588.69	25.13*
Dam Cond. x Week	12	11888.14	1.36

*p<.0001

Table 4A

Duncan's Multiple Range Test for Dam Condition and Test Week

Alpha Level=.05 df=256 MSE=726.77

Dam	N	Mean	Week	N	Mean
UC	59	326 A	5	59	331 A
PF	110	309 A	6	39	330 A
EtOH	108	295 A	4	40	312 B
			3	20	303 B C
			7	19	291 D C
			2	60	286 D
			1	40	285 D

Table 5

Analysis of Variance for Gestation, Number of Pups, Litter Weight, Average Pup Weight, and Pups Born Dead Over Dam Condition

Source	df	SS	F
Dam Condition:			
Gestation	2	10.25	5.61 ++
Number of Pups	2	330.86	68.85 *
Average Pup Weight	2	7906.56	1.98
Litter Weight	2	677934.16	28.81 *
Pups Born Dead	2	6.93	1.94

++ p<.01 * P<.0001

Table 5A

Duncan's Multiple Range Test for Dam Condition

Alpha Level=.05

Gestation			Number of Pups			Litter Weight		
df=71 MSE=0.91			df=285 MSE=2.40			df=139 MSE=1176.47		
Dam	N	Mean	Dam	N	Mean	Dam	N	Mean
EtOH	26	22.8 A	PF	100	12.6 A	UC	46	84.3 A
PF	25	22.2 B	UC	92	12.1 A	PF	50	73.5 B
UC	23	22.0 B	EtOH	96	8.4 B	EtOH	46	50.7 C

Pups Born Dead. An ANOVA for number of pups born dead per litter revealed no reliable differences among Dam Conditions (see Table 5).

Developmental Measures

Initial Ear Unfurling. An ANOVA for the data collected for day of initial ear unfurling (DIEUF) revealed a reliable difference for Pup Conditions ($p<.0001$) (see Table 6). A DMRT showed that PF Pups took longer to achieve initial ear unfurling than did EtOH and UC Pups which did not differ reliably from each other (see Table 6a).

Complete Ear Unfurling. An ANOVA for the data collected for day of complete ear unfurling (DCEUF) revealed a reliable difference for Pup Condition ($p<.0001$) (see Table 6). A DMRT showed that PF Pups took longer to achieve complete ear unfurling than did EtOH and UC Pups which did not differ reliably from each other (see Table 6a).

Incisor Eruption. An ANOVA for the data collected for day of incisor eruption (DIER) revealed a reliable difference for Pup Condition ($p<.01$) (see Table 6). A DMRT showed that EtOH Pups took longer to achieve incisor eruption than did PF and UC Pups which did not differ reliably from each other (see Table 6a).

Eye Opening. An ANOVA for the data for day of eye opening (DEO) revealed no reliable differences among Pup Conditions (see Table 6). By Day 13, 7 of 40 EtOH pups, 15 of 83 PF pups, and 40 of 52 UC pups exhibited eye opening. A 3 x 2 Chi-square for the number of pups per group which achieved eye opening yielded a reliable effect ($X^2=55.8$; df=2; N=175; $p<.0001$). Three 2 x 2 Chi-squares were also performed for these data. The analyses revealed reliable effects for EtOH vs UC Pups

Table 6

Analysis of Variance for DIEUF, DCEUF, DIER, and DEO

Over Pup Condition

Source	df	SS	F
Pup Condition:			
DIEUF	2	12.47	18.36 *
DCEUF	2	8.87	13.41 *
DIER	2	14.17	6.86 ++
DEO	2	0.43	1.65

++ p<.01   * p<.0001

Table 6A

Duncan's Multiple Range Test for Pup Condition

Alpha Level=.05

DIEUF			DCEUF			DIER		
df=256	MSE=0.34		df=254	MSE=0.33		df=234	MSE=1.03	
Pup	N	Mean	Pup	N	Mean	Pup	N	Mean
PF	101	2.7 A	PF	101	2.8 A	EtOH	47	9.6 A
UC	93	2.3 B	UC	93	2.4 B	PF	98	9.2 B
EtOH	65	2.2 B	EtOH	63	2.4 B	UC	92	8.9 B

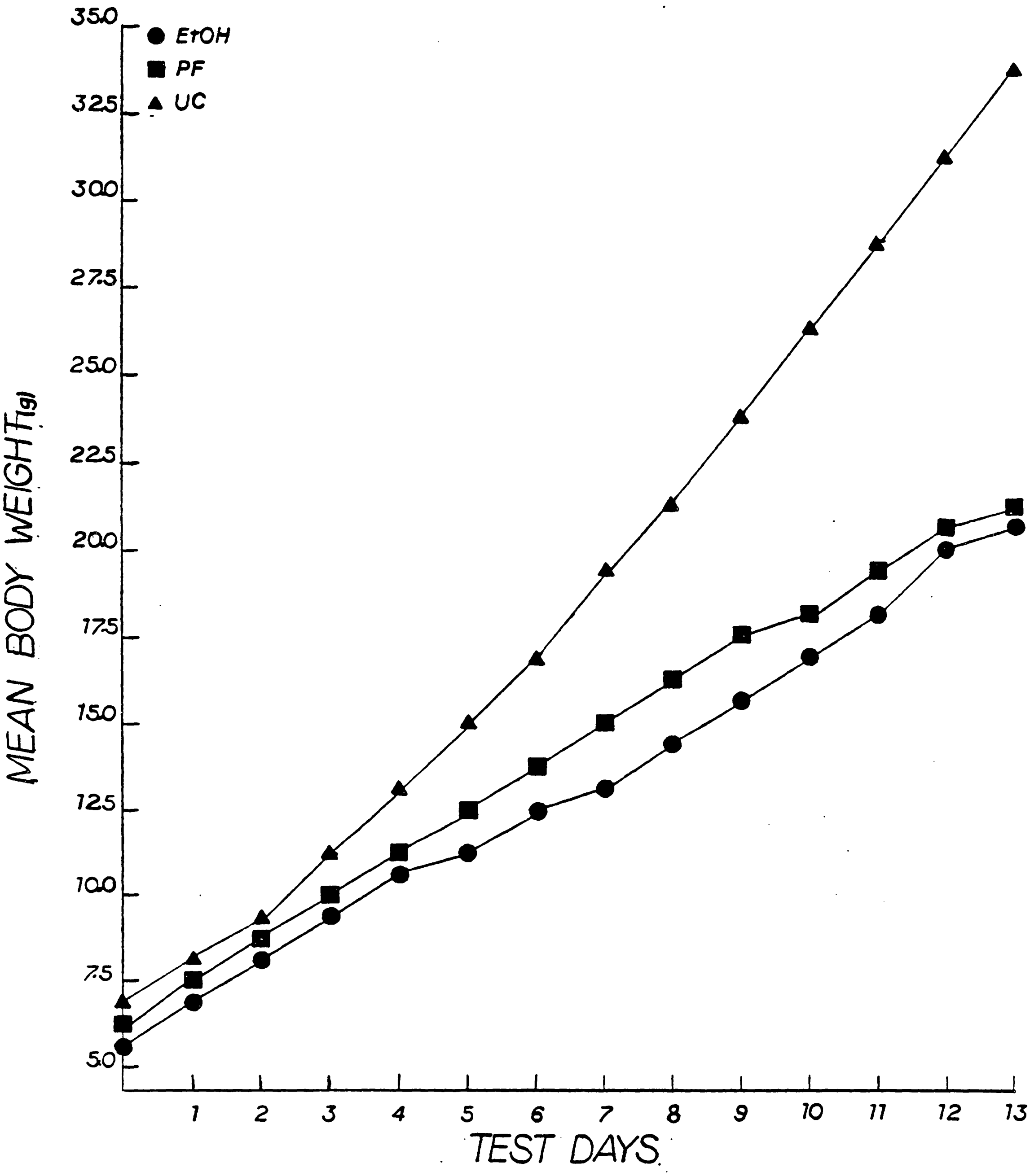
(X^2=31.9; df=1; N=123; $p<.0001$) and for PF vs UC Pups (X^2=45.9; df=1; N=135; $p<.0001$) but not for EtOH vs PF Pups.

<u>Body Weight</u>. Figure 4 shows mean body weight for Pups as a function of days postpartum. Pup weights increased over days. The UC Pups were heavier than PF Pups which were heavier than EtOH Pups. The rate of weight gain for UC Pups was faster than that of PF and EtOH Pups which did not differ reliably from each other. An ANOVA of these data yielded a reliable effect for Pup Condition ($p<.0001$), and Pup Condition x Day ($p<.0001$) (see Table 7).

<u>Body Length</u>. Figure 5 shows mean body length for Pups as a function of days postpartum. Pup body length increased over days. Untreated Control Pups were longer than PF Pups which were, generally, longer than EtOH Pups. The UC Pups rate of growth was faster than that of EtOH and PF Pups which grew at similar rates. An ANOVA yielded a reliable effect for Pup Condition ($p<.0001$), Day ($p<.0001$), and Pup Condition x Day ($p<.0001$) (see Table 8).

<u>Righting Reflex</u>. Figure 6 presents pup's mean latency to achieve the righting reflex as a function of days postpartum. Latency to perform the righting reflex decreased over days. Attainment of the righting reflex occurred more slowly over days for EtOH Pups than for PF and UC Pups. The latter did not differ reliably from each other. An ANOVA yielded a reliable effect for Pup Condition ($p<.0001$) and Pup Condition x Day ($p<.0001$) (see Table 9).

<u>Blood Ethanol</u>



MEAN BODY WEIGHT (g)
35.0
32.5
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
EtOH
PF
UC
1
2
3
4
5
6
7
8
9
10
11
12
13
TEST DAYS

FIGURE 4

Table 7

Analysis of Variance for Pup Body Weight

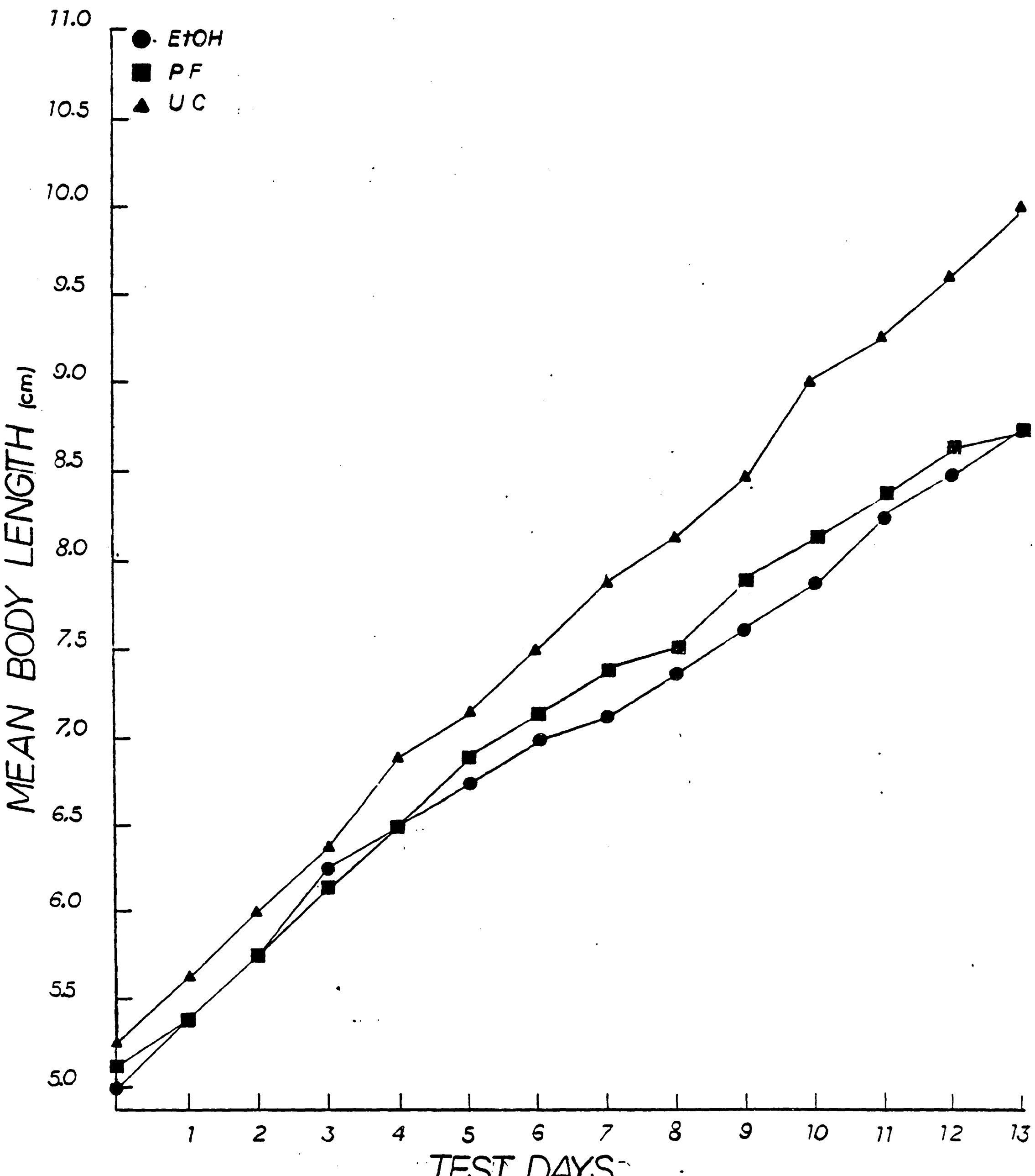
Source	df	SS	F
Pup Condition	2	2365450.96	1559.43 *
Day	13	11494180.44	1165.78 *
Pup Cond. x Day	26	1250944.36	63.44 *

* $p < .0001$

Table 7A

Mean Body Weight for Each Pup Condition

Pup	N	Mean
UC	1325	190.53 A
PF	1417	140.06 B
EtOH	823	124.31 C



11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
EtOH
PF
UC
MEAN BODY LENGTH (cm)
1
2
3
4
5
6
7
8
9
10
11
12
13
TEST DAYS

FIGURE 5

Table 8

Analysis of Variance for Pup Body Length

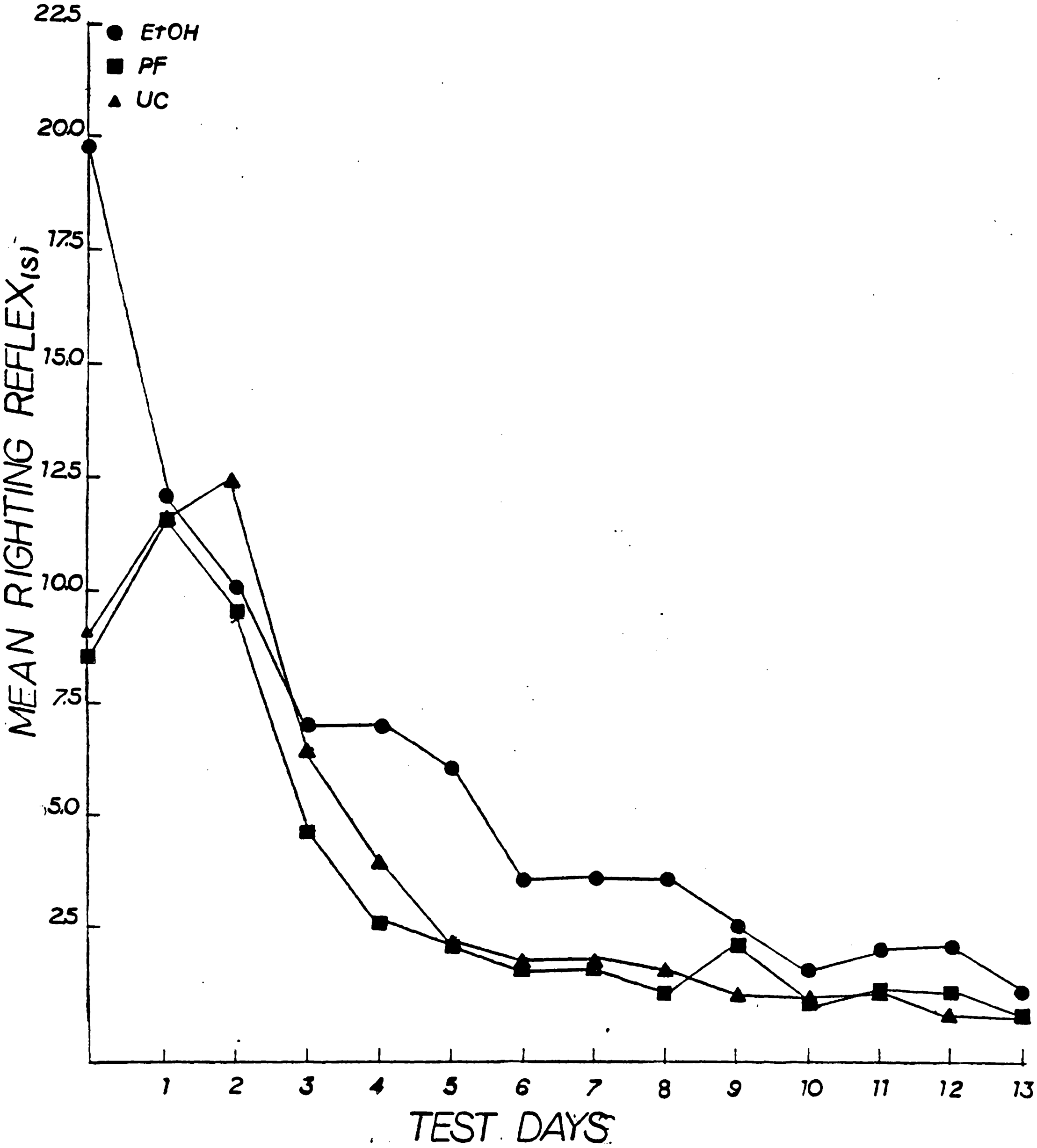
Source	df	SS	F
Pup Condition	2	28805.98	750.76 *
Day	13	492105.21	1973.16 *
Pup Cond. x Day	26	10465.92	20.98 *

* p<.0001

Table 8A

Mean Pup Length for Each Pup Condition

Pup	N	Mean
UC	1309	76.6 A
PF	1383	70.7 B
EtOH	811	68.4 C



22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
EtOH
PF
UC
MEAN RIGHTING REFLEX(s)
1
2
3
4
5
6
7
8
9
10
11
12
13
TEST DAYS

FIGURE 6

Table 9

Analysis of Variance for Righting Reflex

Source	df	SS	F
Pup Condition	2	29853153.12	23.52 *
Day	13	564716442.34	68.45 *
Pup Cond. x Day	26	67017994.44	4.06 *

* p<.0001

Table 9A

Mean Time in Seconds to Perform the Righting Reflex for Each Pup Condition

Pup	N	Mean
EtOH	815	657.76 A
UC	1317	385.54 B
PF	1414	348.81 B

Dam BEC. An ANOVA comparing BEC of EtOH and PF Dams over Test Days yielded a reliable effect of Dam Condition ($p<.0001$) and no reliable effect of Day (see Table 10). A DMRT (see Table 10A) showed that EtOH Dams had a BEC of 107 mg/dl (0.1%). Pair- Fed Dams exhibited a BEC which was not different from the minimum sensitivity of the test.

Pup BEC. An ANOVA for Pup BEC yielded no reliable effects for Pup Condition on days 3, 5, 7, and 9 postpartum (see Table 11).

Retrieval Data

Number of Pups Retrieved. Figure 7 presents mean number of pups retrieved for each Dam Condition over Days. Ethanol Dams retrieved consistantly fewer pups than did PF Dams which retrieved consistantly fewer pups than did UC Dams. A 3 x 2 Chi- square was performed for the number of pups retrieved by each Dam Condition on Days 3, 5, 7, and 9. A reliable effect occurred on each Day: Day 3 ($X^2=15.16$; $p<.001$), Day 5 ($X^2=52.70$; $p<.001$), Day 7 ($X^2=48.02$; $p<.001$), and Day 9 ($X^2=12.12$; $p<.01$).

Initial Attraction. Figure 8 presents mean number of pups chosen first by each Dam Condition as a function of Pup Condition within Pup Pairs. Ethanol Dams chose UC Pups more often than they chose PF Pups in the PF vs UC Pup Pair Condition. Pair-fed Dams chose PF Pups more often than they chose UC Pups in the PF vs UC Pup Pair Condition. Untreated Control Dams chose PF Pups more often than any other pups regardless of Pup Pair Conditions. A Z statistic was performed on these data to see if choice of any given pup within a Pup Pair Condition departed from a probability of 50% (Chance) (see Table 12). The results

Table 10

Analysis of Variance for EtOH and PF Dam's BEC

Source	df	SS	F
Dam Condition	1	45157.91	22.31 *
Day	4	1096.08	0.14
Dam Cond. x Day	4	4791.91	0.59

* $p<.0001$

Table 10A

Duncan's Multiple Range Test for Dam Condition

Alpha Level=.05 df=28 MSE=2023.99

Dam	N	Mean
EtOH	18	107.0 A
PF	20	14.5 B

Table 11

Analysis of Variance for EtOH and PF Pup's BEC

Source	df	SS	F
Pup Condition	1	0.43	0.01
Day	4	349.68	1.79
Pup Cond. x Day	4	345.66	1.77

Table 11A

Mean BEC in g/dl for Each Pup Condition

Pup	N	Mean
EtOH	38	7.0
PF	41	6.8

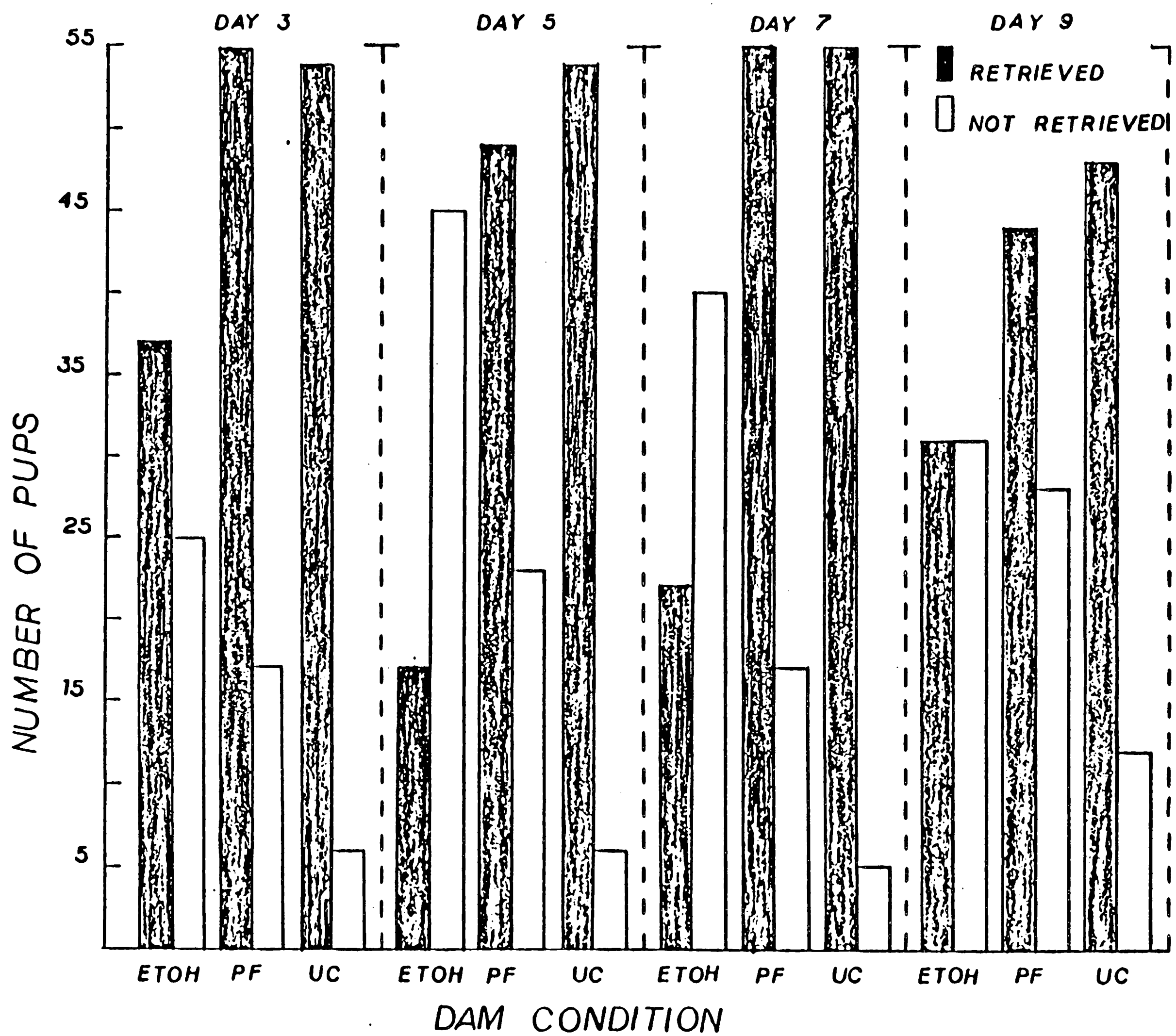

DAY 3
DAY 5
DAY 7
DAY 9
RETRIEVED
NOT RETRIEVED
55
45
35
25
15
5
NUMBER OF PUPS
ETOH
PF
UC
ETOH
PF
UC
ETOH
PF
UC
ETOH
PF
UC
DAM CONDITION

FIGURE 7

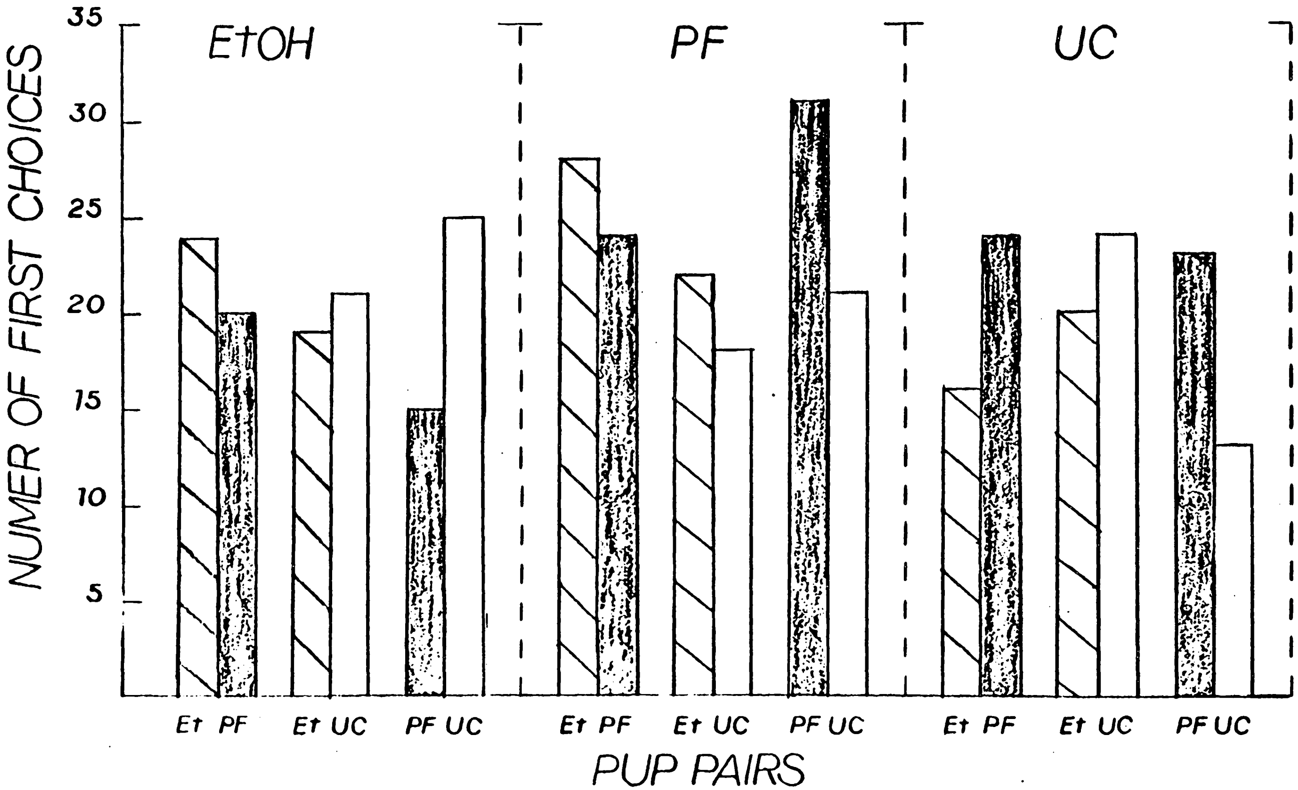

NUMER OF FIRST CHOICES
35
30
25
20
15
10
5
EtOH
PF
UC
Et PF
Et UC
PF UC
Et PF
Et UC
PF UC
Et PF
Et UC
PF UC
PUP PAIRS

FIGURE 8

Table 12

Z Statistic for Initial Attraction of a Pup Within a Pup Pair

Source	N	O	Z
EtOH Dam:			
EtOH vs PF	44	0.08	-0.63
EtOH vs UC	40	0.08	-0.25
PF vs UC	40	0.08	-2.63 +
PF Dam:			
EtOH vs PF	52	0.07	0.71
EtOH vs UC	40	0.08	0.63
PF vs UC	52	0.07	1.43
UC Dam:			
EtOH vs PF	40	0.08	-1.25
EtOH vs UC	44	0.08	-0.63
PF vs UC	36	0.08	1.75

+ $p<.05$

of this analysis yielded a reliable effect only for the EtOH Dam in the PF vs UC Pup Pair Condition ($p<.05$) (see Table 12). The negative Z score reveals that the EtOH Dam's preference was towards the UC Pup.

Pup Vocalizations. Figure 9 presents mean percent calling for each Pup Condition across Days. Percent calling was calculated by dividing the number of pup calls by the pup's retrieval latency and multiplying that quotient by 100. The percent calling data showed that PF Pups called more frequently than did EtOH and UC Pups. The latter did not differ from each other. The biggest difference in mean percent calling among Pup Conditions occurred on Day 3. An ANOVA showed a reliable effect for Pup Condition ($p<.0001$), Day ($p<.0001$), and Pup Condition x Day ($p<.01$) (see Table 13). An ANOVA for each Day yielded a reliable effect for Pup Condition on Day 3 ($p<.0001$) only (see Table 14). A DMRT revealed that PF Pups differed from UC and EtOH Pups which did not differ reliably from each other (see Table 14A).

Prechoice. Figure 10 shows mean Prechoice Latency (s) for each Dam Condition as a function of Days. Dam Conditions generally showed a decrease in Prechoice Latency. Prechoice Latencies for EtOH Dams were longer than those for PF Dams, which were longer than those for UC Dams.

Figure 11 shows Dam's mean Prechoice Latency for each Pup Condition over Days. For EtOH Dams, there was no consistant pattern of Prechoice. For PF Dams, latency to Prechoice decreased over Days. For UC Dams, latency to Prechoice was consistant over Days for all but the EtOH vs PF Pup Pair Condition. Prechoice Latency of the EtOH vs PF Pup Pair

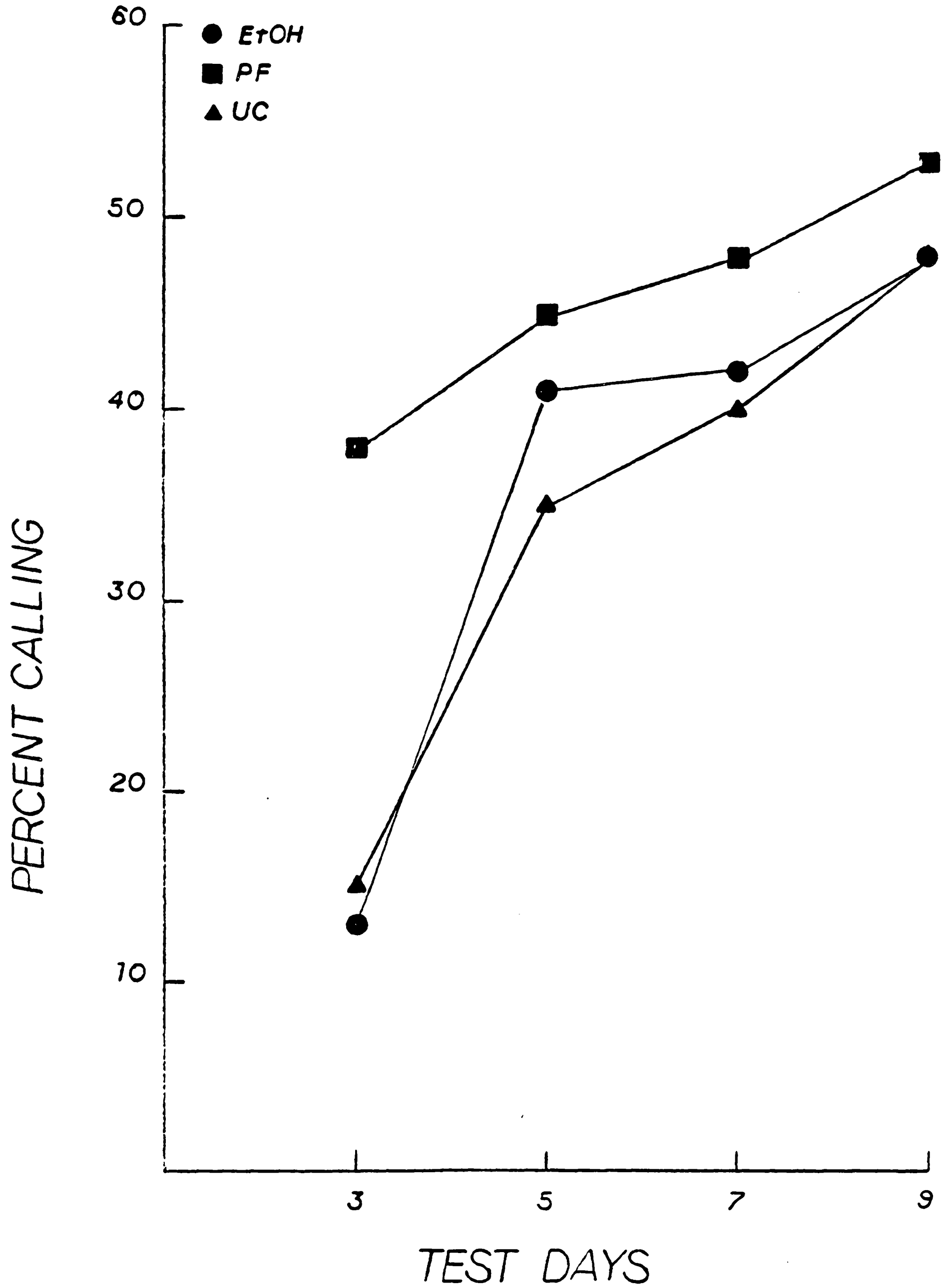


FIGURE 9

Table 13

Analysis of Variance for Amount of Pup Calling

Source	df	SS	F
Pup Cond.	2	20394.39	16.99*
Day	3	83190.63	46.20*
Pup Cond. x Day	6	11753.99	3.26++

*p<.0001 ++p<.01

Table 13A

Mean Number of Vocalizations Over Pup Condition and Test Day

Pup	N	Mean
PF	264	46.11
EtOH	260	36.29
UC	252	34.48

Day	N	Mean
3	194	22.12
5	194	40.70
7	191	43.54
9	194	49.81

Table 14

Analysis of Variance for Amount of Pup Vocalization

for Each Day Over Pup Condition

Source	df	SS	F
Day 3:			
Pup Cond.	2	25497.23	21.07*
Day 5:			
Pup Cond.	2	3482.96	2.52
Day 7:			
Pup Cond.	2	2550.63	2.31
Day 9:			
Pup Cond.	2	910.63	0.81

*p<.0001

Table 14A

Duncans Multiple Range Test for Amount of Pup Vocalizations

Per Trial on Day 3 Over Pup Condition

Alpha Level=.05 df=91 MSE=604.94

Pup	N	Mean	
PF	66	38.06	A
UC	63	14.76	B
EtOH	65	13.08	B

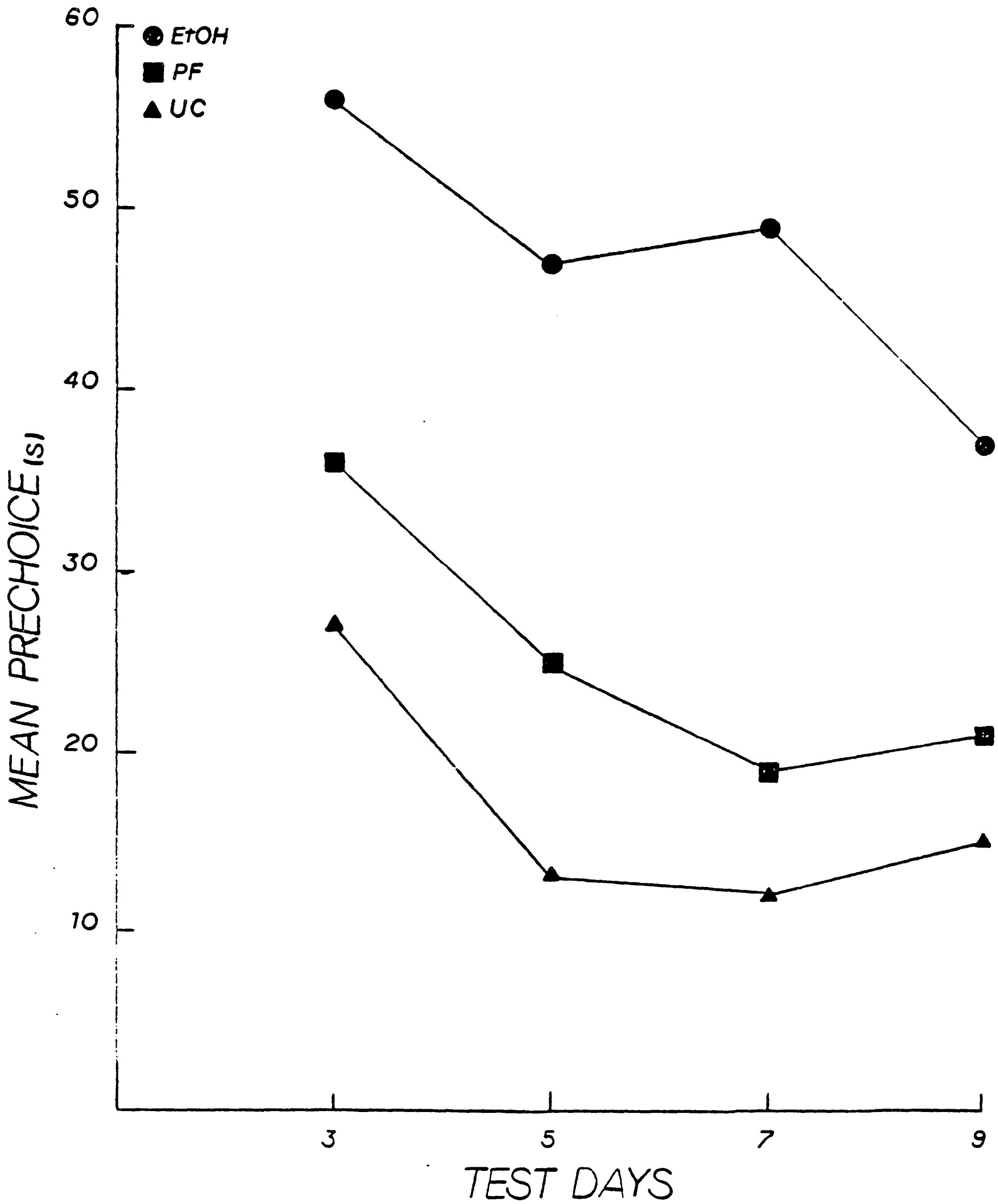


FIGURE 10

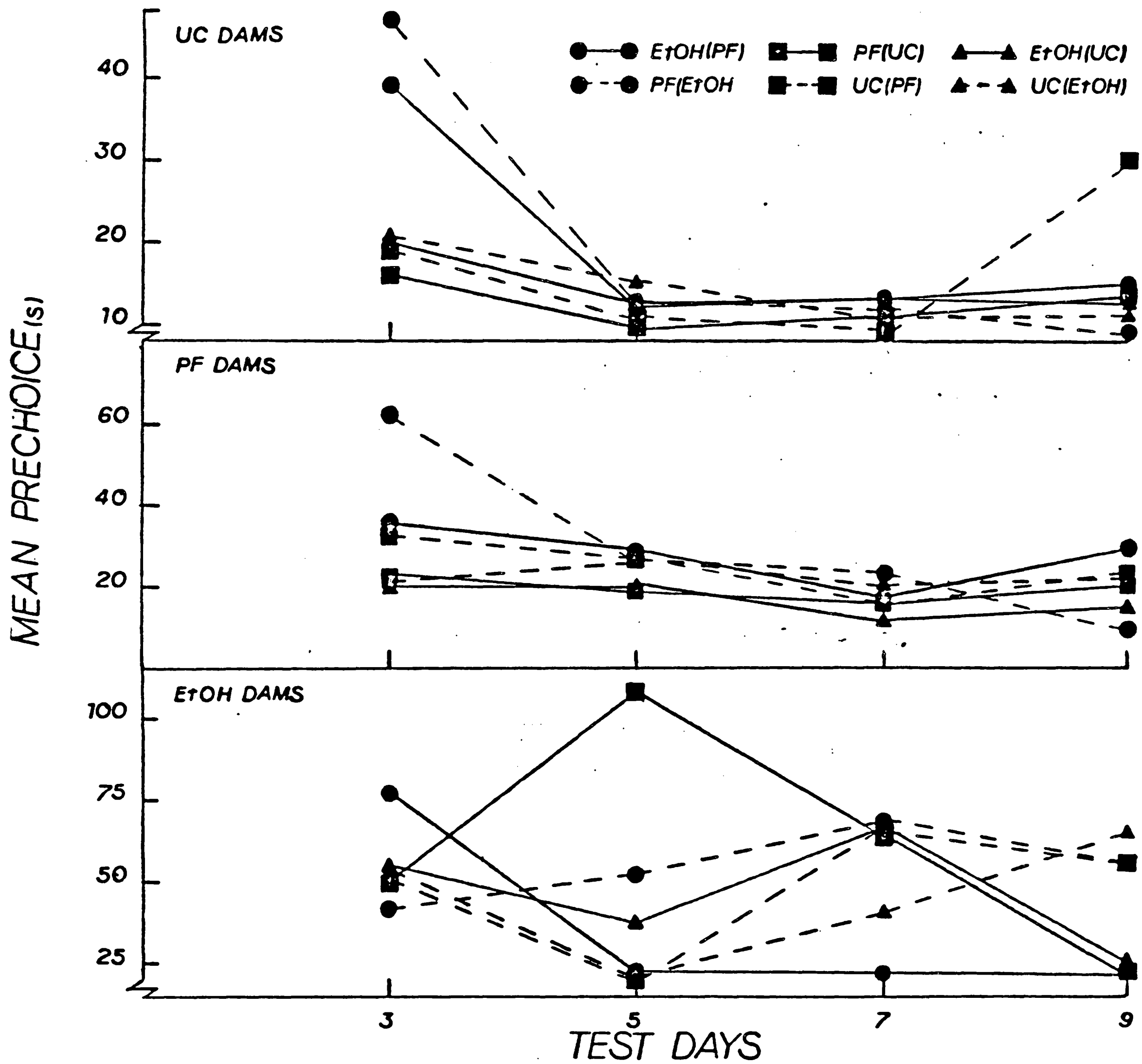

UC DAMS
PF DAMS
EtOH DAMS
EtOH(PF)
PF(EtOH)
PF(UC)
UC(PF)
EtOH(UC)
UC(EtOH)
MEAN PRECHOICE (s)
10
20
30
40
60
100
75
50
25
3
5
7
9
TEST DAYS

FIGURE 11

Condition was slower than that of the other Pup Pair Conditions on Day 3. By Day 5, Prechoice Latency of this Pup Pair was similar to those of the others. On Day 9, Prechoice Latency of the UC Pup in the PF vs UC Pup Pair Condition was slower than that of the other Pup Conditions. An ANOVA yielded a reliable effect for Dam Condition ($p<.01$), Day ($p<.0001$), Dam Condition x Pup Condition ($p<.05$), Pup Condition x Day ($p<.0001$), and Dam Condition x Pup Condition x Day ($p<.01$) (see Table 15).

Simple effects ANOVA for the EtOH Dams yielded a reliable effect of Pup Condition x Day ($p<.05$) (see Table 18). Further analysis of this interaction yielded a reliable effect of Pup Condition on Day 5 ($P<.05$) (see Table 20). A subsequent DMRT revealed that on Day 5 Prechoice Latency for the PF Pup in the PF vs UC Pup-Pair Condition was reliably slower than was Prechoice for all other Pup Conditions (see Table 20A).

An ANOVA of Prechoice Latency for the PF Dams yielded a reliable effect of Day ($p<.0001$) and Pup Condition x Day ($p<.05$) (see Table 21). Analysis for Pup Condition x Day interaction yielded a reliable effect of Pup Condition on Day 3 ($p<.01$) (see Table 22). A DMRT revealed that the Prechoice Latency for the PF Pup in the PF vs EtOH Pup Pair was reliably slower than that of the other Pup Conditions (see Table 22A).

An ANOVA for the UC Dam Condition on Prechoice yielded reliable effects for Pup Condition ($p<.05$), Day ($p<.0001$), and Pup Condition x Day ($p<.0001$) (see Table 23). Simple effects ANOVA for Pup Condition x Day yielded a reliable effect for Pup Condition on Day 3 ($p<.01$) and

Table 15

Analysis of Variance for Prechoice

Source	df	SS	F
Dam Cond.	2	72761.90	6.14++
Pup Cond.	5	1878.56	0.39
Day	3	25920.58	9.07*
Dam Cond. x Pup Cond.	10	22022.54	2.31+
Pup Cond. x Day	15	50753.14	3.55*
Dam Cond. x Pup Cond. x Day	30	53387.05	1.87++

* p<.0001 ++p<.01 +p<.05 Note: All nonsignificant interactions were omitted.

Day 9 ($p<.05$) (see Table 24). A DMRT showed that on Day 3 Prechoice Latency was reliably slower for both Pup Conditions in the EtOH vs PF Pup Pair than it was for the other Pup Conditions (see Table 24A). A DMRT for Day 9 revealed Prechoice Latency for the UC Pup in the UC vs PF Pup Pair was reliably slower than those of the other Pup Conditions (see Table 24A).

Precontact. Figure 12 shows mean Precontact Latency (s) for each Dam Condition over Days. Ethanol Dams had longer Precontact Latencies than did PF Dams which had generally longer Precontact Latencies than did UC Dams.

Figure 13 shows Dam's mean latency to Precontact for each Pup Condition over Days. Ethanol and PF Dams did not show any consistant pattern of Precontact over Days. Untreated Control Dams generally showed a decrease in latency to Precontact. On Day 3 the Precontact Latency to PF Pups was longer than that to the other Pup Conditions. On Days 5, 7, and 9 Precontact Latencies did not differ, the only exception occurred on Day 9. On Day 9, the Precontact Latency to the UC Pups in the PF vs UC Pup Pair Condition was longer than that of the other Pup Conditions. An ANOVA for Precontact yielded reliable effects for Dam Condition ($p<.01$), Pup Condition ($p<.05$), Dam Condition x Pup Condition ($p<.001$), and Pup Condition x Day ($p<.01$) (see Table 16).

An ANOVA of the EtOH Dam Condition showed a reliable effect for Pup Condition ($p<.01$) (see Table 18). A DMRT for EtOH Dam's mean Precontact Latency to each Pup Condition showed that Precontact was fastest

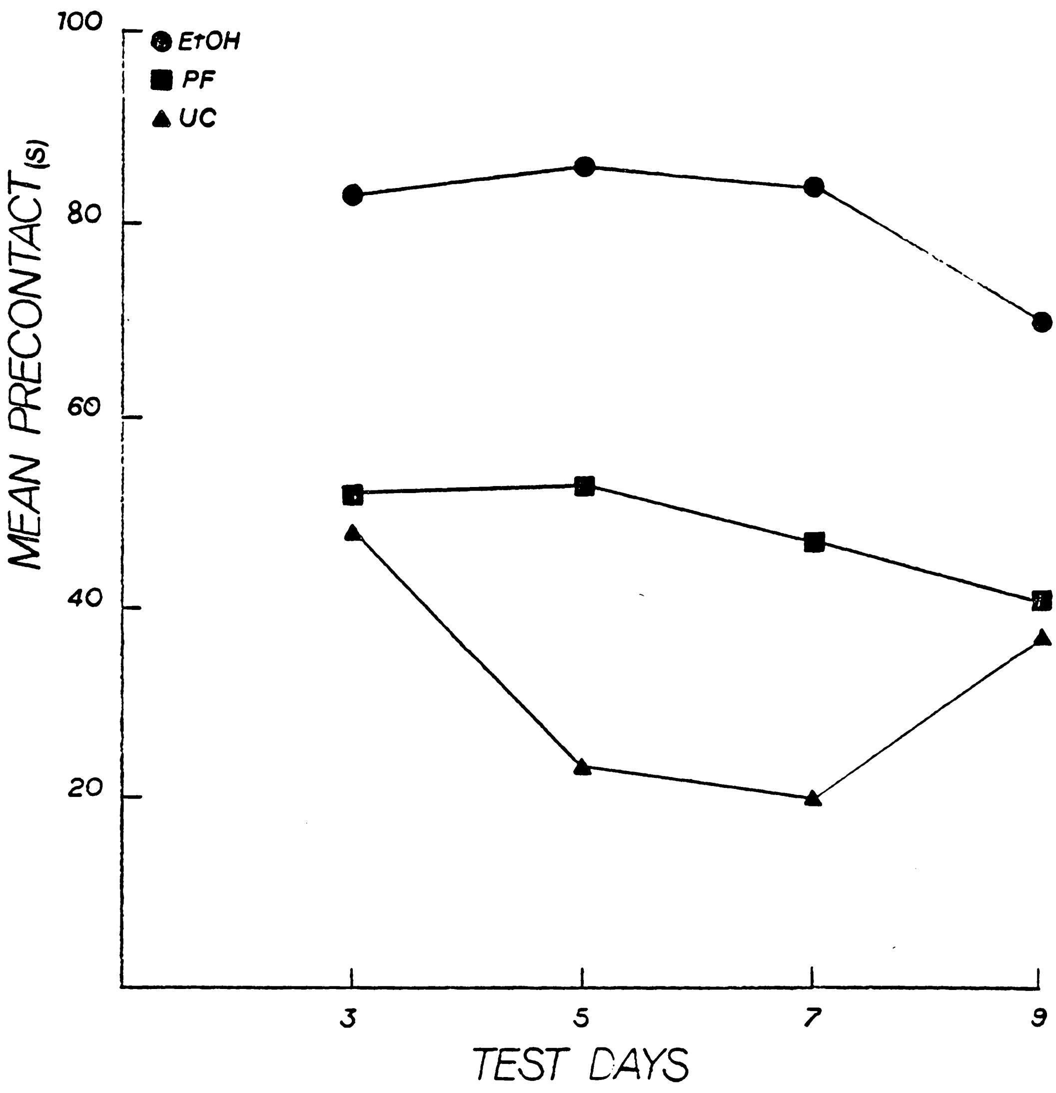


FIGURE 12

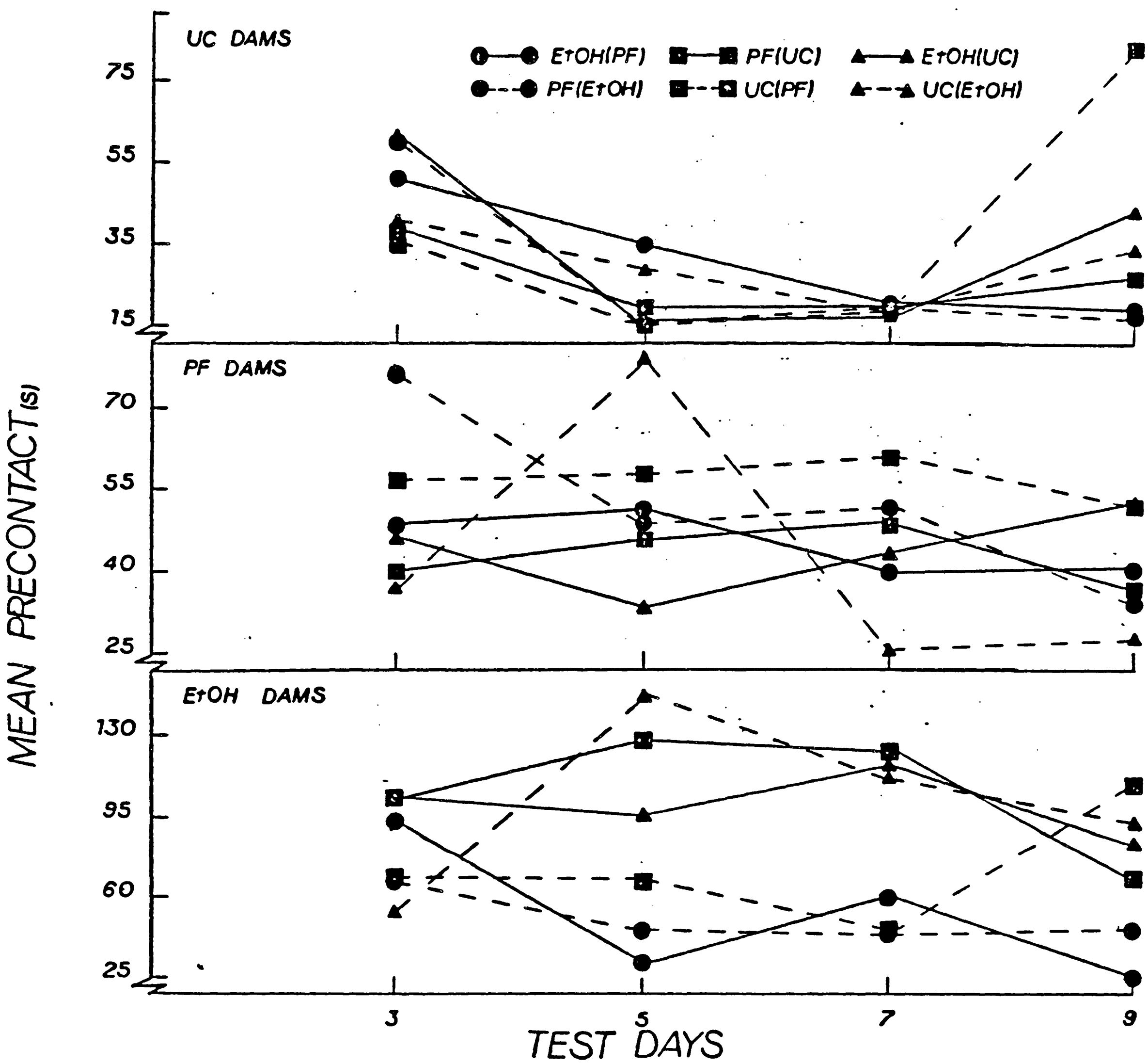

UC DAMS
EtOH(PF)
PF(UC)
EtOH(UC)
PF(EtOH)
UC(PF)
UC(EtOH)
75
55
35
15
PF DAMS
70
55
40
25
EtOH DAMS
130
95
60
25
MEAN PRECONTACT(s)
3
5
7
9
TEST DAYS

FIGURE 13

Table 16

Analysis of Variance for Precontact

Source	df	SS	F
Dam Cond.	2	210845.67	7.32++
Pup Cond.	5	39149.90	2.41+
Day	3	14449.81	1.48
Dam Cond. x Pup Cond.	10	106151.45	3.26**
Pup Cond. x Day	15	109684.89	2.25++

**p<.001 ++p<.01 +p<.05 Note: All nonsignificant interactions were omitted toward the PF Pup in the PF vs EtOH Pup Pair Condition, and slowest for the PF Pup in the PF vs UC Pup Pair Condition, relative to all other Pup Conditions (see Table 19).

An ANOVA for the PF Dam Condition yielded no reliable effects for Pup Condition, Day, or Pup Condition x Day (see Table 21). An ANOVA for the UC Dam Condition yielded a reliable effect for Day ($p<.001$) (see Table 23). A DMRT revealed that UC Dams' Precontact Latencies were reliably longer on Day 3 than on Days 5 or 7 (see Table 23A).

Retrieval. Figure 14 shows mean Retrieval Latency (s) for each Dam Condition over Days. Ethanol Dams had longer Retrieval Latencies than did PF Dams which had a longer Retrieval Latencies than did UC Dams. An ANOVA yielded a reliable effect of Dam Condition ($p<.01$), Day (.05), and Dam Condition x Day (p,.0001) (see Table 17). An ANOVA for the EtOH Dam Condition yielded a reliable effect of Day ($p<.01$) (see Table 18). A DMRT revealed that Retrievel Latencies of EtOH Dams were reliably faster on Day 3 than on Days 5 or 7 (see Table 19).

An ANOVA of Retrieval Latency for the PF Dam Condition yielded a reliable effect of Day ($p<.01$) (see Table 21). A DMRT disclosed that PF Dams exhibited slower retieval Latencies on Day 9 than on any other Test Day (see Table 21A). Mean latency to Retrieve for the UC Dam did not yield any reliable effects (see Table 25).

First Prechoice. Figure 15 presents Dam's mean latency to First Prechoice (s) over Days. First Prechoice Latencies decreased for all Dam Conditions over Days 3-9. Latencies for EtOH Dams decreased more slowly than did those for PF Dams, which decreased more slowly

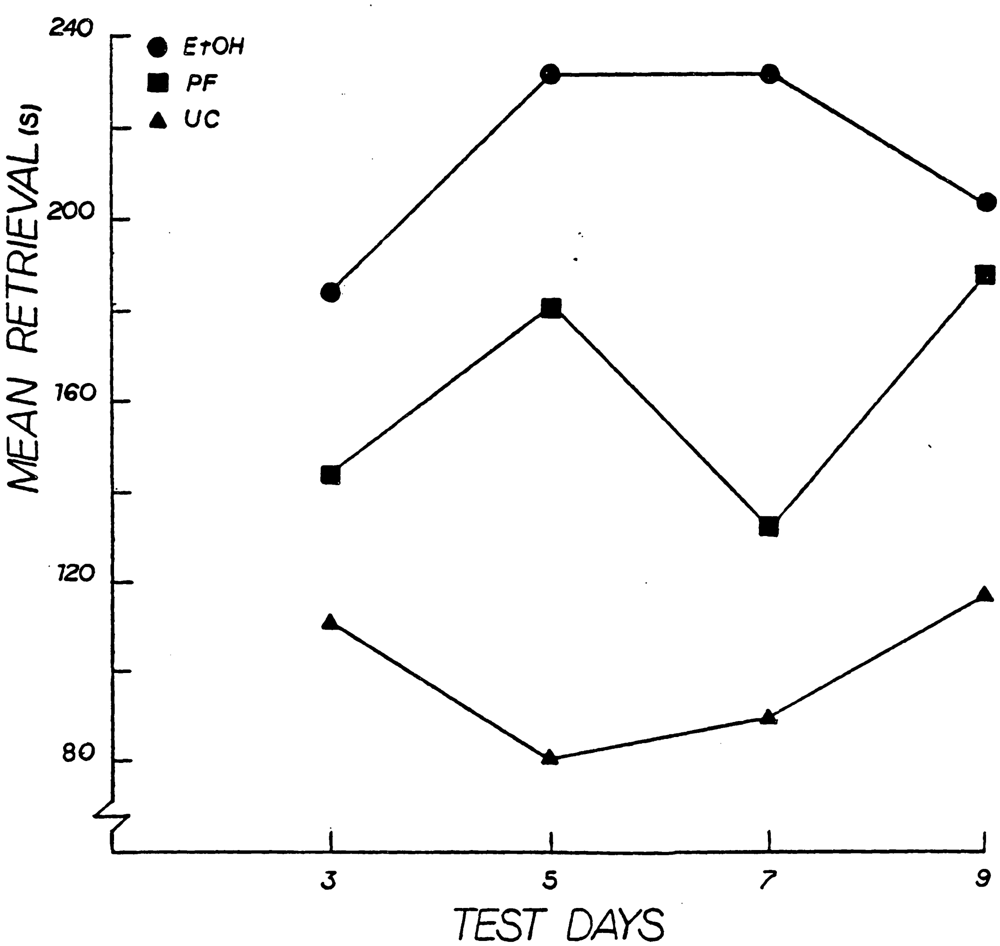


FIGURE 14

Table 17

Analysis of Variance for Retrieval

Source	df	SS	F
Dam Cond.	2	1285766.68	6.90++
Pup Cond.	5	9595.10	0.25
Day	3	80821.58	3.57+
Dam Cond. x Day	6	239854.71	5.30*

*p<.0001 ++p<.01 +p<.05 Note: All nonsignificant interactions were omitted

Table 18

Analysis of Variance for the EtOH Dam Condition on

Prechoice, Precontact, and Retrieval

Source	df	SS	F
Prechoice:			
Pup Condition	5	17909.59	1.17
Day	3	9940.08	1.09
Pup Cond. x Day	15	78733.42	1.72+
Precontact:			
Pup Condition	5	135754.87	3.59++
Day	3	9343.56	0.41
Pup Cond. x Day	15	120045.10	1.06
Retrieval:			
Day	3	108122.89	2.90+

++$p<.01$ +$p<.05$

Table 19

Duncan's Multiple Range Test for Precontact in Seconds for the

EtOH Dam Condition Over Pup Condition

Alpha Level=.05 df=242 MSE=7527.58

Pup	N	Mean	
PF(UC)	40	106.50	A
UC(EtOH)	40	102.52	A
EtOH(UC)	40	102.25	A
UC(PF)	40	70.85	A B
EtOH(PF)	44	53.91	B
PF(EtOH)	44	51.30	B

Duncan's Multiple Range Test for Retrieval in Seconds for the

EtOH Dam Condition Over Days

Alpha Level=.05 df=244 MSE=12407.5

Day	N	Mean	
7	62	233.34	A
5	62	233.24	A
9	62	203.27	A B
3	62	184.24	B

Table 20

Analysis of Variance for the EtOH Dam on Prechoice Over Each Day

Source	df	SS	F
Day 3:			
Pup Cond.	5	6890.94	0.64
Day 5:			
Pup Cond.	5	53428.31	2.88+
Day 7:			
Pup Cond.	5	11879.39	0.89
Day 9:			
Pup Cond.	5	17444.37	1.66

+p<.05

Table 20A

Duncan's Multiple Range Test on Day 5

Alpha Level=.05 df=245 MSE=3707.95

Pup	N	Mean	
PF(UC)	10	108.70	A
UC(EtOH)	10	52.70	B
PF(EtOH)	11	40.73	B
EtOH(UC)	10	37.20	B
EtOH(PF)	11	23.36	B
UC(PF)	10	19.60	B

Table 21

Analysis of Variance for the PF Dam Condition on

Prechoice, Precontact, and Retrieval

Source	df	SS	F
Prechoice:			
Pup Cond.	5	3915.77	1.68
Day	3	12234.26	8.74*
Pup Cond. x Day	15	12627.87	1.80+
Precontact:			
Pup Cond.	5	9547.03	0.74
Day	3	6152.13	0.79
Pup Cond x Day	15	29923.57	0.77
Retrieval:			
Day	3	140674.90	3.90++

*p<.0001 ++p<.01 +p<.05

Table 21A

Duncan's Multiple Range Test for Retrieval in Seconds

Alpha Level=.05 df=284 MSE=12025.90

Day	N	Mean	
9	72	188.39	A
5	72	170.74	A B
3	72	142.74	B
7	72	132.76	B

Table 22

Analysis of Variance for the PF Dam Condition on Prechoice

Over Each Day

Source	df	SS	F
Day 3:			
Pup Cond.	5	12327.46	3.61++
Day 5:			
Pup Cond.	5	1026.98	0.56
Day 7:			
Pup Cond.	5	791.99	0.69
Day 9:			
Pup Cond.	5	2397.19	0.81

++p<.01

Table 22A

Duncan's Multiple Range Test for Pup Condition on Day 3

Alpha Level=.05 df=66 MSE=682.85

Pup	N	Mean	
PF(EtOH)	13	60.85	A
EtOH(UC)	10	38.70	B
EtOH(PF)	13	36.92	B
UC(PF)	13	33.15	B
PF(UC)	13	23.46	B
UC(EtOH)	10	21.60	B

Table 23

Analysis of Variance for the UC Dam Condition

on Prechoice, Precontact, Retrieval

Source	df	SS	F
Prechoice:			
Pup Cond.	5	2094.77	2.39+
Day	3	9436.49	17.98*
Pup Cond. x Day	15	9423.87	3.59*
Precontact:			
Pup Cond.	5	2948.37	0.42
Day	3	30131.80	7.10**
Pup Cond. x Day	15	34109.14	1.61
Retrieval:			
Day	3	62209.35	2.24

*p<.0001 **p<.001 +p<.05

Table 23A

Duncan's Multiple Range Test for UC Dam Precontact Over Days

Alpha Level=.05 df=236 MSE=1452.09

Day	N	Mean	
3	60	48.43	A
9	60	36.62	A B
5	60	23.45	C B
7	60	19.70	C

Table 24

Analysis of Variance for the UC Dam Condition on Prechoice Over Each Day

Source	df	SS	F
Day 3:			
Pup Cond.	5	8054.79	4.44++
Day 5:			
Pup Cond.	5	196.04	0.39
Day 7:			
Pup Cond.	5	214.43	1.04
Day 9:			
Pup Cond.	5	3053.38	3.12+

++p<.01 +p<.05

Table 24A

Duncan's Multiple Range Test for Pup Condition on Days 3 and 9

Alpha Level=.05 df=54

Day 3:
MSE=362.63

Pup	N	Mean	
PF(EtOH)	10	47.40	A
EtOH(PF)	10	39.00	A
UC(EtOH)	11	21.18	B
EtOH(UC)	11	20.00	B
PF(UC)	9	19.22	B
UC(PF)	9	16.44	B

Day 9:
MSE=195.95

Pup	N	Mean	
UC(PF)	9	30.78	A
EtOH(PF)	10	15.40	B
EtOH(UC)	11	12.64	B
UC(EtOH)	11	11.55	B
PF(UC)	9	10.89	B
PF(EtOH)	10	8.10	B

Table 25

Analysis of Variance for First Prechoice

Source	df	SS	F
Dam Cond.	2	4114.92	6.64++
Pup Cond.	5	551.05	0.81
Day	3	6187.93	15.13 *

*p<.0001 ++p<.01 Note: All nonsignificant interactions were omitted

Table 25A

Duncan's Multiple Range Test for Dam Condition and Day

Alpha Level=.05

Dam Condition:

df=385 MSE=191.67

Dam	N	Mean	
EtOH	124	21.07	A
PF	144	15.06	B
UC	120	9.53	C

Day:

df=384 MSE=188.05

Day	N	Mean	
3	97	23.81	A
5	97	13.44	B
7	97	12.65	B
9	97	11.18	B

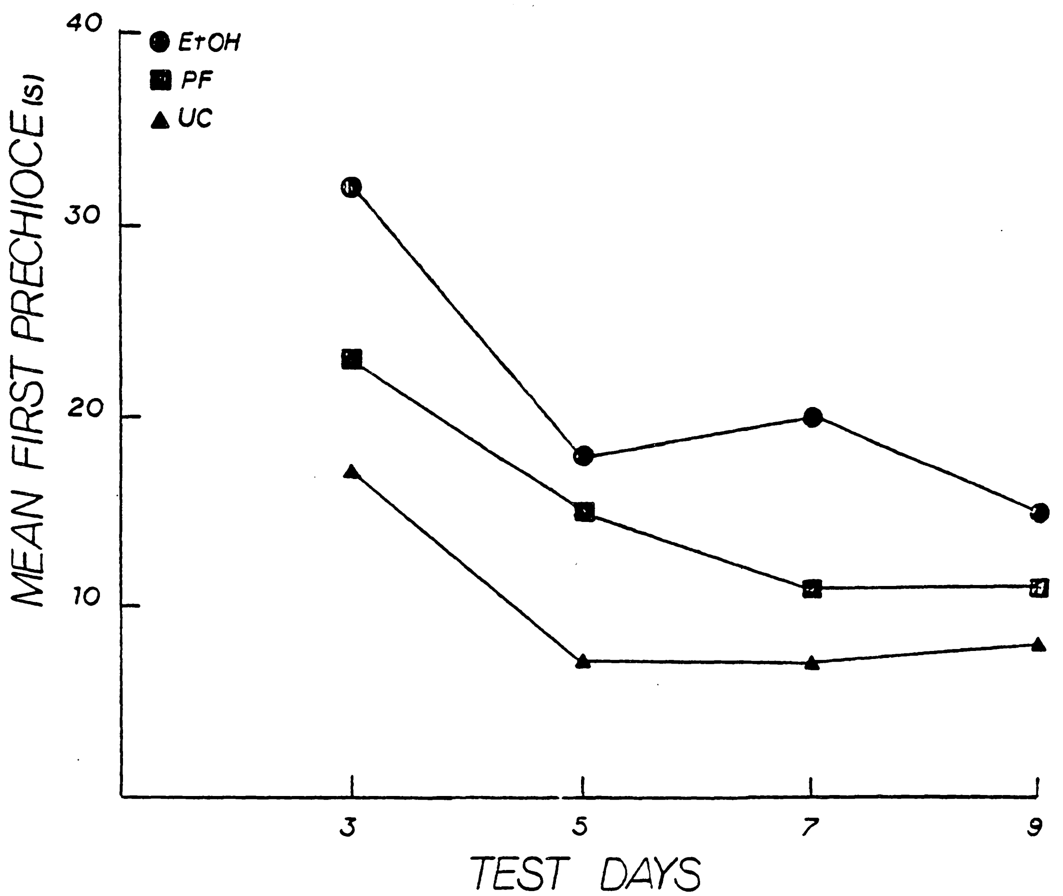


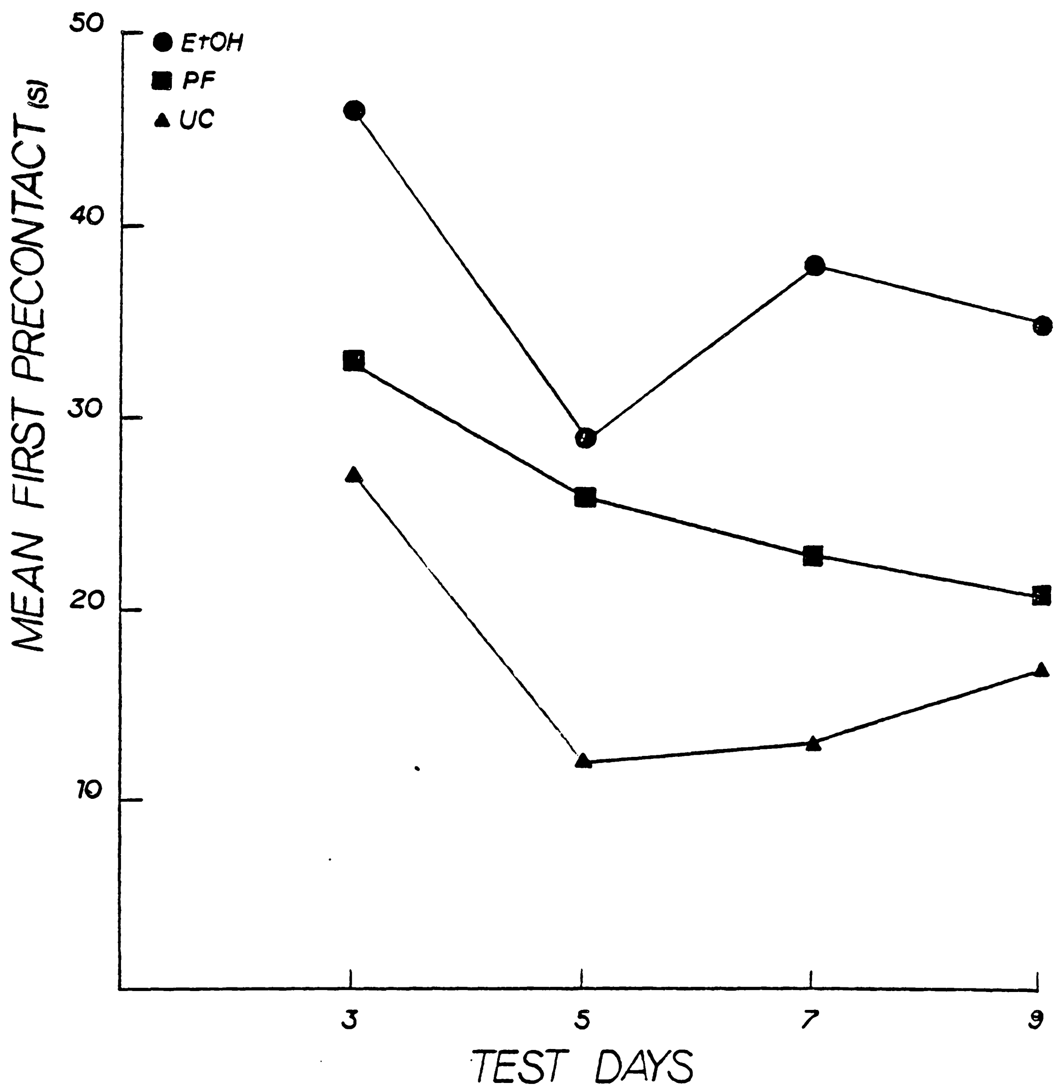
FIGURE 15

than did those for UC Dams. An ANOVA yielded a reliable difference for Dam Condition ($p<.01$) and Day ($p<.0001$) (see Table 25). A DMRT revealed reliable differences among Dam Conditions (see Table 25A). According to the DMRT First Prechoice Latencies were longer on Day 3 than on Days 5, 7, and 9 (see Table 25A). Latencies on Days 5, 7, and 9 were not differ reliably from each other.

<u>First Precontact</u>. Figure 16 presents mean First Precontact Latencies (s) for each Dam Condition over Days. First Precontact Latencies decreased for all Dam Conditions over Days 3-9. Latencies for EtOH Dams decreased more slowly than did those of PF Dams, which decreased more slowly than did those of UC Dams.

Figure 17 shows each Dam Condition's latency to first Precontact for each Pup Condition over Days. Ethanol Dams did not show a clear pattern of behavior over Days. Pair-Fed Dam's Precontact Latencies were slower than were those of UC Dams. On Day 3, PF Dams showed slower Precontact Latencies to the PF Pup in the EtOH vs PF Pup Pair Condition than to any other pups. On Day 3, UC Dams showed slower Precontact Latencies to both pups in the EtOH vs PF Pup Pair Condition than to other pups. On Day 9, UC Dams showed slower Precontact Latencies to the UC Pup in the PF vs UC Pup Pair Condition than to other pups. An ANOVA yield a reliable effect for Dam Condition ($p<.05$), Day ($p<.001$), Dam Condition x Pup Condition ($p<.05$), Pup Condition x Day ($p<.0001$), and Dam Condition x Pup Condition x Day ($p<.05$) (see Table 26).

An ANOVA for the EtOH Dam Condition did not yielded significant effects for any factor (see Table 27). An ANOVA for the PF Dam



EtOH
PF
UC
50
40
30
20
10
MEAN FIRST PRECONTACT (S)
3
5
7
9
TEST DAYS

FIGURE 16

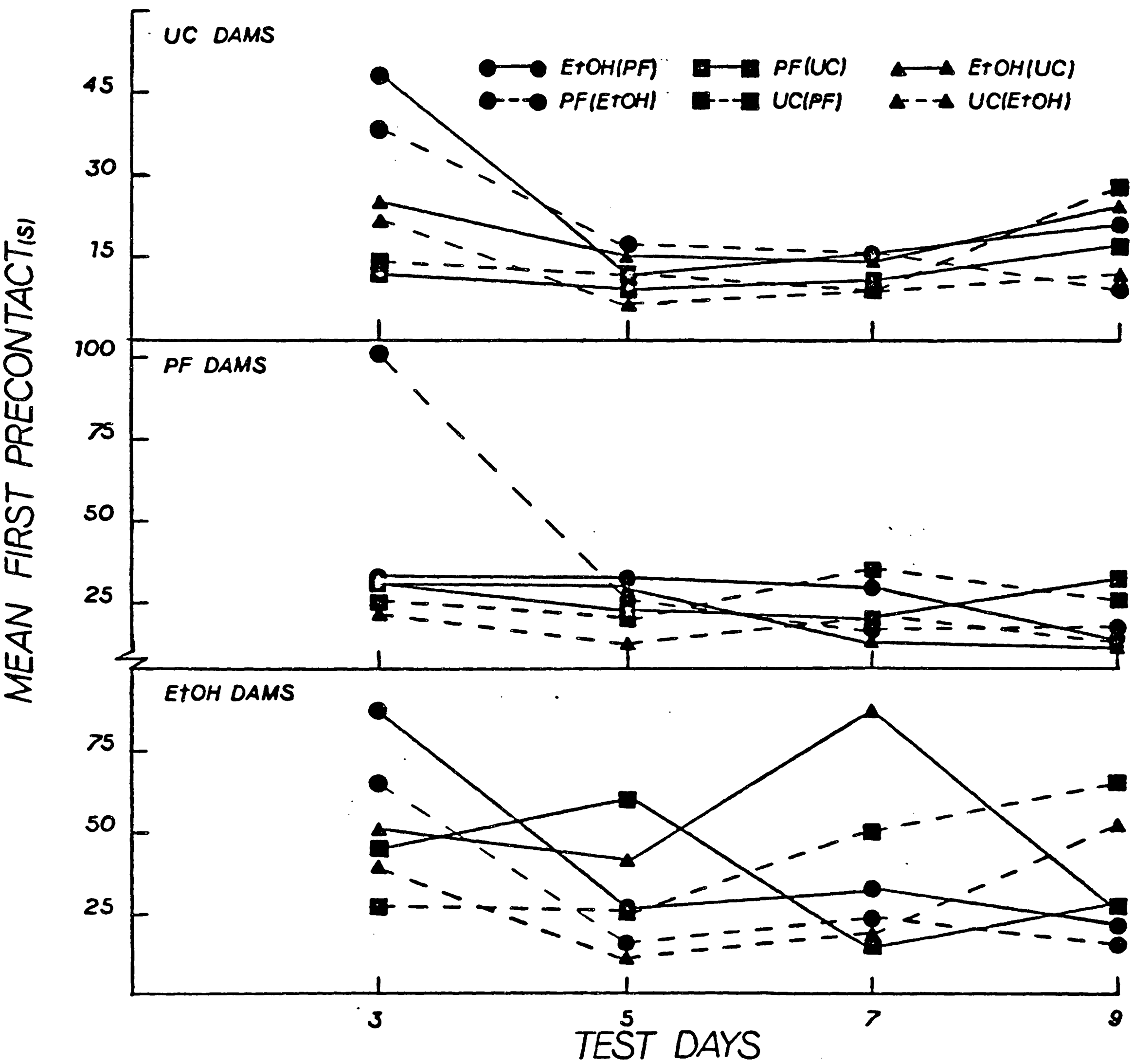

UC DAMS
PF DAMS
EtOH DAMS
EtOH(PF)
PF(EtOH)
PF(UC)
UC(PF)
EtOH(UC)
UC(EtOH)
MEAN FIRST PRECONTACT(S)
45
30
15
100
75
50
25
75
50
25
3
5
7
9
TEST DAYS

FIGURE 17

Table 26

Analysis of Variance for Precontact

Source	df	SS	F
Dam Cond.	2	15296.89	4.64+
Pup Cond.	5	1915.40	0.57
Day	3	12019.14	5.96**
Dam Cond. x Pup Cond.	10	13410.05	1.99+
Pup Cond. x Day	15	32719.81	3.24*
Dam Cond. x Pup Cond. x Day	30	30822.96	1.52+

*$p < .0001$ **$p < .001$ +$p < .05$ Note: All nonsignificant interactions were omitted

Table 27

Analysis of Variance for the EtOH, PF, and UC Dam Conditions on First Precontact

Source	df	SS	F
EtOH Dam:			
Pup Cond.	5	5497.76	0.65
Day	3	4495.48	0.88
Pup Cond. x Day	15	30066.79	1.18
PF Dam:			
Pup Cond.	5	5713.00	2.04
Day	3	7724.34	4.59++
Pup Cond. x Day	15	14226.85	1.69
UC Dam			
Pup Cond.	5	1951.71	2.52+
Day	3	4023.44	8.66*
Pup Cond. X Day	15	4111.72	1.77+

*p<.0001 ++p<.01 +p<.05

Table 27A

A Duncan's Multiple Range Test for PF Dams Over Days

Alpha Level=05 df=120 MSE=560.47

Day	N	Mean
3	36	33.28 A
5	36	25.58 A
7	36	23.11 A B
9	36	20.58 B

Condition yielded a significant effect for Day ($p<.01$) (see Table 27). A DMRT revealed that latency to First Precontact was faster on Day 9 than on Days 3, 5, and 7 which did not differ reliably from each other (see Table 27A). An ANOVA for the UC Dam Condition yielded a reliable effect for Pup Condition ($p<.05$), Day ($p<.0001$), and Pup Condition x Day ($p<.05$) (see Table 27). A simple effects ANOVA for the Pup Condition x Day interaction revealed a significant effect for Pup Condition on Day 3 ($p<.05$) (see Table 28). A DMRT revealed that latency to First Precontact was faster for PF vs UC Pup Pair Condition than for any other Pup Pair Condition (see Table 28A).

First Retrieval. Figure 18 presents mean latency to First Retrieval for each Dam Condition over Days. Ethanol and PF Dams exhibited slower First Retrieval Latencies than did UC Dams. Nevertheless, an ANOVA over these data did not yielded any reliable effects for Dam Condition (see Table 29).

Choice. Figure 19 shows mean Choice (s) for each Dam Condition as a function of Test Days. Untreated Control Dams spent less time in the vicinity of pups than did PF Dams, which spent less time in the vicinity of pups than did EtOH Dams. For each Dam Condition amount of time spent in Choice was relatively stable over Days. An ANOVA yielded a reliable effect for Dam Condition ($p<.001$) and Dam Condition x Day ($p<.05$) (see Table 30). An ANOVA for Dam Condition over each Day yielded a reliable effect for Dam Condition on Day 3 ($p<.01$), Day 5 ($p<.0001$), Day 7 ($p<.0001$), and Day 9 ($p<.0001$) (see Table 31). A DMRT for each Day revealed that EtOH Dams spent more time in the vicinity of pups

Table 28

Analysis of Variance for the UC Dam Condition

on First Precontact for Each Day

Source	df	SS	F
Day 3:			
Pup Cond.	5	4312.98	2.63+
Day 5:			
Pup Cond.	5	337.18	1.00
Day 7:			
Pup Cond.	5	239.99	1.88
Day 9:			
Pup Cond.	5	1078.78	1.09

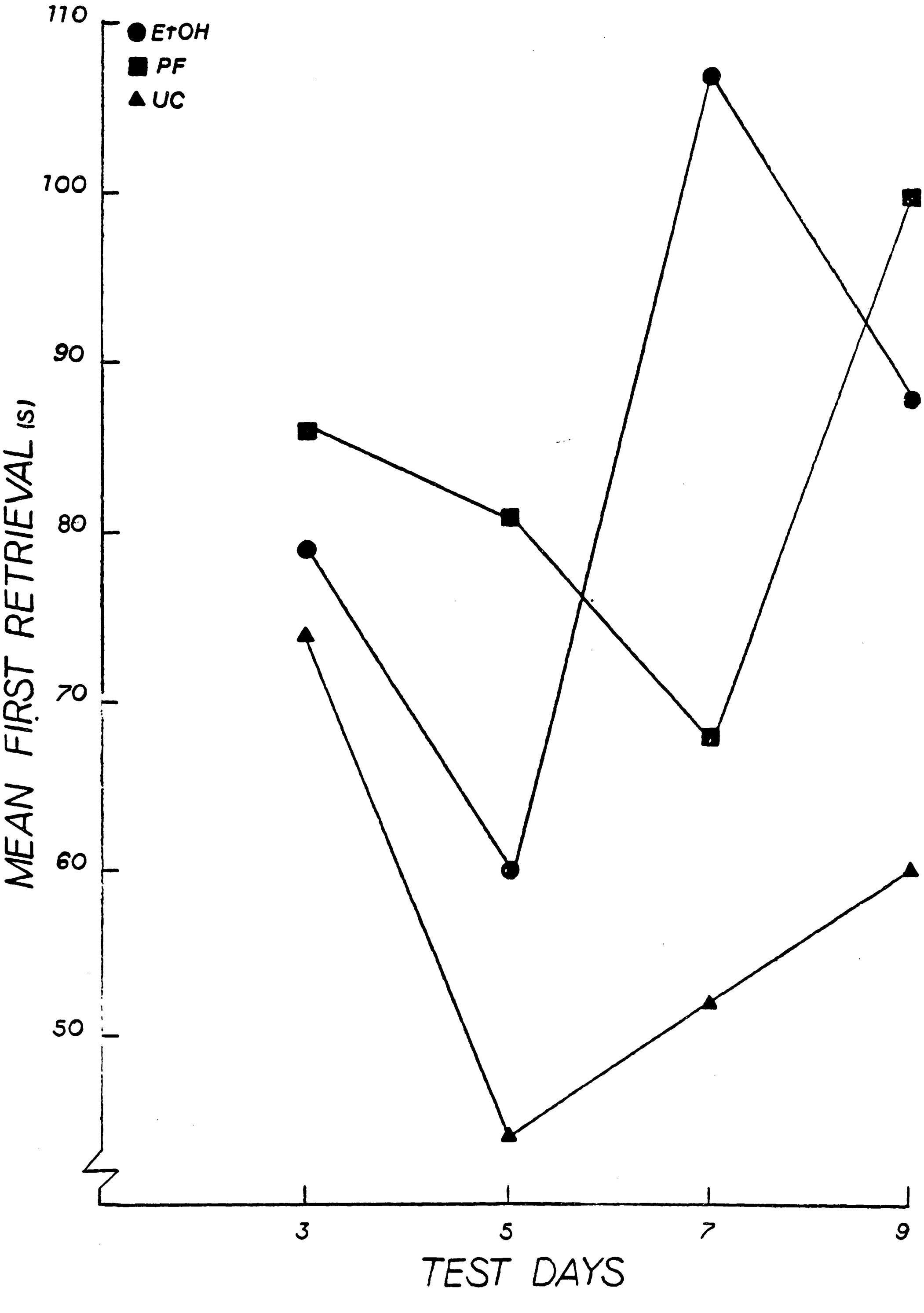
+p<.05

Table 28A

Duncan's Multiple Range Test for Pup Condition on Day 3

Alpha Level=.05 df=24 MSE=328.27

Pup	N	Mean		
EtOH(PF)	4	47.75	A	
PF(EtOH)	6	37.67	A	B
EtOH(UC)	5	25.20	A	B
UC(EtOH)	6	23.00	A	B
UC(PF)	3	14.33		B
PF(UC)	6	12.17		B



110
100
90
80
70
60
50
EtOH
PF
UC
MEAN FIRST RETRIEVAL (S)
3
5
7
9
TEST DAYS

FIGURE 18

Table 29

Analysis of Variance for First Retrieval

Source	df	SS	F
Dam Cond.	2	52037.78	3.20
Pup Cond.	5	13108.02	0.71
Day	3	11486.23	1.04

Note: All nonsignificant interactions were omitted

Table 29A

Mean First Retrieval in Seconds Over Dam Condition

Dam	N	Mean
EtOH	62	84.44
PF	112	83.22
UC	110	57.34

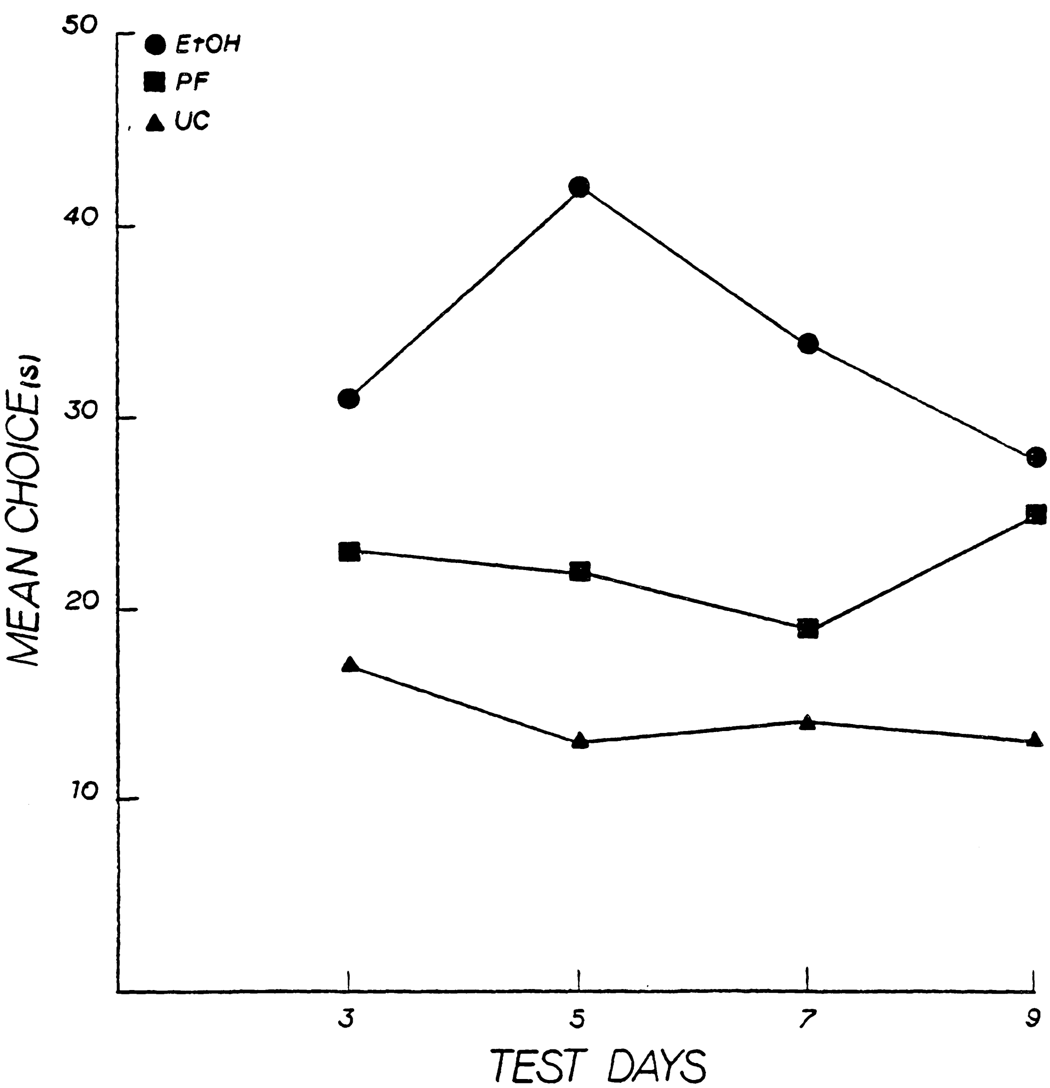


FIGURE 19

Table 30

Analysis of Variance for Choice

Source	df	SS	F
Dam Cond.	2	45174.59	8.70**
Pup Cond.	5	1355.98	0.52
Day	3	1512.06	0.97
Dam Cond. x Day	6	6987.29	2.25+

**p<.001 +p<.05 Note: All nonsignificant interactions were omitted.

than did UC Dams (see Table 31A). Pair-Fed Dams performance was intermediate between that of EtOH and UC Dams.

Contact. Figure 20 shows mean Contact (s) for each Dam Condition as a function of Test Day. Untreated Control Dams spent less time in physical contact with pups than did PF or EtOH Dams which did not differ from each other. An ANOVA yielded a reliable effect for Dam Condition ($p<.05$) (see Table 32). A DMRT revealed that UC Dams spent less time in physical contact with pups than did PF and EtOH Dams, which did not differ reliably from each other (see Table 32A).

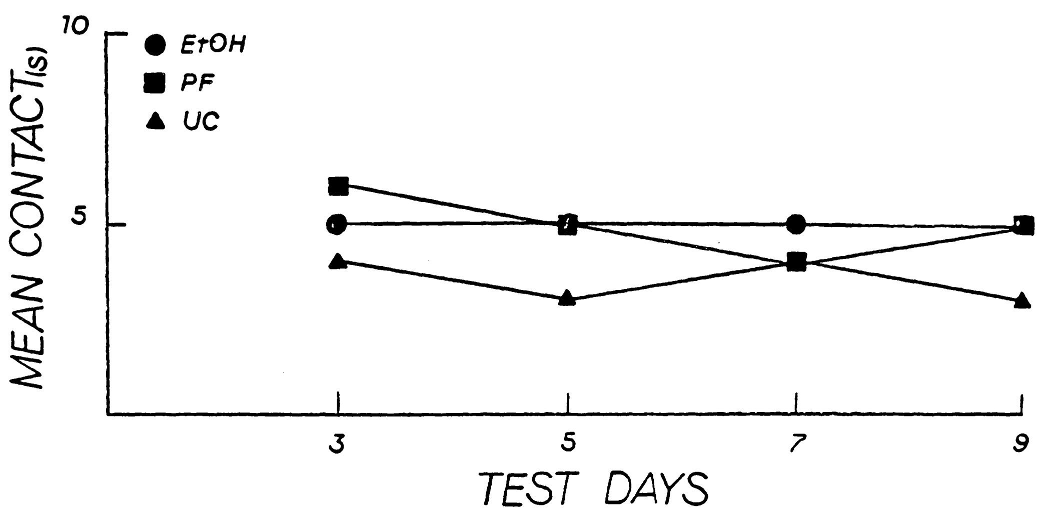


FIGURE 20

Table 31

Analysis of Variance for Dam Condition on Choice for Each Day

Source	df	SS	F
Day 3:			
Dam Cond.	2	6485.73	4.95++
Day 5:			
Dam Cond.	2	27386.92	15.43*
Day 7:			
Dam Cond.	2	13292.08	12.98*
Day 9:			
Dam Cond.	2	7555.49	9.91*

*p<.0001 ++p<.01

Table 31A

Duncan's Multiple Range Test for Dam Condition Over Days

Alpha Level=.05 df=191

Day 3				Day 5				Day 7				Day 9			
MSE=655.46				MSE=887.34				MSE=511.93				MSE=381.10			
Dam	N	Mean		Dam	N	Mean		Dam	N	Mean		Dam	N	Mean	
EtOH	62	31.31	A	EtOH	62	42.27	A	EtOH	62	33.84	A	EtOH	62	28.15	A
PF	72	22.82	AB	PF	62	22.06	B	PF	72	18.71	B	PF	72	24.60	A
UC	60	16.82	B	UC	60	13.18	B	UC	60	14.02	B	UC	60	13.12	B

Table 32

Analysis of Variance for Contact

Source	df	SS	F
Dam Cond.	2	276.21	3.90+
Pup Cond.	5	84.28	0.69
Day	3	124.66	1.77

+p<.05 Note: All nonsignificant interactions were omitted.

Table 32A

Duncan's Multiple Range Test for Dam Condition

Alpha Level=.05 df=773 MSE=25.05

Dam	N	Mean
PF	288	4.97 A
EtOH	248	4.96 A
UC	240	3.41 B

Discussion

The present data are consistent with previous results for FAS in rats (Abel, 1980). Ethanol dams had longer gestation lengths and fewer pups per litter than did control dams. Ethanol pups exhibited delays in physical maturation when compared with control pups. The blood ethanol concentration (BEC) of EtOH dams was 0.1%. The postnatal BECs of EtOH pups were not different from control pups. These results demonstrate that the FAS condition was established in the EtOH group.

Delays in physical maturation for EtOH pups may have resulted from a decrease in available nutrients to the pup during lactation. Chronic ethanol ingestion by dams results in dam's decreased food consumption and impaired nutrient assimilation (Abel, 1980). Ethanol dams consumed reliably less food during gestation and lactation than did UC dams. Though assimilation efficiencies were not collected (e.g., amount of digested calories minus amount of undigested calories), measures of litter characteristics suggest that EtOH dams assimilated nutrients more poorly than did controls. For example, EtOH dams had fewer pups per litter than did PF or UC dams, but average pup weight per litter at birth did not differ among dam conditions. These findings suggest that EtOH dams compensated for the decrease in available nutrients during gestation by producing fewer pups. This lowered produtivity may have been due to lowered conception rates or selective reabsorbtion of pups during gestation. Reliable differences in postpartum pup weights among the pups born to dams in the EtOH, PF, and UC conditions did not appear until day 4. Pup ear unfurling was achieved reliably faster by EtOH pups than by control pups. The reverse was true for developmental measures taken after day 4 postpartuition.

In terms of the dam's caloric requirements, lactation is the most costly period of the reproductive process (Mattingy & McClure, 1982). A sizable portion of the energy acquired by the dam from ingested food or fat reserves must be made available for lactation. A reduction in available energy for lactation retards pup development and in fact, the EtOH pup showed slowed physical maturation. Further, PF pups were similar to EtOH pups in their pattern of slowed physical development. Since PF dams were fed isocalorically to EtOH dams this suggests that these effects are nutritional.

Chronic ethanol ingestion by EtOH dams may have also influenced pup development in utero. The righting reflex, which is an indicator of neuromuscular coordination (Abel, 1981), was achieved at a reliably slower rate for EtOH pups than it was for PF and UC pups, which did not differ reliably. Since PF pups and EtOH pups were essentially similar in physical maturation, this effect could not be a result of decreased nutrient availability to the pup. Further, EtOH pups did not exhibit reliably different BECs from those of PF pups. Thus, ethanol was probably not a direct factor in disrupting pup development postparturitionally. These data suggest that chronic in utero ethanol exposure experienced by the pup, exerted direct pharmocalogical effects on the pup's neuromuscular development.

Ethanol is a central nervous system depressant. The BEC of EtOH dams was 0.1%. This level is sufficient to produce the pharmacological effects of ethanol (e.g. impaired motor coordination). Ethanol dams' retrieval behavior evidenced pharmacological influences of ethanol. Ethanol dams retrieved reliably fewer pups and exhibited reliably longer latencies on all retrieval measures than did PF or UC dams.

Prenatal alcohol exposure did not reliably affect the number of ultrasonic vocalizations produced by the EtOH pup. The PF pups vocalized at a reliably higher rate than did EtOH or UC pups, which did not differ reliably from each other. Inspection of the sonograms of EtOH, PF, and UC pup vocalizations yielded information for interpreting the retrieval data. Untreated Control pup vocalizations were usually continuous whistles of about 100 milliseconds, which gradually decreased in frequency and amplitude. Ethanol pup vocalizations were similar in pattern to those of UC pups but exhibited a discontinuous shift. That is EtOH pup vocalizations would abruptly change to a higher frequency. Pair-fed pup vocalizations were generally similar to those of UC pups. On occasion a PF pup vocalization was similar to that of the EtOH pup.

The altered behaviors produced by the treatment conditions of dams and pups had a synergistic effect on dam-pup interactions. Ethanol dams reliably preferred UC pups in the PF vs UC pup pair condition. Pair-Fed dams demonstrated a preference for PF pups in the PF vs UC pup pair condition. Untreated Control dams demonstrated a preference for PF pups regardless of pup pair condition. These data are partially consistent with Bell's (1979) data. Bell showed that pups which were stressed by handling in order to augment their ultrasonic vocalizations received increased caregiving from the dam. Pair- Fed pups vocalized more than UC or PF pups did. Untreated Control and PF dams prefered PF pups more than UC and EtOH pups. Ethanol dams failed to show a preference for PF pups. This finding suggests that sheer numbers of vocalizations by a rat pup may be a sufficient but not a necessary cause for increased maternal responsiveness. Gottlieb (1984) contends that acoustical properties of a vocalization, such as frequency, harmonics, and amplitude, have varying degrees of communicative significance. Since data on the acoustical properties of the pup vocalizations were not analyzed, the basis for the dam's responding is unclear. It is unclear as to whether the control dams were responding specifically to the increased number of calls produced by the PF pup or to some other acoustical property of the PF pup's vocalization.

The information from the sonographs suggests the following hypothesis concerning the dams retrieval behavior. Due to the narrow frequency range and relatively short duration of an ultrasonic vocalization there is little directional information available to the dam from a single ultrasonic pulse (Elwood & Keeling, 1982; Lewis, 1983). The pup which emits the greater number of calls should be located more easily by the dam. Since PF pups vocalized more than UC or EtOH pups did, there should be quantitatively more directional information provided by the PF pup. Thus, the PF pup should be located more easily than would the EtOH or UC pup. To the extent that calls emitted by EtOH pups vary from species typical calls, the degree of disparity between their calls and species typical calls should determine the ability of these pups to elicit maternal retrieval behavior. As for the dam, PF and UC dams should be able to percieve and respond to vocalizations emitted by the pup more efficiently than EtOH dams because they are not experiencing the pharmacological effects of ethanol. Ethanol dams may be limited in their perception and responsivity to pup vocalizations. They may only be able to respond to the optimal stimulation provided by species typical pup vocalizations. Species typical vocalizations should be emitted by UC pup and thus UC pups should be most prefered by EtOH dams. In sum, PF and UC dams should tend to prefer PF pups whereas EtOH dams should tend to prefer UC pups.

The retrieval data showed that EtOH dams will tend to chose UC pups more often than either PF or EtOH pups. These data also showed that PF and UC dams will typically chose PF pups more often than EtOH or UC pups. Further, EtOH dams will take on the average three times longer to make this choice than will UC dams. This implies that EtOH dams are in general poorer caregivers than UC dams. Thus, given the same pup condition, pups reared by EtOH dams will recieve poorer care than pups reared by UC dams. Conversely, EtOH pups are poorer elicitors of maternal behavior than PF or UC pups. Thus, given the same dam condition, dams rearing EtOH pups should exhibit fewer maternal behaviors towards EtOH pups than towards PF or UC pups. This scenerio has further implications for pup development. A pup receiving poorer caregiving from a dam should develop at a slower rate than one who receives better caregiving. A pup which is a poorer elicitor of maternal behavior should develop at a slower rate than a pup which is a better elicitor of maternal behavior.

This study lends support to the use of the animal model of FAS in discovering and analyzing processes analogous to those found in humans. The human infant cry is reported to be an index of neurological and endocrinological function because of the larynx's close tie with the CNS (Blinick, Tavolga, & Antopol, 1971). Similarly, rat pup ultrasonic vocalizations are produced via a larynx which is closely tied with the CNS (Wetzel, Kelley, & Campbell, 1980). Information from the sonographs of rat pup ultrasonic vocalizations suggest acoustical differences among the calls based on the prepartal condition of the rat pup. Further, the dam's choice of a pup was based on behavioral differences produced by the treatment condition of the dam and the pup. Thus, rat pup ultrasounds may prove to be a useful behavior for studing the effects of various prenatal insults on the developing organism. The study of dam-pup interactions may also prove fruitful in assessing the synergistic relationship between biological and environmental contributors to development. As in the present study, the biological system of the pup can be altered through prenatal exposure to ethanol. The biological condition of the pup can be determined by developmental measures, BECs, and acoustical characterists of ultrasonic vocalizations. The caregiving environment can be altered by exposing the dam to ethanol. The dam's condition can be determined by BECs and measures of maternal behavior. The synergistic relationship between biological and environmental contributors to development can then be assessed by crossfostering varing conditions of pups to varing conditions of dams and noting the differences between groups over time on the aforementioned dependent measures.

This experiment represents a first step toward incorporating the bidirectional model of behavioral development into the study of fetal alcohol syndrome. This study found that prenatal alcohol exposure produced altered behavior and responsiveness in both dam and pup. These modifications had a synergistic effect on dam-pup interactions. The ultrasonic vocalizations of the rat pup, which mediate maternal behavior, most likely differed among pup conditions on some other acoustical property in addition to number of calls. Alterations in these vocalizations could have bidirectional effects on development by changing the responsiveness of the dam to the pup. Future research along these lines could lead to parallel studies of the cries of fetally alcoholic human neonates, and their interactions with their mothers.

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