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Initial Food Selection and Sucrose
Preference in Weanling Offspring
of Rats Fed Starch or Sucrose

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INITIAL FOOD SELECTION AND SUCROSE PREFERENCE IN WEANLING
OFFSPRING OF RATS FED STARCH OR SUCROSE

By
Cynthia Loraine Theall

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ABSTRACT

INITIAL FOOD SELECTION AND SUCROSE PREFERENCE IN WEANLING OFFSPRING OF RATS FED STARCH OR SUCROSE

By

Cynthia Loraine Theall

Preference behavior of rat offspring as a function of different carbohydrate sources in the maternal diet was studied in two experiments. In Experiment I, offspring from dams fed 65 percent cornstarch (CS) or sucrose (SUC) diets were offered a choice of the diets from ages 18 to 23 days. Total intakes of sucrose diet were 101 ± 7.0 and 101 ± 6.4 mg/g body weight for CS and SUC offspring, respectively; intakes of starch diet were 8 ± 1.0 and 10 ± 1.3 mg/g body weight. In Experiment II, weanling offspring were tested for sucrose versus water preference in an incomplete block design; each animal was tested with two of seven concentrations. Analysis of the resulting cubic regression equations revealed no differences in sucrose preference between the offspring groups. Results indicate that sucrose or starch in the maternal rat diet are not factors in offspring sucrose preference or initial food choice between the two diets of this study.

For my family

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	vii
INTRODUCTION	1
REVIEW OF LITERATURE	3
Taste and the Choice of Foods	3
Identification and Selection of Foods	3
Neophobia	4
Conditioned Taste Aversion	4
Taste and the Regulation of Food Intake	9
Preference Behavior	12
Preference Alterations During Development	13
The Rat as a Model	13
Early Experience and Later Preference	15
Changes in Maternal Diet and Offspring Preferences	21
Human Preference Alteration	27
Conclusions	28
Parameters of Sweet Preference	29
Preference and Aversion	29
Preference Tests	31
Ontogeny of Sweet Preference	32
Alliesthesia	33
Rewards of Sweet Taste	35
Medical Implications of Carbohydrate Consumption	37
METHODS	42
Experimental Design	42
Preparation of Diet	43
Treatment of Dams	45
Experiment I	46
Experiment II	48
RESULTS	52
Experiment I	52
Experiment II	52
Sucrose Preference	52
Total Fluid Intake	57

Offspring Food Intake	61
Offspring Solute Intake	64
Solution A Residual Effects	66
Maternal Food Intake	66
DISCUSSION	68
Present Study	68
Experiment I	68
Experiment II	71
General Discussion	75
Taste <u>In Utero</u>	77
Preferences and Neuroendocrine Physiology	79
CONCLUSIONS	82
APPENDIX	83
BIBLIOGRAPHY	91

LIST OF TABLES

TABLE 1.	Diet composition (percent by weight)	44
TABLE 2.	Protocol for food choice test	47
TABLE 3.	Balanced incomplete block design	50
TABLE 4.	Protocol for sucrose preference test	51
TABLE 5.	Coefficient analysis for sucrose preference	56
TABLE 6.	Coefficient analysis for total fluid consumption	61
TABLE 7.	Coefficient analysis for offspring food intake	64
TABLE A1.	Vitamin mix	83
TABLE A2a.	Treatment mean adjustments, sucrose preference, CS offspring	84
TABLE A2b.	Treatment mean adjustments, sucrose preference, SUC offspring	85
TABLE A2c.	Treatment mean adjustments, total fluid intake	86
TABLE A2d.	Treatment mean adjustments, food intake	87
TABLE A2e.	Treatment mean adjustments, solute intake	88
TABLE A3.	Split-plot analysis of maternal food intake	89
TABLE A4.	One-way ANOVA tests, maternal food intake	90

LIST OF FIGURES

FIGURE 1.	Offspring food selection between two maternal diets . .	53
FIGURE 2.	Sucrose preference in CS and SUC offspring	55
FIGURE 3.	Prediction equation for offspring sucrose preference .	58
FIGURE 4.	Total fluid consumption by CS and SUC offspring during sucrose preference test	59
FIGURE 5.	Regression curves for total fluid consumption by CS and SUC offspring during sucrose preference test . .	60
FIGURE 6.	Prediction equation for total fluid consumption during sucrose preference test	62
FIGURE 7.	Food intake in CS and SUC offspring during preference test	63
FIGURE 8.	Prediction equation for offspring food intake during preference test	65

INTRODUCTION

Taste is an important sensory modality for the identification and choice of foods. Afferent information from the oral cavity can serve as a crude sentry to the gastrointestinal tract. Without the gustatory ability to identify and distinguish beneficial from harmful substances, an organism may not survive. In humans, ageusia causes profound dissatisfaction and may lead to anorexia and malnutrition (155).

Taste may also serve as a motivator for feeding. A nutrient source is of no value to an individual if that food item is not consumed because of unacceptable taste. Conversely, sweet tasting substances appear to be innate motivators for feeding and are often used as positive reinforcement for instrumental learning in animals and humans. The hedonic value of foods is not immutable, however. Cultural differences in food choices suggest both an environmental and a genetic component to preference behavior (32, 100). Taste preferences may also be affected by the internal metabolic state of the organism (16).

Several studies have been undertaken in animals to alter taste and food preferences during development. One method has been to pre-expose the animals to taste stimuli early in life and then test for preference behavior at a later age. Another approach has been to

adulterate the maternal diet during gestation and/or lactation and test the offspring for preference behavior (93). Most of these studies do not yield permanent alterations in taste preference, suggesting that the gustatory system is flexible to environmental manipulations.

One study in rats by Galef and Henderson (48) suggested that offspring would choose the diet that their mother had consumed during lactation without direct experience with the diet prior to testing. Galef implied that taste cues in the milk that were derived from the maternal diet elicited the choice behavior in the offspring. One of the two diets in the experiment was considered to be a more naturally palatable diet, yet offspring initially chose the diet that their dam consumed. The two diets, however, differed in several nutritional and flavor parameters.

The present study was designed to investigate the effect of altering one component of the maternal diet, the carbohydrate source. Rat dams were fed either a sucrose-based or starch-based purified diet throughout gestation and lactation. Offspring were tested in two experiments. Experiment I was a diet choice test to determine the initial food selection by the offspring for the two maternal diets. Experiment II was a two-bottle sucrose versus water preference test to determine if the carbohydrate source in the maternal diet affected sucrose preference behavior in the weanling offspring.

REVIEW OF LITERATURE

Taste and the Choice of Foods

Identification and Selection of Foods

Food searching and eating are the major activities of most species (136). In the wild, up to 90 percent of the day's activity is spent searching for food. New foods are selected only by frequent encounters with a sufficiently palatable prey (33). For humans in industrialized society, as little as one hour per week may be engaged in hunting for food at the supermarket. The "search image" (33) is dictated by marketing techniques which exploit the human as predator. Foods in grocery stores are specifically located to ensure frequent encounters with highly palatable items. Though it is most exaggerated in modern man, Epstein (38) has said that, "Foods and fluids are selected for appearance and flavor by all except the nutritionist and laboratory scientist."

Sensory stimulation is necessary for arousal which allows for discrimination and reinforcement (121). Animals must learn to separate potential food sources from non-foods which can only be derived from experience (136). Guinea pigs learn to discriminate between lettuce, paper towel, and clear plastic in a trial-and-error fashion (67).

Omnivores have a wide source of potential food items which is advantageous to their survival but they are also in danger of consuming harmful substances (136). Vomiting is one of the few defenses once a substance has been swallowed and rats, at least, cannot vomit (136). Thus, taste has evolved as a primary sensory system specialized for the identification and choice of appropriate food substances.

Neophobia

Novel prey may elicit both feeding behavior and fear, a conflict phenomenon called "approach-avoidance" (33). Wild rats shy away from novel foods in a familiar environment or familiar foods in a novel environment. Domestic rats, however, are not inhibited by unfamiliar food in a familiar environment (2, 73). The fear of newness, which is not unique to food selection, is called neophobia (2). With experience, foods become categorized by animals according to their familiarity: beneficial, safe, dangerous, or new. The resistance of immigrants to abandon ethnic food habits is a human example of the preference for familiar versus new tastes (136).

Conditioned Taste Aversion

In the wild, unpalatable foods are generally toxic to the consumer. The association of a particular food with post ingestional illness can be learned with only one exposure to the noxious food. Poison-avoidance learning may not be strictly related to taste. In nature, unpalatable prey are often colorful and conspicuous in behavior (33). The distinctly marked monarch butterfly larvae feed on milkweed which contains a digitalis compound poisonous to blue jays

which avoid the insects (84).

Garcia and associates (51, 52) have shown that taste and smell are specifically linked to visceral events. The flavor of a solution will be avoided in future encounters if it is followed by lithium chloride injection or X-irradiation which both produce nausea. Aversive stimulation by foot shock does not produce the flavor-pain association. Garcia termed this the "belongingness" of stimuli to systems which they modulate. Contrary to other forms of classical conditioning, conditioned taste aversion (CTA) is produced in one trial, even without temporally close presentation of taste and poison.

Several theories have sought to explain CTA. The trace decay concept suggests that gustatory memory may be slower to dissipate than other memory traces. Interference theory incorporates the idea of belongingness, that the nausea is identified only with the taste (and possibly olfactory) modality. Finally, the theory of learned safety presents the idea that novel foods are "guilty unless proven otherwise", that is, neophobia will prevail unless there is a lack of aversive consequences upon ingestion (136, 145).

It is thought that conditioned taste aversion may indirectly have a role in food preferences by limiting the available food supply. For most cultures, lactose intolerance deters adult individuals from drinking milk. Chinese cuisine is devoid of milk products, perhaps for this reason. There is also the possibility that the onset of lactose intolerance at weaning serves to stimulate the growing animal to search for more complete food sources (136). Human food aversions are known to develop after food consumption in a rocky boat, even though cognitive factors should dictate that the food was not

responsible for the motion sickness (34).

Animals consuming diets deficient in one or more nutrients often crave those nutrients, termed specific hunger. Metabolic effects on gustatory receptors have been ruled out as the mediator of this response (82). One theory suggests a change in the affective state of the organism to alter the reward value of the required nutrient (82). The specific hunger for sodium in adrenalectomized animals appears to involve a heightened response to the taste of sodium (38, 82, 97). But specific hunger may also be an aversion to a deficient diet effectively acting as a long-delay poison (136). Thiamin deficient rats cannot discriminate between diets with and without thiamin if no other sensory differences exist, such as flavor, color, or texture (82). Rats made deficient on diet A will choose diet B over diet A in a choice situation. If the nutrient in question is removed from diet B, the rats will continue to choose diet B, even though it is no longer beneficial to them (165). It would be expected that, with experience, the rats would develop a preference for a diet C over diet B once the deficiency symptoms reappeared.

For the omnivore, food aversions may not be so critical for survival but in the face of a limited food supply, learned aversions may lead to starvation (165). Though taste aversions are resistant over time, they readily extinguish if future encounters are not paired with illness (136).

It is widely thought that the rejection of bitter substances has been adaptive toward the avoidance of poisonous plants and animals, but Kare states that there is little research to support this contention (108). Quinine, citric acid, acetic acid, and butyric acid

which are used as food adulterants are presumably avoided because of their bitter taste but perhaps also because of post ingestional effects (120).

Gillan (58) explains that a natural rejection of bitter tasting substances precludes conditioned taste aversion learning. Domestic chicks were trained to associate a visual stimulus with quinine and a different visual stimulus with water. The quinine stimulus elicited decreased drinking from a tube containing water in a subsequent test. The effect of the quinine could not be attributed to post ingestional association since infusion of quinine into the crop bypassed the oral receptors and failed to produce the visual stimulus association. Milkulka et al (91) have suggested that in toads, taste aversion learning is accomplished by the aversive taste of food adulterated with two percent hydrochloric acid, which is presumably not toxic.

An unconditioned aversion to bitter substances may be adaptive for birds, reptiles, and amphibians which must deal with a host of potentially painful insects. It has been suggested that natural taste aversions may be responsible for the adaptive success of non-poisonous insects in visually mimicing their poisonous counterparts (58).

A fixed bitter-taste rejection system may be maladaptive for herbivores which potentially can include bitter tasting plants of low toxicity into their food repertoire. Jacobs and Labows (71) have investigated the relative tolerance of bitter, but nontoxic, sucrose octa acetate solutions by guinea pigs. When given various bitter and/or toxic plants to eat, the guinea pigs associated post

ingestional illness rather than bitter taste in future avoidance of toxic plants. Some of the guinea pigs succumbed, however, perhaps because the decreased perception of bitter taste allowed for overconsumption of toxic plants before illness could deter further ingestion.

Detection or repression of bitter tastes may be selectively advantageous to human populations in which individuals can collect and communicate knowledge about the relative dangers of food sources. This may interact with environmental pressures which confer survival to individuals with a particular genome. Phenylthiocarbamide (PTC) is a synthetic thyroid antagonist which tastes bitter to some individuals. The ability to taste PTC is a known genetic trait. "Tasters" are prevalent in iodine-deficient populations living in areas where natural goitrogens exist in potential food sources (turnips, peas, cabbage) (136). Although it is not known whether tasters find these foods aversive because of the perception of a bitter taste not detected by non-tasters, it has been suggested that PTC tasting might serve to protect against ingestion of foods which may be harmful.

In Yucatan, Mexico, Davies (32) has found that the inability to perceive PTC is correlated with an unusually high consumption of coffee. This human population is endemic for pellagra, presumably because of the corn-based diet. Davies suggests that the inability to taste PTC in many of these natives might be generalized to a decreased perception of bitterness in coffee. The potential advantage of coffee consumption lies in the conversion of trigonelline to niacin by roasting. There appears to be sufficient ingestion of niacin by this route to prevent death from pellagra. Whether PTC taste blindness confers an overall increase in bitterness threshold is not

known. Many Americans consume coffee and other bitter substances without apparent consequence of PTC perception. Thus, it is not known whether this study indicates genetic selection or merely the identification of an interesting correlation.

Taste and the Regulation of Food Intake

Teitelbaum (151) has observed that, in 200 years, investigators have completed a circle of thought regarding the regulation of food ingestion. Psychological observations in the 1700's suggested that taste was the primary motivator for eating. Discovery of animal metabolism by Lavoisier directed scientific investigation toward regulation by energy needs. Adolph (1) concluded that rats eat for calories and that the acceptance of food is entirely defined by the metabolizable composition of the food. Glucostatic and lipostatic theories by Mayer (89) centered around hypothalamic modulation of food intake behavior. Destruction or stimulation of specific hypothalamic nuclei disrupted the regulation of food intake. Behavioral observations of ventromedial and lateral hypothalamic lesioned animals have since underscored the importance of palatability in motivating these animals to eat. Young (162) has proposed that food habits are the primary mediators of regulation, based on hedonic response, which is conveyed to the brain by the gustatory system.

Sharma (140) has suggested that the taste versus calories dichotomy arose because sensory physiologists were not involved in early studies of food intake regulation. Classical work by physiologists and nutritionists emphasized the metabolic factors. During the past 20 years, interdisciplinary work has suggested that taste

may indeed be a factor in the physiology of food ingestion.

Gustatory stimulation may serve to trigger certain metabolic and digestive processes (108). Nicolaidis (110) found that food ingestion elevates blood glucose and respiratory quotient one minute after food consumption. The changes were too rapid to be accounted for by post ingestional effects. Animals with esophageal fistulas release insulin after food ingestion even though food is diverted from reaching the stomach (111). This response could also be triggered by bland tasting bulk but did not appear to be a conditioned response to swallowing.

Even though other oral factors besides taste may be in effect, taste may have a reciprocal role in the quantification of the digestive response. The evoked gustatory activity can be modified by gastric distension and electrical and chemical stimulation (140). Sharma (140, 141) found that the gustatory discharge is inhibited by gastric distension in satiated animals but potentiated by distension in hungry animals. The dual autonomic innervation of the stomach may be responsible for this opposing effect.

Naim et al (105) have found, in dogs, that the pancreatic exocrine response to oral stimulation is enhanced by ingestion of palatable over unpalatable foods. In humans (84), consumption of oatmeal gruel by one human patient produced little gastric secretion but a meal of choice dramatically increased gastric fluid production. Hyperphagic animals cannot maintain weight if they must bar press for intragastric infusion of food. The instrumental intragastric feeding is readily accomplished by these animals if sweet fluid is simultaneously infused into the mouth (151). Gentile (53) has found

that for a given deprivation state in normal rats, highly palatable food consumption in one meal will increase the amount of food consumed in the subsequent meal and shorten the intermeal interval.

LeMagnen (82) has suggested that the self-reinforcing effect of some alimentary stimulations may be responsible for the failure of regulation of food intake in some humans. Differences in the perception of pleasure associated with food stimuli may contrast with the general population and indicate a possible cause for disorders such as obesity or anorexia nervosa. Cabanac and Duclaux (17) report that some obese patients find sweet solutions to be equally pleasant whether satiated or full, in contrast to the proposed potentiation of taste pleasure by hunger. Grinker (62) observed that the hedonic ratings of various sweetened drinks by overweight youngsters related to the degree of obesity. Those with higher body fat composition found high sucrose concentrations more aversive than did their leaner friends. Taste thresholds, however, do not appear to be changed in either obese humans or rats (62). Heightened responsiveness to palatable foods may be either a cause or a result of the obese condition (17, 62).

There is evidence for differences in responsiveness to the concentration of sucrose solutions in normal as well as obese subjects, however. Thompson et al (152) have described two types of hedonic response to sucrose solutions in both normal and obese humans. Type I subjects displayed a typical preference-aversion behavior toward increasingly concentrated stimuli. Type II responders, however, had monotonically elevated pleasantness associated with increasing concentration. It appears that hypothalamic damage such as the

ventromedial hypothalamic lesion can alter hedonic responsiveness but that reponse pattern may also be a function of genetic and internal factors. These attributes may be involved in the expression of obesity when palatable foods are readily available.

Sharma (140, 141) has suggested that metabolic and sensory signals are neurally integrated for the regulation of food intake. Stellar (148, 149) has proposed that a neural mechanism must account for the anatomical, physiological, behavioral, and hedonic parameters of food intake regulation. Little is known about the production of hedonic experience. Recent evidence by Norgren (115, 116) suggests that there are distinct anatomical pathways linking gustatory neural afferents to the amygdala and the lateral hypothalamus, the classical seat of regulation. It may thus be impossible to separate or ignore the role of taste in the regulation as well as the selection of food.

Preference Behavior

Though food choices may be shaped by aversions to certain tastes or toxic stimuli, there are naturally palatable foods for any given species. The inherent palatability of a food is not resistant to modification. Excessive consumption of a tasty food leads to depressed preference for that food, or "sensory specific satiety" (82). Several mammals and birds, when fed exclusively on a palatable food for a long time, initially switch to a less preferred item in a choice situation (33). Morrison (98) suggests that this may be the reason why two-choice preference tests are not all-or-none phenomena; a measurable amount of the less preferred food or solution is almost always consumed. Flaherty et al (44) found that rats are more likely

to consume a 4 percent sucrose solution rather than a 32 percent sucrose solution if these solutions followed consumption of a 32 percent sucrose solution. It appears that excessive consumption of a palatable food may overcome neophobia in determining choice behavior between a novel and familiar food.

Beauchamp and Maller (3) suggest that there are four factors determining human preference behavior. Preferences are related to previous experience with the food; early life experiences are important in forming adult preferences. Certain food classes or items appear to be chosen throughout life. Finally, human food preferences can be modified by knowledge about foods. Studies of preference acquisition in animals have demonstrated that some of these factors may indeed determine human preference behavior.

Preference Alterations During Development

The Rat as a Model

The rat is a common experimental animal in many of the studies investigating preference acquisition. The adult rat gustatory receptors are located in fungiform, two foliate, and a single circumvallate papillae on the tongue. Fungiform papillae first appear at 14 to 15 days gestation and by 20 days, fetuses have intraepithelial nerve processes. Taste bud development in the circumvallate papilla also occurs at about 20 days of gestation. Though complete maturation of the taste buds does not occur until 14 days of age, discrimination of taste solutions may require only a few taste buds (92). There is electrophysiological evidence that the rat is responsive to tastant

solutions at six days of age and perhaps at younger ages (66).

Because of the extensive postnatal maturation in the rat, environmental influences on the gustatory system are most likely to occur after birth (95), perhaps as flavors ingested in milk. Milk can derive flavors from both inhalation of odors and ingestion of various feeds by the female. In cows, ragweed, buttercup, onion, garlic, turnip, kale, and silage contribute off-flavors in commercial milk (139, 143).

Jenness (72) reports that pooled samples of rat milk contained approximately 21 percent solids: 10.3 percent fat, 6.4 percent casein, 2.0 percent whey protein, 2.6 percent lactose, and 1.3 percent ash. Luckey et al (86) found milk from Wistar dams contained an average of 9.3 percent fat, 8.7 percent protein, 3.7 percent carbohydrate, and 1.4 percent ash. Milk volume and composition change over the course of lactation (77); these values thus represent only average milk composition.

The rat mammary gland utilizes glucose to synthesize lactose, triglyceride, and protein. Approximately half of the fatty acids are synthesized de novo, the other half derived from circulating plasma triglycerides. Insulin stimulates pyruvate dehydrogenase activity which directs milk lipid production (158). Subtle flavor changes may occur in milk as a result of the amount and type of lipid present in the fluid.

Flavor differences may affect the formation of neural connections in sensory pathways via cell division, migration, growth, differentiation, and cell death. These changes may affect sensory experience in the offspring. Alterations in sensory experience have been shown

to affect food preference behavior (8, 93, 94).

Early Experience and Later Preference

Numerous investigators have sought to alter preference behavior in animals by preexposure to a taste stimuli or food item prior to preference testing. In China during the 1920's and 1930's, Kuo (78) studied food habits of dogs, cats, and Mynah birds raised on specific diets from birth. Puppies were fed diets of either soybean, fruits and vegetables, or meat, dairy products, fruits and vegetables. Kittens were fed diets containing either soybeans, mackerel and rice, or assorted foods. Mynah birds, which eat almost any edible food, were raised on diets with either a variety of foods or restricted to soybeans. All animals were tested for food choices among the diets at six months of age. In all cases, the animals which ate the restricted diets from birth continued to eat only those foods even amid a wide choice of edible items. Kuo concluded that young grow up with particular food habits because of the kinds of foods fed, not from genetic pre-attachment to specific foods.

In 1958, Warren and Pfaffmann (154) raised guinea pigs on a 0.0001 molar solution of the bitter substance, sucrose octa acetate (SOA) as the sole fluid source for the first three weeks of life. In a two-bottle SOA versus water preference test at 21 days of age, the preexposed guinea pigs accepted the SOA solution as readily as water, in contrast to the water preexposed controls. This effect was not permanent, however. After three months on a normal diet with unflavored water, the preexposed guinea pigs rejected SOA solution to the same degree as control animals in a second preference test.

In two separate experiments, Evans (40) and Dethier and Goldrich (36) demonstrated that the composition of the media that blowfly larvae are raised on affects the gustatory sensitivity of the adult blowfly at three to four days of age. Adult blowflies require only sugars and a small amount of protein for sustenance which they deliver to the gut by lowering the proboscis into stimulant solutions. The results of the two studies were contradictory with regard to the direction of the sensitivity change. Evans found that fructose-media decreased adult sensitivity to fructose whereas Dethier and Goldrich found an increase in fructose sensitivity. Some of the discrepancies may be attributed to differences in methodology and definition of absolute versus relative preference in the adult insect.

In 1966, Bronson (10) failed to alter food preferences in rats based on early experience. In this study, there was evidence to support a recency rather than a primacy effect as the factor in the choice behavior of the adult animals. In one of the experiments, rats were fed Mynah bird pellets encapsulated in gelatin containers from 16 to 25 days of age then fed plain pellets until 48 days of age. Another group of rats ate plain pellets from infancy to 35 days of age when they were fed the pellet capsules until 43 days of age. In a three day preference test, the only significant effect was demonstrated by rats consuming capsules as adults; they consumed more capsules. There are several criticisms to this entire study, including the involvement of taste in identifying gelatin capsules as food and the different treatments of the two diet groups.

In the same year, Burghardt and Hess (15) published results supporting a primacy effect of food exposure. Three groups of

hatchling snapping turtles were fed either meat, fish, or worms for 12 days. When offered a choice between the three foods at 13 days of age, each group preferred the diet which it had previously consumed. Turtles were then fed for 12 days on a diet different from the original food and retested at 26 days of age. The turtles still preferred their original food, in spite of an intermediate period on a different diet. The authors suggested that the animals may have imprinted to the foods in a behavior similar to social imprinting in ducks and geese.

Rabinowitch (128) found similar primacy preferences in herring gull and ring-billed gull chicks. Chicks were fed either chopped earthworms, pink canned cat food, or dyed-green cat food from hatching to five days of age. Chicks were tested for preference at six days of age between the initial food and one of the unfamiliar foods and then between the two unfamiliar foods. Preference scoring was based on the first three pecks by the chick at a particular food cup. Familiar foods were chosen in 88 percent of the tests with the herring gull chicks and 96 percent of the tests with the ring-billed chicks. Of the unfamiliar foods, preferences were in the order of acceptability: worms, pink cat food, green cat food. The method of testing does not rule out the interaction of neophobia, however, and these results may not truly represent an imprinting mechanism.

In another study, Rabinowitch (127) conducted several experiments with Zebra finches involving early exposure to canary seeds, red millet, or white millet. These seeds differed in shape, color, and flavor. The principal conclusion was that the foods which the birds ate early in life tended to be selected in a food choice situation

as an adult. The restriction to a nonpreferred seed (canary seed) during the first five weeks of life elicited an adult preference. This preference gradually declined with exposure to the more preferred millet seeds but was retained by some individual birds for two to four months. Rabinowitch suggested that there may be a critical period in the development of the birds during which seed exposure was most likely to have its strongest impact upon later preference.

In 1969, Burghardt (14) also demonstrated a primacy effect in birds. At three days of age, Vantress broiler chicks were fed either rape or canary seeds then given the opposite seed at four days of age. These seeds also differed in color, shape, and flavor. At 12 days of age, the birds were tested for preference. Those first given canary seed preferred the canary seed, though there was a shift toward rape seed over several days of testing. Those originally eating the rape seed did not demonstrate a preference for either seed.

Capretta and Bronstein (22) also have investigated the effect of early food habits in birds. Leghorn chicks were exposed to a mobile food trough containing red or green food mash. Chicks were characterized by their activity in following the trough and eating food. The mobile trough was designed to imitate the motion of the hen in attracting the chicks toward food. In a subsequent preference test, only chicks which actively followed the trough and ate diet during the training preferred that diet in an unmoving trough. In other experiments, Capretta (21) and Capretta and Bronstein (22) failed to demonstrate significant effects of red or green diet preexposure on subsequent preferences. One reason cited was the use of a pale red dye which might not have been distinguished in mash from the plain food.

Also, the initial feeding of chicks at 24 hours of age may not have allowed for the visual association of color with food. There is evidence to suggest that chickens will not associate pecking with food ingestion until yolk sac reserves are depleted, around three days of age (136). Though visual stimuli may be effective in determining preferences, the direct effect of gustation in these experiments is questionable.

Several investigators have suggested that mere exposure to a food or tastant will increase subsequent consumption of the item because the stimulus becomes more familiar. In a study by Siegel (145), one group of rats were preexposed to coffee or vinegar solutions prior to a paired solution/poison injection exposure. Some animals were not preexposed and some animals received a vehicle injection. All animals were tested for choice between coffee and vinegar solutions five days later. Preexposure to the solutions attenuated the aversion to the solution which was paired with the poison. In a sense, there was a greater preference for a solution since previous association had not been deleterious. Rats not preexposed to solutions prior to poisoning demonstrated a typical conditioned taste aversion response. Without poisoning, rats preferred vinegar to coffee and this preference was exaggerated with preexposure to the vinegar solution. Experience with taste stimuli early in life thus affected overall behavior toward possibly aversive stimuli.

Domjan (37) has shown that preexposure to an unpreferred high concentration of saccharin for as little as five minutes for two days significantly increased future intake of the solution. This effect could not be attributed to adaptation to the laboratory environment

or water deprivation. Intraoral infusion of saccharin solution also excluded the necessity of the animal to approach the saccharin container during preexposure. In 1978, Best et al (6) extended this study and found that the ingestion of saccharin solution can be increased by both the number and duration of preexposures and that the experience can influence ingestion 96 days later without interim contact with the solution. The authors suggest that preexposure to tastants diminishes the neophobic response leading to learned safety.

Capretta et al (24) extended this idea when they raised mature and immature rats on either unflavored, single-flavored, or a variety of flavored solutions over a 12 day period. The training fluids were aqueous solutions of rum, black walnut, and vanilla extracts. After a two-day interim period with tap water, rats were tested for ten days for acceptance of a novel, chocolate-flavored solution paired with water. One month later, the animals were retested for six days. There was a greater consumption of the chocolate solution by the immature, varied-flavor exposed rats in both test situations. Mature rats drank less of the novel solution than the immature rats and did not differ in solution consumption during the initial test as a function of preexposure condition. During the second test, the mature rats did increase their novel solution intake but this was probably a result of experience from the initial test and not a function of preexposure condition. Immature rats raised solely on water ("flavor-restricted") never consumed as much chocolate solution as did the varied-flavor rats. The authors suggest that early experience with a variety of flavors, perhaps at a critical developmental stage, may allow an animal to be more accepting of any novel flavor.

In a series of experiments, Revusky has found that food preferences can be affected by the metabolic state of the animal during pre-exposure. In one study (133), rats were fed either grape juice or milk after 20.5 hours of food deprivation, then fed the opposite fluid while satiated. In similar experiments (132, 134), rats were preexposed with coffee, vinegar, anise, or almond-flavored solutions while thirsty or satiated. Tests revealed that the rats consumed more of the flavor associated with thirst than with satiation. Preferences were retained for as long as 60 days between initial exposure and test. Unpreferred stimuli did not evoke maintained choice for more than 10 consecutive days of testing, however. Revusky attributes this to a loss of post ingestional benefit of a flavor when not fluid deprived.

Changes in Maternal Diet and Offspring Preferences

Several investigators have attempted to alter preference behavior in the offspring of several species by changing the maternal diet. Taste changes are thought to affect the offspring either in utero or via the maternal milk. Tastes may directly affect gustatory maturation (93) or may serve as preexposure stimuli for the offspring which may direct the offspring to choose that flavor in subsequent tests.

In one study by LeMagnen and Tallon (83), rat dams received daily subcutaneous injections of citral around the nipples for either 0, 10, or 30 days of lactation. Citral is a potent lemon-odored substance which permeated and flavored the milk. At 40, 60, and 120 days of age, the offspring were allowed to choose between a plain and citral-treated diet. Female offspring from both 10 and 30

day treatments chose the citral diet at 40 and 60 days of age. Males from dams treated for 30 days of lactation chose the citral diet at 60 days of age. None of the offspring preferred the citral diet at 120 days of age.

In 1971, Burghardt (13) fed gravid garter snakes either night crawlers or goldfish for nine weeks. The liveborn offspring were tested at six and seven days of age for preference between the two foods. Snakes were presented with cotton swabs soaked in either fish or worm extract. Preference scores were based on the number of attacks, latency to attack, and the number of tongue flicks toward the swabs. Offspring snakes from both diet groups chose the worm extract regardless of maternal dietary experience. Since snakes do not nurse their young, flavor cues could not be transmitted in milk. Any effect of maternal diet on the preference behavior of the offspring would require in utero changes which were not defined in this study.

In a series of studies, Galef and associates have suggested that taste cues in maternal rat milk do influence the preference behavior of the offspring. Galef and Henderson (48) fed rat dams either powdered Purina Lab Chow or Turtox "fat-sufficient diet", a powdered sucrose and casein-based mix. It is widely assumed that the sweet taste of sucrose is more preferable than the flavor of complex carbohydrate. Dams were fed the diets in separate cages so that offspring would not have direct experience with the diets prior to testing. From 17 to 23 days of age, the offspring were allowed to choose between the two diets in daily 20 minute preference tests. Offspring ate more of the diet that their mother consumed, even though the Turtox diet is presumed to be more palatable. Further experiments suggested that these

results were not a function of offspring consumption of maternal feces or food particles on the dams' coat.

In a fourth experiment by Galef and Henderson (48), offspring were raised for 14 hours per day by a lactating major mother and for six hours per day by a minor mother which was lactating or by a virgin female with induced maternal behavior excluding lactation. All mothers were fed either the Purina or Turtox diets. Offspring were tested for diet choice as described above. There was no effect of the non-lactating minor mother on offspring choices. Lactational minor mothers consuming diets opposite to the major mothers did affect offspring choice. The authors suggest that familiar cues in the milk were responsible for the behavioral differences in the offspring.

Galef and Clark (47) housed female rats and their litters in two enclosures with each colony eating either the Purina or Turtox diet. Females were fed in separate cages and during this time, one bowl of each diet was made available to the offspring in the enclosures. Feeding behavior of the offspring was monitored by closed-circuit television. Offspring ate more of the diet that their mother consumed even though they did not have direct contact with the diets during the dam's presence in the enclosure.

Galef and Sherry (49) fed 21-day-old rat offspring 0.5 ml milk expressed from females eating diets opposite to the natural mothers of the offspring. This ingestion was followed by lithium chloride poisoning. Twenty-four hours later, offspring were tested for food choice in a 24-hour test. Offspring poisoned after ingestion of Turtox-derived milk consumed even less Turtox diet than the non-poisoned control

offspring. The natural dams of these offspring ate the Purina diet and even though previous work (48) suggested that the offspring would choose the Purina diet, the one-time ingestion of milk from a different diet dam followed by poisoning did lower the intake of that diet at weaning compared to control animals. Offspring from Turtox-fed dams were poisoned after ingestion of Purina-derived milk but they did not differ from controls in their consumption of Turtox diet. This result probably was related to the fact that both control and experimental offspring chose almost all of their food from the Turtox diet. The authors attribute the conditioned taste aversion of the diet to chemical cues imparted in the rat milk but it appears that it is not necessarily a recency phenomenon. Control animals which were poisoned after ingestion of milk from dams consuming the same diet as the natural dams did not alter their preferences in comparison to nonpoisoned control animals. Perhaps the preexposure to the same-flavored milk throughout lactation attenuated the effect of the poison, as in the study by Siegel (145).

In a second experiment by Galef and Sherry (49), 18-day-old pups from Purina-fed mothers were manually given milk for three days from either a Purina-fed or Turtox-fed nurse or not given supplementary milk. During the first 24 hours after weaning, pups receiving the Turtox-derived milk showed a weak preference for the Turtox diet when compared to the Purina-derived milk-fed offspring. The authors suggest that there is evidence for the identification of diet by the offspring based on preexposure cues from the mother's milk. Galef concluded, however, that there is insufficient evidence that these cues are relevant in natural weaning food selection (46).

Bronstein et al (12) repeated the study by Galef and Henderson (48) in which rat dams were fed Purina or Turtox diet. Within a litter, the offspring in this study were tested for total consumption of either the Purina or Turtox diet; only one diet was made available in the two food cups of the testing apparatus. This procedure was followed in order to control for possible differential behavioral effects of the mothers as a result of dietary differences. At 23 days of age, food consumption of pups was recorded at one-half and six hour intervals. During the first half-hour, offspring consumed more of the diet that their mothers ate. After six hours, offspring had decreased consumption of the Purina diet and had greater intake of the Turtox diet, regardless of maternal dietary experience.

Capretta and Rawls (25) fed lactating dams either water or a garlic-flavored solution for 21 days. Offspring received either garlic solution or water for five days post-weaning. After two days of tap water for all groups, the offspring were offered a choice of garlic solution or tap water for eight hours daily from 29 to 40 days of age. The test was repeated at 73 to 78 days of age. Offspring whose mothers drank garlic solution and which were reared on garlic solution post-weaning drank more garlic fluid than control offspring from water-fed dams. Intermediate preferences were exhibited by offspring from dams consuming garlic fluid at nursing or by offspring at weaning only. Preferences were stable for all groups during the retest period.

In 1975, Bronstein and Crockett (11) fed rat dams garlic-laced food or exposed the dams to a colony room containing garlic which permeated the air. Offspring from both conditions preferred garlic

food at weaning but this effect did not persist beyond the first day of testing. The differences in results from this study versus the Capretta and Rawls study (25) may be due to differences in the concentration of garlic fed to the dams. Capretta has acknowledged that the liquid garlic solution used in his experiments is only weakly rejected by water-fed animals whereas the garlic powder produces a strong aversion in naive rats that becomes more pronounced with time (20). Garlic concentration in the milk may also have differed because of the different methods of ingestion.

In a series of experiments by London et al (85), rat dams were given quinine, citric acid solution, or water as the sole fluid source at weaning. On days seven and eight postweaning, offspring were allowed to choose between quinine and water or citric acid and water. Post-weaning exposure to quinine solution and pre-weaning exposure to citric acid solution led to greater consumption of these fluids by offspring in the preference tests. Three-month-old adult rats preexposed in a similar manner did not alter their preferences as a function of preexposure period. These investigators suggest that the normally aversive bitter and sour tastes can be modified by experience at critical times in development. Whether these taste changes are lasting is not apparent in this study since the rats were not tested at an older age to determine the permanency of the effects.

In 1979, Martin and Alberts (88) have also suggested that taste cues in maternal milk are identifiable by the offspring. Rat dams were fed a diet containing geraniol which survives metabolic degradation and is present in the milk in detectable amounts. When 20-day-old

pups were poisoned with lithium chloride after ingestion of geraniol-flavored milk, there was a decreased preference for the geraniol diet when compared to non-poisoned controls.

Human Preference Alteration

There are few research studies with humans involving early exposure to a tastant and subsequent preference. One epidemiological study does suggest that experience with certain tastes may modulate preference in adult life. Moskowitz et al (100) have found that laborers from the Karnataka region of India eat a diet containing many sour foods, including the tamarind seed. When offered solutions of citric acid and quinine, the subjects found citric acid to increase in pleasurability with rising concentration. Quinine was especially pleasant at low concentrations and unpleasant only at high concentrations. The investigators did suggest that the population may have been sufficiently isolated to develop a group of genetically sour-preferring individuals. The authors also stressed the importance of the dietary experience in contributing to preferences which contrasted sharply from urban Indians and Westerners.

Rozin (136) has suggested that human cuisines are an example of the effect of early experience upon adult food choices. Cuisines are defined to have four basic components: basic foods such as breads; methods of preparation such as stewing or stir-frying; flavor principles like sweet-and-sour; and rules such as the Kosher principles. Individuals become accustomed to the habits and flavors of their culture which dictate food choices. Unfamiliar foods are often prepared with familiar flavors such as tomato sauce in order to

make them more acceptable. It thus appears that cultural food preferences are tenacious habits which develop from environmental influences at an early age.

Conclusions

These studies indicate that preference behavior of animals can be altered by preexposure of the animal to a stimulus, either directly or by addition to the maternal diet. There appears to be a critical period in development in order for preference changes to occur. Though a few studies suggested that a food imprinting behavior determined the primacy effects of food experience, the reversibility of most studies contradicted imprinting as a universal mechanism for food preference acquisition. Mistretta and Bradley (93) have postulated that the results of these studies may indicate that the gustatory system is plastic, an advantageous mechanism for organisms with changing food supplies. The results of both Kuo (78) and Capretta et al (24) support a concept that early experience with a diversity of foods or flavors allowed for greater acceptance of novel flavors later in life. Conversely, restriction of food experience in early life led to narrow food choices, not so much because the food had been imprinted but because the organism was more aversive to novel food stimuli.

In a critique of many of these studies, Mistretta and Bradley (93) have identified the stimulus quality and concentration as important factors in the final outcome of the experiments. Without quantitative comparisons, it is difficult to interpret the contradictory results. Other factors which were selected as variable in these studies

were the method of preference testing, timing of early exposure, choice of species, and the interaction of many novelty effects. None of the studies claiming taste cues in the milk of dams fed certain diets analyzed the milk for composition or concentration of the suspected tastant(s). Many of these studies require further investigation under more controlled conditions.

Capretta et al (23) have stated that early training with naturally preferred foods is more resistant to change than training with non-preferred foods. This may explain the failure of many of the studies to permanently alter the preferences of an animal for a generally non-preferred food. The concept raises the question as to why some foods evoke "natural preferences" in various species which appear to be unaffected by nonaversive dietary experience.

Parameters of Sweet Preference

Preference and Aversion

Sugar is readily ingested by humans, dogs, horses, cattle, pigeons, rabbits, and some 200 other animals including the baboon. In nature, honey attracts bees and maple sap draws bears, deer, squirrels, birds, and insects (114). Though cats are electrophysiologically unresponsive to sugar solutions (122), they can distinguish sugar-containing milk from milk without sugar (45). Nevertheless, cats appear to be behaviorally indifferent to aqueous sucrose solutions (26, 123). Chickens are also unreactive to all but concentrated sucrose solutions which they appear to reject because of the taste and not as a function of viscosity (54). Both rats and humans have similar thresholds for the perception of sweet taste: 0.5 percent

and 0.4 to 0.7 percent, respectively (135).

In 1949, Young (163) recognized that the optimally preferred sucrose concentration was not the concentration which yielded the greatest amount of sugar to the recipient, suggesting that the metabolic effects of sucrose were not the determinants of the preference behavior. In psychophysical studies with humans, Moskowitz et al (99) have demonstrated that the intensity and pleasurability of a sweet stimulus are different. There is a linear rise in sweetness intensity with increasing sucrose concentration. The pleasantness of sweetness in both foods and fluids rises then falls with increasing concentration. A constant sensory level and not a fixed concentration of sucrose determine the peak of this preference-aversion curve for the different foods and fluids.

In animal experiments, the shape of a preference-aversion curve is determined by taste and post ingestional effects. In sham drinking, esophageal fistulas prevent the post ingestional effects of sucrose consumption and animals continue to demonstrate preference-aversion behavior (97). In a two-choice preference test, preferences will disappear if fluids are infused intragastrically (97) or if gustatory nerves have been severed (114, 123). The volume of the fluid ingested is a linear function of osmotic pressure which may affect the shape of the curve at high sucrose concentrations (194). Taste, however, appears to be the primary determinant of sucrose preference-aversion. This behavior is limited to sucrose (and other tastants) paired with water; when animals are offered a choice between two sucrose solutions, the higher concentration will be chosen (82).

Preference Tests

In the 1940's, Richter and Campbell (135) developed the two-bottle 24-hour preference procedure in which it was common to test a single group of animals with ascending concentrations of sucrose for days or weeks. This method has been criticized since it confounds the responses of nutritive taste stimuli with short and long term post ingestional effects (18). Short interval tests (2 to 10 minutes) are more accurate measurements of consumption based on taste. Preference-aversion curves are produced but peak preferences are shifted upward in comparison to the 24-hour choice test (18). Short interval presentation may require training or deprivation procedures and should be conducted over several trials or days to control for neophobia (93). Intermediate tests (10 minutes to one hour) are measures of both taste and early post ingestional factors while long term tests (24 hour) are affected by osmotic and metabolic consequences of the stimulus (82). Nevertheless, 24-hour two-bottle choice tests are still prevalent as measurements of preference behavior even though taste factors may be obscured. Long-term tests may be valid in comparing the results of different manipulations using solutions with similar osmotic pressure.

Single-bottle tests often present one or more solutions in consecutive, random order and the volume of ingested fluid is the measure of preference. This method requires short interval presentation to avoid the interaction of fluid satiety with taste preference. There are studies, however, utilizing this method for fairly long test intervals (35, 87). These tests may be desirable for organisms

functionally unable to choose between solutions.

Ontogeny of Sweet Preference

In order to determine the development of sweet preference behavior, one or two bottle choice tests are inappropriate for an animal which lacks motor integration for performance. This is most evident in young animals. In order to characterize adult preference behavior in rats, Grill and Norgren (61) developed the taste reactivity test. Fifty microliters of a fluid stimulus are infused into the oral cavity and the animal's response is videotaped. Analysis of the facial responses yields a stereotypic pattern of gusto-facial reflexes for particular tastants. Ganchrow et al (50) have utilized this technique to determine taste preferences in neonatal rabbits. Characteristic responses were different for sucrose, saccharin, citric acid, quinine hydrochloride, and distilled water, suggesting the ability to distinguish the solutions based on taste even without dietary experience.

Human neonatal taste responses have been ascertained by the gusto-facial reflex technique and various modifications of the single-bottle preference test (146, 147). Maller and Desor (87) and Desor et al (35) found that in both three-hour and three-minute feedings, human infants consume more glucose solution than water and more formula than water when the two are presented consecutively in a balanced fashion. Nowlis and Kessen (117) showed that one to three-day-old infants can differentiate between glucose and sucrose solutions at two concentrations by the number of tongue pressures to a specialized nipple. Jacobs et al (70) cite work that human neonates accept sweet substances, presumably because of the taste. These studies have led

Desor to suggest that human preference for sweet is inherent rather than learned (35).

Though older infants and adults have similar response to sweet compared to nonsweet, there are differences in sweet preference in human populations (35). Younger subjects prefer a high concentration of sucrose to a greater extent than adults. Both black adolescents and adults demonstrate preference for a higher sucrose concentration than whites (34). Heritability factors are not considered to be a primary factor in overall food selection since food preferences are poorly correlated for both mono- and dizygous twins (3). But there may be population differences in the preference for a single taste quality based on genetic character. Nachman (104) has bred rats for high and low saccharin preference. Both Muto (102) and Desor et al (34) recognize the individuality of sweet preference in animals and humans.

Alliesthesia

Preferences for sweet and other substances are not constant within individuals. Sheffield and Roby (142) observed that hungry rats drink more saccharin fluid than satiated rats, even though the saccharin was energy deficient. Collier and Bolles (29) noted that preferences for a sucrose solution were affected by hunger and thirst. LeMagnen (82) described changes in the preference-aversion curve after insulin administration, food deprivation, water loading, or water deprivation. Cabanac (16) called this phenomenon alliesthesia, a change in sensation based on changes in the internal milieu. Several investigations have supported this theory.

When human subjects were offered a choice of 5 or 30 percent sucrose solution for a long drink, 5 percent was chosen but after insulin injection, 30 percent was chosen because it was no longer "sickeningly sweet" (84). Kutscher et al (79) found that five units per kilogram of insulin increased saccharin preference whereas 2-deoxyglucose, lithium chloride, and hypertonic saline injections decreased preference. In one experiment, Briese and Quijada (9) found increased pleasurability of five sugar solutions after insulin but it did not correlate with the blood glucose level. Desor et al (35) found that hunger and thirst increased the volumes of tastants consumed by neonates but not necessarily the shape of the preference-aversion curve. Mower et al (101) reported that changes in internal state are responsible for changes in preference for some but not all stimuli and the changes are dependent upon the perceived pleasantness without deprivation. Preference alteration almost always is in the direction of homeostatic restoration.

In a wider scope, hunger seems to potentiate taste (69). Under deprivation conditions, individual food dislikes disappear (126) and predators will hunt prey generally ignored in times of plenty (33). Jacobs and Sharma (69) suggest that the approach component of approach-avoidance behavior may be sensitized by hunger. Sweet and salty tastes may be more readily perceived and accepted without a concomitant decrease in the aversive component of the stimulus. Only hungry rats differentiate glucose from unsweetened corn solids in quinine solution, presumably due to this mechanism. It is recognized that starving humans will devour foods usually repulsive or considered inedible (38). More than a cognitive desire to survive is required

to provide humans with the constitution to eat human flesh or axle grease (129). Both Wetzel (156) and Revusky (132, 133, 134) have identified the changes in reward value of stimuli under conditions of deprivation.

Rewards of Sweet Taste

Sweet stimuli are recognized as powerful reinforcers for ingestive behavior and for instrumental performance. At preferred concentrations rats will learn to bar press for a sugar solution reward in a Skinner box even when the animal is not hungry or thirsty (123). The sweet taste, which is potentiated in deprivation, persists in reward value after satiation. The sweet taste is pleasurable even if it is non-nutritive. Rats will perform without extinction in a T-maze for a saccharin reward even though hunger is not reduced (142). This behavior appears to be at odds with the concept of reinforcement and need reduction. Pfaffmann (121) has called this "sweet for sweet's sake."

The reinforcement properties of sucrose make it a particularly difficult substance to avoid in modern society. Overindulgence is associated with other immediately reinforcing commodities such as opiates or sex. Overuse does not necessarily indicate addiction, however, and there is no evidence to suggest tolerance or withdrawal effects as a result of excessive sucrose consumption (41). A popular belief that early ingestion of sucrose leads to addiction to the sugar still prevails, however.

Several studies have demonstrated that experience with sucrose in the diet is ineffective in altering preference or consumption of

sucrose food. Young (163) fed rats dry diets with sucrose solution and water as fluid sources. When given a two-bottle test, sucrose preference was stable after ad libitum access to sucrose solution and after 20 days without sucrose solution intake. Muto and Miyahara (103) fed rats either high or low protein diets coupled with the presence or absence of a 40 percent sucrose solution. Experience with the sucrose solution from birth to 21 days, 22 to 43 days, or not at all was not correlated with ad libitum intake of sucrose solution from 29 to 42 days of age. Wurtman and Wurtman (160) have recently demonstrated that adult preference from 30 to 63 days of age for a 0, 12, or 48 percent sucrose diet was not correlated with consumption of one of the three diets from ages 16 to 30 days of age. It appears that in rats preexposure to a highly preferred substance will not affect the subsequent ingestions of that substance if no aversive stimuli are paired with the initial consumption.

In some human cultures, the neonate is fed sucrose or honey-based prelacteal solutions because of a taboo against colostrum consumption (74). The effect of this treatment upon future sweet preference in the individual is unknown. There are no records of non-sugar tradition cultures rejecting sugar, however. Some natives of Northern Alaska are physiologically intolerant to sucrose but continue to eat sucrose despite a malaise that should cause a conditioned aversion (74).

It is recognized that infants and adults from other cultures tend to change their eating habits when exposed to the "Western dietary" (74). This may be due to the hedonic exaggeration of palatable foods. Capretta et al (23) have stated that nonaversive

procedures of altering food preferences are less effective than aversive techniques, especially in regard to highly preferred foods. It is thus necessary to investigate the metabolic and medical consequences of the reward value of sucrose ingestion.

Medical Implications of Carbohydrate Consumption

Carbohydrate consumption in humans primarily involves starch and sucrose (106). Nondigestible cellulose constitutes the fiber portion of the diet but does not contribute to the nutrient pool. Amylose and amylopectin are the primary carbohydrates derived from starch. Amylose is an unbranched polymer of glucose molecules linked by alpha 1,4 bonds. Amylopectin is an alpha 1,4 polymer of glucose units with chain branches at alpha 1,6 linkages. Grains, legumes, and vegetables are primary sources of starch (81, 157).

Sucrose is a primary sugar consumed by Americans. It is a disaccharide composed of a glucose and fructose molecule linked by an alpha 1, beta 2 bond (81, 157). The refining of sugar cane and sugar beet has made sucrose a readily available commodity that is added to many prepared foods. Fructose is also consumed to some degree. It is present in fruits in a concentration of one to seven percent and in honey, 37 percent (130). Lactose is consumed in milk and other sugars are incorporated into many commercial products.

There is limited starch digestion in the mouth by action of salivary amylase. Once food has been swallowed, the enzyme is inactivated by the low pH of the stomach. In a less technological era, salivary amylase may have been important in releasing glucose to the oral cavity as a gustatory stimulus.

In the duodenum, pancreatic amylase and alpha 1,6 glucosidase hydrolyze the alpha 1,4 and alpha 1,6 bonds of starch yielding maltose and glucose (81, 157). The hydrolysis is an extracellular process and is accomplished in minutes (31). There is no oral, gastric, or duodenal digestion of sucrose in humans (31).

Glucose is thought to be absorbed into the intestinal cell by a sodium-dependent cotransport system in the jejunum and ileum (157). In humans 50 to 70 percent of the maltose is taken up in the jejunum, the remainder in the proximal ileum. Sucrose is absorbed in the lower jejunum and mainly in the ileum. At the brush border of the intestinal cells, maltases I through V hydrolyze maltose to glucose units. Maltases III and IV hydrolyze sucrose to glucose and fructose molecules (157). Small quantities of sucrose may escape the action of the disaccharidases and be absorbed into the intestinal cell and to the blood by nonmediated diffusion (90). There is a possibility that serosal to mucosal permeability exists as a regulatory mechanism for the absorption of sucrose, glucose, and fructose (125). Absorption of disaccharides is rare in normal individuals, however. Disaccharides are not metabolized by body cells and are excreted in the urine (90).

Glucose and fructose are transported across the serosal membrane of the intestinal cell to the portal blood via sodium-dependent and independent active transport systems, respectively (157). Kiyasu and Charkoff (77) demonstrated that 30 to 70 percent of labeled fructose is recovered as fructose in the portal blood of the rat. In the guinea pig, only 10 to 30 percent of labeled fructose remains as fructose. A substantial portion of intestinal cell

fructose is converted to glucose prior to portal blood transport. In humans, intrajejunal infusion of fructose does not elevate arterial blood glucose which, without insulin secretion, suggests little intestinal fructose conversion (138). Reiser (131) also cites evidence that fructose in humans appears primarily as fructose and lactate in the portal blood. Thus, there is support for similar fructose transport in rats and humans.

Hexokinase initiates glucose entry into liver cells which is insulin independent. Glucose is committed to phosphorylation to glucose-6-phosphate to enter the glycolytic cycle. Products are ATP, NADPH, glycogen, carbon skeletons for biosynthesis, and blood glucose which is available to other tissues for metabolism. Unlike glucose, fructose is largely metabolized in the liver. Though fructose can enter glycolysis by conversion to glucose via sorbitol, there is insufficient evidence that fructose is significantly metabolized by this route (130). Fructose is largely converted to fructose-1-phosphate by the action of fructokinase which is not affected by fasting or insulin (64, 119). Fructose does not stimulate insulin secretion by the pancreas, perhaps because it is not utilized in insulin-dependent tissues. Fructose-1-phosphate is primarily converted to dihydroxyacetone phosphate and glyceraldehyde by action of an aldolase enzyme (64).

In the rat, fructose has been found to increase hepatic and plasma lipids by increasing fatty acid synthesis and esterification of fatty acids by fructose conversion to glycerol-3-phosphate (113). Naismith (106) counters this evidence with the idea that the enzyme induction of fatty acid synthesis is the result of continuous flooding

of liver cells with any monosaccharide. But, since fructose is principally metabolized in the liver and is not diverted to other sites of action, it is the monosaccharide most likely to be in excess in the liver. Fructose entry into glycolysis occurs below the action of the regulatory enzyme, phosphofructokinase. Without insulin and glucagon control of fructose uptake, it does appear that fructose units could be readily shuttled into fatty acid synthesis without negative feedback inhibition by citrate. However, the lipogenic properties of the fructose moiety of sucrose are entirely dependent on individual and species conversion of fructose to glucose prior to absorption into the portal blood.

There is much controversy regarding the exchange of sucrose and starch as carbohydrate sources in the diet. In an extensive review by Bender and Damji (4) conflicting evidence is cited which correlates sucrose intake with food consumption, life span, liver weight, lipids, glycogen content, liver and kidney metabolism, serum lipids in man and animals, insulin levels, respiratory quotient, vitamin status, dental decay, and enzyme and metabolic changes. During the past 60 years, United States total carbohydrate consumption has decreased with a concomitant rise in fat intake. The proportion of carbohydrate consumed as sucrose, however, also has risen. These changes in food consumption parallel economic development as well as an increase in mortality from degenerative diseases (19). Yudkin (164) has supported a role of sucrose in the etiology of coronary heart disease but Bierman (7) has proclaimed that there is little documentation for any health hazard except dental caries (137).

The USDA-DHEW have recently presented seven dietary guidelines

among which is the recommendation that Americans increase their consumption of complex carbohydrate to displace energy intake from both sugar and fat (153). Though the dietary guidelines seek to minimize the nutritional factor in multidimensional diseases, their success may be dampened by the gustatory pleasures associated with sweetened foods. It is thus essential to identify the risks and benefits of modern food consumption patterns on both health risks and affective pleasures. Since sweet foods appear to be universally pleasurable, the initiation of the preference for sweet must be investigated with regard to possible maternal dietary effects upon initial preference behavior of the young.

METHODS

Experimental Design

The studies by Galef and colleagues (47, 48, 49) have shown that rat offspring initially prefer Purina Lab Chow to the presumably more palatable Turtox diet when the dams consumed the Purina food during lactation. Since the Turtox diet contains sucrose, it would be expected that the sweet taste would be preferred by all offspring regardless of the dietary experience of the dams. The present study was designed to control the differences in dietary composition between two maternal diets. These experiments investigated the influence of sucrose versus starch as carbohydrate sources in maternal rat diets upon the initial selection of these diets by weanling offspring and upon offspring sucrose preference behavior.

Rat dams were fed either a cornstarch-based or sucrose-based purified diet prior to breeding and throughout gestation and lactation. The dams and litter groups were designated CS (cornstarch) and SUC (sucrose) corresponding to the diet consumed by the dams. The dams were meal-fed in separate cages to prevent the offspring from consuming their mother's diet prior to testing.

The offspring were tested for preference behavior in two experiments. Experiment I was a food preference test in which the offspring were allowed to choose between the diet their mother consumed

and the opposing maternal diet. These test diets were identical to the maternal diets. Offspring food choices were designated starch and sucrose. Thus, there were two litter groups, CS and SUC, and their food choices, starch and sucrose. The hypothesis for this experiment was that if the offspring were familiarized to taste cues that resulted from the maternal diet and expressed in the milk, then the offspring should choose more of the diet their mother consumed in the food preference test.

In Experiment II, a different group of CS and SUC offspring were tested for sucrose preference behavior in a two-bottle sucrose versus water preference test. Since the two maternal diets contained either cornstarch or sucrose, the hypothesis for this experiment was that the sucrose preference-aversion curve may differ between the CS and SUC offspring.

Preparation of Diet

The cornstarch and sucrose diets were prepared separately on the same day. The composition of the two diets and the sources of ingredients are described in TABLE 1. For each diet, the following dry ingredients were mixed together in a Hobart mixer (Hobart Manufacturing Company, Troy, OH) for 10 minutes: casein, d,l-methionine, fiber, mineral mix, vitamin mix, and choline bitartrate. For each diet, cornstarch or sucrose and the above dry ingredients were added to a Wenger food mixer (Wenger Manufacturing, Sabetha, KS) and blended for about five minutes. Alpha tocopherol acetate (Sigma Chemical Company, Saint Louis, MO) was blended with the corn oil in two flasks (one for each diet) and the liquid mixture added to the dry ingredients while

TABLE 1. Diet composition (percent by weight)

<u>Ingredients</u>	<u>Starch Diet</u>	<u>Sucrose Diet</u>
Casein ^a	20.0	20.0
D,L-methionine ^b	0.3	0.3
Fiber ^c	4.5	4.5
Corn oil ^d	5.0	5.0
Mineral mix ^e	4.0	4.0
Vitamin mix ^f	1.0	1.0
Choline bitartrate ^g	0.2	0.2
Cornstarch ^h	65.0	- -
Sucrose ⁱ	- -	65.0

^aHigh nitrogen casein; U.S. Biochemical Corporation, Cleveland, OH.

^bU.S. Biochemical Corporation, Cleveland, OH.

^cCelufil non-nutritive bulk fiber; U.S. Biochemical Corporation, Cleveland, OH.

^dCapri, Anderson-Clayton Company, Dallas, TX.

^eJones-Foster salt mixture; U.S. Biochemical Corporation, Cleveland, OH (75).

^fPrepared from stock ingredients purchased from U.S. Biochemical Corporation, Cleveland, OH (TABLE A1).

^gCrystalline; U.S. Biochemical Corporation, Cleveland, OH.

^hClinton, Clinton Corn Processing Company, Clinton, IO.

ⁱD+ crystalline sucrose; U.S. Biochemical Corporation, Cleveland, OH.

the machine was in operation. Diets were mixed for about 45 minutes, then packaged in polyethylene lock-top bags for storage in a walk-in freezer. One bag of each diet for current use was stored in a cold room.

The calculated gross energy for each diet was 3.9 kcal per gram.

Treatment of Dams

Thirty-five virgin female Sprague-Dawley rats (Spartan Research, Haslett, MI) were obtained at 90 days of age weighing 210 to 245 grams. They were housed in hanging, stainless steel group cages, four to five per cage. The females were maintained for ten days on a commercial closed formula diet (Wayne Lab Blocs, Wayne Laboratory Foods, Chicago, IL) and provided with water ad libitum. A 7 AM to 7 PM light/dark schedule was provided in the animal quarters.

At 100 days of age, the females were randomly assigned to one of the two diet groups, CS or SUC, and identified by marking the tails with a waterproof pen. In order to accustom the female rats to meal-feeding, which would be necessary during lactation to prevent the offspring from consuming the maternal diet, the rats were transferred to individual wire-bottomed hanging cages and given access to their respective diets from 8 to 11 AM daily. Newsprint was taped underneath each meal-feeding cage to collect spillage which was returned to the food cup before weighing. Food cups were weighed before and after feeding sessions on a Mettler toploading balance (Mettler, Des Plaines, IL) to the nearest 0.1 gram.

Prior to breeding, the female rats contracted a self-limiting infection, sialodacrioadenitis (corona virus). The animals recovered

within five days and it was assumed that this event would not adversely affect the study.

Female rats were bred with eight Sprague-Dawley male rats which weighed between 380 to 535 grams. Breeding was initiated when the females were 112 days old. Mating was confirmed by taking vaginal smears and inspecting the fresh slides for live sperm. A total of ten pregnant females in each diet group were meal-fed throughout gestation and lactation and weighed twice per week. The rats were housed in closed-bottom stainless steel breeder cages with drinking water provided ad libitum. Wood chip bedding was changed regularly.

Eighteen dams delivered litters ranging from 5 to 14 pups. All litters were reduced to eight pups within 24 hours of birth with the exception of one CS dam which delivered only five pups. She was given three pups from another CS dam which delivered 12 pups the same day. No other cross-fostering procedures were done.

A total of 16 litters were assigned to either Experiment I or Experiment II based on delivery date.

Experiment I

Five litters from each diet group were selected for Experiment I. The general protocol for the experiment is depicted in TABLE 2. Offspring were habituated to the apparatus at 15, 16, and 17 days of age. The apparatus was a square plywood box, 10 inches wide by 6 inches high with open top and bottom. Inner surfaces of the box were painted black and newsprint was used to cover the top during the experiment to simulate the dark environment of natural feeding conditions. Each litter was tested in its own apparatus which was placed on clean

TABLE 2. Protocol for food choice test

<u>Age of Rat (Days)</u>	<u>Procedures</u>
15	Habituate as a litter - no food
16	Habituate as a litter - with food
17	Habituate individually - with food
18 to 23	Measure individual food intake

newsprint on a laboratory bench. Franke animal feeding jars (Fisher Manufacturing, Pittsburgh, PA) were used as food cups, which were placed in opposite diagonal corners of the box. According to the testing schedule, the food cups were either empty for habituation or filled with the maternal diets, one food cup for starch and one for sucrose.

The 15-day-old offspring were habituated as a litter to the apparatus without food present in the food cups for one half hour. On the next day, the litters were habituated in the apparatus for one half hour with food present in the food cups. On the next day, the offspring were individually habituated to the apparatus with food present in the food cups, twenty minutes per rat. Food intake was not recorded since previous literature (48) indicates that 17-day-old offspring do not consume a measurable amount of food.

From ages 18 to 23 days, the offspring were randomly assigned a testing order within each litter for each test day. Food cups with the two diets, starch and sucrose, were weighed to the nearest 0.01 gram on a Mettler toploading balance. Rats were allowed to consume food for twenty minutes then returned to their litter cage. Spillage was returned to the food cups which were reweighed and food intake

recorded to the nearest 0.01 gram. Newsprint was changed and the apparatus wiped clean after each rat was tested. The food cups were refilled, reweighed, and moved clockwise to the next corner of each box to prevent position association by subsequent rats. Animals were tested on six consecutive days. Body weights were recorded at 18 and 23 days of age.

Multivariate analysis of variance was proposed as the statistical method of analysis.

Experiment II

The effect of maternal diet on sucrose preference in the offspring was tested utilizing a two-bottle sucrose versus water preference test. The following concentrations of sucrose (weight/weight percent) were employed: 0.3125, 0.625, 1.25, 2.5, 5, 10, 20 (76, 124). Sucrose solutions (D+ crystalline sucrose, U.S. Biochemical Corporation, Cleveland, OH) were mixed fresh each morning of the experiment in double distilled water. All fluids were presented at room temperature in Richter tubes. Fluid intake was measured by weighing the tubes on a toploading Mettler balance prior to presentation and at the end of each 24 hour test period. Tubes were rinsed and fresh fluid given every 24 hours, just prior to 7 PM.

Long-term preference testing with sucrose solutions presents the problem of metabolic and osmotic post ingestional effects versus energy satiation (18). Since weanling rats gain a great deal of weight in three to four weeks, energy demands might differ through the course of the experiment and confound the taste versus metabolism problem. The three to four weeks required to test all animals with all

concentrations would also be sufficient time for the offspring to mature sexually. Sucrose preference behavior appears to differ between males and females after puberty (63, 160). To avoid these complications, which might arise with a testing period of several weeks, each offspring in this experiment was tested with only two of the seven sucrose concentrations.

A balanced incomplete block design (28) was utilized to determine the two concentrations each rat would receive as test solutions (TABLE 3). The block design contained 21 blocks ($b=21$) which represented individual rats, with a block size of two ($k=2$), which were the two test solutions, SOL A and SOL B. Each concentration was replicated in six different animals in the design ($r=6$). Block design numbers, 1 to 7, represented codes for the sucrose fluid concentrations, from lowest to highest percent. Since the original design always coded for the lower concentration as SOL A, the order of the concentrations for SOL A and SOL B was randomized. Twenty-one offspring from each diet group were randomly assigned a block in their respective design. Due to a restriction in the number of available Richter tubes and the differences in the age of the offspring, the animals were actually tested in two periods based on proximal ages, Period 1 and Period 2. The complex design necessitated ignoring any period effects in the analysis of the data. Each diet group block design was treated as if all animals were tested simultaneously.

At 22 days of age, offspring were transferred to individual stainless steel wire-bottomed cages and weaned to the starch diet. Double distilled water was presented in two Richter tubes wired side by side to the exterior of the cage. Food and water were provided ad libitum.

TABLE 3. Balanced incomplete block design^aSUC Offspring

SOL			SOL			SOL		
RAT	A	B	RAT	A	B	RAT	A	B
1	1	2	8	1	3	15	1	4
2	2	6	9	4	2	16	2	3
3	3	4	10	3	5	17	6	3
4	7	4	11	4	6	18	5	4
5	5	1	12	5	7	19	2	5
6	5	6	13	1	6	20	7	6
7	3	7	14	2	7	21	7	1

CS Offspring

SOL			SOL			SOL		
RAT	A	B	RAT	A	B	RAT	A	B
1	1	2	8	1	3	15	1	4
2	6	2	9	4	2	16	2	3
3	4	3	10	3	5	17	6	3
4	4	7	11	6	4	18	5	4
5	1	5	12	5	7	19	5	2
6	5	6	13	1	6	20	6	7
7	3	7	14	7	2	21	1	7

^aFor each design: (28)

b=21	number of rats
k=2	number of solutions
t=7	number of concentrations
r=6	number of replications of concentrations
$\lambda=1$	number of times each concentration is paired with every other concentration
n=42	total number of animals

Rats were allowed to adapt to this environment for two days. At 24 days of age, SOL A was presented in one Richter tube and water in the second tube. After 24 hours, the tubes were weighed, refilled, and the position on the cages reversed. After 24 hours, fluid intake was again recorded. Because of possible residual effects of SOL A, both tubes were thoroughly cleaned and filled with water for presentation at 26 days of age. Water intake during the subsequent 24-hour period was recorded. Twenty-seven-day-old rats received SOL B in one tube and water in the second tube. After 24 hours, fluid consumption was measured, tubes were rinsed and refilled with SOL B or water and the position of the tubes was reversed. After 24 hours, fluid intake was recorded (TABLE 4). Food intake was recorded daily. Offspring body weights were recorded at weaning and at the conclusion of the experiment.

TABLE 4. Protocol for sucrose preference test

<u>Age of Rat (Days)</u>	<u>Procedures</u>
22	Habituate to tubes, water vs water
23	" "
24	SOL A vs water presented
25	Position of tubes reversed (SOL A)
26	Water vs water presented
27	SOL B vs water presented
28	Position of tubes reversed (SOL B)

Incomplete block analysis and polynomial regression were proposed as methods of analysis to determine treatment means and the best-fitting curve for the responses of each diet group.

RESULTS

Experiment I

In the food choice test, food intake was expressed in terms of offspring body weight to control for the effect of body size on food consumption. Intakes for 18, 19, and 20-day-old rats were based on the 18-day-old body weight; 21, 22, and 23-day-old rat intakes were based on the 23-day-old body weight. Individual rat food intake was totaled over the six days of testing.

The CS offspring consumed a mean of 101 ± 7.0 mg per gram body weight of the sucrose diet and 8 ± 1 mg per gram body weight of the starch diet. The SUC offspring consumed a mean of 101 ± 6.4 mg per gram body weight of the sucrose diet and 10 ± 1 mg per gram body weight of the starch diet (FIGURE 1). Because of the overlap in standard errors of the mean within the CS and SUC offspring and the disparate standard errors of the mean between the starch and sucrose diets, no formal statistical analyses were applied to these data.

Experiment II

Sucrose Preference

In the sucrose versus water preference test, the fluid consumption was recorded in grams; values for the two days were averaged within

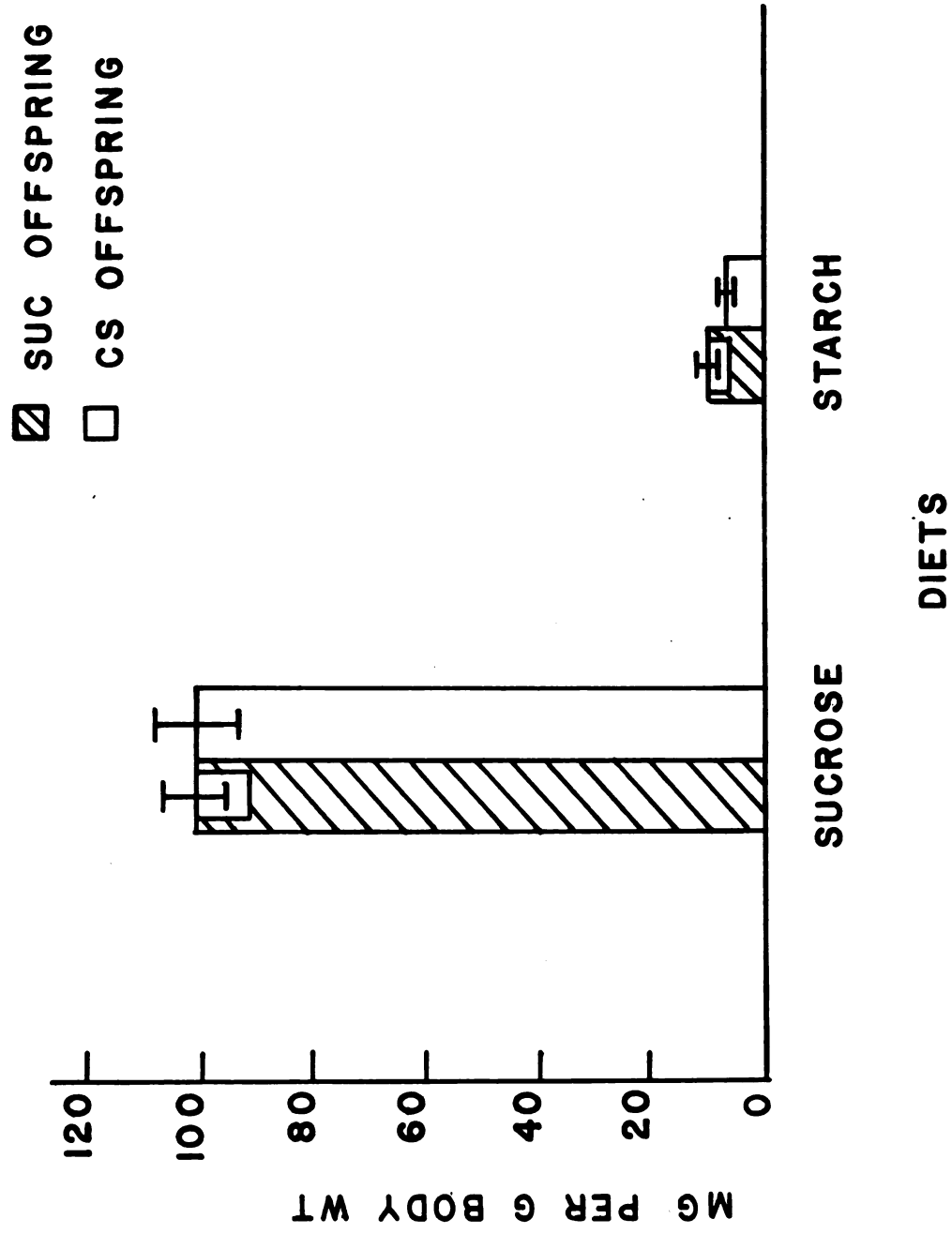


FIGURE 1. Offspring food selection between two maternal diets

each solution. Mean intakes for SOL A and water, SOL B and water, and total water intake for the interim test day were obtained. Intakes of SOL A and B were based on body weights at ages 22 and 29 days, respectively. Fluid consumption was expressed as the sucrose fluid percent:

$$\frac{\text{mg sucrose solution consumed per gram body weight}}{\text{total mg fluid consumed per gram body weight}}$$

Treatment means for each of the seven sucrose concentrations were calculated based on six animals per concentration in each diet group. These concentration means were adjusted for block effects by incomplete block design statistical procedures (TABLE A2a and A2b) (56). A plot of sucrose fluid percent versus sucrose concentration for the adjusted treatment means shows two sucrose preference-aversion curves, one for each diet group (FIGURE 2). These data points were analyzed by polynomial regression to determine the best-fitting curve as defined by a polynomial equation (56, 112).

The regression equation for the CS offspring was found to be: $Y=0.025641868 x^3 - 1.1245788 x^2 + 12.276299 x + 46.297619$. The regression equation for the SUC offspring was found to be: $Y=0.047351709 x^3 - 1.7466899 x^2 + 15.361631 x + 53.702381$. Differences between the unstandardized coefficients of the two equations were tested using Student's t test (55). There were no significant differences between the two equations in any of the four coefficient parameters (TABLE 5). Thus, the data points for the two diet groups represented the same sucrose preference-aversion curve. There was no significant difference in sucrose preference for these weanling offspring as a function of the carbohydrate source in the maternal diet.

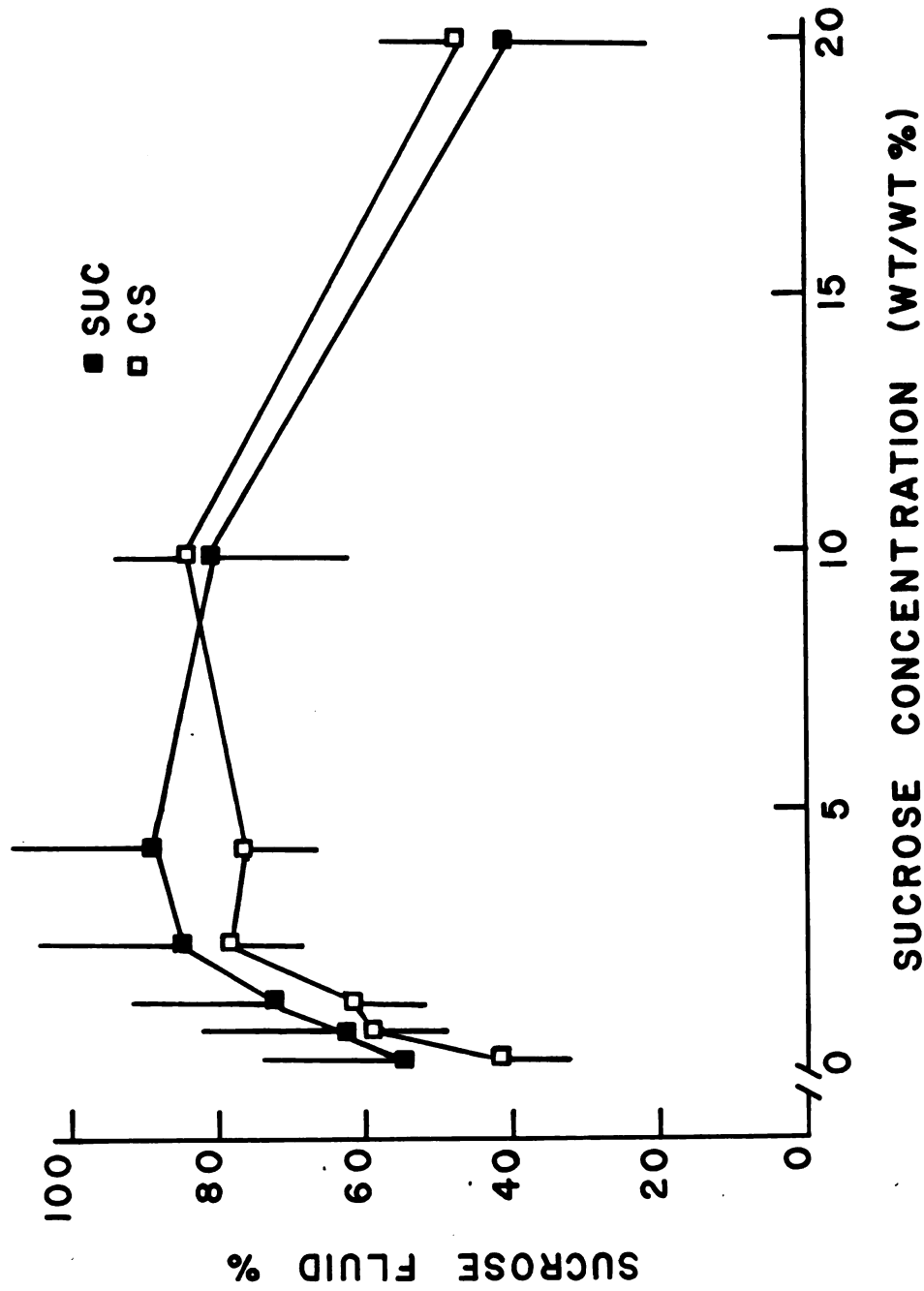


FIGURE 2. Sucrose preference in CS and SUC offspring

TABLE 5. Coefficient analysis for sucrose preference^a

<u>Coefficient (B)^b</u>	<u>Student's t value^c</u>
B ₀	-0.14997
B ₁	0.54999
B ₂	0.78785
B ₃	-0.6325

^aDerived from $t = \frac{B_x^a - B_x^b}{\sqrt{(SE B_x^a)^2 + (SE B_x^b)^2}}$ where a and b are the values derived from the two equations, B values are the unstandardized regression coefficients, and SE are the standard errors of the coefficients.

^bFrom $y = B_0 + B_1x + B_2x^2 + B_3x^3$

^cDegrees of freedom = $n_1 - B_a + n_2 - B_b$ where n is the number of treatments (7) and B_a and B_b are the number of significant regression coefficients (3, 3, respectively). Critical value for $\alpha = 0.05$ is ± 2.447 (57).

Data from the two diet groups were pooled and a single prediction equation for sucrose preference was determined via polynomial regression (112). The regression equation was determined to be: $Y = 0.036496788 x^3 - 1.435644 x^2 + 13.818965 x + 50$. The peak consumption of sucrose solution was determined mathematically to occur at a concentration of 6.3 percent which yielded a maximum sucrose fluid percent intake of 89.2. Variances for the prediction equation were determined mathematically for selected sucrose concentrations (56).

The regression equation was entered into the PLOT/DRAW MSU STAT program for plotting (30). Actual treatment means were also plotted by the computer as squares. Variances were added to the plot by hand (FIGURE 3).

Total Fluid Intake

Average amounts of sucrose fluid and water consumed during the presentation of SOL A and SOL B were totaled for each solution and each animal. Results were expressed as mg fluid consumed per gram body weight. A graph of total fluid consumed versus sucrose concentration was made by plotting the adjusted treatment means (FIGURE 4, TABLE A2c). Total fluid consumption as a function of sucrose concentration was analyzed by polynomial regression which yielded two equations (112). For the CS offspring, $Y = 0.12202874 x^3 - 4.9927023 x^2 + 50.894235 x + 196.36518$. For the SUC offspring, $Y = -1.3831333 x^3 + 234.02759 x^2$. There were no differences in any of the coefficient parameters as determined by Student's t test (TABLE 6). The two equations were plotted on one graph with the adjusted treatment means (FIGURE 5) so as to visually compare the regression curves with FIGURE 4.

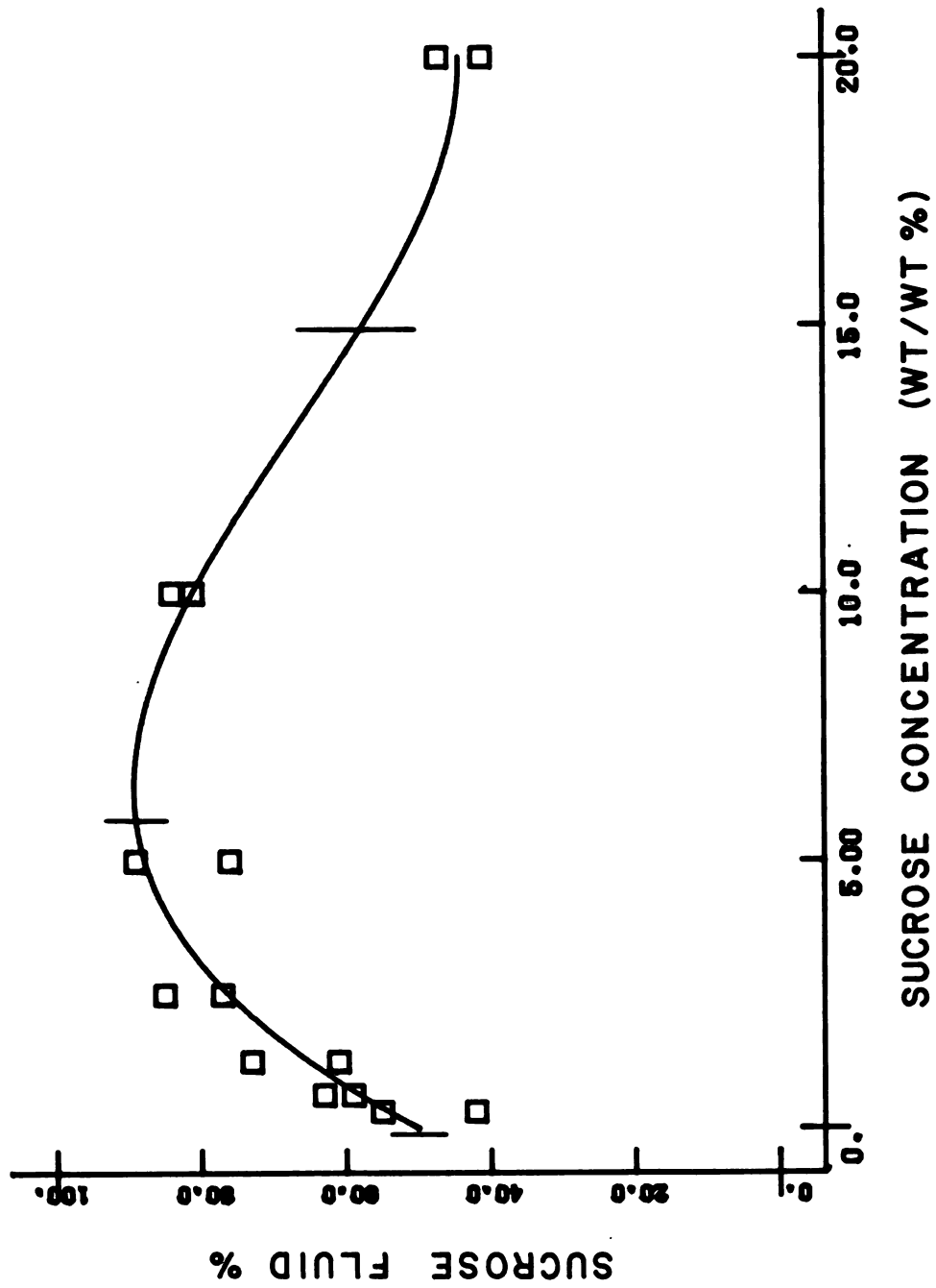


FIGURE 3. Prediction equation for offspring sucrose preference

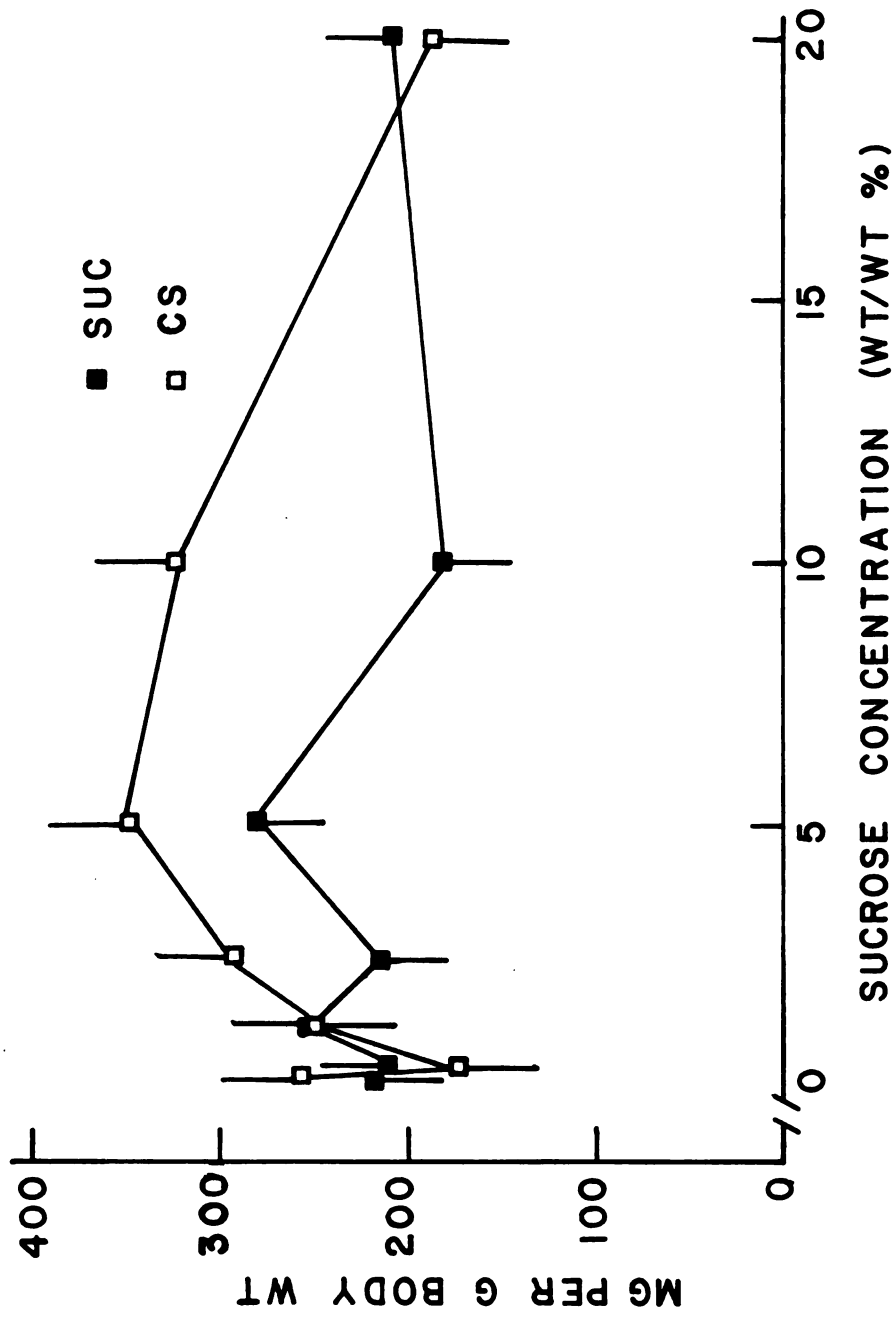


FIGURE 4. Total fluid consumption by CS and SUC offspring during sucrose preference test

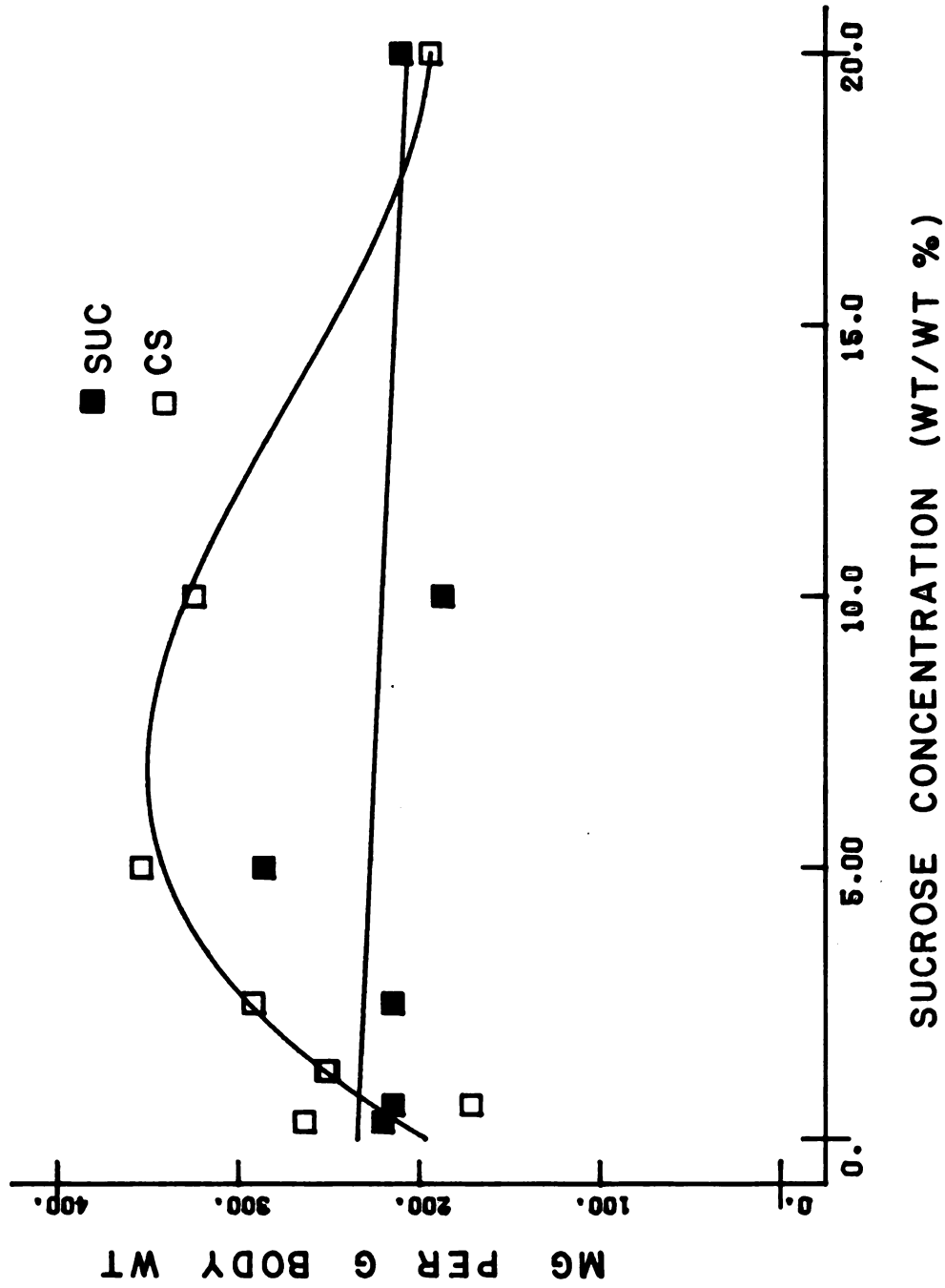


FIGURE 5. Regression curves for total fluid consumption by CS and SUC offspring during sucrose preference test

TABLE 6. Coefficient analysis for total fluid consumption

<u>Coefficient (B)</u>	<u>Student's t value^a</u>
B ₀	0.9254
B ₁	1.8416
B ₂	-1.2172
B ₃	0.8663

^aCritical value is ± 2.228 for $\alpha=0.05$, degrees of freedom = 10 (57).

Data were pooled and a prediction equation for total fluid intake was obtained (112): $Y=0.14820649 x^3 - 5.0470502 x^2 + 41.776301 x + 199.13839$. The equation was plotted via the MSU STAT program (30) together with the actual treatment means (FIGURE 6). These results suggest that there is no difference in total fluid consumption by the offspring in the sucrose preference test as a function of sucrose or starch in the maternal diet. There does appear to be a difference in total fluid consumption for all offspring as a function of sucrose concentration consumed during the preference test.

Offspring Food Intake

Food intake for the offspring was recorded in grams during the testing period, ages 24 to 29 days. Amounts were expressed such that mean food consumed with SOL A and SOL B were based on body weights at 22 and 29 days of age, respectively. Treatment means were calculated and adjusted for block effects (TABLE A2d). A plot of mg food consumed per gram body weight versus sucrose concentration demonstrated two curves (FIGURE 7). Two equations were determined via polynomial

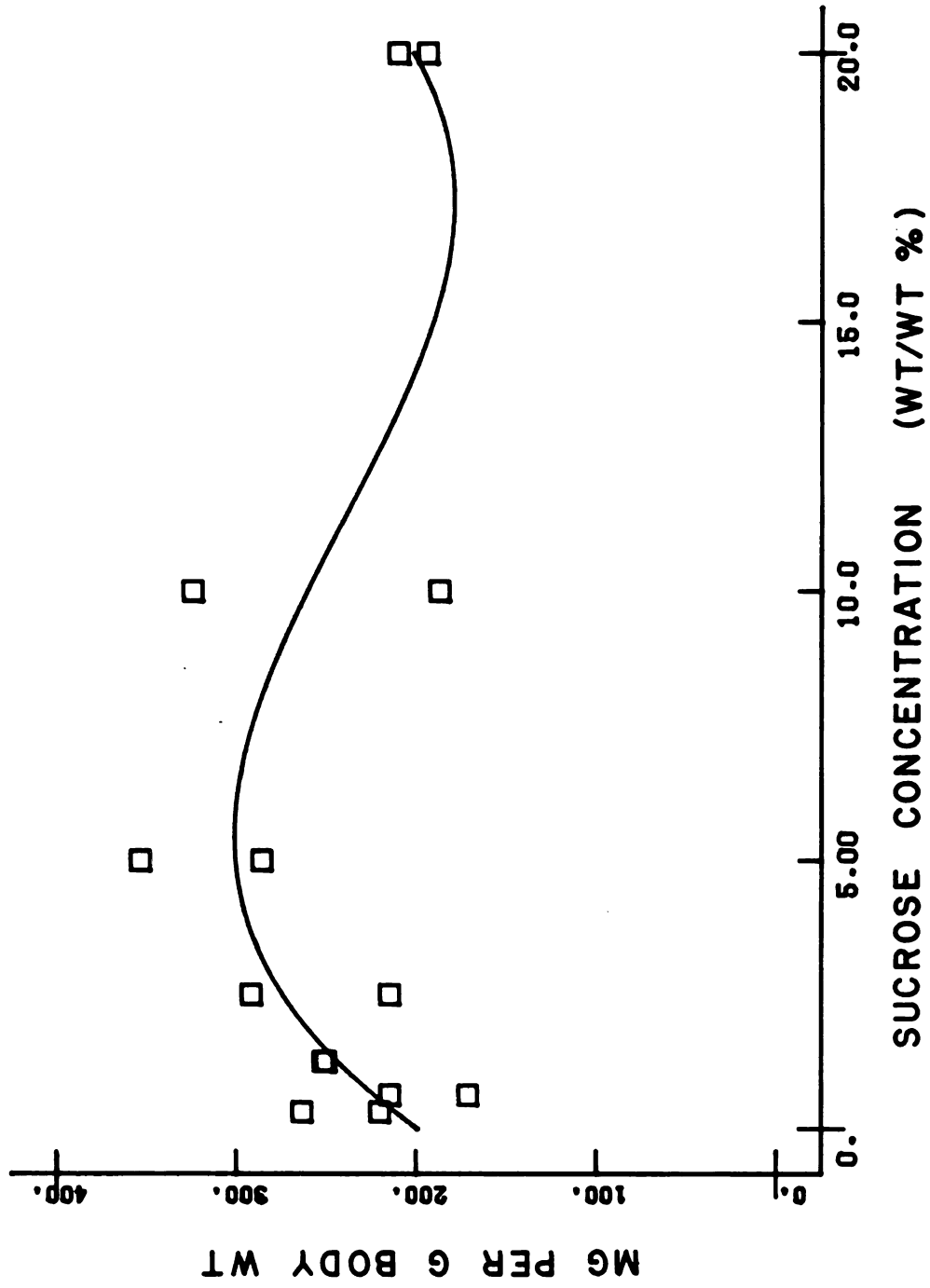


FIGURE 6. Prediction equation for total fluid consumption during sucrose preference test

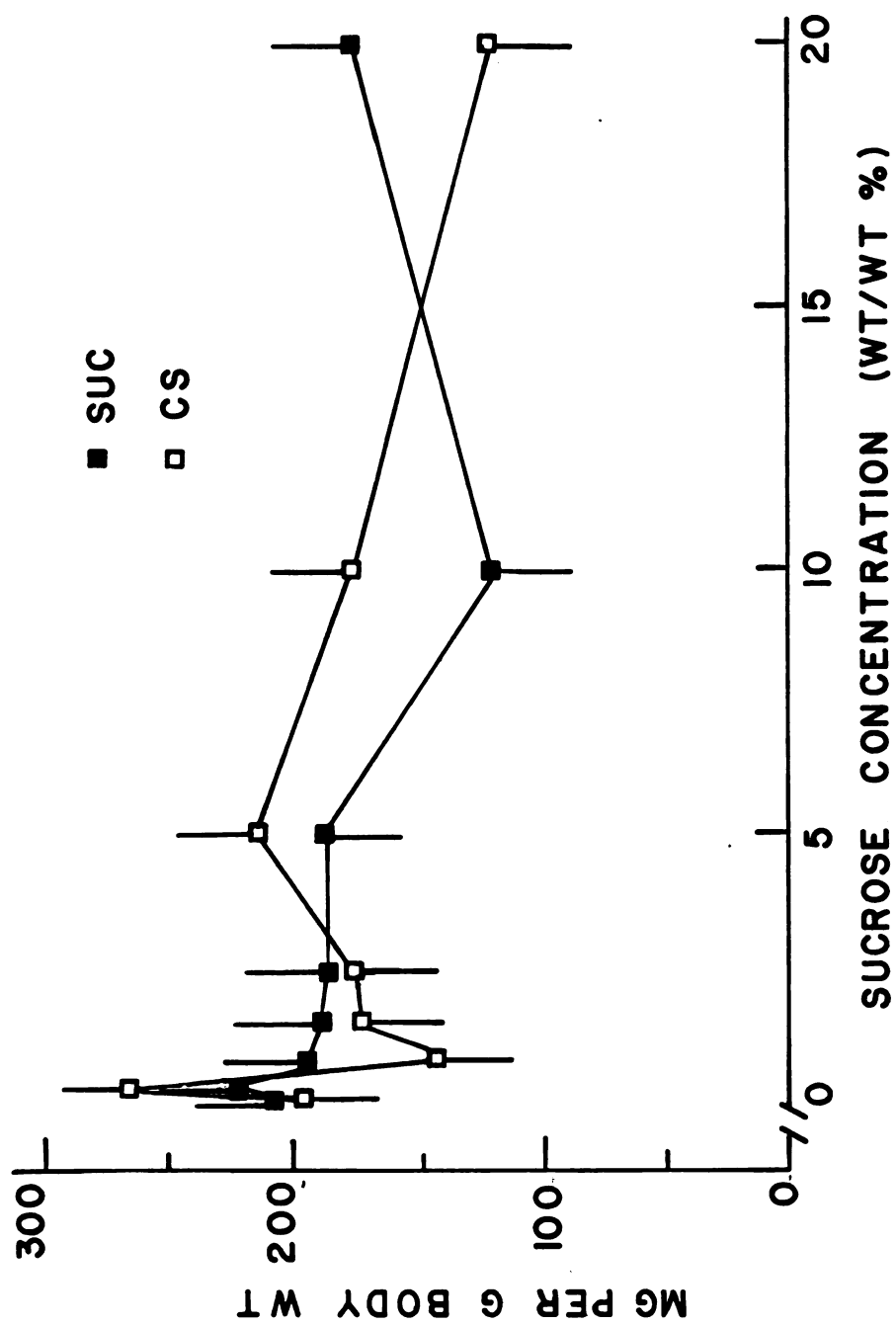


FIGURE 7. Food intake in CS and SUC offspring during preference test

regression. For CS offspring, $Y = -0.2360896 x^2 + 1.148407 x + 192.79118$. For SUC offspring, $Y = 0.47061978 x^2 - 11.659194 x + 209.71780$. Differences between the unstandardized coefficients of the two equations were tested using Student's t test (55). There were no significant differences between the two equations in any of the coefficient parameters (TABLE 7).

TABLE 7. Coefficient analysis for offspring food intake

<u>Coefficient</u>	<u>Student's t value^a</u>
B ₀	-0.683
B ₁	1.354
B ₂	-1.104

^aCritical value is ± 2.228 for $\alpha = 0.05$, degrees of freedom = 10 (57).

Data from the two groups were pooled and a prediction equation for food intake versus sucrose fluid concentration was determined by polynomial regression (112). The regression equation was found to be: $Y = 0.11726507 x^2 - 5.2553947 x + 201.25449$. The regression equation was entered into the PLOT/DRAW MSU STAT program (30) and plotted for visual inspection (FIGURE 8).

Offspring Solute Intake

Solute intake was derived from the amount of sucrose fluid consumed and the concentration of the fluid. Intake was expressed as mg sucrose consumed per gram body weight. In the mathematical adjustment of the treatment means, some negative values were derived which were meaningless (TABLE A2e). Polynomial regression analysis for solute

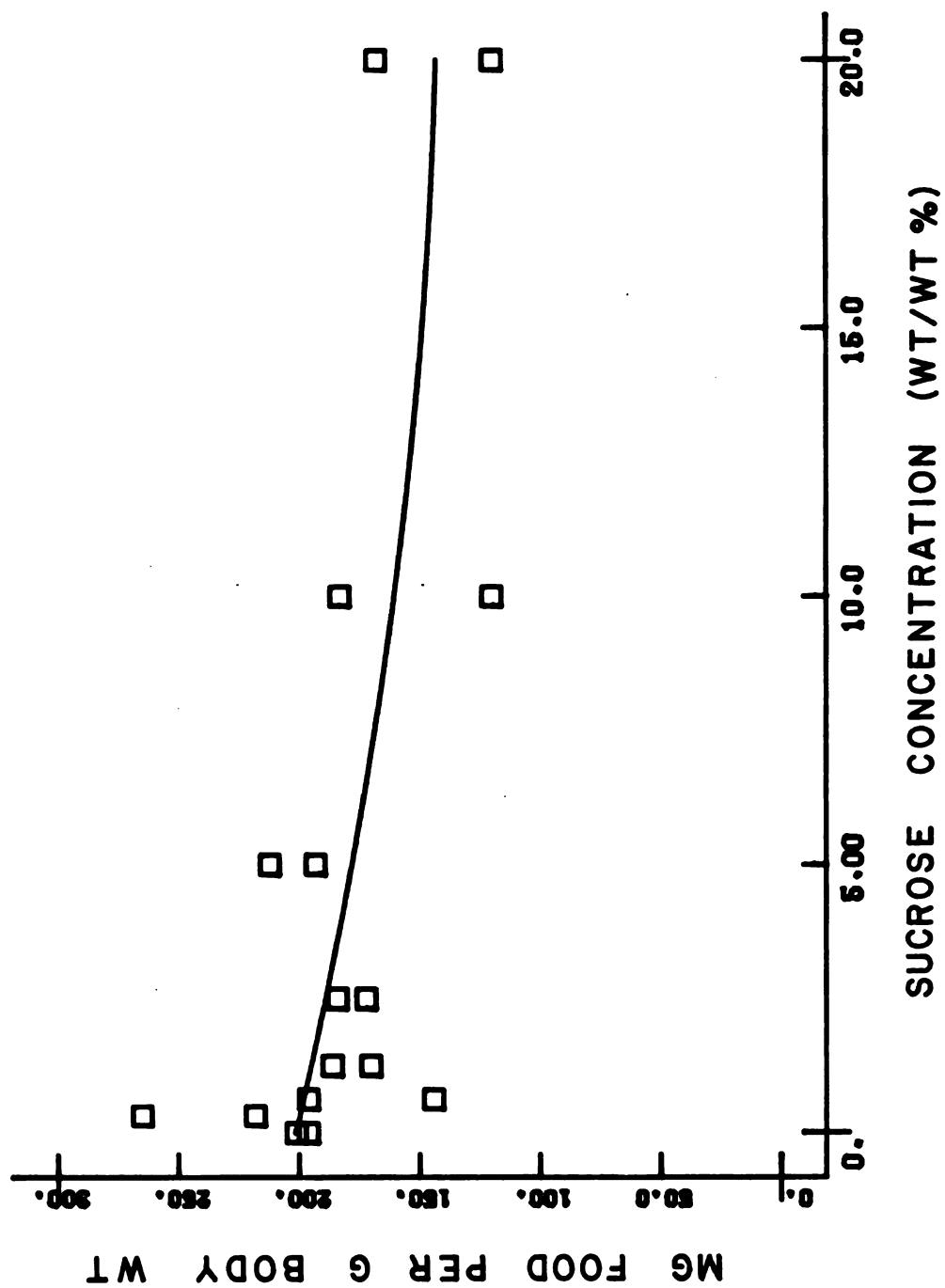


FIGURE 8. Prediction equation for offspring food intake during preference test

intake could not be interpreted with these negative treatment means; therefore, further calculations with the data were abandoned.

Solution A Residual Effects

Because of the possible residual effects of SOL A on both water and SOL B intake, the SOL A concentration was compared with the amount of water consumed on the interim test day when both tubes contained water. If there were residual effects of SOL A, there may be a correlation between the concentration of SOL A and the amount of water consumed during the interim test day. A Pearson correlation test for each diet group demonstrated that there was no significant correlation for either diet group ($r=0.2468$, $p>0.05$ for CS; $r=0.0437$, $p>0.05$ for SUC) (57, 112). Since there was no apparent effect of SOL A on subsequent water intake, it is presumed that the SOL A concentration did not confound SOL B consumption.

Maternal Food Intake

Maternal food intake was recorded prior to breeding and throughout gestation and lactation. Since there may be a maternal preference for the sucrose diet, a comparison of food intakes by CS and SUC dams was vital. Non-pregnant intake was determined for each female by averaging consumption for the seven days prior to conception. Intake during gestation was averaged from the 22 days prior to delivery and during lactation from the 23 days beginning with the delivery date. A split-plot, repeated measures analysis of variance was performed (TABLE A3) (56). There was a significant interaction between the diet groups and the stages of reproduction ($F(2, 28) = 11.2065$, $p<0.01$) (57).

There was also a significant difference in food intake among the stages of reproduction ($F(2, 28) = 187.64, p < 0.01$) (57). These results were expected since food intake dramatically increases during pregnancy and lactation. There was no significant difference between the two diet groups in this overall analysis of variance ($F(1, 14) = 3.894, p > 0.05$) (57).

Because of the significant interaction of diet groups with reproductive stages, three one-way analysis of variance tests (55) were performed for each of the three reproductive stages to determine if a particular stage elicited differences in food intake between the two diet groups (TABLE A4). The CS females ate significantly more food than the SUC females during the seven days prior to conception and during lactation ($F(1, 14) = 5.8577, p < 0.05$ and $F(1, 14) = 7.65, p < 0.05$, respectively) (57). There were no significant differences in food intake between the two diet groups during pregnancy ($F(1, 14) = 1.0, p > 0.05$) (57).

DISCUSSION

Present Study

Experiment I

The results of this experiment demonstrate that offspring from both cornstarch- and sucrose-fed mothers consume more of the sucrose diet in a choice test between cornstarch and sucrose diets. The offspring had no previous dietary experience with the two diets prior to preference testing. The assumption from these results is that the offspring chose the sucrose diet for its sweet taste. A test period of twenty minutes should have been short enough to measure primarily taste and perhaps some osmotic factors. Water was not provided in the testing apparatus which may have limited food intake but a clear preference was established. Other sensory factors may have entered into this preference, however. The diets differed in texture since the cornstarch was powdery and the sucrose granular. This variable should have been controlled for by utilizing powdered sucrose in the preparation of the sucrose diet.

The adaptation of the animals to the apparatus prior to testing and the six day test duration should have reduced any effects of neophobia in the offspring (73, 93). No evaluation of habituation was made such as recorded observations of animal location at the

conclusion of each adaptation session. It is not known whether the rats recognized food in the food containers during the two days of adaptation to the apparatus but the food intake recorded during the preference test was evidence that the offspring did associate the diets as foods. It was noted that the offspring ate progressively more food over the six days, probably because of growth as well as familiarization with the diets.

In contrast to the results of the present experiment, Galef and Henderson (48) showed that rat offspring preferred the diet consumed by their dams during lactation when offered a choice of the two diets at weaning. These results may have been due to other factors besides the carbohydrate sources in the diets, such as the level of carbohydrate, level and type of fat, dietary consistency, or some synergistic effect of many differences. Bronstein et al (12) demonstrated that there was maternal dietary preference by the offspring from dams consuming the same diets as employed by Galef and Henderson (48) but only during the first half hour of testing. When tested for longer than one half hour, the rat offspring consumed a greater amount of the presumably more palatable diet, which showed that the initial preference for the maternal diet was not fixed. The present study suggests that even in 20 minute tests, offspring do not demonstrate a preference for the maternal diet when dietary composition other than carbohydrate source is identical and one of the carbohydrate sources is thought to be more palatable than the other.

The results of the present experiment complement work by Wurtman and Wurtman (160) which was published during the course of this study. These investigators showed that experience with 0, 12, or 48 percent

sucrose diets by rats from 16 to 30 days of age did not affect the percentage of sucrose consumed from the choice of the three diets by the rats at 30 to 63 days of age. The conclusion of the authors was that the amount of sucrose consumed during early life is not a factor in determining the amount of sucrose consumed in later life. The present experiment suggests that sucrose or starch as carbohydrate sources in the maternal diet, at a fixed percent of total diet, are not factors in determining the initial selection between the two diets by the offspring; the sucrose diet was preferred to the cornstarch diet. Whether these results are due to a natural preference for the sucrose diet, some unknown aversion to the cornstarch diet, or the lack of flavor cues in the dam's milk is not determined by this experiment. The literature (3, 35, 74, 87, 117, 160) suggests that sweet preference is inherent, thus it is tempting to state that these results support the hypothesis that preference for sweet foods is unaffected by dietary experience. The hypothesis for this experiment was that maternal dietary experience may have served as preexposure gustatory stimulation for the offspring by transfer of dietary flavors into the maternal milk (48)

Though subtle compositional changes may have occurred in the dam's milk due to the carbohydrate source, there is no proof that such changes existed and contributed to milk flavor. Chemical analysis of the rat milk by thin layer chromatography was originally planned in order to determine whether sucrose was a component of the milk, which was alluded to in a study by Italian investigators (80). Translation of this Italian article revealed that sucrose was only found in certain commercial infant formulas, not human milk. In the present experiment,

analysis of the dam's milk for sucrose was delayed until the results of the behavioral experiments demonstrated whether preference differences warranted the expense and effort of the analyses. Without obvious behavioral sequelae in the offspring, it was decided that analysis of the milk would reveal no new information regarding the response of the offspring. In retrospect, it may have been advisable to determine the effect of sucrose versus starch consumption on milk composition without regard to behavioral outcome in the offspring. There is a possibility that high sucrose consumption by the dams may have overloaded the intestinal enzyme system so as to allow absorption of intact disaccharide which may have been expressed in the milk. Even though the present experiment does not suggest that such flavor cues existed, it is not known whether compositional differences were evident but had no behavioral effect.

Experiment II

The results of this experiment demonstrate that sucrose or starch as carbohydrate sources in the maternal diet have no effect upon the sucrose preference behavior of the offspring weaned to a starch diet. Church and Randall (27) have defined preference as the consumption of sucrose fluid greater than 60 percent of total fluid intake. Sucrose fluid intake from 40 to 60 percent of total fluid intake was defined as nondiscrimination and aversion as sucrose fluid consumption less than 40 percent of total fluid intake. With these definitions of preference and aversion, the pooled results of the present experiment showed that offspring from both diet groups preferred sucrose over water at 1.25, 2.5, 5, and 10 percent concentrations, with

nondiscrimination at 0.3125 and 20 percent concentrations and borderline preference/nondiscrimination at 0.625 percent concentration. There was no aversion for sucrose solution in this preference-aversion curve using these definitions (FIGURE 3). Higher sucrose concentrations may have elicited an aversion response.

Offspring in this experiment were weaned to the starch diet. Consumption of a diet other than the starch diet during preference testing may have produced different sucrose preference behavior. Sucrose preference testing of offspring weaned to a high fat diet may have resulted in a conflict in reward availability since high fat diets have been shown to be palatable to rats (151). Preference behavior for sucrose may change in comparison to the present experiment but there is no reason to believe that offspring response would be an effect of the carbohydrate source of the maternal diet. This hypothesis would have to be tested in future experiments.

The use of a modified 24-hour two-bottle preference test by incomplete block design was effective in determining the sucrose preference-aversion curve over a range of seven sucrose concentrations without the excessive duress of long term testing. Though the 24-hour method does measure post ingestional as well as taste factors, the results of this experiment indicate that neither factor in the offspring was affected by the dietary experience of the mother. The use of the short-term preference tests measuring only taste effects may have revealed differences between the offspring diet groups but it would still be expected that any differences should also have been apparent in the 24-hour test. Polynomial regression was a useful tool in mathematically defining the preference-aversion curve and

may be beneficial in quantitatively comparing results from different manipulations in future studies.

The incomplete block design of the experiment presented some limitations. It was necessary to adjust concentration means mathematically to control for the fact that every animal was not tested with every concentration. Adjusted treatment means represented six animals but raw data from the individual animals could not be analyzed by polynomial regression. The adjusted treatment means were utilized to determine differences between the offspring diet groups. Computation of standard errors of the mean for these data were laborious compared to complete block or factorial design methods. In the sophistication of the design, some information may have been lost because mathematical transformations presented three negative treatment means for offspring solute intake which were not interpretable (TABLE A2e).

There is also a possibility that statistically significant differences between the offspring groups would have been shown if another method of analysis had been employed. This idea might especially be postulated for the two curves obtained for total fluid intake. CS offspring fluid intake was represented by a cubic equation, SUC offspring by a linear equation. Though the shapes of the curves were different, the two regression equations did not differ in their coefficient parameters. Regression equations are more likely to represent the overall population response because of the incomplete block design and the quantitative nature of the treatments. For these reasons, Student's *t* tests between diet group responses for each concentration were not thought to be valid procedures for

these data.

Postdata analysis of offspring food and solute intake was undertaken to evaluate how these factors might be influenced by the sucrose concentration of the test solutions. Food intake was found to decrease slightly at higher sucrose concentrations. It was expected that there would be a concomitant rise in solute intake from the sucrose solutions in order to maintain energy intake. The inability to determine the regression equation for solute consumption as a factor of sucrose concentration prevented a meaningful interpretation of these parameters. Changes in energy requirements in the growing offspring may have precluded a constant regulatory effect which may explain the variability in the solute data. Subject variability may also have been a major factor in the incomplete block design analysis of solute intake. Food and solute intake and energy maintenance might best be studied in young animals in an incomplete block design, however. Perhaps larger group size would alleviate the intersubject variability in future investigations.

The major post ingestional effect of sucrose fluid consumption after 24 hours is thought to be related to osmotic pressure of the fluid (149). This effect may serve to diminish intake of sucrose solution at high concentrations and may affect fluid consumption in the subsequent 24 hours. Results of this experiment indicated that the post ingestional effects were not residual. Twenty-four hour water consumption was not correlated with the concentration of sucrose solution consumed in the previous 24 hours.

General Discussion

In the analysis of maternal diet consumption, significant group differences in food intake were recorded prior to conception and during lactation. The direction of the differences was opposite to the preferences of the offspring in Experiment I. Dams prior to conception and during lactation ate more food if they were fed the corn-starch diet rather than the sucrose diet. There was no difference in food consumption between the two groups of dams during gestation. The dams were not fed in a choice situation, however. Food consumption by dams eating the sucrose diet may have been depressed because of post ingestional osmolarity effects of the sucrose diet. Also, because of the three-hour meal-feeding regimen, the dams consuming sucrose diet may have exhibited "sensory specific satiety" (82) for the sweetness of the diet.

The amount of food consumed by the dams was qualitatively compared to anticipated ad libitum intakes based on literature values to determine whether meal-feeding significantly altered food consumption during gestation and lactation. Compared to ad libitum intakes of 10 to 15, 20, and 35 grams for non-pregnant, pregnant, and lactating female rats (109), the CS dams ate a mean of 14.2, 14.9, and 26.3 grams, respectively. SUC dams ate a mean of 12.4, 15.7, and 21.6 grams, respectively. Dams were weighed twice per week according to the day of the week and not the interval of gestation thus it was impossible to compare weight gain between the two groups of dams. Weight records based on day of gestation should be maintained in future experiments.

Fecundity, which may have been depressed because of

undernutrition (5), was not a problem since the 18 dams delivered 5 to 14 pups with an average litter size of 10. Mean litter size for Sprague-Dawley dams is 11 ± 2 (109); thus the litter size was within normal range. Offspring birth weights were not recorded. Weanling weights at 23 days of age ranged from 17.1 to 46.6 grams with a mean weight of 31.7 grams for 120 offspring tested in both experiments. Average weanling weights for Sprague-Dawley rats are 46 grams for males and 44 grams for females (109). The offspring in this study were underweight compared to the literature average weights and it is noted that two offspring died during Experiment I, possibly for this reason. Nevertheless, most of the offspring were healthy throughout the study and it is not expected that the meal-feeding pattern of the dams adversely affected offspring performance in these experiments. Future experiments utilizing the meal-feeding regimen may split the three-hour feeding block into several intervals with the possibility that dams may consume more food during the course of several small meals.

This study investigated the effect of only sucrose and cornstarch as exclusive carbohydrate sources in the maternal diet upon offspring preference. The carbohydrate sources also were studied at only one dietary level. Although it would not be expected that maternal consumption of diets containing glucose or fructose would affect offspring food preference, there may be different choices by offspring for glucose or fructose diets compared to the present study. It is not expected, however, that the preferences would be a result of maternal consumption of glucose or fructose diets. Mixtures of carbohydrate sources and at levels other than 65 percent in maternal diets may also contribute to differences in offspring food selection. For

these reasons, the carbohydrate component of the maternal diet cannot be ruled out as a possible factor in offspring preference behavior. In light of the statement by Capretta (23) that preferences for palatable foods are unaffected by nonaversive manipulations, it would not be expected that any changes in carbohydrate composition of the maternal diet would affect dietary choice of the offspring and the results of the present experiment support this hypothesis.

It is recognized that most other studies which have introduced detectable flavor changes in the maternal milk have failed to produce permanent alterations in offspring preference behavior (11, 12, 83). Rats were the experimental animal in these studies and in the present study; it is recognized that the gustatory maturation in the rat occurs primarily after birth (95). Significant changes in preference behavior as a function of maternal diet may occur as a result of in utero stimulation in species with gustatory system maturation prior to birth (8). Long gestational species may be better models of human preference acquisition.

Taste In Utero

Bradley and Mistretta have stated that birth is not the beginning of environmental influence, especially in long-gestational species. They have suggested that in utero experience may contribute to the neural development and maturation of the gustatory system in humans (8). Taste buds in the human appear in the seven-week-old fetus and are mature during the last six months of gestation (94). Mistretta has postulated that the chemical environment of the growing fetus may influence the gustatory system and play a role in

establishing later preferences (92).

Mistretta and Bradley have utilized the fetal sheep as a model for their studies (96). Taste buds appear at about one-third gestation in sheep and are morphologically mature before birth. Electrophysiological studies have shown that the taste buds are also functionally mature. Whether the sensory apparatus is behaviorally important to the fetus is not known. Fetal sheep and human fetuses are known to swallow amniotic fluid (94, 95). Amniotic fluid contains glucose, fructose, lactic and pyruvic acids, citric acid, fatty acids, phospholipids, creatinine, urea, uric acid, amino acids, polypeptides, proteins, and salts (95). The discontinuous swallowing and contact of amniotic fluid by the taste receptors in the fetus may be vital for normal sensory stimulation and development and may serve as a preexposure stimulus, perhaps modulating initial food preferences.

Sheep have been utilized in sucrose preference studies (27) but may be difficult to house in many laboratory animal care facilities. The guinea pig is born with mature sensorimotor function and has a fairly long gestation: 58 to 75 days (43). A future experiment utilizing the guinea pig in a design similar to the present study may demonstrate some effect of maternal dietary experience upon offspring preference. Behavioral changes in food and taste preferences may require stimulation at a developmental stage corresponding to gustatory neural development.

Preferences and Neuroendocrine Physiology

The present study has shown that weanling offspring choose a sucrose diet over a starch diet and prefer sucrose solution to water over a range of concentrations, irrespective of maternal dietary experience with sucrose. The apparent innateness of sucrose preference in a majority of animal species (114, 122) suggests that there may be a physiological mechanism for this ubiquitous behavior.

The instrumental performance by rats for a sweet reward resembles the behavior elicited by rats trained to bar press for an electrical stimulation of discrete brain areas, called intracranial self-stimulation (ICSS) (118). In fact, the lateral hypothalamus, classically considered to be the "feeding center", is also one of the sites for electrode placement in ICSS (161). Preference for a normally unpreferred coffee-flavored water by rats has been shown to increase following association with lateral hypothalamic ICSS (150). This phenomenon has been dubbed "conditioned taste preference" (39). Unlike "natural preferences", conditioned taste preferences rapidly extinguish without associative ICSS reward. Perhaps naturally preferred substances, such as sucrose, elicit a "natural" reward via the same system as ICSS which reinforces the palatable characteristics of the food substance. Such a physiological system, mediated by neural connections from the gustatory system to the limbic brain (115, 116) may explain the sweet desire throughout the animal kingdom.

Feeding, satiety, and pleasure "centers" as models are being replaced by neurohumoral system models. Wise (159) has proposed that the limbic pleasure centers described by Olds (118) are, in effect, dopaminergic systems. Dopamine is recognized as a possible mediator

of mood and affective state (60). Recent work (65) has shown that rats increase dopamine output while feeding. Further work may determine the role of dopamine metabolism in taste preference behavior. It may be worthwhile to monitor dopamine metabolism in animals consuming starch versus sucrose diets or after consumption of a preferred sucrose solution versus an aversive-tasting quinine solution.

Nance and Kilbey (107) have shown that the pharmacological depletion of serotonin by para-chlorophenylalanine (pCPA) increases sucrose preference in rats. This effect can be reversed by the administration of agents which return serotonin to control levels. Since serotonin is an inhibitory neurotransmitter, depletion may disinhibit other neural pathways to increase the hedonic response of sucrose as a stimulus. Depletion of serotonin may also trigger an overall carbohydrate preference so as to restore serotonin levels to normal via insulin-mediated diffusion of tryptophan into the brain (43, 160).

Other neuroendocrine systems may be functional in the determination of preference reward. Sucrose preference was elevated in animals tested in the dark phase compared to the light phase of the circadian cycle (68). The neophobia associated with a novel stimulus, in this case, saccharin solution, was attenuated with administration of melatonin (59). Hormonal changes may be responsible for the taste preferences associated with pregnancy (136) which may explain the food consumption patterns of the dams eating the sucrose diet in the present experiment. It may be interesting to monitor sucrose preference in pregnant rats to determine if there are differences between nonpregnant and pregnant animals.

With regard to preference alterations affecting the sucrose

preference-aversion curve, there is a need to standardize the definition of increased preference. Increased preference may be interpreted as preference for a higher concentration of sucrose solution or an increased volume of sucrose solution consumed for a given concentration. These results may be interpreted as changes in threshold for the perception of the sweet taste or changes in the reward value of the solutions. Comparison of studies would be more valuable if consistent definitions of increased or decreased preference are utilized.

Preference behavior is dependent upon initial rewards or displeasures associated with flavors, post ingestional consequences, and internal metabolic needs. The "sweet tooth" may represent a common mechanism to ensure food for the brain which has an obligate requirement for glucose (64). The present study is further evidence for the innateness of sweet preference, at least in neonatally immature species. The tenacity of sweet preference may present an obstacle to the public acceptance of the USDA-DHEW dietary guidelines (153) recommending reduction in sucrose intake. Since sweet foods are no longer a luxury item for humans, they may be chosen to the exclusion of other nutrient sources because of their sweet taste. If sucrose intake is to be reduced, the nature of sweet preference must be understood. Brain chemistry and anatomy must be the next targets of investigation to unravel the mysteries of food preference acquisition and the pleasures associated with palatable food consumption.

CONCLUSIONS

The present study found that sucrose preference in weanling rat offspring and choice between cornstarch and sucrose diets by the offspring were not a function of maternal consumption of cornstarch or sucrose diets. There also does not appear to be any effect of the maternal dietary experience upon offspring total fluid consumption or food intake during the sucrose preference test.

With the rat as a model, these results support the hypothesis that preference for a sweet-tasting diet over a non-sweet diet is an inherent behavior which is not influenced by sucrose or starch consumption by the mother during gestation and lactation.

APPENDIX

TABLE A1. Vitamin mix^a

<u>Ingredient</u>	<u>Amount Per Kg Diet</u>
D-calcium panthothenate	64 mg
Folic acid (pteroylglutamic acid)	40 mg
Niacinamide	120 mg
Pyridoxyl HCL	56 mg
Vitamin B-12 ^b	96 mg
D-biotin (crystalline)	2 mg
Menadione sodium bisulfate	400 ug
Riboflavin	20 mg
Thiamin HCL	10 mg
Vitamin A ^c	16,000 IU
Vitamin D ^d	8,000 IU

^aAll ingredients were purchased from U.S. Biochemical Corporation, Cleveland, OH.

^b0.1 percent trituration with mannitol.

^cAdded as premix made from 0.01 percent trituration of vitamin A powder, 20,000 IU per microgram, with dextrose.

^dAdded as premix made from 0.25 percent trituration of vitamin D₃ (activated 7-dehydrocholesterol) with dextrose.

TABLE A2a. Treatment mean adjustments, sucrose preference, CS offspring^a

Concentration (Sucrose wt/wt %)	(A) Unadjusted Mean (Sucrose fluid %)	(B) Sum Block Means (Sucrose fluid %)	(C) $r(A)-(B)$	Adjusted Mean (Sucrose fluid %)
0.3125	58	426.5	-78.5	42
0.625	55	348.0	-18.0	59
1.25	66	405.5	- 9.5	61
2.5	70	373.0	47.0	77
5.0	88	485.0	43.0	76
10.0	65	319.5	70.5	84
20.0	48	346.5	-58.5	47

^aAdjustment of the treatment (concentration) means controls for the block effects of the incomplete block design. Replication (r) is six for each mean. The adjusted mean is derived from $\bar{y} + (k/t\lambda)$ (C) where $\bar{y}$ is the overall unadjusted mean; k is the number of blocks (2); t is the number of treatments (concentrations, 7); λ is the number of times each treatment (concentration) is paired with every other treatment (1); (C) is derived from this table. $\bar{y} = 64$ percent for these offspring. The values for k , t , and λ are the same for all treatment adjustments in this experiment (56).

TABLE A2b. Treatment mean adjustments, sucrose preference, SUC offspring

Concentration (Sucrose wt/wt %)	(A) Unadjusted Mean (Sucrose fluid %)	(B) Sum Block Means (Sucrose fluid %)	(C) $r(A)-(B)$	Adjusted Mean ^a (Sucrose fluid %)
0.3125	57	393.0	-51.0	55
0.625	71	451.5	-25.5	63
1.25	78	456.0	12.0	73
2.5	83	445.0	53.0	85
5.0	84	436.5	67.5	89
10.0	75	410.0	40.0	81
20.0	38	330.0	-102.0	41

^a $\bar{Y}$ = 70.0 percent.

TABLE A2c. Treatment mean adjustments, total fluid intake

<u>CS Offspring</u>		(A) Unadjusted Mean ^a (mg/g bwt)	(B) Sum Block Means (mg/g bwt)	(C) r(A)-(B)	Adjusted Mean ^b (mg/g bwt)
Concentration (Sucrose wt/wt %)					
0.3125		283.3	1701.5	- 1.7	263.4
0.625		170.7	1349.0	-324.8	171.1
1.25		262.2	1621.5	- 48.3	250.1
2.5		265.3	1495.0	96.8	291.6
5.0		338.2	1716.0	313.2	353.4
10.0		319.7	1707.0	211.2	324.2
20.0		207.7	1492.0	-245.8	193.7
<u>SUC Offspring</u>					
0.3125		209.7	1279.5	- 21.3	220.1
0.625		235.5	1454.0	- 41.0	214.5
1.25		232.5	1306.0	89.0	251.6
2.5		212.3	1314.5	- 40.7	214.6
5.0		283.8	1494.5	208.3	285.7
10.0		211.7	1408.0	-137.8	186.8
20.0		197.8	1243.5	- 56.7	210.0

^aFor mean total fluid intake during sucrose preference testing.^b $\bar{y}$ =263.9 mg/g bwt, CS: 226.2 mg/g bwt, SUC.

TABLE A2d. Treatment mean adjustments, food intake

<u>CS Offspring</u>		(A)	(B)	(C)	
Concentration (Sucrose wt/wt %)	Unadjusted Mean ^a (mg/g bwt)	Sum Block Means (mg/g bwt)	r(A)-(B)	Adjusted Mean ^b (mg/g bwt)	
0.3125	236	1123.0	293.0	265	
0.625	146	1006.5	-130.5	144	
1.25	175	1088.5	- 38.5	170	
2.5	175	1082.0	- 32.0	172	
5.0	204	1116.0	108.0	212	
10.0	187	1114.0	8.0	183	
20.0	141	1058.0	-213.0	120	
<u>SUC Offspring</u>					
0.3125	207	1110.5	131.0	218	
0.625	201	1154.5	51.0	196	
1.25	176	1039.5	16.0	186	
2.5	181	1075.0	11.0	184	
5.0	186	1075.0	41.0	193	
10.0	147	1096.0	-214.0	120	
20.0	165	1035.5	- 45.0	168	

^aFor mean food intake during sucrose preference testing.^b $\bar{y}$ = 181.0 mg/g bwt, CS; 181.0 mg/g bwt, SUC.

TABLE A2e. Treatment mean adjustments, solute intake

CS Offspring		(A)	(B)	(C)	
Concentration (Sucrose wt/wt %)	Unadjusted Mean ^a (mg/g bwt)	Sum Block Means (mg/g bwt)	r(A)-(B)	Adjusted Mean ^b (mg/g bwt)	
0.3125	0.52	50.60	-47.48	- 4.23 ^c	
0.625	0.57	27.79	-24.37	2.38	
1.25	2.10	36.915	-24.315	2.39	
2.5	5.04	31.775	- 1.535	8.90	
5.0	15.18	82.515	8.565	11.79	
10.0	20.76	77.34	47.22	22.83	
20.0	21.25	85.565	41.935	21.32	
SUC Offspring					
0.3125	0.39	28.425	-26.085	- 0.10 ^c	
0.625	0.90	34.33	-28.93	- 0.91 ^c	
1.25	2.30	37.155	-23.355	0.69	
2.5	4.85	29.155	- 0.055	7.34	
5.0	12.10	52.165	20.435	13.20	
10.0	16.83	69.30	31.68	16.41	
20.0	14.19	58.810	26.33	14.88	

^aAmount of solute consumed expressed in terms of body weight (solute is sucrose)

^b $\bar{y}$ = 9.34 mg/g bwt, CS; 7.36 mg/g bwt SUC.

^cNegative adjusted treatment means which are interpretable

TABLE A3. Split-plot analysis of maternal food intake

Source	df	SS	MS	F	
Among <u>S</u>					
Trt ^a	1	45.825	45.825	3.894 ^b	
<u>S</u> /trt ^c	14	164.764	11.769		
Within <u>S</u>					
Stages ^d	2	1023.578	511.758	187.64 ^e	*
AB	2	61.133	30.566	11.206 ^f	*
Error b	28	76.370	2.727		

^aTreatments are the diet groups, CS and SUC

^bDerived from MS(trt)/MS(error a). Not significant.

^cError a.

^dStages of reproduction: non-pregnant, gestation, lactation.

^eDerived from MS(stages)/MS(error b). Significant $p < 0.01$.

^fDerived from MS(AB)/MS(error b). Significant $p < 0.01$ (56, 57).

TABLE A4. One-way ANOVA tests, maternal food intake

Non-pregnant stage

Source	df	SS	MS	F	
Trt ^a	1	13.5056	13.5056	5.8577	*
Error	14	32.2788	2.3056		

^aTreatments are the CS and SUC diet groups.

^bSignificant for $p < 0.05$ (1,14). Means: CS, 14.2 g; SUC, 12.4 g.

Gestation stage

Source	df	SS	MS	F	
Trt	1	2.25	2.25	1.0 ^c	
Error	14	42.02	3.00		

^aNot significant. Means: CS, 14.9 g; SUC, 15.7 g.

Lactation stage

Source	df	SS	MS	F	
Trt	1	91.2025	91.2025	7.65 ^d	*
Error	14	166.835	11.916		

^dSignificant for $p < 0.05$, (1, 14). Means: CS, 26.3 g; SUC, 21.6 g (55, 57).

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