# Flavor Chemistry of Animal Foods

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# FOREWORD

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# PREFACE

The vast accomplishments in human food technology during the past two decades have stimulated a more recent revolution in animal foods. As a result, higher quality, more palatable foods for domestic pets and food producing animals are being developed. As knowledge and expertise expand, they are being applied to new problem areas.

The most recent efforts have been with nondomestic animals. We are encroaching steadily on wildlife habitat to the extent that increasingly intensive management is required to prevent serious declines in animal populations. With our growing recreational needs for an expanding human population, more wildlife resources are sought, and greater emphasis is being placed on wildlife refuges, game preserves, and zoos. Consequently, there is an escalating need to improve or supplement the food supplies of many diverse species. However, it is difficult to improve the quantity or quality of the food supply without affecting the behavior or physiology of wildlife. The solution will come through an integrated effort involving several scientific disciplines.

In another area, we are finding better ways to protect our human food supply from vertebrate pests by using animal attractants and repellents in management methods. Attractants entice animals to encounter items used in animal damage control or items used in census techniques to determine the population of problem species. Repellents are applied directly to plants or grains to protect them from birds or mammals.

Flavor chemistry and food technology for animals is more complex than the mere adaptation of existing technology. Unlike humans, animals cannot appraise food or food additives directly. This problem requires an interdisciplinary research program that is structured far differently than those for human food research. For example, biologists and ecologists are often needed, and the behaviorist assumes a different and much expanded role.

This volume presents a broad coverage of the subject, both from the standpoint of animal species and of the various scientific disciplines involved. Many different domestic and nondomestic animals are discussed by specialists in organic and analytical chemistry, biochemistry, behavior, biology, nutrition, and physiology. Together they provide a clearer understanding of the interrelationships among these various disci-



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plines and how each contributes to the field of flavor chemistry of animal foods.

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# Progress in Animal Flavor Research

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For a terrestrial animal, flavor may be defined as the composite sensation resulting from placing something in the mouth. Therefore, flavor may include taste, olfactory, vomeronasal, trigeminal and other chemical sense inputs as well as tactile, temperature and proprioceptive cues. Thus, the subjective sensation we call flavor is the result of interactions of a complex of receptors. The bulk of experimental work in this field has focussed upon one class of receptors and associated CNS processes, the taste system. There are powerful arguments that the taste system is a uniquely important component in regulating flavor perception and food intake (e.g., 1,2), but other sensory components significantly influence flavor perception (e.g., 3, 4).

Humans regard flavors as qualities which add to the aesthetics of dining. Applied flavor researchers devote most of their efforts toward making foods and beverages more acceptable to humans or domestic animals. However, flavor sensations are not confined to domestic species' analyses of food substances. Examples of non-food-related flavor perception can be found in the responses of male hamsters to female conspecifics' vaginal secretions and the responses of guinea pigs to conspecific and congeneric urines. These secretions are both sniffed and licked (5, 6), and there is evidence for both olfactory and vomeronasal involvement in the case of the hamster (7). Guinea pig urine appears to include a complex of active substances (8) and codes a great deal of information including species, sex, diet of donor and individual identity (5,9,10). The inputs of several chemical sense systems and hence perception of the flavor of the urine may be required for the processing of all of the information present. As yet, however, there is no experimental evidence that the taste system is crucial in the processing of information contained in mammalian secretions and excretions. Instead, taste is apparently rather specifically concerned with food and liquid intake. Consequently, taste responses are emphasized in this paper in a comparative-evolutionary approach toward flavor and its relation to food selection by wild and domestic species.



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## Taste Stimuli and Responses to Them: Overview

The sensory reception and response systems of organisms, like their other features, are shaped by selection forces and are thus tuned to meet the pressures of the ancestral environment. Chemical detection systems are found in very primitive life forms including bacteria, protozoans and coelenterates (11, 12, 13, 14). These species respond to a variety of stimuli including some of those identified by humans as having one of the presumed (15) four basic tastes (sweet, salty, sour and bitter). It is striking that similar classes of substances are behaviorally active and that certain types of compounds elicit rather similar responses cross-phyletically.

Some compounds in the environment have had great influences upon survival of animal life since it first appeared. It is reasonable to surmise that receptor systems for the detection of these compounds would be widely distributed in the animal kingdom. To evaluate this possibility for compounds falling into the four classic taste qualities reported in the human literature, a listing has been made of substances reportedly yielding each of these qualities to humans (Table I). Of the naturally occurring chemicals, it can be seen that many sugars, some D-amino acids, as well as L-alanine and glycine are identified as sweet. Such substances are found in many foods. For example, free sugars (especially glucose, fructose and sucrose) are present in substantial amounts in many fruits and vegetables (20).

The list of salty tasting substances contains numerous organic and inorganic salts including some toxic substances (e.g., lithium chloride), but also some nutritionally important cations and anions. Most abundant of all of these compounds is sodium chloride which, in addition to its physiological importance, was also part of the medium (sea water) in which many phyla evolved. For marine and terrestrial animals alike, the most common salty compound is sodium chloride with the toxic ones much less common. The distribution of NaCl is more patchy for terrestrial than for marine animals, however (21). Since NaCl is an absolute requirement for complex organisms, it is not surprising that nearly all species tested respond to it.

Sour tasting substances are all acids (but not all acids taste sour) and are present in many potential food items, notably fruits. Acids, however, also push the organism toward physiological acidosis under experimental conditions and are not generally preferred to water at any detectable concentration. In the liquid medium in which most phyla evolved, the ability to detect a departure from normal environmental pH could adaptively trigger the avoidance of conditions with which many species are not physiologically able to cope. Nevertheless, there are a few species, the chicken for example (22), which tolerate and perhaps prefer solutions of high acidity.

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Table I. Partial listing of compounds tasting sweet, salty, sour or bitter to humans.

| Sweet | Salty | Sour | Bitter |
|---|---|---|---|
| Sucrose | Sodium chloride | Hydrochloric acid | Caffeine |
| Glucose | Lithium chloride | Nitric acid | Nicotine |
| Fructose | Ammonium chloride | Acetic acid | Quinine compounds |
| Raffinose | Potassium chloride | Butyric acid | Strychnine |
| Galactose | Magnesium chloride | Succinic acid | Other alkaloids |
| Lactose | Sodium fluoride | Lactic acid | Urea |
| Maltose | | Citric acid | Brucine |
| Xylose | | | Theobromine |
| Saccharin (Na) | | | Naringin |
| Cyclamate (Na) | | | Glucoside |
| L-aspartyl-L-phenylalanine | | | Coumarins |
| L-alanine | | | Terpene hydrocarbons |
| D-histidine | | | Terpenoids |
| D-phenylalanine | | | L-leucine |
| D-leucine | | | L-tryptophan |
| D-tyrosine | | | L-tyrosine |
| Glycine | | | L-phenylalanine |
| Beryllium chloride | | | Magnesium sulfate |
| Thaumatin | | | Denatorium benzoate (Bitrex) |
| Monellin | | | Sucrose octaacetate |

References - 16, 17, 18, 19.

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Many naturally occurring bitter tasting substances are toxic, including secondary compounds used as defenses by plants and animals against depredation. Such substances, when detected, are generally rejected by organisms. It is not clear in every instance that rejection of bitter substances is independent of their non-sensory effects, however (see later sections).

This brief listing of compounds for which humans report one of the four basic taste sensations supports the notion that these compounds have great ecological relevance for many species. This does not mean that each has equal relevance for all species, or that other stimuli do not have relevance for some species (c.f., 2, 23). Indeed, within one taxonomic group, the responses of individual species to various chemical stimuli may differ considerably. The degree to which these differences represent adaptations to the unique ecological conditions encountered by each species has not been thoroughly studied in mammals.

Taste and food preference studies have been conducted on a variety of species, for a variety of reasons and with a variety of procedures. The most frequently used design has been that of Richter (24) which compares the acceptance of a test solution with that of water for a 24-hour period. Modifications of this design include shortening or lengthening the period of exposure, changing the composition of the solvent and reversing the location of test and standard bottles in an attempt to control for position preferences. An advantage of long test durations may be that animals' responses over periods of 24, 48, 72 or 96 hours are not subject to brief whims of animals or to time-of-day effects. Short test proponents argue that these factors can be controlled somewhat by careful design and that the results of short-duration tests (10 min.- 6 hr.) are less likely to be confounded by post-ingestional feedback effects (e.g., 25).

Some studies have presented only one or two concentrations of a given stimulus. Others have tested whole series, but may have presented concentrations in ascending, descending or random orders, each of which may affect results differently (26). Common procedures have seldom been used by different authors studying different species. Thus, cross-species comparisons are difficult at best. The following discussion will center on three species for which data have been collected on a variety of stimuli and under several different exposure times.

## Taste Preferences: Comparisons Among Rats, Cats and Guinea Pigs

Summaries of the taste responses of laboratory rats (Rattus norvegicus), domestic cats (Felis cattus) and domestic guinea pigs (Cavia porcellus) appear in Table II. These forms represent three distinct feeding types: herbivore (guinea pig), carnivore (cat) and omnivore (rat). They are also important research animals; thus there is need to provide them with acceptable diets. Finally,

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data are also available on taste responses of wild relatives of each of these species, providing the opportunity to draw a few cautious inferences concerning the evolution and lability of taste preferences.

Sweet Substances. Rats (Table II) show preferences for many substances tasting sweet to humans. (Hereafter, such responses will be called "sweet preferences" for the sake of brevity). Cavies show preference for the only sugar (glucose) with which they have been tested but do not prefer it at as high concentrations as do rats. Cats, on the other hand, either are indifferent to or reject sugars under most conditions of testing (c.f., discussion in 29). This indifference to sucrose and other sugars makes the cat (along with some other species, 2, 41) an exception to the generality of sweet preferences among mammals (42) and is in accord with electrophysiological studies which report finding very few fibers sensitive to sucrose in cats (43,44,45). We may conclude, therefore, that a sweet taste is, for all practical purposes, inert as an appetitive cue for domestic cats, whereas rats and guinea pigs can use this cue in food selection.

The responses to sweets by the domestic forms are very similar to those of their wild relatives. Maller (46, 47) found very similar acceptance percentages (percent of total fluid intake that is test solution in solution vs. water choice test) of single concentrations of a variety of sugars by wild and laboratory Norway rats (Rattus norvegicus). Laboratory rats increased fluid consumption greatly during these tests whereas the wild type did not. Shumake, et al (48) found wild and laboratory rats to be very similar in acceptance of solid diets treated with sucrose in a mechanized design in which consumption differences appear to have been "controlled out." Therefore, the domestication, or more properly laboratorization, of Rattus has not altered the sweet preferences of this species but has altered consumption, a related response.

Wild cavies showed higher acceptance and a broader preference range for glucose than did their domestic counterparts (33). The domestic cavies' acceptances became like the responses of the wild type (which remained consistent) upon retesting (34). Between-type consumption differences also were found, particularly during the animals' initial exposures to glucose. However, unlike the situation with rats, it was the wild cavies who showed the greater increases in consumption of sweet solution. These differences between types also diminished with repeated testing. These and other data indicate that the wild cavy orients more quickly to solution cues than does the domestic form but fail to provide any evidence for sensory differences between them (34).

Beauchamp, et al (29) have recently tested taste responses of twelve wild cats of Genus Panthera (lions, P. leo; tigers, P. tigris; leopards, P. pardus; jaguars, P. onca) in short-term preference tests. The results were not substantially different from

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Table II. Summary of responses of three familiar mammals to some natural sweet, salty, sour and bitter stimuli in solution vs. water choice tests.

| | Laboratory Rat | Domestic Cat | Domestic Guinea Pig |
|---|---|---|---|
| Sweet | | | |
| Sucrose | Pref(.006*-1.6M)[26] Pref(.011-.9M) Rej(>1.6M)[27] | Indif[28,29]@ | |
| Glucose | Pref(.055-1.6M)[26] Pref(.055-1.4M)[31] | Indif[29] | Pref(.2M only)[32,33] Pref(.1-.4M†)[34] |
| Fructose | | Indif[29] | |
| Maltose | Pref(.22-1.39M†)[27] Pref(.006*-1M†)[26] | | |
| Galactose | Pref(.0067-.61M) Rej(>1.05M)[31] | Rej(.60M†) | |
| Salty | | | |
| Sodium chloride | Pref(.008*-.05M†)[35] Pref(.0092-.052M)[24] Rej(.10*-.15M†)[36] | Pref(.1M) Rej(.5M)[28] Indif(.015*-.12M†)[37] Rej(≥.5M)[28] | Pref(.008-.25M)[34] Rej(≥.5M)[34] |
| Sour | | | |
| Citric acid | | Rej(.06*-.48M†)[37] | Rej(≥.031M)[34] |

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Table II. (continued)

| | Laboratory Rat | Domestic Cat | Domestic Guinea Pig |
|---|---|---|---|
| Bitter | | | |
| Quinine sulfate | Rej(.000008M*†)[35] | Rej(≥.00025M*)[37] | Indif(.00016*-.00124M†)[34] |
| Quinine hydrochloride | Rej(≥.000021M)[38] | Rej(≥.000005M*)[28] | Rej(≥.002M)[40] |
| | Rej(≥.000275M)[39] | | |

NOTES:

1) Pref = Preference
2) Indif = Indifference
3) Rej = Rejection
4) Preference, Indifference and Rejection were determined either from statistical tests presented in the cited references or by visual evaluation of tables and figures if statistical data were lacking. Assignment of terms in the latter case was based upon our experiences with conducting this sort of testing.
5) Exposure times varied between studies cited.

* Indicates that these concentrations were the lowest used in the study cited.

† Highest concentrations used.

@ A preference for .25-.375M sucrose dissolved in .03M NaCl has been reported for cats.[30] A subsequent study failed to find such a preference.[29]

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those with domestic cats: Panthera preferred no sweet substances.

Salty Substances. All three domestic species show a preference for at least one hypotonic (<.15 M) concentration of sodium chloride (Table II). Rats' preferences cease at concentrations below isotonia while guinea pigs' extended into mild hypertonia on a second series of exposures (34). Wild cavies preferred .008-.25 M NaCl to water from the first series onward. That cavies were tested for 4-hr. periods on two consecutive days while rats were given 24- or 48-hr. continuous exposures may account for cavies' higher acceptance of more concentrated saline solutions. Wild and laboratory rats' responses to salt-treated diets were also similar (48).

Since free-ranging terrestrial herbivores tend to have chronic salt deficiency problems (49), a preference for salinity approaching isotonia even in the absence of a deficiency might be adaptive. Rabbits (28) and squirrel monkeys (36) appear to have such preferences. However, some herbivores have rejected isotonic and even mildly hypotonic saline solutions in laboratory tests (North American porcupine - 50 ; sheep, "normal" goats, cattle - 51, 52). The question of salt preferences in herbivores needs careful research including control of sodium content in the maintenance diet to identify the role of the sodium hunger (21, 24) in influencing these results. An orientation toward salt in the absence of deficiency may also be adaptive for omnivores in selecting their diets from a broad range of potential nutrients. Cats preferred only one concentration of NaCl in Carpenter's (28) study and none in that by Beauchamp and Maller (37). These carnivores may generally receive sufficient sodium from the meat they eat and thus may not show any sodium appetite nor manifest strong hedonic preferences for NaCl solutions in the laboratory.

Sour Substances. Data on the acceptance of sour solutions are sparse. Where they are found, very few preferences for acids have been reported. Acids are found in many fruits; yet there are no reported sour preferences among frugivores (a poorly studied herbivorous subset), and the armadillo rejected .001 N formic acid (53) which is found in high concentrations in its natural diet, ants. Wild and domestic cavies preferred no concentration of citric acid, but did not reject any until concentrations of .062 M and .031 M, respectively, were reached. Cats also rejected .06 M citric acid, the lowest concentration tested (37). This concentration has a very strong taste to humans. The addition of citric acid to diets lowered acceptance by both wild and hooded rats (48).

Bitter Substances. Quinine compounds are rejected at very low concentrations by cats, at higher concentrations by rats and are tolerated to a considerable extent by guinea pigs. Thus, of the species under discussion, the one most likely to encounter

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bitter tastes in its dietary pursuits, the guinea pig, is the one least likely to reject them. This relative tolerance of the bitter substance is not the result of domestication since wild cavies are equally inclined to accept quinine (sulfate - 34). It may be, however, that bitter substances occur in so many plants, including many not toxic to cavies, that the bitter taste is a poor discriminative cue as to the toxicity of an item. Additionally, if bitter tastes are tied anatomically into a primitive innate rejection mechanism, cavies may have, in the course of evolution, broadened their menus by tuning down the sensitivity of the system (see below). Interestingly, rabbits (ecological analogues of cavies - 54), are also rather tolerant of quinine (hydrochloride - 28). Other herbivores (meadow voles - 55, porcupines - 50, assorted farm ruminants - 56, 57) are less tolerant of QHCl than cavies and rabbits.

Synthetic Taste Compounds. Of the several synthetic sweeteners available, sodium saccharin has been used in taste preference studies with the three species which are our chief concern. A synthetic bitter substance, sucrose octaacetate, has also been offered to these three forms. Both compounds are relatively inert nutritionally and toxicologically, thus responses to them may be relatively independent of non-sensory factors which frequently confound responses to natural compounds.

Rats', cats' and cavies' responses to saccharin (Table III), unlike those of some other forms (2), indicate that they generally treat it as they treat sugars listed in Table II: rats and cavies prefer it at some concentrations; cats show indifference changing to rejection at higher concentrations. Table III also shows that

Table III. Responses of three familiar mammals to two synthetic tastants, sodium saccharin (sweet) and sucrose octaacetate (bitter) in two-bottle, solution vs. solvent choice tests.

| Species | Na Saccharin | Sucrose Octaacetate |
|---|---|---|
| Laboratory Rat | Pref(.0003-.01M†)[58]<br>Pref(.01*-.044M†)females[59]<br>Pref(.01*-.015M)males<br>Rej(≥.031M)males | Rej(≥.000001M*)[60] |
| Domestic Cat | Rej(≥.02M)[28] | Indif(.0000625*-.0005M†)[37] |
| Guinea Pig | Pref(.002-.016M)[34] | Rej(≥.0005M)[3]<br>Rej(.001M†)[40] |

NOTE: Abbreviations, symbols and procedures for assigning terms are identical to those for Table II.

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rats and domestic cavies respond to SOA similarly to the way they respond to quinines; rats reject SOA at low concentrations while cavies are more tolerant of it. Cats, on the other hand, are indifferent to SOA. Other species respond still differently to SOA. Mice, depending upon strain and individual, may reject, prefer, or be indifferent to SOA (61, 62) with genetic factors apparently responsible for rejection and indifference. Another pattern of response to SOA is that of mild rejection followed by significantly increased acceptance upon continued exposure. This pattern has been found in wild cavies (34)and cattle (63). A comparison of SOA and quinine responses within species suggests that different kinds of "bitterness" may exist for nonhuman animals.

It is clear from the foregoing that cats with their indifference to sweets and low rejection thresholds for bitter substances and cavies, with avidity for sweet and sodium chloride, and tolerance of bitter, have vastly different responses in simple taste tests. These two types are differently equipped for sampling food substances and select different diets in nature. More data are needed on other carnivores and herbivores to determine whether these responses are characteristic of feeding type. The data already reviewed indicate that some herbivores respond differently to saline and quinine solutions than do cavies, and it may be that the term "herbivore" is too inclusive to reveal adaptive relationships between taste responses and different strategies of herbivory (e.g., rumination vs. non-rumination, effects of body size on ability to be a selective eater). The taste profile of the rat is uniquely similar to that of man (2), who is not only another omnivore, but is inadvertantly the chief provider of food for the rat.

Cat, rat and cavy data clearly indicate that taste preferences have been modified during phylogeny. In contrast, available data suggest that these responses have changed little during domestication in comparison to other behaviors (e.g., 64, 65). One possible explanation for this conservatism is that although animals must eat to survive at every stage of both natural evolution and domestication, natural selection operates on large populations (with a wide range of individual variation) while the initial stages of domestication efforts have probably involved small numbers of individuals. Present-day wild species represent the successes of evolution: the offspring of individuals (perhaps small subsets of the total population) that were able to adapt to changing food supplies. Since the fate of each animal is more important during early domestication, it is suggested that man has been forced to cater to the preferences of his captive animals and thus has not selected for greatly altered taste response systems.

A problem in interpreting taste preferences' implications in food selection is that animals do not eat "pure" tastes as sources of nutrition. Recalling the taste responses of cats (Tables II and III), it is noted that they appeared to prefer one NaCl concentration and rejected or were indifferent to virtually

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every other stimulus listed. Recent experiments (e.g., 4, 29, 66) have demonstrated that cats prefer solutions of protein, certain amino acids and fat emulsions. In short, cats prefer what one might expect a carnivore to desire and require. The extent to which these substances are stimulating the taste system as opposed to other sense inputs is not definitely known. White and Boudreau (66) provide evidence for taste involvement while Mugford (4) has demonstrated importance for olfaction in food selection by cats. Our observations of rats and cavies suggest that these species also use olfactory inputs to modulate their acceptance of foods.

## Modification of Flavor Preferences by Individual Experience

Thus far, preferences, aversions and indifferences have been treated as though they were species traits with only a suggestion that experiential factors might be involved in acceptances of either "pure" taste stimuli or of foods. However, the role experience plays in determining the acceptability of a flavor is a major question for flavor research and one that has profound practical implications.

Young (67) has pointed out that the concept of experience is ambiguous. When animals are maintained on one diet, physiological adaptations to that diet may occur which make change to a new diet difficult. This is not the same as learning (in the usual sense) to prefer or avoid a food as the result of previous experience. In the following, the major concern will be with learning rather than with physiological adaptations to familiar diets.

Difficulty in introducing new foods to mature animals is a common problem. Many experimental studies have demonstrated extreme diet neophobia in rats and other species. However, for many species, palatable and nutritious new foods are gradually taken in quantity (cf., 21, 68). The rat's ability to select an adequate diet from an assortment of nutrients is well documented (e.g., 69, 70). If a rat is deficient in a nutrient, foods newly available will be sampled in such a way that the source supplying the needed substance is usually identified (71). Rozin (21, 71) explains the sampling and rapid shift in preference as deriving from an aversion to the old food formed through an association of adverse physiological consequences with its ingestion rather than from a learned association between the new food and positive nutritional effects. The rat merely selects a food or food combination which does not produce a negative physiological condition. The flavors of the foods serve as markers for what the rat has learned about them.

Specific hungers (except for sodium hunger, 21, 72) are thus viewed as arising from a conditioned food aversion process, that is, the pairing of characteristics of food (usually taste or flavor for mammals) with illness (73). The discovery of conditioned food aversions has been an important breakthrough for the understanding of diet selection by wild animals, for providing

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new strategies in reducing animal damage to crops and range animals (68), and in leading to a greater understanding of the role of the chemical senses in the behavior and evolution of animals. The medicine effect (73), the association of a flavor with physiological well-being, has been much more difficult to demonstrate experimentally (74, 75) than conditioned aversions. Data are still insufficient, however, for the conclusion that medicine effects cannot have practical consequences in food selection.

The experimental evidence indicating that previous experiences are of strong significance in determining preferences among safe foods (those not producing illness or deficiencies) is not overwhelming (76). Studies of the influence of early experience with foods upon later preferences have been stimulated by research on imprinting, the development of young-parent attachments in precocial birds and perhaps in other species as well (77). Imprinted young form and attachment to one stimulus and subsequently prefer it to other stimuli. Studies of some non-mammalian species (e.g., snapping turtles, 78; chickens, 79; blowflies, 80) have indicated that foods experienced early in life are preferred over those experienced later. Hess(79) called this phenomenon "food imprinting." Subsequent work (e.g., 81) has demonstrated that these preferences are not as irreversible as filial imprinting can be (77).

A number of studies using tastes, odors and whole foods have examined the role of early experience in determining subsequent preferences in mammals. Warren and Pfaffmann (40) treated the nipples of mother guinea pigs with sucrose octaacetate and found a decreased aversion to .001M. SOA (which is avoided by domestic cavies, Table III) as a result. When the animals were retested 3-4 months later, they avoided the higher SOA concentrations as completely as did the controls. In another guinea pig study (82), groups of neonates were fed either of two types of green vegetables daily for the first 3 weeks of life. The animals were then offered choices between the familiar vegetable and a novel one. The familiar vegetable was the only one consumed for the first 3-5 days, but novel and familiar greens were consumed equally by day 7. Drickamer (83) found that early experiences (days 21-35) with artificially odorized diets resulted in significant preferences by two species of mice (Peromyscus maniculatus bairdi and P. leucopus) for the familiar odorized diet when these animals were tested one day after the cessation of selective experience. Thirty days later, only P. m. bairdi subjects exhibited this preference and no evidence was presented to indicate how well these preferences would have held up under repeated testing.

In three studies, one case (40) involves temporary depression of an apparent innate rejection as the result of repeated exposures to the non-toxic tastant and two cases (82,83) involve the effects of experience and thus familiarity being confounded with a potential neophobic response. These findings are compatible with the learned safety of foods hypothesis discussed above. They show no evidence that a lasting preference for a particular taste or

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flavor was formed by these animals on the basis of early experience (cf. also 84).

Yet early experience clearly does play an important role in determining initial food choices in some species and these shaping mechanisms may have clear survival value. In a series of important experiments, Galef and associates (85, 86, 87, 88, 89, 90) not only demonstrated ways in which young rats may acquire their food preferences but also provided explanatory mechanisms for the pest control operator's suspicion that more rats avoid baits than actually sample them (cf. 91). Galef found that young rats come to prefer foods through cues passed to them via their mothers' milk and/or by following older rats to food sources. Older rats' selection of foods would tend to be passed on to the young. Since the young do not actually learn to avoid the bait (cf. 86), it is likely that some of them would eventually sample it. Those not killed would form conditioned aversions to the bait and live to lead other rats to alternate food sources.

In contrast to these findings concerning early experience with one food, Capretta et al (92) found that early exposure of laboratory rats to a variety of flavors induced a greater likelihood in these rats to accept a novel flavor subsequently. It thus appears that laboratory rats' (rather weak) neophobic tendencies can be overcome by repeated exposure to both novel and safe flavors. Long-term variety in diet (and consequently in flavors as well) has also led to increases in absolute intake in both laboratory rats (93) and cats (4).

Mugford (4) has also noted observations by some (e.g. 94) that in some circumstances flavor preferences are not fixed on familiar foods and instead novel foods are taken preferentially. Working with food preferences of cats and dogs, Mugford (4) found that after 16 weeks of post-weaning feeding on one diet, both species tended to prefer a novel diet in a choice test. If the novel diet was a less palatable one (as determined in preference tests with naive animals), this neophilia was transient. These data and others discussed by Mugford suggest the phenomenon to be a short-term adaptation effect of immediately previous intake experiences.

## Interaction of Inherent Preferences and Effects of Experience

The studies reviewed above indicate that experiences can have short-term effects upon food preferences and long-term effects upon food rejection. They do not, however, provide support for presumptions that early exposure to excessive sweets, for example, will create a sensory addiction in an organism. Instead, the sweet preferences shown by many species including humans appear to be present at birth (95). Likewise, sodium chloride detection and the sodium appetite and the rejections of sour and bitter stimuli appear to be built-in, adaptive response tendencies which derive from sensory hedonics (96). Conditioned food aversions, though seemingly representing a rather

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primitive type of learning (21) may be a later addition phylogenetically as well as ontogenetically. Hedonic responses and conditioned aversions may turn out to be the determinants of food selection with response modulations caused by the organism's neophobic or neophilic tendencies.

Studies reviewed indicate that preferences for familiar over novel diets were transient when nutritional and toxicological differences were not present. The flavors of the foods exert paramount influence on selection. When one diet presents toxicological problems or is nutritionally deficient, however, conditioned aversions may reverse preferences originally based upon innate hedonics. For example, Naim et al (97) presented rats with a choice between a saccharin-flavored, poorly nutritious diet and a sucrose octaacetate-flavored, nutritious diet. As might have been expected (Table III), the rats originally preferred the saccharin-flavored diet but preferences gradually changed so that after 7 days of continuous choice, the SOA-flavored diet was preferred. When the experiment was repeated using quinine sulfate as the bitter stimulus, preferences did not change. Quinine sulfate may be "more intensely bitter" to rats, but the discrepant results can be explained in another way. Naim et al (97) have suggested that the pharmacological properties of quinine, especially the reduction of gastric secretion levels, might reduce the nutritional benefit derived from the formerly adequate diet and thus render it aversive.

Sensory information is crucial to the food selection process whether hedonic factors are operating alone or under modification due to experience. When stimulus complexes are presented, as in the feeding situation, certain aspects are more conspicuous (salient) to the animal than are others. The relative salience of the components of a given complex may vary from species to species. Taste has been shown to be a more potent stimulus than color for the formation of conditioned aversions by guinea pigs (98, 99), but the opposite may be true for birds (100). Salience of stimuli can also vary within one sensory modality. In reviewing the taste responses of three common mammals (Table II), it was noted that cats were indifferent to glucose and responded to very low concentrations of quinines while the converse was true for cavies. The two types might be expected to respond quite differently to mixtures of sweet and bitter tasting substances: cavies with high acceptance, cats with rejection (note responses to saccharin in Table III).

Cavies' and cats' respective indifferences to bitter and sweet stimuli could arise from difficulty in detecting the concentrations not responded to or from psychological indifference to detected stimuli. If the former were the case, those stimuli would have little salience in affecting food choices while in the latter possibility, psychological importance could be easily acquired. The first alternative is more likely. The observation that cats continue to consume sucrose after becoming sick from it (28, 30)

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suggests that they cannot detect it. Similarly, conditioned aversion experiments with wild cavies indicate that they have difficulty in detecting SOA. When cavies were made ill by lithium chloride injections after drinking .00025M SOA (clearly identified as bitter by humans) consumption upon a second exposure to the SOA solution was reduced by only 26%. When .008M saccharin was used as the conditioned stimulus, consumption was reduced 99% with only one subject drinking a measurable quantity (101).

Since animals' selection of foods is based upon their evaluation of sensory information, it is not surprising that the salience of stimuli for different species has been modified during evolution. For taste in mammals, the basic ancestral response tendencies seem to have been modified mainly by altering the sensitivities of existing systems rather than by evolving new ones. It has been suggested here that systems which are perhaps irrelevant (sweet in cats) or uninformative (bitter in cavies) are reduced in sensitivity during phylogeny. It is also hypothesized that the sensitivities of response systems for other stimuli in these and other species have been enhanced during evolution.

In evaluating the roles of inherent preferences, experience, neophobia and neophilia in food selection, it is important to remember the challenges presented to free-ranging species. Unlike animals kept under laboratory conditions, most wild species eat a variety of foods. The availability and abundance of foods undergo seasonal fluctuations. Animals must, therefore, shift foods to survive. This process involves sampling new items and responding to the sensory and physiological effects of their ingestion. Aversions acquired in previous years may hasten avoidance of toxic substances subsequently. The animals themselves also undergo seasonal and/or cyclical hormonal changes which, in some instances, have been shown to alter taste responses (e.g., 102, 103, 104) and food selection (2). For external and internal reasons, animals continually exercise their food selection faculties.

## Conclusions

This paper has stressed factors involved in the exhibition of preferences and aversions by animals to potential nutrients. Perhaps the biggest advance in animal flavor research over the past forty years has been the identification and experimental isolation of these factors: inherent preferences, conditioned aversions, neophobia and neophilia. While each of these factors has been shown to affect choices, the manner in which they interact for a given species must be determined experimentally in each case. The identification of these factors, however, provides the tools needed to answer applied questions concerning the feeding of pets, livestock and zoo animals and the control of animal damage. In doing such research, the subjects' phylogenetic and ontogenetic histories must always be considered.

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ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

## LITERATURE CITED

1. Hankins, W.G., Rusiniak, K.W. and Garcia, J., Behavioral Biology (1976) 18, 345-358.
2. Kare, M.R. and Beauchamp, G.K., In: Swenson, M.J. (ed.) "Dukes' Physiology of Domestic Animals," 713-730, Cornell University Press, Ithaca, N.Y., 1977.
3. Mozell, M., Smith, B., Smith, P., Sullivan, R. and Swender, P., Archives of Otolaryngology (1969) 90, 367-373.
4. Mugford, R.A., In: Kare, M.R. and Maller, O. (eds.) "Chemical Senses and Nutrition, Vol. II," Academic Press, New York, in press.
5. Beauchamp, G.K., Physiology and Behavior (1973) 10, 589-594.
6. Johnston, R.E. and Zahorik, D.M., Science (1975) 189, 893-894.
7. Powers, J.B. and Winans, S.S., Science (1975) 187, 961-963.
8. Beruter, J., Beauchamp, G.K. and Muetterties, E.L., Biochemical and Biophysical Research Communications (1973) 53, 264-271.
9. Beauchamp, G.K., Nature (1976) 263, 587-588.
10. Beauchamp, G.K., unpublished data.
11. Adler, J., In: Carlile, M.J. (ed.), "Primitive Sensory and Communication Systems," 91-100, Academic Press, London, 1975.
12. Beidler, L.M., In: Denton, D.A. and Coughlan, J.P. (eds.), "Olfaction and Taste: 5th International Symposium," 71-76, Academic Press, New York, 1975.
13. Garcia, J. and Hankins, W.G., In: Denton, D.A. and Coughlan, J.P. (eds.) "Olfaction and Taste: 5th International Symposium," 39-45, Academic Press, New York, 1975.
14. Hodgson, E.S., In: Kare, M.R. and Maller, O. (eds.) "The Chemical Senses and Nutrition," 7-18, Johns Hopkins Press, Baltimore, 1967.
15. McBurney, D., Chemical Senses and Flavors (1974) 1, 17-28.
16. Brand, J.G., Naim, M. and Kare, M.R., In: Rechcigl, M. (ed.) "Handbook Series in Nutrition and Food," CRC Press, Cleveland, in press.
17. Furia, T.E. , In: Furia, T.E. and Bellanca, N. (eds.) "Fenaroli's Handbook of Flavor Ingredients, Vol. I.," 8-11, CRC Press, Cleveland, 1975.
18. Moskowitz, H., In: Sipple, H.L. and McNutt, K.W. (eds.), "Sugars in Nutrition," 37-64, Academic Press, New York, 1974.
19. Solms, J., Journal of Agricultural and Food Chemistry (1969) 17, 686-688.
20. Shallenberger, R.S., In: Sipple, H.L. and McNutt, K.W. (eds.), "Sugars in Nutrition," 67-80, Academic Press, New York, 1974.
21. Rozin, P., In: Hinde, R.A., Shaw, E. and Beer, C. (eds.) "Advances in the Study of Behavior, Vol. 6," 21-75 Academic Press, New York, 1976.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

22. Fuerst, W.F., Jr. and Kare, M.R., Poultry Science (1962) 41, 71-77.
23. Kare, M.R., In: Kare, M.R. and Halpern, B.P. (eds.) "Physiological and Behavioral Aspects of Taste," 6-15, University of Chicago Press, Chicago, 1961.
24. Richter, C.P., Endocrinology (1939) 24, 367-371.
25. Cagan, R.H. and Maller, O., Journal of Comparative and Physiological Psychology (1974) 87, 47-55.
26. Kare, M.R. and Ficken, M.S., In: Zotterman, Y. (ed.) "Olfaction and Taste," 285-297, Pergamon Press, Oxford, 1963.
27. Richter, C.P. and Campbell, K.H., American Journal of Physiology (1940) 128, 291-297.
28. Carpenter, J.A., Journal of Comparative and Physiological Psychology (1956) 49, 139-144.
29. Beauchamp, G.K., Maller, O. and Rogers, J.G., Journal of Comparative and Physiological Psychology, in press.
30. Bartoshuk, L.M., Harned, M.A. and Parks, L.H., Science (1971) 171, 699-701.
31. Richter, C.P. and Campbell, K.H., Journal of Nutrition (1940) 20, 31-46.
32. Bauer, F.S., Physiology and Behavior (1971) 6, 75-76.
33. Jacobs, W.W. and Beauchamp, G.K., Physiology and Behavior (1977) 18, 491-493.
34. Jacobs, W.W., in preparation.
35. Bernard, R.A., Halpern, B.P. and Kare, M.R., Proceedings of the Society of Experimental Biology (N.Y.) (1961) 108, 784-786.
36. Harriman, A.E., American Midlands Naturalist (1968) 79, 396-401.
37. Beauchamp, G.K. and Maller, O., unpublished data.
38. Benjamin, R.M., Journal of Comparative and Physiological Psychology (1955) 48, 119-122.
39. Young, P.T., Burright, R.G. and Tromater, L.J., American Journal of Psychology (1963) 76, 205-217.
40. Warren, R. and Pfaffmann, C., Journal of Comparative and Physiological Psychology (1959) 52, 263-266.
41. Kare, M.R., In: Beidler, L.M. (ed.) "Handbook of Sensory Physiology: Chemical Senses Part 2: Taste," 277-292, Springer-Verlag, Berlin, 1971.
42. Pfaffmann, C. American Scientist (1964) 52, 187-206.
43. Boudreau, J.C., Bradley, B.E., Bierer, P.R., Kruger, S. and Tsuchitani, C., Experimental Brain Research (1971) 13, 461-488.
44. Cohen, M.J., Hagiwara, S. and Zotterman, Y., Acta Physiologica Scandinavia (1955) 33, 316-332.
45. Pfaffmann, C., Journal of Neurophysiology (1955) 18, 429-440.
46. Maller, O., In: Kare, M.R. and Maller, O. (eds.) "The Chemical Senses and Nutrition," 201-212, Johns Hopkins Press, Baltimore, 1967.
47. Maller, O. and Kare, M.R., Proceedings of the Society for

Experimental Biology and Medicine (1965) 119, 199-203.
48. Shumake, S.A., Thompson, R.D. and Caudill, C.J., Journal of Comparative and Physiological Psychology (1971) 77, 489-494.
49. Abraham, S.F., Blaine, E.H., Denton, D.A., McKinley, M.J., Nelson, J.F., Shulkes, A., Weisinger, R.S. and Whipp, G.T., In: Denton, D.A. and Coughlan, J.P. (eds.), "Olfaction and Taste: 5th International Symposium," 253-260, Academic Press, New York, 1975.
50. Bloom, J.C., Rogers, J.G. and Maller, O., Physiology and Behavior (1973) 11, 95-98.
51. Goatcher, W.D. and Church, D.C., Journal of Animal Science (1970) 30, 777-783.
52. Goatcher, W.D. and Church, D.C., Journal of Animal Science (1970) 30, 784-790.
53. Maller, O. and Kare, M.R., Animal Behavior (1967) 15, 8-10.
54. Jacobs, W.W., "Social Behavior of the Domestic Guinea Pig: The Male-Female Association." Unpublished doctoral dissertation, University of Chicago, 1973.
55. Laughlin, M.E., Donovick, P.J. and Burright, R.G., Physiology and Behavior (1975) 15, 185-189.
56. Goatcher, W.D. and Church, D.C., Journal of Animal Science (1970) 31, 364-372.
57. Goatcher, W.D. and Church, D.C., Journal of Animal Science (1970) 31, 373-382.
58. Fisher, G.L., Pfaffmann, C. and Brown, E., Science (1965) 150, 506-507.
59. Valenstein, E.S., Kakolewski, J.W. and Cox, V.C., Science (1967) 156, 942-943.
60. Cote, J. and Brand, J.G., unpublished data.
61. Warren, R.P., Science (1963) 140, 808-809.
62. Warren, R.P. and Lewis, R.C., Nature (1970) 227, 77-78.
63. Chalupa, W., personal communication, 1977.
64. Hale, E.B., In: Hafez, E.S.E. (ed.), "The Behavior of Domestic Animals," Williams and Wilkins Co., Baltiomore, 1962.
65. Boice, R., Psychological Bulletin (1973) 80, 215-230.
66. White, T.D. and Boudreau, J.C., Physiological Psychology (1975) 3, 405-410.
67. Young, P.T., Psychological Review (1968) 75, 222-241.
68. Rogers, J.G., this symposium.
69. Harris, L.J., Clay, J., Hargreaves, F. and Ward, A., Proceedings of the Royal Society, Series B (1933) 113, 161-190.
70. Richter, C.P., Harvey Lecture Series (1942-1943) 38, 63-103.
71. Rozin, P., Journal of Comparative and Physiological Psychology (1969) 69, 126-132.
72. Richter, C.P., In: Grasse, P.P. (ed.) "L'Instinct dans le Comportement des Animaux et de l'Homme," Masson et Cie, Paris, 1956
73. Garcia, J., Hankins, W. and Rusiniak, K., Science (1974) 185, 824-831.

74. Revusky, S.H., Psychonomic Science (1967) 7, 109-110.
75. Revusky, S.H., Journal of Comparative and Physiological Psychology (1968) 66, 777-779.
76. Beauchamp, G.K. and Maller, O., In: Kare, M.R. and Maller, O. (eds.) "Chemical Senses and Nutrition, Vol. II," Academic Press, New York, in press.
77. Hess, E.H., "Imprinting," Van Nostrand Reinhold Co., New York, 1973.
78. Burghardt, G. and Hess, E.H., Science (1966) 151, 108-109.
79. Hess, E.H., Science (1964) 146, 1128-1139.
80. Dethier, V.G. and Goldrich, N., Science (1971) 173, 242-244.
81. Fuchs, J.L. and Burghardt, G.M., Learning and Motivation (1971) 2, 271-279.
82. Beauchamp, G.K., unpublished data.
83. Drickamer, L.C., Behaviour (1972) 41, 269-287.
84. Bronstein, P.M. and Crockett, D.P., Behavioral Biology (1976) 18, 387-392.
85. Galef, B.G., Journal of Comparative and Physiological Psychology (1971) 75, 358-362.
86. Galef, B.G. and Clark, M.M., Journal of Comparative and Physiological Psychology (1971) 75, 341-357.
87. Galef, B.G. and Clark, M.M., Psychonomic Science (1971) 25, 15-16.
88. Galef, B.G. and Clark, M.M., Journal of Comparative and Physiological Psychology (1972) 78, 220-225.
89. Galef, B.G. and Henderson, P.W., Journal of Comparative and Physiological Psychology (1972) 78, 213-219.
90. Galef, B.G. and Sherry, D.F., Journal of Comparative and Physiological Psychology (1973) 83, 374-378.
91. Steiniger, F. von, Zeitschrift fur Tierpsychologie (1950) 7, 356-379.
92. Capretta, P.J., Petersick, J.T. and Stewart, D.J., Nature (1975) 254, 689-691.
93. Sclafani, A., In: Novin, D., Wyrwicka, W. and Bray, G.A. (eds.) "Hunger: Basic Mechanisms and Clinical Implications," 281-295, Raven Press, New York, 1975.
94. Morrison, G.R., Journal of Comparative and Physiological Psychology (1974) 86, 56-61.
95. Desor, J.A., Maller, O. and Turner, R.E., Journal of Comparative and Physiological Psychology (1973) 84, 496-501.
96. Young, P.T., Psychological Review (1966) 73, 59-86.
97. Naim, M.. Kare, M.R. and Ingle, D.E., Journal of Nutrition, in press.
98. Braveman, N.S., Learning and Motivation (1974) 5, 182-194.
99. Braveman, N.S., Behavioral Biology (1975) 14, 189-199.
100. Wilcoxon, H.C., Dragoin, W.B. and Kral, P.A., Science (1971) 171, 826-828.
101. Jacobs, W.W. unpublished data.
102. Michell, A.R., Physiology ad Behavior (1975) 14, 223-226.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

103. Wade, G.N. and Zucker, I., Journal of Comparative and Physiological Psychology (1969) 69, 291-300.
104. Zucker, I., Physiology and Behavior (1969) 4, 595-602.

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# 2

# Food Preference Behavior in Birds and Mammals

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The higher vertebrates survive in the wild through elaborate and eloquent systems of food searching, prey finding, sampling, and selection activities (1, 2). Some omnivorous species have a tremendous adaptive advantage in meeting their energy and nutritional requirements. For example, as one primary food source declines, the omnivores tend to sample new food sources or previously less palatable foods in order to survive (3, 4).

Many migratory bird species including the African weaver finch or quelea (5) are able to survive and proliferate in spite of seasonal changes in the abundance of seeds and grains. The behavior and physiology of migratory birds appear to be directly correlated with environmental changes. For example, the quelea lay down fat stores before migrating from dry season areas (6). These birds also migrate with the rainy season to obtain enough food for themselves (seeds and grains) and for their young (green plant material and raw protein from termites and other insects). Certain herbivores such as antelope, mountain sheep, and elk are able to maintain themselves throughout the winter seasons of low food availability by moving to lower elevations (7) or by changing their microflora and microfauna to allow digestion of normally less nutritious plants such as locoweed or sagebrush.

These observations point out the physiological and behavioral adaptiveness of wild birds and mammals and their ability to adopt new food habits. Partially for this reason, it is highly unlikely that any commercially available flavoring agents or flavor enhancers that have been developed for humans will have strong and durable flavor preference effects in wild species (8, 9). Instead, most wild animals appear to be adapted or conditioned to associate certain food odors, tastes, and flavors with beneficial effects (i.e., more energy, less nervousness, weight gain, and general health). Other flavors become associated with injurious sublethal poisoning (10) effects such as vomiting, diarrhea, or unconsciousness.

Still, there are certain innate flavor preferences that exhibit themselves widely among the omnivores. For example, man,



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rodents, hogs, and most other mammals (11, 12) all show a preference for glucose, sucrose, and even non-nutritive saccharin that is independent of nutritional wisdom or experience. Bitter substances including quinine, sucrose octaacetate, and cyanide are avoided by birds (13), coyotes (14), rodents (15), and herbivores (16). For most species this rejection behavior may be regarded as unlearned. The vast majority of food items consumed by omnivores contain extremely complex mixtures of flavors, and experience with the food is probably the single most important factor controlling preference behavior.

Pure flavor sensation and animal preference for it are extremely difficult to measure. P. T. Young's (17) approach to this problem of measuring hedonic (pleasurable) or aversive (repellent) taste stimuli has been widely adopted and proposed as a standard method for evaluating taste preference in rats (18). Unfortunately, very few natural flavors have only sensory content. Past associations with flavors can be just as, if not more, important than their sensory content (3, 19). Birds and mammals do not simply sample a new food item and then either continue or discontinue feeding on the basis of sensory (hedonic) content alone. Typically, the flavors or chemical components of the food items initially act as signals for either nutritional or toxic effects. Later, palatability may actually be changed (20). In this respect, the chemical senses are similar to the visual or auditory senses. For example, if gerbils are reared in their natural tunnel systems, they tend to quickly flee and hide from moving visual stimuli. If they are reared, like most laboratory rodents, in wire mesh cages with no opportunity to construct burrow systems, the gerbils show very little avoidance of the same stimuli (21). There are exceptions--if, for example, an animal is hormonally primed and presented with a "super normal" stimulus, the sheer intensity may be enough to evoke a genetically fixed response (22).

Regardless of the wild species to be considered or the food flavor under study, the chemist involved in animal food flavor research and development should be aware of the numerous factors that ultimately influence and determine food preference behavior of birds and mammals.

This review will not be an attempt to report all that is known about food preference behavior of birds and mammals. Such information could be of limited benefit to the chemist working on animal food flavor problems, but, as I will point out to the reader several times, each bird or mammal in a specific habitat is a somewhat unique entity. Laboratory data do not necessarily generalize to field situations and field experiments are often difficult if not impossible to duplicate in the laboratory. Instead of an exhaustive review, I have elected to cover some important factors that can often influence flavor preference behavior. Next, a brief review is given of attempts to modify preference behavior by the empirical screening approach. Finally, an example of one approach to the problem of intensifying rice flavor to increase

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rodent bait consumption is covered in some detail. A number of suggestions in regard to future animal food flavor research are then covered in the conclusions section.

## Factors Influencing Food Preference Behavior

Nutritional Factors. Birds and mammals are efficient at food regulation. Even man, whose flavor preference behavior is largely determined by social, economic, and cultural patterns (23) tends to generally increase fat intake in cold environments, protein content in temperate zones, and carbohydrate content in tropical zones. Much of this food selection behavior, however, is distorted by food availability whether it be related to socioeconomic status or food crops prevalently grown in specific climates.

From the standpoint of animal health, nutritional quality of foods ingested have a direct bearing on survival of mammals. By definition, the flavor qualities of animal foods tend to take on meaning and can lead to nutritional wisdom. As P. T. Young (24) and M. Kare (25) have often pointed out--the chemoreceptors stand as guards to the alimentary canal. But, just how efficient are mammals and birds at achieving nutritional balance?

The taste or flavor of certain essential nutrients such as glucose and sodium are immediately sought when a need arises in mammals. Morrison and Young (26) and Nachman (27) believe there is an automatic increase in the palatability for salts or sugars as they become deficient in the diet. Some data (28) indicate that there may be glucoreceptors in the liver that send neural information back to feeding and appetite centers in the brain. Maller et al. (29) have proposed a direct pathway to the brain from the oral-pharyngeal area of rats that regulates intake of certain nutrients such as NaCl and glucose. The exact nature of this pathway and its projection area into the lower brain centers have not yet been described. Pilcher and Jarman (30) in attempting to investigate this original finding, believe that the hepatic system is more important than the direct pathway hypothesis. Contreras & Hatton (31) have cited some evidence that the sodium appetite and palatability change toward salty tasting foods or liquids in albino rats may be controlled by the K/Na ratio in their blood. Morrison and Young (26) found that rats made sodium deficient by formalin injection drank equal amounts of either 0.3 M NaCl or .03 M $Na_2CO_3$ solution even though NaCl contains five times more sodium per unit volume. Again, they conclude from these data that sodium depletion produces palatability changes and that the amount of "salty" tasting solution drunk signals satiety rather than sodium repletion. Thus, for some nutrients, such as salts or sugars, palatability changes automatically regulate nutritional balance in rats.

For thiamine (Vitamin $B_1$) deficiency, Rozin (32, 33) cites rather convincing evidence that the flavor of the diet leading to vitamin $B_1$ sufficiency is associatively learned by rats. Food

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odors may play a dominant role in vitamin enriched dietary selection (3) or glucose injection (34). This same general finding is implicated in the early reports concerning specific appetite for essential vitamins (35, 36). For example, the vitamin deficient rats did not cue in on the "flavor" of vitamin B. Rather, they selected a new diet with a distinctive (new) flavor when the old flavored diet led to continued deficiency symptoms. Diets containing calcium appear to affect rats in a similar manner (27). Although no specific appetites for vitamins A and D have been demonstrated (27), Bernard et al. (37) have shown that vitamin A deficiency leads to decreased taste sensitivity. Apparently, vitamin A is essential for normal taste sensitivity of the mammalian taste buds (38) and both quinine rejection and NaCl selection are retarded in vitamin A deficient rats.

There appear to be several differences and some similarities between taste preference responses of birds when compared to mammals in nutritional deficiency preference tests. For example, Hughes and Wood-Gush (39) were unable to demonstrate any evidence of specific appetite for sodium in chickens fed either a sodium deficient diet or given formalin injections. Young chicks were particularly susceptible to sodium chloride toxicity. In rats, specific appetite for sodium is readily established (40, 41, 42), probably because rats are more capable of sodium excretion than most birds, except for, perhaps certain marine species that possess specialized salt-secreting glands (43). Hughes and Wood-Gush (39) were able to easily demonstrate a specific appetite for thiamine in chickens similar to that found in rats (32). Thus, dietary deficiencies can sometimes greatly influence preference test results in both birds and mammals.

Physiological Influences. Animals ingest foods primarily to satisfy energy requirements, metabolic needs, and for reproductive function. In short, food flavors become associated with, or are correlated with, adequate diet under given environmental conditions. Animals that do not select adequate diets do not survive and reproduce. Thus, foods that correct physiological deficiencies or need states are reinforced and food habits are then established (27). For example, the effects of infused nutrients into the stomachs of rats have a marked influence on feeding behavior. Adair, Miller, and Booth (44) found that amino acids influsion produced an overall decreased food consumption by albino rats. D-glucose alone, however, only specifically suppressed consumption of D-glucose solutions and not overall consumption. Thus, the willingness of an animal to eat any available food cannot be simply explained by a blood glucose-homeostasis mechanism (i.e., the glucostatic theory).

Mixtures of certain high calorie nutritive and non-nutritive sweeteners in water solution can lead to excessive drinking by laboratory rats (45). When offered a mixture of 3% glucose and 0.125 or 0.250% saccharin in distilled water, the rats consumed

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fluid amounts approximately equal to their body weights every 24 hours. This excessive drinking (polydipsia) was explained as a synergistic action of the mixture that led to a highly palatable sweet taste without an associated high caloric content, while the slightly bitter taste of saccharin was masked by glucose.

Maller (46) has indicated that wild Norway rats (Rattus norvegicus) are calorically selective in their food habits. Thus, palatability factors could play a lesser role in food selection by wild rodents as compared with laboratory rats. I have found, however, that wild Norway rats exhibit polydipsia when offered the glucose and saccharin mixture. A group of six wild Norway rats drank an average of 362 ± 21 ml of fluid per day. Six wild rice-field rats (Rattus rattus mindanensis), on the other hand, did not show such extreme polydipsic behavior, drinking on the average 78 ± 8 ml of the fluid mixture per day. The exact nature of this species difference was not clear from tests with various two-choice combinations of water, saccharin solution, glucose solution, and the mixture. Apparently, both glucose and saccharin and their interaction at either the receptor or caloric regulating level, contribute to the species difference.

As indicated above, wild rats do not always select calorically balanced diets, especially under laboratory conditions. In attempting to further understand caloric regulating and feeding systems of rats, Piquard et al. (47) have found that a 25% daily calorie glucose infusion into the rat's circulatory system via intracardiac catheter produced an 80% decrease in intake of glucose-treated food. Amino acid and lipid infusion produced decreases in intake of proteins, lipids, and glucids as well. Booth and Campbell (48) found that insulin infusion into the circulatory system of rats produced increased food consumption but they could not show that infusions of fatty acids (the breakdown product of fats) had any effects on the food intake of albino rats. Campbell and Davis (28) have shown that both duodenal and portal glucose influsions will reduce licking rates for glucose solutions. Thus, at least for glucose, sweet taste palatability and subsequent caloric regulation may be controlled to some degree by chemoreceptors in the liver.

Other physiological variables such as circadium rhythms (49), estrus cycle (50, 51), sexual hormones (52), and insulin level (53) can have influences on odor detection and taste preference or perception in rats. A detailed review of these effects is beyond the scope of this report. However, flavor researchers dealing with improving the food acceptance of livestock, domestic pets, or wild animals should be aware that these variables can affect flavor preference test results. Postingestional factors such as level of circulating blood glucose (54), bulk or fiber content of the food (7), and sublethal illness effects (33) also often play some role in preference test results especially when the animals are offered continuous access or long-term access (>1-2 hrs) to the test food or foods.

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Genetic Factors and the Effects of Domestication. Domestication of the wild Norway rat has led to certain subtle changes in flavor preference behavior and the reactions of different rat strains to new foods. Mitchell, Beatty, and Cox (55) found that two different wild Norway rat populations produced $F_1$ progeny that differed in their initial acceptance of a new food in a new feeder. Norway rat $F_1$ progeny from a pig farm also showed a higher incidence of poison-elicited pica after cyclophosphamide injection than did the progeny from Norway rats captured on a coastal island. These differences in feeding behavior could be the product of selection pressure because rats in farming areas would be much more likely to contact rodenticides and traps than rats living on an isolated island. Water, as a partial physical barrier, could have also led to more inbreeding among the island rat population. In any event, the results tend to reinforce the notion that longitudinal studies are needed to evaluate domestication effects.

Brief-exposure preference tests as described by Young and Kappauf (56) have not demonstrated genetic changes in taste preference behavior of Long-Evans hooded versus laboratory bred-and-reared wild Norway rats (57). Thus, food familiarity and rearing conditions may be, in some instances, more important determiners of taste preference behavior and food acceptance than the more subtle genetic effects. Jackson (58), for example, reported that roof rats in central Florida feed extensively on oranges. Other Southern roof rats do not show much acceptance of citrus as a food item. In the Pacific islands, roof rats readily take coconut on some islands and ignore this source of food on other islands.

There is, however, little doubt that genetic influences can play a role in determining taste preference behavior. For example, mice of different species show different preference responses to 10% glucose versus 10% fructose (59), different conditioned aversion responses to various sugars (60), and different saccharin preference behavior (61).

Wenzel (62) cautions researchers that domesticated and laboratory animals (albino rats, pigeons, and chickens) may be much less sensitive to taste and odor stimuli than are wild mammals and birds. For example, domestic chickens are able to compensate for reduced caloric content in their diet by drinking larger quantities of 10% sucrose in water solution. Normally, however, chickens are indifferent to sucrose. Kare and Maller (63) found, on the other hand, that Red Jungle fowl tend to prefer sucrose solutions at all times and appear to be superior to the domestic chicken in caloric regulation. Likewise, there is some evidence (46) that caloric regulation in wild rodents is usually superior to that demonstrated by laboratory rats.

In terms of animal food flavor development as an applied endeavor, these variables of domestication and genetic effects do not generally require separate analyses. The reported data do

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imply, however, that the flavor research bioassay should incorporate the target species, and should duplicate certain aspects of the natural ecosystem of the target mammal or bird.

Behavioral and Ecological Influences. Much of the ecological literature related to food habits, preferences, and aversions of mammals deals with complex predator-prey relationships. Pearson (64) observed a cyclical relationship between microtine rodent population levels and the abundance of mammalian predators (e.g., skunks, foxes, weasels, and feral cats). Because these predators rely on rodents as a primary food source, they tend to delay recovery of lower rodent population cycles. As rodent populations reach high density levels, disease, parasites, and stress along with predation pressure are thought to cause rodent population declines. When the microtine prey population has ebbed, predators will either starve or emigrate.

Macdonald (65) has studied the prey preference behavior of red foxes (Vulpes vulpes) in great detail in the field. The particular prey species taken can depend on several factors such as prey availability and its antipredator behavior, the relative energy costs of hunting the prey, and the size and nutrient value of the prey. Foxes took field mice in preference to bank voles and wood mice. They also tended to prefer Microtus to Peromyscus. Moles and common shrews tended to be avoided as prey by the red fox. Fresh carcasses of other carnivores (e.g., weasels, badgers, and foxes) were inspected by foxes but were not eaten. Instead, foxes would often mark their locations with urine or feces. The general pattern seemed to indicate that foxes preferred the flesh of herbivores and avoided the flesh of insect- and meat-eating animals. The study could provide some clues to the food flavor chemist as to possible starting points in developing food flavors for foxes, dogs, or other canids.

Apfelback (66) has observed that prey-catching behavior of polecats is highly dependent on olfactory cues. He found that polecats fed either dead chicks or dead rodents for their whole lives showed no interest in the odor of the other prey and refused to eat it. Searching behavior is apparently only shown by polecats when they contact the odor of the familiar prey item. These demonstrated effects, although primarily shown in pen tests, may indicate a sensitive period (i.e., through the fourth month of life) during which certain carnivores imprint on certain prey items as food.

With rodents, such olfactory or food flavor imprinting is difficult to demonstrate under both laboratory and semi-field conditions. Some of the earlier attempts (15, 67, 68) failed to show any effects of early feeding experience (i.e., post weaning) with laboratory rats or guinea pigs. More recent reports (69, 70, 71) have shown that temporary effects can be demonstrated for food odor and food flavors if rats are exposed to the stimuli very

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early in life (i.e., preweaning or during the first postnatal week of life).

Perhaps more important than possible flavor imprinting effects, resulting from the first foods eaten by newly weaned rats, are their maternal feeding experiences with specific flavors from lactating dams (72). Galef and Henderson (73) demonstrated that weanling rats would actively seek and preferentially feed on the diet fed to lactating dams during the nursing period, even when the diet was normally less palatable. Presumably, the flavor of the dam's diet will affect the flavor of her milk to some degree. Another important factor that determines which food a rat pup is most likely to eat is the feeding site selected by parent rats (74). This appears to be a form of social imitation that the pups display toward adults that are actively feeding. A third factor that can influence rat pup feeding preference is the existence of a maternal pheromone (75, 76). The diet of the lactating rat can determine the odor and flavor quality of this pheromone (i.e., caecotrophe) excreted following parturition, and rat pups from such mothers tend to prefer the odor and flavor of the mother's diet. Stimulus familiarity (77) appears to be the most important aspect of these socially transmitted flavor preference effects. Finally, a fourth factor that leads to feeding site selection by rat pups (78) appears to be other olfactory cues that result from urine deposits left by adult rats at specific feeding sites.

Flavors of the most familiar and normally preferred food items can, of course, determine which foods are most frequently selected by the adult rats. Ricefield rats (*R. r. mindanensis*) show rice varietal preference (79) and tend to prefer Palay over Glutenous rice in field rodent baiting programs. We have confirmed this rice varietal preference effect in closed colony semi-field environments with small groups of adult ricefield rats. As indicated in Table I, the rats showed the highest consumption of FK-178A rice ($P < .01$) and Milagrosa was preferred to the other two tested varieties, IR-20 and C-4 ($P < .05$). Under actual field conditions, most rats would only contact one or two similar varieties so that these data do not allow for predictive value in forecasting damage by the rats. However, the data do imply that subtle changes in the natural rice flavors of baits could potentially influence the extent of bait acceptance.

Some food habit studies with stomach content or fecal matter analyses as measures take relative food availability (80, 81) into account. Fellows and Sugihara (82), by this method, were able to determine that Norway and Polynesian rats in and near Hawaiian sugarcane fields showed high acceptance of broad-leaved fruits (melastoma, passion fruit, guava, thimble berry, and popolo). The young (< 7 mo) of both rat species showed avoidance of grass vegetation, whereas adults (7-24 mo) showed some passive acceptance. Such changes in food acceptance could reflect either ontogenetic (e.g., development of a more adequate digestive system for exploiting nutrients from more food sources) or learning effects (e.g.,

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associating the nutritive value of grasses with their flavors). Field investigations that consider what is being eaten in relation to abundance can provide some of the most useful preference data for wild birds and mammals. However, these studies can be extremely time-consuming and undoubtedly require a great deal of systematic assessment.

Table I. Rice bait consumption (g) of eight rat colonies for four varieties of rice (Mean ± S.E.).

| Day | Rice variety | | | |
|---|---|---|---|---|
| | Milagrosa | FK-178A | IR-20 | C-4 |
| 1 | 18.7 ± 4.8 | 26.4 ± 5.7 | 7.4 ± 2.4 | 7.4 ± 2.5 |
| 2 | 15.1 ± 4.2 | 34.6 ± 5.0 | 5.9 ± 1.7 | 9.4 ± 1.9 |
| 3 | 14.4 ± 3.3 | 33.9 ± 7.3 | 10.1 ± 2.6 | 7.0 ± 1.5 |
| 4 | 8.9 ± 1.5 | 38.7 ± 4.2 | 7.8 ± 1.6 | 8.6 ± 1.4 |
| 5 | 15.6 ± 5.2 | 37.4 ± 5.1 | 10.5 ± 4.2 | 8.8 ± 1.4 |
| 6 | 6.6 ± 2.3 | 49.9 ± 6.7 | 4.6 ± 1.3 | 9.9 ± 3.2 |
| 7 | 4.9 ± 2.1 | 49.1 ± 7.5 | 9.1 ± 3.1 | 6.6 ± 2.2 |
| 8 | 4.0 ± 1.4 | 49.4 ± 8.6 | 8.3 ± 3.6 | 10.2 ± 3.3 |
| 9 | 10.1 ± 3.9 | 23.5 ± 3.0 | 9.8 ± 4.0 | 8.5 ± 2.0 |
| 10 | 6.9 ± 2.7 | 36.6 ± 4.8 | 5.6 ± 1.3 | 12.5 ± 5.5 |
| 11 | 5.0 ± 2.6 | 34.9 ± 3.4 | 11.1 ± 3.0 | 2.5 ± 1.0 |
| 12 | 3.9 ± 0.9 | 36.6 ± 5.7 | 8.1 ± 1.1 | 6.6 ± 2.5 |

Behavioral and ecological effects on flavor acceptance or rejection in birds are more difficult to characterize than those found in mammals. For example, Kare and Ficken (25) reviewed the early work concerned with taste preference responses of chickens with treated versus untreated water over relatively long exposure periods. It was generally found that chickens were indifferent to a large number of carbohydrates (e.g., sucrose, glucose, lactose) except for xylose that was strongly rejected. Chickens also consistently rejected chloride salts (ammonium, calcium, and ferric) at the higher concentrations. Likewise, most sour solutions (organic and inorganic acids) were rejected by chickens. The effects of prior experience on taste preference behavior of the fowl was demonstrated (25) by the fact that ascending versus descending series of preference test concentrations yield vastly different data. The importance of prior experience was confirmed by Davidson (83) who reported on preference behavior of various bird species toward berries and seeds in natural settings. He has suggested that low consumption of a new food item by many bird species for 1-10 days is of no particular significance. Thus, certain avian species may be extremely slow to change their normal feeding habits.

Detailed and systematic laboratory flavor preference evaluations in wild birds are somewhat scant. Working with feral

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pigeons, Duncan (84) demonstrated that both acetic and hydrochloric acids (sour to man) produce pronounced rejection even at low concentrations (e.g., .005N acetic acid). With sodium chloride, Duncan has indicated that the preference - aversion function is similar to that found in the rat. That is, preference was indicated at wt/vol concentrations below 1.0% and the birds tended to reject concentrations greater than 1.0%.

Some of the discrepancies found for preference behavior of the same general species (e.g., chickens, quail, pigeons) could be a function of different methods of measuring preference. Gentile (85), for example, found that chickens preferred certain concentrations of sucrose and glucose (1-5%) when brief-exposure (< 3 minutes) preference tests are used. Gentile believes that the Kare and Medway (86) preference data for carbohydrates reflect not only taste but also postingestional factors.

As summarized by Wenzel (62), salts and bitter tasting stimuli (e.g., quinine hydrochloride, sucrose octaacetate) are generally rejected by the pigeon, quail, chicken, and Great Tit. Sour stimuli (acetic and hydrochloric acids) are also rejected by most bird species tested except for quail that prefer slightly sour tastes. The Great Tit and quail also prefer glucose, but the pigeon and chicken are less predictable.

Using more natural taste stimuli, Yang and Kare (87) reported that certain arthropod defensive secretions could greatly affect red-winged blackbird preference behavior. Both salicylaldehyde and p-benzoquinone were rejected at very low concentrations (0.025% wt/vol) in water solutions. The data may indicate certain insect species gain protection from bird predation by virtue of their chemical secretions that taste unpleasant or irritating to birds.

Brower's (88) mimicry model of selective predation proposes that birds are initially influenced to feed on insects by such factors as movement, sight, shape, and other nontaste cues. However, after an unpleasant experience with these insect substances, certain aspects of the food item become avoided. These can include taste as well as color, shape, texture, and size of specific insects. Some interesting possibilities for the development of improved bird repellent chemicals are discussed along these lines by Rogers (89).

Little work has been done in an attempt to increase bait acceptance by pest birds. Bullard (90) has provided some evidence that bait formulation could be a critical factor in bird-baiting programs. Surface-coated grain baits (corn, wheat, or oats) containing a bird repellent (methiocarb) were shown to vary greatly in chemical concentration (c.v. = 21-48%). Uniformly mixed and tableted baits had much less chemical concentration variability (c.v. = 4.6-8.0%). These data would indicate that a more uniform repellent dosage can be achieved with tableted material.

Most of the emphasis in research and development of bird control agents has been directed at the development of improved repellents (89). Recent efforts into this research include an

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evaluation of the mode of action of commercially marketed "bird-resistant" varieties of sorghum (91). Although the data are not yet fully analyzed, it appears that a rough negative correlation exists between polyphenolic content of the seed variety and seed preference by quelea and red-winged blackbirds. It is hoped that the research into the natural secondary plant substances [e.g., alkaloids (92) and astringents (93)] will eventually allow improved crop protection from destructive bird species. It is also hoped that this research can lead to the development of improved bird-resistant grain varieties or the isolation and eventual marketing of extremely safe, effective, and low-cost taste repellent chemicals.

## Empirical Screening of Flavoring Agents, Enhancers, and Spices

Obviously, the flavor chemist and his co-workers cannot be expected to research all of the factors (nutritional, physiological, behavioral, etc.) that will affect the preference behavior of a given bird or mammal. Some researchers have therefore taken an empirical screening approach to the problem. For example, Hilker et al. (94) attempted to alter food preferences of rats using a mixture of black pepper, cloves, and cinnamon each at 0.5% concentration. The spiced diet was fed to weanling rats for 5 weeks followed by a 2-week free-choice period. The young rats consumed significantly less of the spiced diet compared with the untreated diet, whereas adults showed no particular preference between the diets. Spices and flavorings are thought (95) to stimulate appetite and gastrointestinal readiness for receiving food. However, a more recent (23) viewpoint on this subject is that the flavors of additives primarily serve as distinctive taste and odor cues for the safety and familiarity with certain foods eaten by man. If this viewpoint is correct, the screening of flavoring agents and spices to increase bait consumption by rodents in agricultural areas may be valueless.

Bait additives that enhance naturally preferred food flavors could offer a potential means of improving baiting programs by attracting larger numbers of rats to the baits and by increasing toxic bait consumption. We have not been able to demonstrate that protein flavor enhancers (i.e., 5'-ribonucleotides) (96, 97), potentiators such as monosodium - L - glutamate, or sweetness enhancers added to ground rice bait have any particularly strong effects on the choice behavior of ricefield rats from the Philippines (Table II). These findings tend to support the views that the taste and odor perceptual systems of man versus rodent are vastly different (1, 98), and that man's acceptance of some of these products may be, in large part, culturally determined (23).

Food odors as area attractants to rodents for control purposes have been postulated for many years (99, 100, 101, 103). Reiff (99) took a rather analytic approach to the flavor preference problem in rodents by investigating certain chemical compounds

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Table II. Mean percent preference ± S.E.M. for 12 bait additives each tested at three concentrations.

| No. | Additive[a] name | Concentration[b] Control | $C_1$ | $C_2$ | $C_3$ |
|---|---|---|---|---|---|
| 1 | V-50 | 47.4±1.0 | 47.0±2.7 | 45.3±4.2 | 29.7±6.5 |
| 2 | Dried humen | 49.2±1.0 | 45.1±5.6 | 39.9±5.3 | 48.6±4.8 |
| 3 | Zymino | 50.3±1.1 | 42.0±4.8 | 47.3±6.8 | 43.1±3.8 |
| 4 | Canned food flavor | 51.7±1.8 | 42.1±4.2 | 30.6±7.8 | 38.6±4.8 |
| 5 | Cereal flavor | 50.0±0.8 | 51.1±1.7 | 39.4±5.0 | 37.7±5.2 |
| 6 | Vegamine #1 | 48.7±2.0 | 46.3±2.5 | 50.1±2.9 | 38.3±6.6 |
| 7 | Vegamine #28 | 50.7±1.2 | 43.8±4.7 | 41.5±6.3 | 49.6±5.2 |
| 8 | Vegamine #69 | 52.1±0.8 | 43.1±6.3 | 41.4±4.9 | 44.6±6.1 |
| 9 | V 84T | 48.2±0.8 | 44.1±2.1 | 38.0±4.0 | 42.1±3.1 |
| 10 | Soy sugar | 50.1±1.0 | 46.1±5.1 | 52.9±2.1 | 49.8±4.0 |
| 11 | Veltol plus | 51.9±1.7 | 51.8±0.7 | 46.1±0.8 | 44.7±1.7[c] |
| 12 | Sugar | 51.7±0.8 | 57.6±3.2 | 51.9±2.6 | 60.2±3.7 |

[a] Additive numbers 1-10 are protein hydrolysates; number 11 is a sweetness enhancer; number 12 is a nutritive sweetener.

[b] Additive numbers 1-8 were tested at: $C_1$ = 0.3%, $C_2$ = 0.6%, $C_3$ = 1.2%; number 9 tested at: $C_1$ = .15%, $C_2$ = 0.3%, $C_3$ = 0.75%; numbers 10 and 11 tested at: $C_1$ = .10%, $C_2$ = 0.2%, $C_3$ = 0.4%; number 12 at: $C_1$ = 10%, $C_2$ = 20%, $C_3$ = 40%.

[c] $P\ (C_3 > \text{Control}) < .05$.

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commonly found in food items eaten by rats and mice. Certain tertiary amines, essential oils, and aldehydes are found naturally in meats, vegetables, cereal grains, and dairy products known to be readily eaten by rats. Although these elemental flavor compounds can directly contribute to the total flavor of certain food items for rats, their combined action is generally much stronger than the action of any single compound. We have found this same general effect with ricefield rats (18). Of 20 candidate food odor compounds evaluated with an automated odor test device (102), none produced as much investigation as the food odor (Purina Laboratory Chow) associated with their normal laboratory diet (see Table III). Bull (103) came to a similar conclusion in his evaluation of more general food odors (fish, beef, dog food, coconut oil) and flavoring agents (raspberry, aniseed oil). Even with "repellent" odors, strong odor repellent effects were only observed when the active agent (e.g., Rotran 55) was added to the food in the hoppers. Again, familiarity with the food flavor, along with texture and particle size, proved to be more influential on rat feeding behavior than the presence or absence of novel food odors. For wild rodents, and perhaps other macro-osmic mammals, food odor attractants are heavily dependent upon the animals' past experience in tasting, ingesting, and deriving nutritional benefits from the food.

Hansson (104) noted that field voles, bank voles, and wood mice showed increased gnawing on sticks impregnated with various vegetable oils. Part of this response appeared to be related to texture changes in the wood as the oils penetrated it. Some of the response, however, also appeared to be related to the presence of certain long-chain fatty acids (e.g., oleic, linoleic) in the oils. We have compared the effects of adding 10% vegetable or grain oils to rice bait with independent groups of R. r. mindanensis. Rats in all groups showed reliable preference for soybean, corn, peanut, linseed, palm kernel, safflower, coconut, and rice oil over untreated bait material. As can be seen in Table IV, Freon-11 extracted rice oil produced the most consistent effect on rice bait consumption. All oils changed the texture as well as taste and odor of the bait. This texture change effect was evaluated by comparing the preference responses for the safflower oil (high-oleic type) with the other oils since this material has been reported (105) to be odorless and tasteless to man. Supposedly, even with a very low taste or odor component to rats, safflower oil still produced almost 90% preference after 6 exposure test days. The rice oil was the most consistently preferred material, probably because it acted to intensify the flavor of a normally highly preferred and familiar food (i.e., rice).

## Intensifying Flavor with Extracted or Volatilized Compounds

The empirical screening approach can, at times, lead to successful development of improved animal food flavoring agents as

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Table III. Odor preference test results when 20 candidate materials were compared with investigation time toward the familiar food odor (Purina Laboratory Rat Chow).

| Candidate food odor attractant | Percent response (mean ± S.E.) |
|---|---|
| Soybean oil | 94.6 ± 23.0 |
| Isovaleric aldehyde | 94.3 ± 21.6 |
| N-butyldiethanolamine | 87.5 ± 38.6 |
| N-proplyamine | 80.0 ± 56.4 |
| Peanut oil | 80.0 ± 18.3 |
| 2-Furaldehyde | 77.5 ± 31.3 |
| Linseed oil | 76.2 ± 24.1 |
| Ethyldiethanolamine | 75.6 ± 11.5 |
| Sassafrass oil | 74.4 ± 23.9 |
| Dihydroxyethylaniline | 72.5 ± 58.8 |
| Wintergreen oil | 72.2 ± 16.9 |
| Corn oil | 67.5 ± 16.3 |
| Hexanoic acid | 65.9 ± 25.9 |
| N-octylamine | 61.9 ± 26.6 |
| N-amylamine | 60.0 ± 42.9 |
| Isobutylamine | 52.4 ± 19.7 |
| Cod liver oil | 50.0 ± 19.3 |
| N-(n-propyl)-benzylamine | 40.0 ± 28.1 |
| Valerone | 36.4 ± 4.5 |
| N-hexylamine | 35.6 ± 3.6 |

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Table IV. Mean ± S.D. percent preference of ricefield rats for rice containing oil-treated versus untreated rice.

| Day | Oil | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Soybean | Corn | Peanut | Linseed | Palm kernel | High-oleic-safflower | Coconut | Freon-11-extracted-rice oil |
| 1 | 54.6±32.8 | 61.8±30.3 | 55.6±24.9 | 71.3±21.6 | 64.8±19.3 | 65.9±38.4 | 25.9±32.8 | 83.9± 6.8 |
| 2 | 60.8±29.3 | 67.1±34.8 | 79.9±12.3 | 88.1±14.4 | 78.8±12.4 | 80.2±23.7 | 66.8±35.8 | 97.9± 2.3 |
| 3 | 76.6±36.1 | 76.8±38.6 | 83.0±12.6 | 88.4±12.9 | 77.5±11.7 | 82.3±25.0 | 71.1±38.5 | 98.3± 3.9 |
| 4 | 75.0±34.2 | 75.3±38.6 | 84.9±15.0 | 90.3±17.2 | 79.7±10.3 | 82.3±25.0 | 69.9±35.7 | 96.2± 7.0 |
| 5 | 72.6±35.1 | 78.8±38.9 | 88.1±13.9 | 88.5±18.0 | 71.0±15.2 | 88.3±21.5 | 87.2± 7.3 | 93.8±14.9 |
| 6 | 79.3±36.0 | 78.2±39.1 | 89.3±14.8 | 86.0±19.5 | 72.3±20.2 | 89.6±22.2 | 64.1±37.1 | 94.1±13.1 |
| Mean ±S.D. | 69.8±33.9 | 73.0±36.7 | 80.1±15.6 | 85.4±17.3 | 74.0±14.9 | 81.4±26.0 | 64.2±31.2 | 94.0± 8.0 |

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indicated above. However, a more direct approach to the problem involves the study of what the animal normally or naturally prefers in its native habitat. This most preferred (or least preferred) food is then chemically analyzed in conjunction with a series of behavioral bioassays. To illustrate this approach, I have outlined the following examples.

Rice and Church (106) presented evidence that black-tailed deer prefer distilled water treated with low concentrations of water or ethanol extracts from foods normally accepted by deer (bitter brush, Douglas-fir, and hemlock). In response to certain organic acids commonly found in plants browsed by ungulates, there were pronounced sex differences. Bucks strongly preferred the intermediate concentrations of malic acid but showed weaker preference for succinic and citric acids. Does, on the other hand, showed weak to strong rejection of these same acid concentrations. This report indicated that extracted flavor compounds found in natural food sources of black-tailed deer can greatly affect their consummatory drinking behavior.

Mugford (107) reported that both the duration and the size of meals eaten by domestic cats can be increased by perfusing normally preferred meat odors (cooked rabbit) through their maintenance diet (dry food). Le Magnen (108) and Larue (109) have also noted that food odors can influence feeding patterns in rats. For certain predatory mammals such as foxes (65), food odors may elicit food seeking regardless of experience. For many other mammalian species [e.g., Norway rats (4), polecats (66), and roof rats (58)] food flavor preferences are highly dependent upon experience with the food or prey species.

As another example of an alternate approach to empirical screening, Bullard and Shumake (110) evaluated eight potential flavor components of whole grain, uncooked rice with ricefield rats in an attempt to increase the palatability of ground rice baits. Rice bran oil, the acetonitrile extract of rice bran oil, the ether extract of ground rice, endosperm, rice polish, rice bran, rice bran volatiles, and whole grain rice volatiles were evaluated as additives to ground rice with a group of 12 rats. A brief exposure taste preference device (18, 111) was used in these evaluations to ensure that postingestional factors related to caloric content or digestability would not confound preference test results. The whole grain rice volatiles material was the only one found to reliably increase both consumption and feeding time. In a later semi-field pen test, rice volatiles treated bait was preferred ($P < .05$) over whole-grain, granulated, and soy bean oil (1%) treated rice.

The effect of the rice volatiles additive on toxic bait consumption was then evaluated (110). Two groups of rats were given a choice between either 0.2% zinc phophide treated rice and untreated rice, or 0.2% zinc phosphide treated rice with the rice volatiles added and untreated rice. This latter group showed almost double the toxic rice bait consumption and a mortality of

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88%. The control group rats (without rice volatiles on toxic rice bait) showed only 50% mortality ($P < .005$). This experiment indicated to us that flavors from a preferred food for rats could be further intensified to increase palatability. This increased palatability had the advantage of increasing toxic bait consumption, presumably because we were working with highly familiar rice flavors preferred by ricefield rats (112, 113).

## Conclusions

It appears to be unlikely that food flavoring agents, spices, and enhancers developed for humans will effect strong and long-lasting preference behavior modifications in birds and mammals. Food flavor familiarity may be the single, most important factor controlling food preference behavior of wild species (8, 23, 77). Mammals show highest acceptance of familiar foods, but they also tend to sample small amounts of any new food item placed in their environment (4). Some bird species are extremely resistant to changing their preference behavior for certain food items (83), but there is no compelling evidence proving that food flavor is less important to birds than to mammals (62, 85, 89). For mammals (and possibly birds also), food texture, particle size, and feeder location can sometimes produce as much, or more, effect than odor and taste of food (103, 104).

Preference test results for a given flavor additive and species will vary with methodological factors such as: length of test, previous experience of the test animal, and test fluid or food base used. In general, taste preference evaluations with standard compounds (e.g., NaCl, sucrose, HCl, and quinine) do not yield data that allow prediction of the kinds of food flavors wild animals will most readily accept and prefer in a given ecosystem. Food habit studies that take into account the relative abundance of the food items under study, can sometimes yield the most productive and predictive information. Empirical screening as a method of searching for animal food attractants or repellents can be an expensive effort and is, at times, not worthy of the researcher's time. Attempts to correlate chemical structure with repellent effects elicited by compounds from an empirical screening program in rodents have led to some degree of prediction (114), but the effort and expense of such activity may not be easily justified. Correlation of chemical structure with biologically active compounds isolated from natural sources (e.g., secondary plant substances) may be a more fruitful approach.

Our approach to developing an improved and intensified rice flavor for baiting rats with rice has been as follows. First, the natural or normal food habits of the ricefield rat were evaluated under field conditions (113). Second, this preference response was then confirmed for rice under brief-exposure, two-choice laboratory test conditions. Third, the most familiar and preferred food, rice, was separated into gross chemical components (e.g.,

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bran, endosperm, oil, flavor volatiles, etc.) (110), and each of these was separately added back to ground rice. Additional behavioral tests were then conducted to evaluate potential application of the most preferred flavor component (e.g., increasing toxic bait consumption, increasing bait palatability, and attracting rats from a distance). If all laboratory and semi-field evaluations continue to be positive and consistent, we will then proceed to small- and large-scale field testing. After some positive confirmation from the field tests, we will then either synthesize the natural rice volatiles flavor or devise a commercial extraction process for producing this bait additive.

The approach to developing improved bird repellents can proceed along these same lines. There are numerous seeds, berries, and plants that birds normally avoid. Part of this avoidance could be due to conditioned aversion (89) or to aversive taste (93). Regardless of the physiological and behavioral mechanisms involved in the repellent effects, I believe the trend toward exploitation of natural food items as a source for developing more attractive or repellent animal food flavors will continue.

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## Literature Cited

1. Kare, M. R. J. Agric. Food Chem. (1969) 17:677-680.
2. Watson, A. "Animal Populations in Relation to Their Food Resources," Blackwell Scientific Press, Oxford, 1970.
3. Le Magnen, J. "Handbook of Sensory Physiology," IV, Beidler, L. M., Ed., pp. 463-482, Springer-Verlag, Berlin, 1971.
4. Barnett, S. A. Brit. J. Anim. Behav. (1953) 1:159.
5. Crook, J. H. and Ward, P. "The Problems of Birds as Pests," Murton, R. K. and Wright, E. N., Eds., pp. 211-230, Academic Press, New York, 1968.
6. Ward, P. Ibis (1965) 107:173-214.
7. Geist, V. "Mountain Sheep," Univ. Chicago Press, Chicago, 1971.
8. Barnett, S. A. "The Rat: A Study in Behavior," Univ. Chicago Press, Chicago, 1975.
9. Kare, M. R. "The Physiological and Behavioral Aspects of Taste," Kare, M. R. and Halpern, B. P., Eds., pp. 6-15,

Univ. Chicago Press, Chicago, 1961.
10. Nachman, M. and Hartley, P. L., J. Comp. Physiol. Psychol. (1975) 89:1010-1018.
11. Sheffield, F. D. and Roby, T. B. J. Comp. Physiol. Psychol. (1950) 43:471-481.
12. Kennedy, J. M. and Baldwin, B. A. Anim. Behav. (1972) 20: 706-718.
13. Rogers, J. G., Jr. J. Wildl. Manage. (1974) 38:418-423.
14. Sterner, R. T. and Shumake, S. A. "Coyotes: Biology, Behavior, and Management," Bekoff, M., Ed., pp. 297-325, Academic Press, New York (in press).
15. Warren, R. P. and Pfaffmann, C. J. Comp. Physiol. Psychol. (1959) 52:263-266.
16. Arnold, G. W. and Hill, J. L. "Phytochemistry and Ecology: Proceedings of the Phytochemical Society Symposium," Harborne, J. B., Ed., pp. 71-101, Academic Press, London, 1972.
17. Young, P. T. Psychol. Rev. (1966) 73:59-86.
18. Thompson, R. D., Shumake, S. A. and Bullard, R. W. Proc. Fifth Vert. Pest Conf. (1972) 5:36-42.
19. Pager, J. Physiol. Behav. (1974) 12:189-195.
20. Garcia, J., Kover, R. and Green, K. F. Psychon. Sci. (1970) 20:313-314.
21. Clark, M. M. and Galef, Jr., B. G. Anim. Behav. (1977) 25:298-316.
22. Eibl-Eikesfeldt, I. "Ethology, the Biology of Behavior," Holt, Rinehart, and Winston, New York, 1970.
23. Rozin, P. Life Sci. Res. Rep. (1976) 2:285-312.
24. Young, P. T. "Handbook of Physiology, Sect. 6: Alimentary Canal," I, Code, C. and Heidel, W., Eds., pp. 353-366, American Physiological Society, Washington, 1967.
25. Kare, M. R. and Ficken, M. S. "Olfaction and Taste," Zotterman, Y., Ed., pp. 285-297, Macmillan, New York, 1963.
26. Morrison, G. R. and Young, J. C. Physiol. Behav. (1972) 8:29-32.
27. Nachman, M. and Cole, L. P. "Handbook of Sensory Physiology," IV, Beidler, L. M., Ed., pp. 337-362, Springer-Verlag, Berlin, 1971.
28. Campbell, C. S. and Davis, J. D. Physiol. Behav. (1974) 12:357-365.
29. Maller, O., Kare, M. R., Welt, M. and Behrman, H. Nature (1967) 213:713-714.
30. Pilcher, C. W. T. and Jarman, S. P. J. Com. Physiol. Psychol. (1974) 87:56-61.
31. Contreras, R. J. and Hatton, G. I. Physiol. Behav. (1975) 15:569-576.
32. Rozin, P. J. Comp. Physiol. Psychol. (1965) 59:98-101.
33. Rozin, P. and Kalat, J. W. Psychol. Rev. (1971) 78:459-486.
34. Pain, J. F. and Booth, D. A. Psychon. Sci. (1968) 10:363-364.
35. Scott, E. M. and Quint, E. J. Nutr. (1946) 32:113-119.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

36. Harris, L. J., Clary, J., Hargreaves, F. J. and Ward, A. Proc. Roy. Soc., B, Biol. Sci. (1933) 113:161-190.
37. Bernard, R. A., Halpern, B. P. and Kare, M. R. Proc. Soc. Exptl. Biol. Med. (1961) 108:784-786.
38. Bernard, R. A. and Halpern, B. P. J. Gen. Physiol. (1968) 52:444-464.
39. Hughes, B. O. and Wood-Gush, D. G. Physiol. Behav. (1971) 6:331-339.
40. Richter, C. P. Am. J. Physiol. (1936) 115:155-161.
41. Scott, E. M., Verney, E. L. and Morissey, P. D. J. Nutr. (1950) 41:173-186.
42. Wolf, G. and Steinbaum, E. A. J. Comp. Physiol. Psychol. (1965) 59:335-339.
43. Schmidt-Nielsen, K. Circulation (1960) 21:955-967.
44. Adair, E. R., Miller, N. E. and Booth, D. A. Commun. Behav. Biol. (1968) 2:25-37.
45. Valenstein, E. S., Cox, V. C. and Kakolewski, J. W. Science (1967) 157:552-554.
46. Maller, O. Proc. Soc. Exptl. Biol. Med. (1965) 119:199-203.
47. Piquard, F., Schaffer, A. and Haberey, P. Physiol. Behav. (1975) 15:41-46.
48. Booth, D. A. and Campbell, C. S. Physiol. Behav. (1975) 15:523-535.
49. Ter Haar, M. B. Horm. Behav. (1972) 3:213-219.
50. Pietras, R. J. and Moulton, D. G. Physiol. Behav. (1974) 12:475-491.
51. Wade, G. N. and Zucker, I. Physiol. Behav. (1970) 5:269-273.
52. Valenstein, E. S., Kakolewski, J. W. and Cox, V. C. Science (1967) 156:942-943.
53. Hoff, L. A. Dissert. Abst. (1968) 28:4314.
54. Booth, D. A. and Davis, J. D. Physiol. Behav. (1973) 11: 23-29.
55. Mitchell, D., Beatty, E. T. and Cox, P. K. Behav. Biol. (1977) 19:206-216.
56. Young, P. T. and Kappauf, W. E. Amer. J. Psychol. (1962) 75:482-484.
57. Shumake, S. A., Thompson, R. D. and Caudill, C. J. J. Comp. Physiol. Psychol. (1971) 77:489-494.
58. Jackson, W. B. Pest Cont. (1965) 33:12, 13, 50, 52.
59. Wagner, M. W. Psychol. Repts. (1971) 29:799-804.
60. Ader, R. Bull. Psychon. Soc. (1973) 1:253-254.
61. Pelz, W. E., Whitney, G. and Smith, J. C. Physiol. Behav. (1973) 10:263-265.
62. Wenzel, B. M. "Avian Biology: III," Farner, D. S., King, J. R. and Parkes, K. C., Eds., pp. 389-415, Academic Press, New York, 1973.
63. Kare, M. R. and Maller, O. J. Nutr. (1967) 92:191-196.
64. Pearson, O. P. J. Anim. Ecol. (1966) 35:217-233.
65. Macdonald, D. W. Mammal Rev. (1977) 7:7-23.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

66. Apfelback, R. Zeit. Tierpsychol. (1973) 33:270-273.
67. Krishnakumari, M. K. Pest Control (1973) 41:36, 38, 43.
68. Bronson, G. J. Comp. Physiol. Psychol. (1966) 62:162-164.
69. Capretta, P. J. and Rawls, L. H. J. Comp. Physiol. Psychol. (1974) 86:670-673.
70. Bronstein, P. M. and Crockett, D. P. Behav. Biol. (1976) 18:387-392.
71. Cornwell, C. A. Behav. Biol. (1976) 17:131-137.
72. Galef, B. G., Jr., and Sherry, D. F. J. Comp. Physiol. Psychol. (1973) 83:374-378.
73. Galef, B. G., Jr., and Henderson, P. W. J. Comp. Physiol. Psychol. (1972) 78:213-219.
74. Galef, B. G., Jr., and Clark, M. M. J. Comp. Physiol. Psychol. (1972) 78:220-225.
75. Leon, M. and Moltz, H. Physiol. Behav. (1971) 7:265-277.
76. Leon, M. and Moltz, H. Physiol. Behav. (1972) 8:683-686.
77. Leon, M., Galef, B. G., Jr., and Behse, J. H. Physiol. Behav. (1977) 18:387-391.
78. Galef, B. G., Jr., and Heiber, L. J. Comp. Physiol. Psychol. (1976) 90:727-739.
79. Kuehnert, G. Plant Prot. News (Manila) (1976) 5:24-27.
80. Reichman, O. J. J. Mammal. (1975) 56:731-751.
81. Turkowski, F. J. J. Wildl. Manage. (1975) 39:748-756.
82. Fellows, D. P. and Sugihara, R. T. Hawaiian Plant Rec. (1977) 59:67-86.
83. Davidson, V. E. Audubon (1962) 64:346-350.
84. Duncan, C. J. Anim. Behav. (1960) 8:54-60.
85. Gentile, M. J. "Neural and Endocrine Aspects of Behavior in Birds," Wright, P., Caryl, P. G. and Vowles, D. M., Eds., pp. 305-318, Elsevier Scientific Publishing Co., Amsterdam, 1975.
86. Kare, M. R. and Medway, W. Poult. Sci. (1959) 38:1119-1127.
87. Yang, R. S. H. and Kare, M. R. Ann. Entomol. Soc. Am. (1968) 61:781-782.
88. Brower, L. P. Sci. Am. (1969) 220:22-29.
89. Rogers, J. G., Jr. "Flavor Chemistry of Animal Foods," Bullard, R. W., Ed., American Chemical Society, Washington (in press).
90. Bullard, R. W. J. Wildl. Manage. (1970) 34:925-929.
91. Bullard, R. W. Personal communication, Denver Wildlife Research Center, 1977.
92. McKey, D. Am. Nat. (1974) 108:305-320.
93. Bate-Smith, E. C. "Phytochemistry and Ecology: Proceedings of the Phytochemical Society Symposium," Harborne, J. B., Ed., pp. 45-56, Academic Press, London, 1972.
94. Hilker, D. M., Hee, J., Higashi, J., Ikehara, S. and Paulsen, E. J. Nutr. (1967) 91:129-131.
95. Mukerji, B. Fed. Proc. (1961) 20:247.
96. Yamaguchi, S., Yoshikawa, T., Ikeda, S. and Ninomiya, T. Agri. Biol. Chem. (1968) 32:797-802.
97. Sato, M. and Yamashita, S. Jap. J. Physiol. (1965) 15:

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

570-578.
98. Halpern, B. P., Bernard, R. A. and Kare, M. R. J. Gen. Physiol. (1962) 45:681-701.
99. Reiff, M. Acta Tropica (1956) 13:289-318.
100. Steinbrecher, W. Zeit. Ange. Zool. (1962) 49:301-349.
101. Long, C. J. and Tapp, J. T. Psychon. Sci. (1967) 7:17-18.
102. Shumake, S. A., Thompson, R. D. and Bullard, R. W. Behav. Res. Methods Instrum. (1973) 5:279-282.
103. Bull, J. O. Proc. Fifth Vert. Pest Conf. (1972) 5:154-160.
104. Hansson, L. OIKOS (Copenhagen) (1973) 24:417-421.
105. Flath, R. A., Forrey, R. R. and Guadagni, D. G. J. Agric. Food Chem. (1973) 21:948-952.
106. Rice, P. R. and Church, D. C. J. Wildl. Manage. (1974) 38:830-836.
107. Mugford, R. A. "Chemical Senses and Nutrition," Kare, M. R. and Maller, O., Eds., pp. 3-43, Academic Press, New York (In press).
108. Le Magnen, J. Probl. Actuels d'Endocrinol. Nutr. (1963) 7:147-171.
109. Larue, C. G. and Le Magnen, J. Physiol. Behav. (1972) 9:817-821.
110. Bullard, R. W. and Shumake, S. A. J. Wildl. Manage. (1977) 41:290-297.
111. Thompson, R. D. and Grant, C. V. J. Exp. Anal. Behav. (1971) 15:215-220.
112. Shumake, S.A. "Chemical Signals in Vertebrates," Müller-Schwarze, D. and Mozell, M. M., Eds., pp. 357-376, Plenum Publishing Co., New York, 1977.
113. Tigner, J. R. Ph.D. Thesis (1972) University of Colorado.
114. Bowles, W. A., Adomaitis, V. A., De Witt, J. B. and Pratt, J. J., Jr. "Relationships between Chemical Structure and Rat Repellency. II. Compounds Screened between 1950 and 1960," U.S. Army Natick Lab. Tech. Rep. (75-11-FEL), Natick, Mass., 1974.

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# 3

# Methodology of Behavioral Testing Associated with Development in Animal Foods

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New animal foods should be nutritionally sound, economically competitive, and readily accepted by the target-animal population. These goals are typically approached by drawing upon the expertise of nutritionists, economists and flavor chemists. It is our contention that animal food development can also benefit from the expertise of those psychobiologists whose interests relate to variables which influence food acceptance by animals. The present paper will discuss the nature of the psychobiologists' approach to the problem of food acceptance and will outline some findings which we feel illustrate the potential contribution of this group of scientists to the development of animal foods.

At the 2nd International Symposium on Olfaction and Taste, Dr. Carl Pfaffmann and his collaborators presented a good example of the psychobiological perspective on the study of food preferences in animals (1). They pointed out that the key questions concern the variables which lead an animal to initiate, maintain and terminate behavior directed towards nutritive and non-nutritive substances. They classified these variables as physiological and behavioral.

The physiological variables included: (1) afferent stimulation from olfactory gustatory and somatosensory receptors in the head, (2) both immediate and long-term consequences of ingestion (i.e., sensory and/or metabolic effects), and (3) the interaction of afferent stimulation and postingestive consequences which modify preference and ingestive behavior.

The behavioral variables are concerned primarily with the arrangement of the preference test situation itself, which under many circumstances, may profoundly affect reactions to the food stimuli being tested.

Our principal emphasis here will be on the behavioral variables although we will devote some attention to interactions of the physiological and behavioral variables. It will be evident that the experimental evidence on which our discussion is based is wholly derived from studies with common laboratory species (e.g., rats, pigeons) and from the study of only a few foods.

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Despite the present absence of data from non-laboratory species and the limited number of foods studied, we are confident that the methodological points we make are broadly applicable in the testing of food acceptance.

The most obvious way to assess acceptability is to employ an ingestional method in which a food is presented to a group of animals and they are allowed to consume it for a period of time. The ingestional method is familiar as a technique for assessing the acceptability of animal foods and, in fact, the ultimate arbiter of acceptability is the manner in which a food initiates, maintains and terminates ingestional behavior. Nevertheless, a second method for the study of food acceptance has evolved from the work of psychobiologists concerned with learning processes in animals, the instrumental method. In this method the indicator of acceptability is a motor response which the animal is required to perform before it gains access to the food. Skillful utilization of either of these methods requires that the role of certain variables be recognized. In the present paper we will review the most important of these variables for each methodology.

## Ingestional Method

It probably should be pointed out that animal psychophysics differs from human psychophysics in one profound way. If we want to know whether a human detects a food substance, discriminates one food substance from another or prefers one food substance over another, we ask the person. We have verbal report as an indicator response. With animals we have no verbal report, so we infer detection, discrimination, acceptability and preference from the non-verbal behavior of the animal. The most common measure resulting from these non-verbal behaviors is the amount of food ingested. The ingestion of foods by animals depends on a variety of physiological and behavioral variables. P.T. Young (2) has stated that "food acceptance is a complex process regulated by at least four groups of determinants...organic conditions, peripheral stimulation, previous experience and bodily constitution". Pfaffmann (1) has pointed out the importance of both the immediate and long term "organic conditions" and the interaction of these states with the peripheral stimulation. He has also stressed behavioral variables including the past history of the animal and the importance of the arrangement of the preference test situation itself. Building on the statements of Pfaffmann (1) and Young (2), we have outlined in Table I what seems to us to be the important determinants of the ingestion of foods.

We have selected only a limited amount of data from this and other laboratories to illustrate the manner in which these factors may influence ingestion of foods.

Bodily Constitution. Little need be said here. It is widely recognized that there are profound differences among species in

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Table I. Factors influencing the acceptability of food.

I. Bodily Consitiution
Inter and intra species differences

II. Organic conditions
Deprivation states
Special organic states
Immediate consequences of ingestion
Long term consequences of ingestion

III. Peripheral Stimulation

Taste
Olfaction
Touch
Vision
Audition

IV. Interaction of Peripheral Stimulation and Organic Conditions

V. Previous Experience
Learned Preferences and Aversions

VI. Conditions of the acceptability test

| | Single Food | Miltiple Food |
|---|---|---|
| Long term test | | |
| Short term test | | |

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food ingestion, dietary needs and tastes. The "sweet tooth" of the rat is not to be found in the cat. In addition, one need not look far to find vast differences between strains of the same species. The C57 and the DBA mice provide good examples of differences in intakes of saccharin, alcohol and morphine (3).

Organic Conditions. One of the most widely studied influences on food ingestion is the deprivation state of the animal. Inferences we would make about preferences are strongly influenced by the length of time the animal has gone without food and water. For example, under a variety of conditions sucrose preferences increase as a function of deprivation (4). In our own laboratory we have recently completed a study of food preferences in the pigeon. A non-deprived pigeon shows approximately equal intake of Canadian peas and white millet in a 24 hour 2 pan test. Birds deprived to 80% of normal body weight will eat only Canadian peas in a short term 2 pan test.

Special Organic States. Pregnancy, lactation, experimental states induced by surgical operations, implanted electrodes, drugs etc., all influence acceptances of foods and liquids.

Immediate Consequences of Ingestion. There are a number of consequences of ingesting food that are immediate and do not fall in the category of the longer term metabolic states of the animal. In 1952, McLeary (5) differentiated between taste and postingestion factors influencing the ingestion of sucrose and concluded that in five minutes or less, the animal has already committed itself to taking some particular volume of the solution. His evidence led him to conclude that hypotonic fluid is rapidly withdrawn into the stomach following ingestion of high concentrations of sugar and this transitory hypotonic state is the physiological mechanism which leads to limiting the further ingestion of sugar. More recently, Puerto et al., (6), have shown that when a nutrient is intubated into the rat's stomach, as the rat ingests a non-nutritive solution, the animal will develop a preference for a fluid paired with that intubation within a 10 minute session. The mechanism underlying this effect is unknown. Valenstein (7) has shown that drinking 0.25% sodium saccharin potentiated the effect of insulin in rats. The results of this experiment show that although saccharin is not nutritive it is not physiologically inert. It seems likely the taste of the sweet saccharin initiates a "preparative metabolic reflex" i.e., possibly insulin released in response to the sweet taste of the saccharin. Finally, Kare (8) at the Third International Symposium on Olfaction and Taste presented data which suggests a direct pathway from the oral cavity to the brain. Glucose labeled with $^{14}C$ applied to the oral cavity for as short as 4 minutes went by a non-circulatory route to the brain in about half of the animals tested. This rapid response could be related to food-intake

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metering.

Long Term Consequences of Ingestion. As we shall see soon, there is a profound difference in the inference we would make about the acceptability of sugars if we use a short term (less than one hour) or a long term (usually about one day) test. This difference results from long term consequences of ingestion which can operate in the long term test. Collier and Bolles (9) state that "constant caloric intake and balance of dietary components, suggest that the typical preference test paradigm should be re-examined in terms of the role of the test item in the total nutritional economy of the animal".

Peripheral Stimulation. This is Young's term (2) and he is referring to the role of "head receptors" (taste, smell, touch, vision, audition) in metering food intake. Obviously taste would be the more important of these sensory inputs, but one cannot ignore the others. The odor of a food and its texture certainly will be involved in at least the initiation of eating. In some species vision may also play an important role. In the experiment referred to earlier where we studied pigeons' food preference and found that the deprived bird always chose Canadian peas over millet, we were able to abolish that preference by crushing the Canadian peas so they were the same size as the millet. It seems most certain that vision plays the critical role in the pigeon's choice of grains.

So much emphasis has been placed on the "body wisdom" of the laboratory-animal and how it selects an appropriately balanced diet, one sees little evidence in the literature that an animal might "go out to dinner and eat for fun".

Collier and Bolles' (9) work on sucrose intakes in the rat could be an example where there is poor body wisdom. Although their animals maintained constant caloric intake while drinking concentrated sucrose solutions, the proportion of total calories ingested from the sugar rose as high as 60%. This would mean a reduction in laboratory chow intake, resulting in a reduction in protein, fat and minerals. It is quite possible that over a long period of time this diet of high sugar intake would result in significant health problems.

A more profound example of drinking "for fun" was first described by Valenstein and colleagues in 1967 (10). They found that laboratory rats drinking glucose and saccharin mixed in the proper proportions (30 grams of glucose + 1.25 grams of sodium saccharin in one liter of water) would exceed their own body weight in fluid intake in a 24 hour period. In our laboratory, we subsequently observed that if the glucose and saccharin were presented in separate bottles that many animals learned to "mix their own cocktail", alternating licking on the two tubes presumably to get the good taste in their mouths (11).



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Interaction of Peripheral Stimulation and Organic Conditions. Deprivation or disease-induced changes in body state are known to affect intakes of foods. There is a question as to whether such changes result in changed taste sensitivity. DeWys and Walters (12), for example, report an elevated sweet threshold and a lowered bitter threshold in cancer patients. Although adrenalectomy causes enhanced intake of NaCl in the rat and insulin injection causes an increased intake of sucrose, the electrophysiological threshold to these two substances are not altered in these two physiological states (1). It is possible that the saliva of the animal is altered, thereby leaving the taste receptor in a modified adaptation state. This does little to explain the lowering of the threshold to bitters as described by DeWys and Walters (12) in the cancer patient.

Probably the most interesting study showing altered taste thresholds as a function of body state is that of Bradley (13). Perfusing the head of the rat with an artificial blood and simultaneously recording from the chorda tympani nerve, Bradley studied the interaction of the state of the body as reflected by the blood and the electrical response in the taste nerve. He concluded that the taste nerve has a dual nature, responding in different ways to chemicals applied on the tongue and to the composition of the blood, monitored by the intero-receptors.

Previous Experience. Lack of experience, or prior experience with a food can markedly affect the results of a preference test. Barnett (14) presents a detailed analysis of "neophobia" where there is an interruption of feeding in the presence of new foods, new objects, and other unfamiliar situations. This fear of the new seems to be more pronounced in wild than in domestic animals, but can be seen in the latter if close observation is made of their initial contacts with the food (15).

Much more profound in their effects on food preferences are the learned taste aversions which have been studied experimentally in the past 20 years. Garcia and co-workers (16) first demonstrated a conditioned taste aversion to saccharin which had been previously paired with ionizing irradiation. Because taste aversions were also conditioned when taste stimuli were paired with drugs (such as LiCl) and poisons (such as Red Squill), the early researchers concluded that the organism avoided the flavored solution because a) it associated the flavor with the aversive gastric upset which follows poisoning or irradiation, b) it learned that the flavor is not safe, or c) a hedonic shift had occurred (17). Recent evidence that taste aversions have been conditioned with amphetamines, barbiturates, tranquilizers and a variety of other drugs in the absence of any signs of toxicity casts doubt on the idea that an aversive event must occur for a taste aversion to be learned. The importance of this for our discussion here is that profound and long lasting changes in preference can be conditioned with brief prior exposures of the

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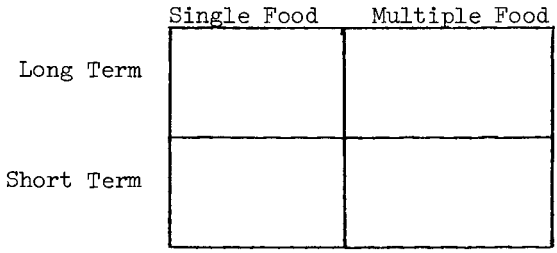
animal to what seems to be "not too aversive" events.

In our own laboratory we have observed rats in a two bottle preference test where the choice was between .12 M LiCl and water. At the end of the first 24 hours they had consumed an average of about 4 ml of LiCl and 36 ml of water. On subsequent days the LiCl consumption dropped to zero. In a more detailed observation of the first 24 hours in a similarly treated new group of rats, we observed that they consumed the 4 ml of LiCl in the first and only drinking bout they had with the substance. Thus, with 500 licks or less the behavior was modified so that LiCl was never consumed again. In fact, this aversion generalized to NaCl since in subsequent two bottle tests the rats totally avoided isotonic solutions when paired with water. This provides us with further evidence that only a brief experience with a small quantity of certain foreign substances is sufficient to result in a long lasting change in eating behavior.

Conditions of the Acceptability Test. It is well known that the procedures we use to measure acceptability of foods will strongly affect our conclusions about preferences. Many of the common procedures can be classified in one of the cells of this matrix:




Numerous authors (1, 18, 19, 20) have addressed themselves to the acceptability of foods and liquids using variations of these single vs. multiple pan tests and long (days, weeks) vs. short (minutes, seconds) term testing periods. These investigations clearly indicate that there are no necessary relations among the conclusions about food acceptability as one tests with these four procedures. Furthermore, it is well established that the immediate and past history of the animal and the "physiological variables" referred to earlier by Pfaffmann (1) and Young (2) interact in a complex way with the cells of this matrix.

Now consider this cell of the matrix for the case where sucrose acceptability is ascertained.

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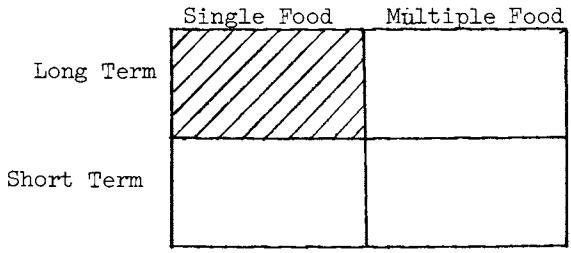


When an animal is given a single bottle test, we usually observe the amount consumed in a certain period of time. If the rat is presented a bottle of .1 M sucrose for several days followed by several days of testing on .2 M, .4 M, .8 M, and 1.6 M sucrose, we would expect a curve as shown in Figure 1. Because of the higher intake of the .4 M sucrose, one might infer that this would be the concentration of choice. In many such tests a water bottle may have been given also, but it matters not since the rat does not touch it. Data such as these have been reported by numerous investigators (9, 21, 22).

If, on the other hand, we paired each of these concentrations with all the others in long term (24 hour) 2 bottle tests, we would find that the 1.6 M is consumed more when paired with each of the others and .8 M is consumed more than the lower concentrations. The rank ordering here is 1.6 M > .8 M > .4 M > .2 M > .1 M.

Because these are long term tests, long term postingestional factors are operating. Collier (9) would say that if the rat is drinking for solute or calories it is more efficient to drink from the higher concentrations suggesting a "principle of least consummatory effort."

With the sugars, the solution of choice appears to be different with the one bottle and the two bottle tests. With saccharin, a non nutritive substance, this difference between the one and two bottle tests is not apparent.

Typical saccharin intakes in a one bottle test (e.g., 23) are presented in Figure 2.

In two bottle tests we paired saccharin concentrations of .03%, 0.1%, 0.3%, 0.9%, and 2.7% in 24 hour tests and found the following rank ordering: 0.3% > .1% > .03% > .9% > 2.7%. In a more refined test we paired each concentration of .033%, 1.%, .2%, .3%, .4%, .5%, .6%, .7%, .8%, .9%, and 1.0% against all others in a long term 2 bottle test. Fig. 3 shows the preference inferred from these data.

The short term tests give the opportunity to look more carefully at factors which initiate feeding and probably are more closely related to taste and the immediate ingestion consequences. An interesting example of the overall difference between the short and long term tests has been the studies of the relative acceptabilities of the sugars. In short term exposures the rat accepts

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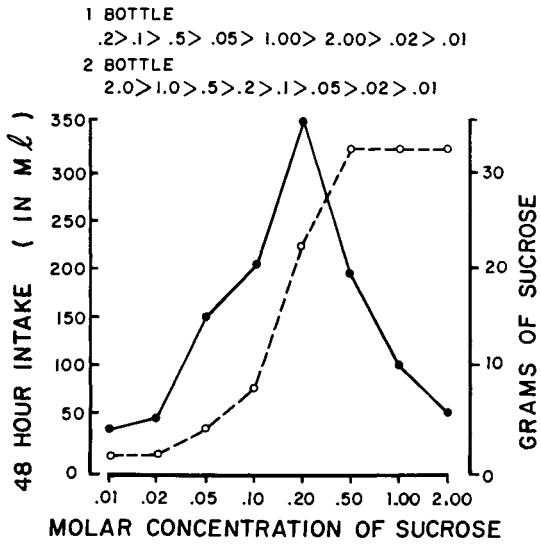


*Figure 1. Intake of sucrose (ml) plotted as a function of molar concentration for single-bottle, 24-hr tests. The solute (g) is also plotted as a function of molar concentration.*

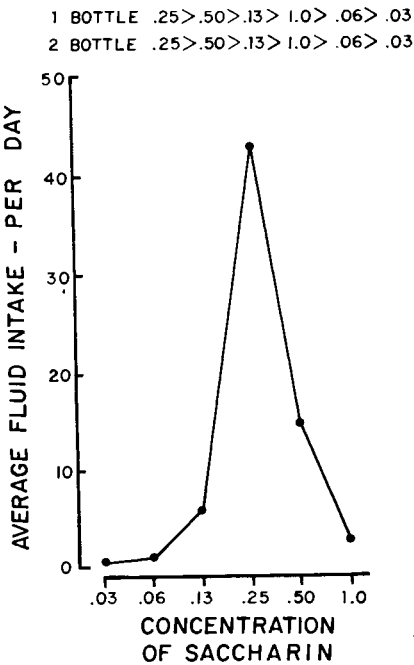


*Figure 2. Intake of saccharin (ml) plotted as a function of percent concentration for single-bottle, 24-hr tests*

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fructose > sucrose > glucose > maltose. If long term tests are used the relative acceptance is reversed, i.e., maltose > glucose > sucrose > fructose. Systematic comparisons between single bottle and two bottle procedures using short term tests have not been made. One should, however, be very cautious in generalizing from the single bottle test to the two bottle test even if the testing period is short. Quite often short term tests involve deprivation schedules, which we have already seen affect preferences. Hesitation in eating and drinking (neophobia) can be a problem in short term tests (15). Finally, if the test is too short different results can occur. Beck et al., (4) have shown that preference for sucrose drops from 90% to 50% with progressively shorter intervals of repeated stimulus presentation. Cohen and Tokieda (24) and Cohen et al., (25) have shown that water is preferred over sucrose if the two choice test is restricted to a few licks.

While the above discussion has focused on only one and two bottle tests, there are examples in the literature of tests with multiple foods (e.g., 26). In addition, procedures have been described where two bottles are alternately presented to the animal in a sequential manner as frequently as once a minute (1).

Most investigators make inferences about the acceptability of the food from the amount consumed. Knowledge of the patterns of eating can be quite helpful in making inferences about acceptability. For example, in studying the initiation of eating both latency to begin ingestion and early rate of eating are important. We use special lickometer circuits in our investigations of neophobia in the rat and can make a "microscopic analysis" of the first approach and hesitancy to drink a novel solution. In our studies with the glucose and saccharin mixture we know that from the first contact with this solution there is a difference in the overall pattern of responding (11). Within two minutes, there is a distinct difference in the pattern of licking the mixture as compared to licking for glucose or saccharin alone.

In studying the maintenance of eating behavior one should know something of meal size, eating responses that occur without interruption (i.e., the "eating bout"), the number of bouts per unit of time and the distribution of interbout intervals. Finally, it is important to know the influence of the biological clocks (daily, estrus, seasonal, etc.) on eating and drinking behavior.

## Instrumental Method

It is well established that skeletal responses are influenced by their consequences. Responses which produce pleasant consequences tend to be repeated; those which produce aversive consequences tend to be suppressed (27). During the past 75 years psychobiologists have refined this simple assertion about the effects of consequences on responses in a program of research and

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theory on the learning processes of animals (28, 29). One spin-off of their work has been the development of rather sophisticated laboratory procedures for studying the ways in which different consequences influence responding. A modern laboratory training apparatus for the rat is shown schematically in Figure 4.

The apparatus is a small enclosure with a metal lever protruding from one wall, and an opening into which small pellets of food can be dispensed. The lever and the food dispenser interface with electronic circuitry which senses all lever presses and dispenses food according to a pre-arranged schedule. The schedules of greatest interest for our purposes are those in which the lever-press response is "instrumental" in producing food for the rat. There are schedules in which a lever press must be made before food will be dispensed. In schedules of this sort we can study how the measureable aspects of the lever-press response (e.g., its frequency of occurrence, the amount of pressure the animal exerts in pressing the lever, etc.) vary as a function of the food obtained. It should be understood that the rat/lever-press arrangement is only one of the many possible species/response arrangements which have been studied with the instrumental methodology. A sample of the other common arrangements would include fish/disc-nosing, birds/disc-pecking, dogs/panel-pushing and monkeys/lever-pressing.

It turns out that lawful functions between the strength of responding and the nature of the consequence are readily obtained in devices of the sort shown in Figure 4, provided that certain methodological precautions are observed. These functions have encouraged many scientists to use the instrumental method as a supplement to, or in place of, the ingestional method to study animals' reactions to various foods. For example, it would be possible to compare the strength of the instrumental response when it produces Food A with that when it produces Food B. The logic of the instrumental method is that if the foods are differentially valued by the animal they should support different strengths of the instrumental response.

But one might ask whether the instrumental methodology has anything special to recommend it. After all, the ingestional method provides a perfectly straightforward way to compare the acceptability of different foods, and the instrumental methodology simply measures a response one step removed from ingestion to estimate the value an animal places on a given food. In our opinion, the instrumental methodology provides a useful supplement to the ingestional method for investigating certain aspects of the animal's reactions to foods. This method is particularly useful when it is applied with an understanding of modern developments in the instrumental technique. In the remainder of this paper we will discuss two of these developments in some detail, including cautions which research indicates must be observed in successfully applying this methodology.

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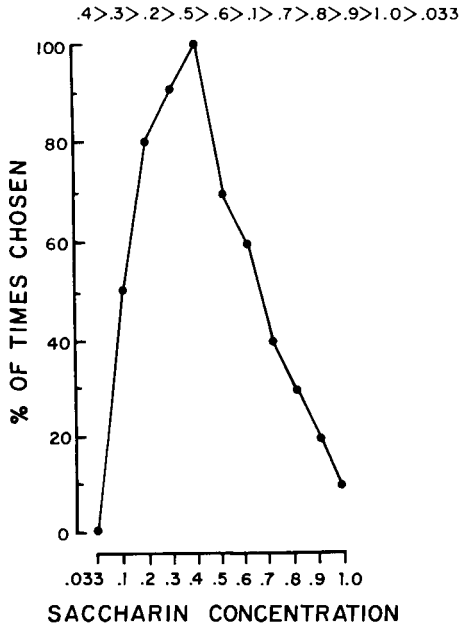


*Figure 3. Saccharin concentrations from .03 to 1.0% were each paired with all other concentrations in 2-bottle, 24-hr tests. The percent of the pairings in which each of the concentrations was preferred is plotted.*

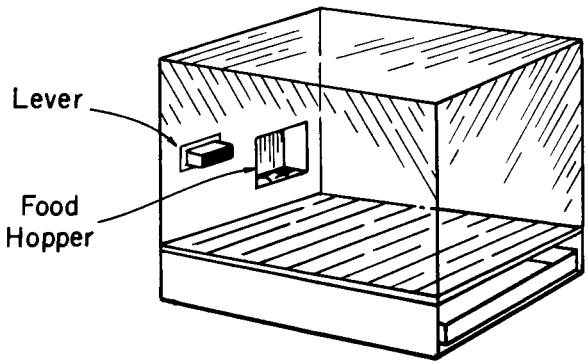


*Figure 4. Instrumental testing apparatus for the rat*

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Simple Reinforcement Schedules. A distinctive characteristic of the instrumental methodology is that the "indicator" response which permits inferences about the value of a food is performed independently of the act of ingesting the food. In the case of the rat in Figure 4, the indicator response is the lever press which occurs at a separate time and place from the eating of the food pellet. Because the indicator response and the ingestion response are separate in this methodology, the investigator has considerable latitute in tailoring some features of the test situation to his needs. In particular, the investigator can readily pace the animal's exposure to the food during a test session, and can arrange for an extended test session in which the indicator response is performed many times, perhaps even in a specific way designed to enhance the sensitivity of that response to certain aspects of the food being tested.

The means by which an investigator gains latitude in arranging his test situation is through the use of schedules of reinforcement which evolved from B. F. Skinner's early attempts to study the eating behavior of rats (30, 31). The distinguishing feature of a schedule of reinforcement is that it imposes a criterion which must be met before the animal's response can produce the food-consequence (32). We will discuss the criteria which constitute the simple reinforcement schedules below, and then will illustrate how these simple schedules can be put to use in evaluating foods.

Figure 5 illustrates the criteria employed in the four simplest reinforcement schedules and the way in which these criteria influence responding. There are four basic criteria. When a "ratio" criterion is employed the animal is required to perform a specified number of responses (e.g., lever presses) before the food-consequence is produced. In the fixed-ratio (FR) cell the criterion is shown as the heavy line intersecting the axis on which cumulative responses are plotted, but not intersecting the axis on which cumulative time since the last feeding is plotted. This means that whenever the animal performs the number of responses required by the criterion, irrespective of how much time it takes to do so, the next response produces food. In the variable-ratio (VR) cell, the criterion varies unpredictably from one food presentation to the next (shown by the light lines intersecting the response axis) but, on the average, the criterion falls at the dark line. After a moderate amount of training the behavioral effects of these schedules on responding between successive food presentations is strikingly different. The hypothetical response data in the fixed-ratio cell show that after a period without responding immediately after a feeding, there is an abrupt change to a high rate of responding which is maintained until the response-criterion is reached and food is presented again. The variable-ratio cell shows that responding continues steadily and at a high rate throughout the interfood interval.

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When an "interval" criterion is employed a response will produce food only if a designated period of time has elapsed since the last food presentation. All responses made before that criterion time have no programmed consequences. The fixed- and variable-interval cells of the table in Figure 5 show that when the criterion interval is a fixed duration the animal does not respond immediately after a feeding, and then responds at a gradually increasing rate until the temporal criterion is reached, whereupon the next response produces food. The cell labelled "VI" shows that when the criterion interval varies unpredictably around an average value, responding is maintained at a steady and moderate rate throughout the interval between feedings.

Consider some applications of these reinforcement schedules in the study of foods. A particularly useful feature of the interval schedules is that they allow the investigator to space periods of food ingestion throughout a lengthy test session without sacrificing a continuous record of the indicator response. As an extreme example, Ferster & Skinner (32) trained pigeons to peck a plastic disc on a Variable Interval 3-min schedule. This schedule allowed brief access to a mixed grain reinforcer following a peck-response at unpredictable intervals whose average duration was 3-min. They reported that the animals pecked at a rate of about 1.5 pecks/sec throughout test sessions which lasted from 9 to 14 hours, often pecking more than 87,000 times per session to obtain only about 250 presentations of grain!

Because the investigator can jointly vary the interval between food presentations and the volume of food presented per reinforcement on fixed-interval schedules he can easily achieve control over the rate at which the animal becomes satiated during an individual test session. Collier & Myers (33) illustrated how these factors can interact in subtle ways to influence the indicator response. Recall that Figure 3 showed that in a single-bottle long-term test rats drink more of a 1M sucrose solution than of a 2M solution. When Collier and Myers (33) used these same concentrations in the instrumental methodology they found that the ranking of the two concentrations depend upon the schedule of reinforcement used. The rats were tested in 30-min daily sessions of lever pressing. When a Fixed-Interval 1-min schedule was used, the rats pressed the lever more often when the reinforcing solution was 1M (32% by weight) than when it was 2M (64%). However, the opposite result was obtained when a Fixed-Interval 4-min schedule was used: the rats pressed more often for the 2M solution than for the 1M solution. Thus, simply by lengthening the scheduled time between successive presentations of the sucrose solutions the schedules yielded an opposite ranking of the two concentrations of the solution.

Additional research by Collier & Myers (33) indicated that this result is best understood as a simple consequence of the two schedules allowing certain physiological variables to act differentially. They concluded that lever pressing was deter-

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mined by the joint action of two independent processes, taste and mometary satiation. Responding was directly related to taste (i.e., concentration) when satiation was low, and the animals satiated more quickly when the schedule allowed frequent access to the solutions (i.e., FI 1 min.). A variety of evidence supported this conclusion. For example, in the early part of each daily test session (when satiation was necessarily low) lever pressing was higher for the higher concentration irrespective of the schedule employed. Furthermore, in the later parts of each test session the rate of lever pressing on the FI 1-min schedule declined, implicating a satiation effect. Finally, manipulation of satiation by adjusting the volume of solution per reinforcement on the two schedules provided results consistent with the taste/satiation account given above.

The manipulation of reinforcer volume in the Collier & Myers (32) experiment also demonstrated the importance of one of the behavioral variables which influence the indicator response in the instrumental methodology. That is, some minimal volume of reinforcement is necessary for the instrumental response to occur at all. Larger minimal volumes are required when the schedule arranges lengthy temporal spacings of reinforcers.

Our discussion of the Collier and Myers experiments is intended to illustrate that when foods are evaluated with the instrumental methodology it is important to consider the role of both the behavioral variables (29,32) and the configuration of physiological variables which the various schedules of reinforcement bring into play.

Before closing this discussion of simple reinforcement schedules we wish to make a brief comment on the ratio schedules. These schedules arrange a direct relation between the number of instrumental responses made and the reinforcement, they have some obvious applications for evaluating foods. For example, using a single-response test we could compare different foods by determining how many responses an animal will make to obtain each food. Beginning with a low-valued fixed-ratio schedule such as FR10 (where food is obtained after every 10th response), the criterion number of responses for reinforcement would increase by, say, 5 responses after successful completion of each ratio. In this incrementing fixed-ratio schedule we could then determine the "break-point" at which the animal would refuse to make the criterion number of responses to obtain that food. Comparison of break-points for different foods would provide one estimate of the relative values of the foods.

One caution in using ratio schedules to evaluate foods might be noted. On ratio schedules the individual responses tend to become "chained" together so that performance of any one response is determined by performance of the immediately preceding response. Consequently, the rate of response on the ratio schedules may not be a sensitive indicator of the value of a reinforcer. However, other measures of performance reflect a rein-

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forcer's effectiveness, particularly the length of the pause after each reinforcer (32). In the space available we can only draw attention to this one nuance of the different reinforcement schedules.

Concurrent Reinforcement Schedules. Preference between foods is studied with the ingestional method by confronting the animal with two foods (as in the two-bottle test discussed earlier) and computing the ratio of the amount consumed of one food to the total amount consumed. Such "preference ratios" provide the bases for inferences about relative value of the two foods.

When the instrumental methodology is employed in preference testing the animal is confronted with two response alternatives, as in the apparatus shown for pigeons in Figure 6. In this case, two plastic discs are mounted on one wall of the test chamber, above a hopper where food reinforcers can be presented. Pecks on the left-hand disc might produce Food A and pecks on the right disc Food B. The figure shows that a preference ratio could be computed as the ratio of pecks on the left disc to the total number pecks on both discs (i.e., L/L + R). In this case a strong preference for the left disc (and its associated food) would be expressed by a preference ratio near 1.0.

While it is easy to arrange an instrumental preference test by requiring the animal to peck once on either disc to obtain the food associated with that disc, the potential of the instrumental method is most fully realized when responding on the two discs is reinforced on the simple reinforcement schedules. Consider the well-studied case of concurrent schedules in which pecking on the left and right discs is reinforced on separate and independent variable-interval schedules (i.e., conc VI VI schedule). Figure 5 illustrated that VI schedules yield continuous responding at moderate rates throughout the interval between food presentations, so that on a conc VI VI schedule we can expect a steady stream of pecking throughout the test session. The question of interest is how the pigeon distributes its responses on the two discs as a function of the properties of the foods associated with each disc.

Figure 7 summarizes some aspects of concurrent VI VI schedules which are important for the present discussion. The top panel illustrates that when both schedules are identical in length and both produce identical foods the sequence of responses is likely to approach a simple alternation pattern. In fact, the animal can maximize the number of reinforcers it obtains per unit time by alternately "checking" in this way whether the VI schedule on each disc is ready to provide a reinforcer. While this is an efficient way for the bird to procede, and yields a preference ratio of approximately 0.5 (which would be expected if, in fact, the bird is indifferent to the two identical foods), this alternating pattern of responding proves not to be optimal when foods with different properties are compared. Consequently, it is

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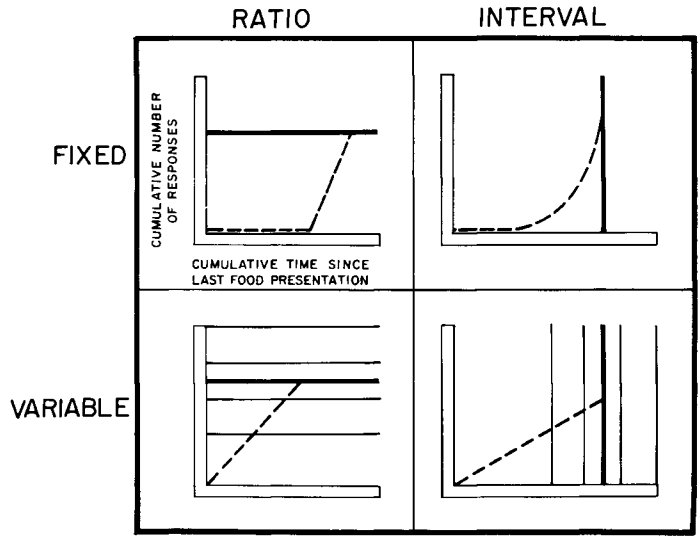
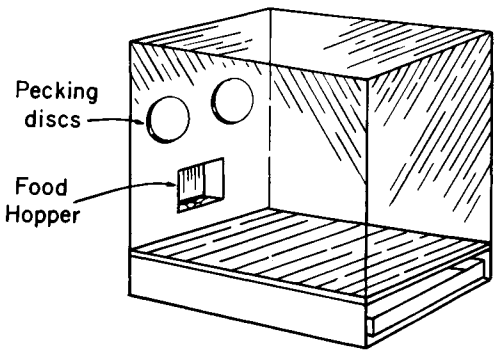



*Figure 5. Criteria for making food available following a response on fixed- and variable ratio and interval schedules. Each cell of the table shows the criterion for that schedule and the typical pattern of responding in the interval between successive food presentations. The criterion is shown by the straight lines which intersect the response axis on the time axis. Responding is shown by the dotted line curve in each cell. The axes in each cell should be read as labeled in the fixed ratio cell.*




Apparatus for training pigeons to choose between food alternatives by pecking on discs.

$$\text{Preference Ratio} = \frac{L}{L+R} = \frac{\text{Total pecks on left disc}}{\text{Total pecks on left + right disc}}$$

*Figure 6. Instrumental test apparatus for studying preference of pigeons*

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1. Hypothetical sequence of responses to left and right alternatives which produce the same food reinforcer ( no C.O.D. imposed ):

LRLRLRLLRLRRL

2. Hypothetical sequence of responses when C.O.D. imposed:

LLLRRRRLLRRRLLLLLLRR

3. Hypothetical data showing "matching" function between preference ratio ( $L / L+R$ ) and reinforcement ratio ( $r_L / r_L + r_R$ ):

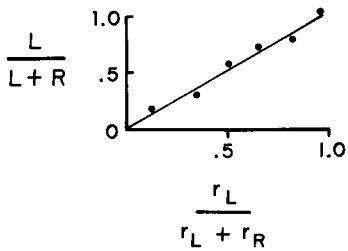


*Figure 7. Relevant features of concurrent schedules*

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desirable to eliminate the alternation pattern of responding before undertaking preference studies with the concurrent schedules methodology.

An effective method for breaking up the alternation pattern is to impose a so-called "change-over-delay" (C.O.D.). Put simply, a C.O.D. penalizes the animal for "changing over" from responding on one disc to responding on the other. The penalty is in the form of a "delay" period in which pecks on one key are ineffective in producing any reward which might have been scheduled there. This delay period is initiated by any peck on a disc which is preceded by a peck on the other disc. The net result of this diabolical scheme is that the pigeon can never obtain food by responding in strict alternation from one key to the other. Consequently, the pigeon changes its pattern of responding and the investigator achieves his purpose. The second panel of Figure 7 shows that when a C.O.D is imposed animals tend to peck several times on each disc before changing over to sample the other one.

The importance of the C.O.D. is concurrent schedules becomes evident when we consider the bottom panel of Figure 7 which illustrates a relationship found when a C.O.D. is employed. Each data point in the figure is hypothetical, the function shown is representative of a large number of findings obtained with concurrent VI VI schedules in which a C.O.D. is employed (34). Each data point represents a preference ratio which our hypothetical pigeon would produce in choosing between foods which differ in quantitative or qualitative ways.

For example, suppose the pigeon obtained the same type of grain reinforcer by pecking on each disc, but a larger amount per reinforcer for pecking on one of the discs. Suppose further that the pigeon was tested with various combinations of different amounts. If for each combination we computed the ratio of the amount obtained by pecking on the left disc to the total amount obtained by pecking both discs, we would calculate the entity,

$$\frac{r_L}{r_L + r_R}$$

where $r_L$ and $r_R$ represent, respectively, the total amounts of food obtained by pecking the left and right discs in a test session. And, if the preference ratio obtained when each combination of amounts was available were plotted against the reinforcement ratio calculated for that combination we would produce a graph very similar to that shown in the bottom panel of Figure 7. This graph illustrates the most striking aspect of the data produced by concurrent schedules: the value of the preference ratio matches the value of the reinforcement ratio. This function is summarized as the so-called matching law which is ex-

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pressed as

$$\frac{L}{L + R} = \frac{r_L}{r_L + r_R} ,$$

where L and R designate the number of responses to the left and right alternatives, respectively, and $r_L$ and $r_R$ designate some measure of the reinforcers available for responding on the two alternatives. The matching law was formulated by R. J. Hernnstein (35, 36, 37).

There are four points to be emphasized about the matching law. First, matching is obtained more reliably when a C.O.D. is imposed. It is presently unknown whether different lengths of C.O.D. will be required to demonstrate matching in different species (34). Second, matching has been demonstrated when the reinforcers differ in such properties as their amount, frequency of occurrence and quality (34). Third, there have been several reformulations of the matching law with the goal in mind of making it more comprehensive (34). Finally, matching has been found in a number of variations on the conc VI VI procedure discussed here but it is importantly influenced by the parameters of the reinforcement schedules employed (34, 38). Discussion of these developments is beyond the scope of this paper.

How might the discovery of matching on concurrent schedules be useful in evaluating animal foods? H. L. Miller recently provided one example in an experiment concerned with scaling the hedonic value of qualitatively different grains for pigeons (39). Using a concurrent VI VI schedule on which pigeons pecked different keys to obtain different grains, Miller first established that the pigeons preferred wheat to buckwheat. Then, that they preferred buckwheat to hemp. He finally predicted and demonstrated that the pigeons preferred wheat over hemp. The special feature of his work was that through a mathematical analysis of his data inspired by the matching law, Miller was able to construct an empirically based scale of grain quality for the pigeon. His calculations indicated that if buckwheat serves as the standard and is arbitrarily assigned a value of 10 quality units, wheat would fall at 14 and hemp at 9 quality units.

In developing animal foods it would seem to be of some importance to determine the ranking of qualitatively different foods on a empirically based scale. The concurrent schedules methodology provides one way of achieving this goal. In fact, the concurrent schedules procedure seems particularly well suited for scaling qualitatively different foods because it employs a common indicator response (e.g., key pecking) for both foods. In this way these schedules literally make it feasible to undertake the proverbial comparison of apples and oranges. Such comparisons are fraught with difficulties when the ingestional method is used because qualitatively different foods often evoke

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greatly different ingestional behavior, thereby complicating comparisons of the foods.

## Conclusion

In this brief review of the psychobiological approach to food acceptance in animals we have attempted to provide both a warning and a temptation to those who develop animal foods. While there is nothing new in the warning that the variables influencing food acceptance in animals are complex, perhaps you have found something new in our emphasis on the behavioral variables in food acceptance tests. Certainly, variables such as the length of the test period, the number of alternative foods available, and the animals' past history are important determinants of ingestive behavior. Likewise, in the instrumental method, the type of reinforcement schedule, the number of schedules concurrently available, and the change-over delay in concurrent schedules procedures importantly influence instrumental responding. Since one's inferences about a food's acceptability are determined both by the behavior we observe in our test situation and our knowledge of the variables which control that behavior, it seems important that these behavioral variables be given their due, along with the physiological variables, when tests for food acceptability are designed.

With regard to temptation, we hope that our limited remarks will tempt you to bite more fully into the "fruit of knowledge" which is the large experimental and theoretical literature on the psychobiological approach to food acceptance in animals.

## Literature Cited

1. Pfaffmann, C.; Fisher, G. and Frank, M. K. In "Olfaction and Taste II", T. Hayashi, Editor, 361-382, Pergamon Press, Oxford, (1976).
2. Young, P. T. Psychological Review, (1966) 73 59-86.
3. Horowitz, G. P.; Whitney, G.; Smith, J. C. and Stephan, F.K. Psychopharmacology (1977) 52 119-122.
4. Beck, R. C.; Nash, R.; Viernstein, L. and Gordon, L. Journal of Comparative and Physiological Psychology (1972) 78 40-50.
5. McLeary, R. A. Journal of Comparative and Physiological Psychology (1953) 46 411-421.
6. Puerto, A.; Deutsch, J. A.; Molina, F. and Roll, P. L. Science (1976) 192 485-487.
7. Valenstein, E. S. and Weber, M. L. Journal of Comparative and Physiological Psychology (1965) 60 443-446.
8. Kare, M. R. In "Olfaction and Taste III", Carl Pfaffmann, Editor, 586-592, Rockefeller University Press, N.Y. City, (1969).
9. Collier, G. and Bolles, R. Journal of Comparative and Physiological Psychology (1968) 65 379-383.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

10. Valenstein, E. S.; Cox, J. W. and Kakolewski, J. W. Science (1967) 157 552-554.
11. Smith, J. C.; Williams, D. P. and Jue, S. S. Science (1976) 191 304-305.
12. DeWys, W. D. and Walters, K. Cancer (1975) 36 1888-1896.
13. Bradley, R. M. Doctoral Dissertation, Florida State University, Tallahassee, Florida 32306 (1970).
14. Barnett, S. A. "The Rat", Aldine Publishing Company, Chicago Ill. (1963).
15. Carroll, M. E.; Dinc, H. I.; Levy, C. J. and Smith, J. C. Journal of Comparative and Physiological Psychology (1975) 89 457-467.
16. Garcia, J.; Kimeldorf, D. J. and Koelling, R. A. Science (1955) 122 157-158.
17. Gamzu, E. In "Learning Mechanisms in Food Selection", Barker, L. M.; Best, M. R. and Domjan, M., Editors, 477-502. Baylor University Press, Waco, Texas (1977).
18. Young, P. T. Psychological Review (1968) 75 222-241.
19. Stellar, E. and McCleary, R. A. American Psychologist (1952) 7 256.
20. Collier, G. and Bolles, R. Journal of Comparative and Physiological Psychology (1968) 65 379-383.
21. Richter, C. P. and Campbell, K. E. H. Journal of Nutrition (1940) 20 31-46.
22. Hagstrom E. C. and Pfaffmann, C. Journal of Comparative and Physiological Psychology (1959) 52 259-262.
23. Young, P. T. and Greene, J. T. Journal of Comparative and Physiological Psychology (1953) 46 288-295.
24. Cohen, P. S. and Tokieda, F. K. Journal of Comparative and Physiological Psychology (1972) 79 254-258.
25. Cohen, P. S.; Wier, J. and Granat, M. B. Physiology and Behavior (1975) 14 383-386.
26. Fuller, J. L. Journal of Comparative and Physiological Psychology (1964) 57 85-88.
27. Thorndike, E. L. Psychological Monographs (1898) 2 (4, Whole No. 8).
28. Kimble, G. A. "Hilgard and Marquis' Conditioning and Learning 2 Edition", Appleton-Century-Crofts, New York (1961).
29. Mackintosh, N. J. "The Psychology of Animal Learning". Academic Press, London (1974).
30. Skinner, B. F. In "Psychology: A Study of a Science, Vol. 2" S. Koch, Editor, 359-379, McGraw-Hill, New York (1959).
31. Collier, G.; Hirsch, E.; and Kanarek, R. In "Handbook of Operant Behavior". W. K. Honig and J. E. R. Staddon, Editors, 28-52 Prentice-Hall Inc., Englewood Cliffs, N.J. (1977).
32. Ferster, C. B. and Skinner, B. F. "Schedules of Reinforcement". Appleton-Century-Crofts, New York (1957).
33. Collier, G. and Myers, L. Journal of Experimental Psychology (1961) 61 57-66.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

34. deVilliers, P. In "Handbook of Operant Behavior", W. K. Honig and J. E. R. Staddon, Editors, 233-287, Prentice-Hall Inc., Englewood Cliffs, N. J. (1977).
35. Herrnstein, R. J. Journal of the Experimental Analysis of Behavior (1961) 4 243-266.
36. Herrnstein, R. J. Journal of the Experimental Analysis of Behavior (1970) 13 243-266.
37. Rachlin, H. "Behavior and Learning", W. H. Freeman & Co., San Francisco (1976).
38. Fantino, E. and Navarick D. In "The Psychology of Learning and Motivation, Vol. 8". G. H. Bower, Editor, 147-185 Academic Press, N. Y. (1974).
39. Miller, H. L., Jr. Journal of the Experimental Analysis of Behavior (1976) 26 335-347.

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# 4

# Isolation, Identification, and Biological Activity Assay of Chemical Fractions from Estrus Urine Attractive to the Coyote

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Most of the sheep killed by predation in the United States are by coyotes. Ranchers or farmers who have recently quit the business of raising sheep most often blame the lack of control of coyotes as being the precipitating cause for their decision. The greatest problem of using a device or chemical to affect coyote populations is to get the attention of the coyote. Excessive numbers of chemical baits must be dropped or a great number of "Coyote Getters" must be set to reach one coyote. Treating with an effective attractant would significantly reduce the cost and work involved. Further, it is very desirable that the attractant be specific for coyotes to reduce the injury and death to non-target species such as skunks, bobcats, eagles and pet dogs.

Many attempts have been made to concoct brews or mixtures to be irresistably attractive for the coyote as he passes a dropped bait or trap. Mixtures of blood, animal organs and urine brewed or fermented in numerous ways have been used for years by ranchers and trappers with varying success. One ingredient common to many scent bait mixtures has been coyote urine. Several researchers have noted that significantly more coyotes have been caught with estrus than with non-estrus urine. This paper reports the initial efforts to isolate and identify an attractant from estrus urine which might prove specific for the coyote.

## Experimental

Estrus Urine Collection. Female coyotes were confined singly in small cages with wire bottoms below which was fitted a stainless collection tray which sloped to empty into a one pint glass canning jar. In order to reduce fecal and drinking water contamination of the collected urine, the coyotes were fed in the morning and in the afternoon all food scraps and water were removed and the cages and collection trays cleaned. The jars were then put in place and urine collected overnight. In the morning the jars of urine were collected, frozen and stored at -20°C. The coyotes were then fed and the collection cycle repeated. For the purposes



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of this study urine was considered estrus urine when collected in a period between the appearance and disappearance of vaginal bleeding.

Chemical Fractionation. The frozen urine was thawed and pooled in one 50 ℓ batch for chemical fractionation. It was made basic (pH 12) with potassium hydroxide and the basic and neutral compounds extracted with diethyl ether according to the extraction scheme outlined in Figure 1. The gel fraction separated from the coyote urine after it had been made basic and was recovered by filtration of the basic urine. The basic compounds were separated by extraction with 6N hydrochloric acid leaving the neutral compounds in the ether residue. The original ether extracted basic urine was then made acid (pH 2) and the acidic compounds extracted with diethyl ether. The acidic, basic and neutral fractions were stripped of ether solvent by distillation.

Bioassay of Urine Fractions. The degree of attraction of the estrus urine fractions, acidic, basic, neutral and urine residue, was estimated by presenting each fraction with its solvent control in a randomized sequence to each of six coyotes in replicated trials. Each coyote was released for thirty minutes into a large pen, 6m x 20m, Figure 2, containing the test urine fraction with its solvent control in separate portable scent stations on the ground and its behavior recorded by a hidden observer as it approached each scent station (1). Time at each scent station as well as behavior such as rub-rolling, chewing, sniffing, scratching and scent marking was recorded by the observer. Three male and three female coyotes were used.

Component Identification. In preparation for gas chromatography the acidic urine fraction was reacted with diazomethane produced from DIAZALD, N-methyl-N-nitroso-p-toluenesulfonamide, Aldrich Chemical Company (2). As the diazomethane was generated by the addition of potassium hydroxide, the ethereal diazomethane solution was distilled into an ice bath-cooled reaction flask which contained the urine acids fraction. The production of diazomethane was continued until a bright yellow color persisted in the reaction flask. The reaction mixture was allowed to sit in a hood overnight during which time the ice melted allowing the reaction mixture to come to room temperature (23°C). The ether solvent was then removed by distillation on a steam bath. Preliminary gas chromatographic runs were made on a Hewlett/Packard, Model 5831 A, gas chromatograph. Separation of the methylesterified components in the acidic urine fraction was made on a 0.03 in. i.d. X500 ft stainless steel open tubular column coated with methyl silicone oil, SF 96-50 (General Electric) containing 5% Igepal CO-880 (General Aniline and Film) using a flame ionization detector. After sample injection, the columns were held at 76°C for 30 min; then the oven temperature was

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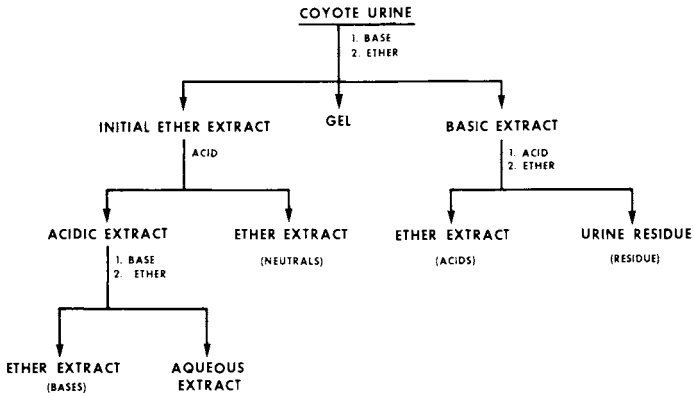


*Figure 1. Urine fractionation procedure*

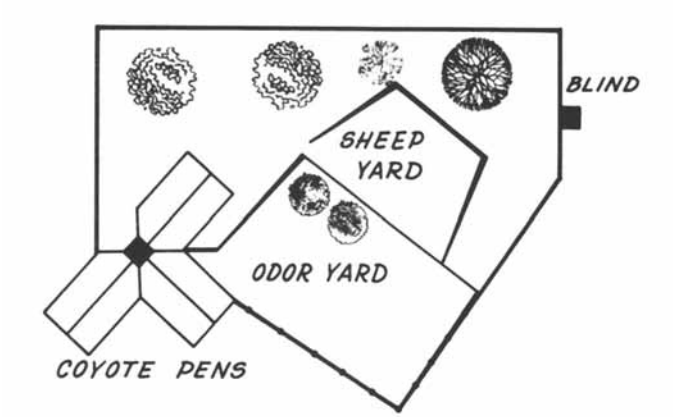


*Figure 2. Coyote test area*

programmed at 2°/min to 210°C.

Tentative identifications were made from mass spectral data obtained by coupling the effluent end of the gas chromatograph column to a quadrupole-type mass spectrometer through a single stage silicone membrane interface (Quad 300, Electronic Associates Inc.) as described by Flath, Forrey and Guadagni (3), and Flath and Forrey (4).

## Results and Discussion

An error which is difficult to avoid in the collection method described is the introduction of artifact chemical compounds into the urine during collection which show up in the later chemical analysis. Contamination of the collected urine by hair, feces, drinking water, food scraps, metabolic byproducts of microbial fermentation and air oxidation can be reduced by design of the collection procedure. To reduce chemical and microbial contamination further, urine can be collected under a thin layer of toluene in a reservoir with continuous urine flow into a refrigerated collection jar where it is rapidly frozen. Experience with human urine collection indicated little change in chemical composition of frozen urine after several months storage.

When whole undiluted coyote urine is gas chromatographed on high resolution capillary columns with sophisticated computer comparison of the recorder tracings, no significant differences can be demonstrated between estrus and non-estrus urine. Therefore, there must be an extensive reduction in the number of chemical compounds by prefractionation before identification of the individual chemical components can be attempted. One of the oldest and most common approaches for the isolation and identification of the chemical cause of an activity is the step by step chemical or physical separation of a natural products mixture while following the activity by an effective bioassay. At each step the chemist must wait before proceeding with the next separation for the results of the bioassay (often conducted by other members of a multi-discipline scientific team) to identify the chemical fraction which contains the major portion of the activity. The chemical separation, Figure 1, produced four coyote urine fractions for testing: acids, bases, neutrals and urine residue.

The design and operation of a suitable assay for the measurement of biological activity almost always poses the greater problem for the chemist rather than the various chemical separations. Because it is a part of his training and he has the necessary specialized equipment, the chemist should participate in the preparation of the test samples. He can advise as to choice of solvents, exact concentrations, volatility in transport and placement, stability to heat and oxygen of the air as well as in possible sources of contamination. But at the test pen, using test animals as variable and complex as the coyote, the chemist gives way to the specialist in wildlife biology as to animal care and

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handling, test design, accurate and objective observation and most important, the interpretation of behavior.
Figures 3 and 4 (5) illustrate that of the four test fractions, the acids fraction elicited the most time spent by the coyotes in sniffing or rub-rolling behavior. With consideration as to both total behavioral events, Figure 5, and time spent at the test odor, Figure 6, all coyotes were attracted more by the acids fraction. Comparison of rub-rolling behavior of the acids fraction with the urine residue, which is almost background, indicates that the extraction procedure removed most of the compounds responsible for this behavior. It is interesting that the acids fraction exhibits greater rub-rolling stimulation than the commercially available coyote trap scent. Both bases and neutrals fractions were lower in sniffing or rub-rolling response. Scent marking (Figure 7) and scraping (Figure 8) behavior was demonstrated by the coyotes longer in response to the bases fraction (5). This is clearly a different behavior to another fraction of the urine and is difficult to explain, other than that this fraction may contain one or more chemicals associated with territorial marking. In all cases, the coyotes paid very little more attention to the gel fraction than to the ether control or urine residue.

Pen tests have several disadvantages for testing urine. The close restraint of even a large observation pen and the necessary occasional movement of personnel undoubtedly produce differences in the behavior of penned coyotes as compared to the animal in its natural habitat. Further, pens tend to become urine saturated from scent marking from previous testing such that the urine fractions are being tested against a high background "noise level". Again test design may be used to compensate for some of the difficulties. The test coyotes should be housed and tested in a remote area free from excessive vehicle and human traffic. Some coyotes like some people are anosmic; therefore, only animals should be chosen for testing which readily respond to some standard odor of proven attractancy and fail to demonstrate significant interest in a blank test sample of low odor content. Test stations or locations of test samples should be moved about in the pen. Test samples should be presented in a randomized sequence and replicated with as many animals as permitted by the resources and test objectives of the experiment.

The chromatogram by gas-liquid chromatography, Figure 9, indicated over thirty major peaks or chemical compounds in the acids fraction of the estrus coyote urine. Mass spectral data permitted tentative identification of the methyl esters of a series of short chain fatty acids, $C_2$-$C_{10}$, together with aromatic compounds as present. Table 1 lists 19 tentative identifications of compounds in the acids fraction. Because other investigators (6) have reported that an artificial mixture of similar fatty acids demonstrated significant attraction of coyotes it will be interesting to prepare a mixture of the fatty acids identified in coyote urine in the exact ratio that they are in

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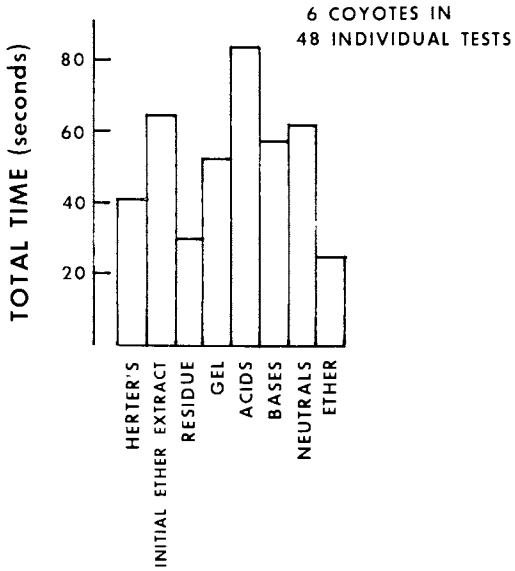


*Figure 3. Sniffing*

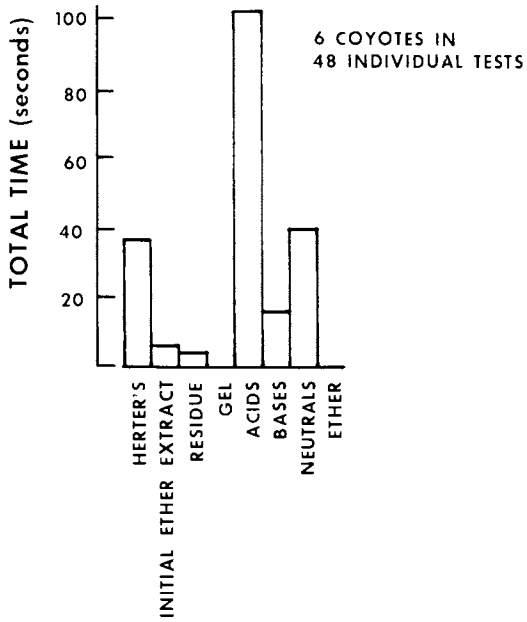


*Figure 4. Rubbing–rolling*

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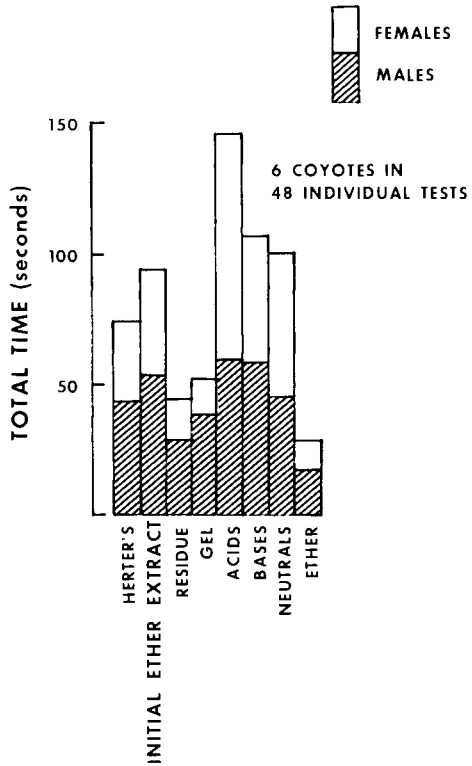


*Figure 5. Total behavioral events exhibited. Time spent at odor.*

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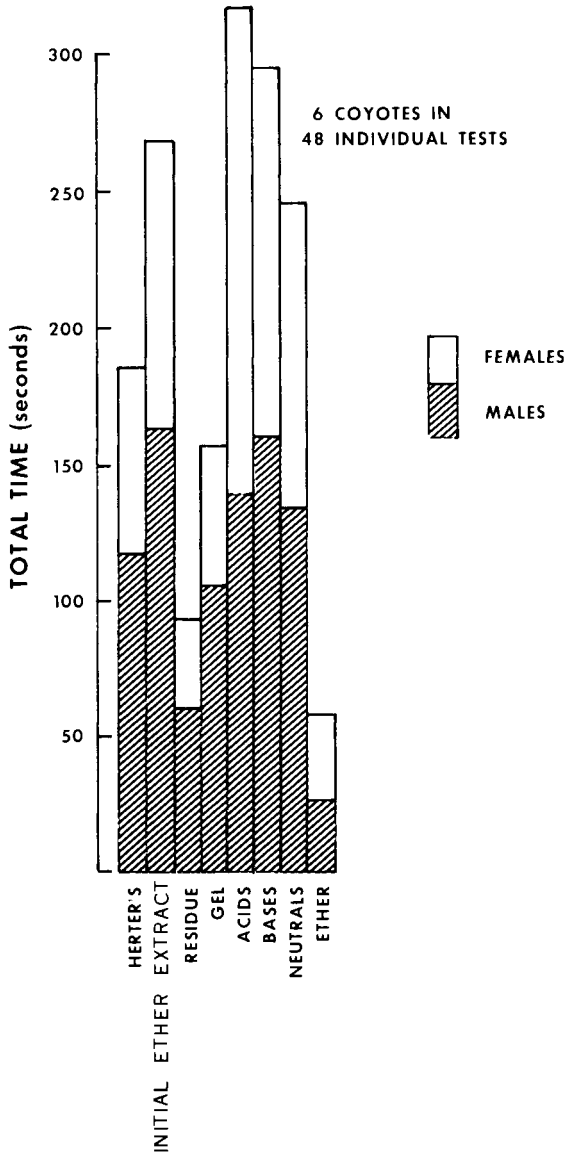


*Figure 6. Total behavioral events exhibited. Time spent at odor.*

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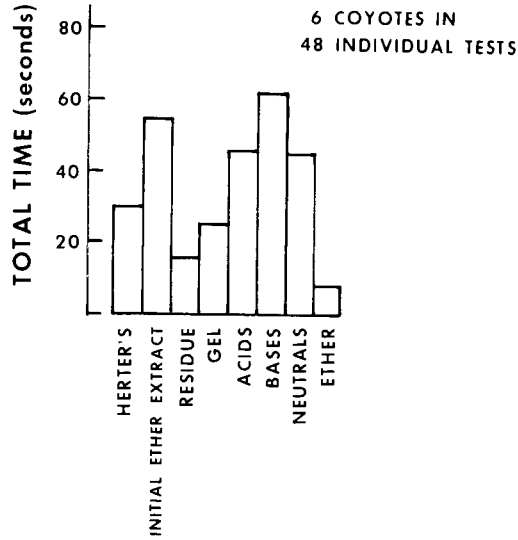


*Figure 7. Scent-marking*

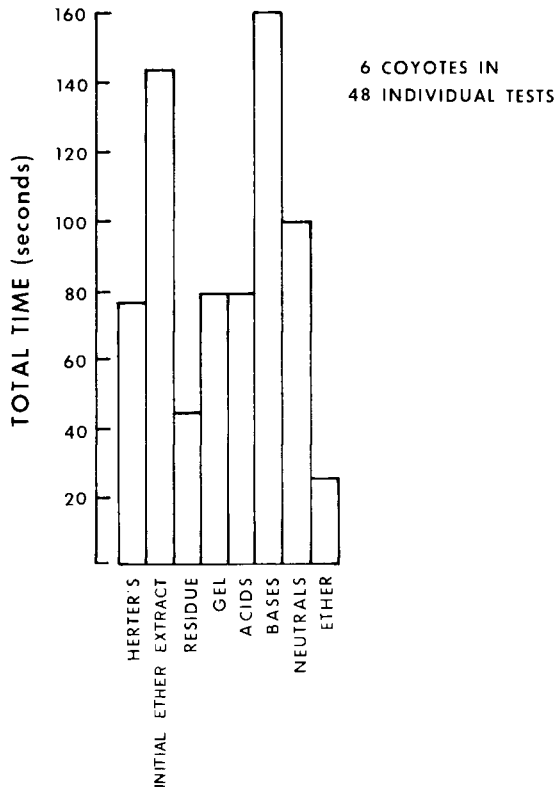


*Figure 8. Scraping*

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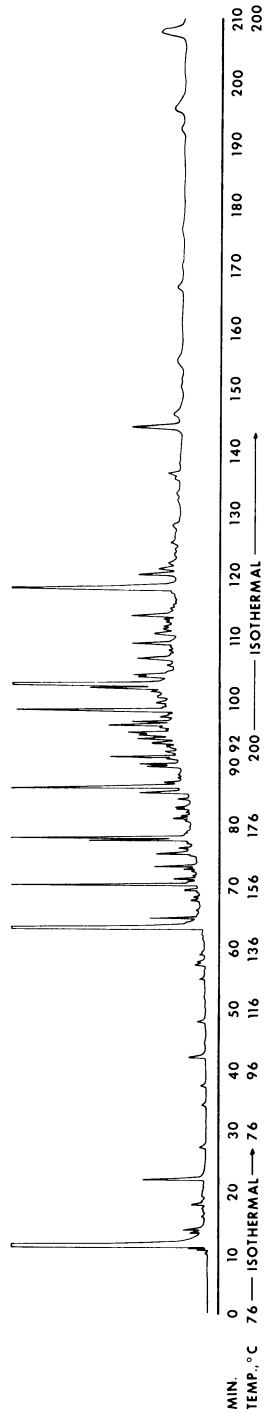


*Figure 9. Chromatogram of acids fraction*

Table I. Methyl Esters of Coyote Estrus Urine Acids*

| | |
|---|---|
| Methyl Butyrate | Methyl 2-Methylheptanoate |
| Methyl 2-Methylbutyrate | Methyl Phenylacetate |
| Methyl Pentanoate | Methyl β-Phenylproprionate |
| Methyl 4-Methylpentanoate | Methyl Nonanoate |
| Di-Methyl Pyrazine | Methyl Decenoate |
| Methyl Hexanoate | ρ-Methoxy Acetophenone |
| Methyl Heptanoate | Methyl Anthranilate |
| Acetophenone | Methyl 3-Methoxybenzoate |
| Methyl Benzoate | Methyl 4-Methoxyphenylacetate |
| Methyl Octanoate | |

*All compounds are tentative identifications from mass spectral patterns.

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estrus urine to measure not only the degree of attraction but also the specificity of the mixture for coyotes with respect to skunks, bobcats and dogs.


## Abstract

For many years coyote urine and especially estrus urine has been reported to attract coyotes when used in scent baits. Estrus urine was collected and separated chemically into acid, base, and neutral fractions and tested for degree of attractancy by presentation to pairs of coyotes. In replicated trials activity such as licking or chewing, neck-rubbing or rolling and scent marking were recorded by a hidden observer. The chemical composition of the active acid fraction was determined by gas liquid chromatography interfaced with a mass spectrograph.


## Literature Cited


1. Timm, Robert M., Connolly, Guy E., Howard, Walter E., Longhurst, William M., Teranishi, Roy, and Murphy, E.L., Sci. of Biol. J., (1975) 1, 87-91.
2. Boer, T.J. and Backer, H.J., Org. Syn., Coll. Vol. 4, John Wiley and Sons, New York, N.Y. (1963).
3. Flath, Robert A.,Forrey, Ralph R., and Guadagni, Dante G., J. Agr. Food Chem., (1973) 21, 948-952.
4. Flath, Robert A., and Forrey, Ralph R., J. Agr. Food Chem., (1977) 25, 103-109.
5. Timm, Robert M., Ph.D. Thesis, Univ. of Calif., Davis, Calif., (1977).
6. Linhart, S.B., Dasch, G.J., Roberts, J.D., and Savarie, P.J., Test Methods for Vertebrate Pest Control and Management Materials, ASTM STP 625, W.B. Jackson and R.E. Marsh, Eds., pp. 114-122. American Society for Testing and Materials, 1977.


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# 5

# Bacterial Action and Chemical Signalling in the Red Fox *(Vulpes vulpes)* and Other Mammals

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The products of microbial action play an important part in food attractancy. Man has long employed micro-organisms to contribute characteristic flavor qualities to foodstuffs (1,2,3). For various carnivores also, odors substantially of microbial origin can be attractive. The attractancy exhibited by the scent of decomposing carrion may be related to the use of odor cues to locate food in the natural environment. However, the phenomenon is complex. Inedibly rotten meat constitutes an effective lure to trap fox and, although edible carrion can provide an important food source for the red fox, highly putrefied carcasses possessing great attractancy are not consumed but instead frequently become important scent marking sites (4).

In addition to such "environmental" scent sources, microbial activity is also associated with the body surfaces of living mammals themselves. Recently it has been suggested that microbially generated odors arising from such sources could serve a communicative function in certain species. This concept has been discussed by Albone et al, 1977 (5). Although in the context of mammalian chemical communication generally, volatile substances have received major attention, involatile materials could also be important in cases where physical contact occurs(6). Examples of such contact are many and include the licking responses to vaginal secretion in the golden hamster (7), to urine in the red fox (4), and to the tarsal scent organ in the black-tailed deer (8). Further, involatile components of guinea pig urine have been implicated in sexual recognition (9), while the puberty-accelerating pheromone in male mouse urine is also thought to lack volatility (10).

The interdisciplinary study of communication by chemical signals in mammals is itself new (11, 12), while an exploration of the role of bacteria in generating such signals is only now commencing. In this paper, we offer a necessarily incomplete discussion based on the results of experiments, undertaken in a variety of disciplinary contexts, on microbially generated chemical signals of environmental and mammalian origin, with

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particular reference to the case of red fox. Such studies, while being of fundamental importance to a proper understanding of the biological function of olfactory communication, are also of potential applied importance for predator control and public health programs. An example of the latter would be in the development of effective baits for the oral administration of rabies vaccine to wild fox populations as part of an anti-rabies program (13, 61).

## Fermentative Scent Sources: I) Mammalian; the Anal Sac

In mammals, microbial odor production is most likely to result from incomplete substrate oxidation associated with anaerobic processes, the end products of aerobic catabolism being principally carbon dioxide and water. As a result, external anatomical structures such as pouches or cavities, particularly if fluid-filled, are of major importance as these hinder access of air and maintain warm, moist (or wet) conditions favoring microbial growth. The anatomical literature reveals numerous such sites, although very few have yet been studied in this context. Examples include the infra-orbital and interdigital glands of ungulates, the preputial diverticulum of the pig, the anal sacs of carnivores and the perfume pockets of viverrids (14,15). The vagina is another important fermentative scent source which has been studied in relation to chemical signalling in primates, principally by Michael and his associates (16).

It is interesting that although very few compounds have yet been identified to which a specific mammalian communicatory function can be assigned, a number of these are substances commonly occur in nature as microbial products. Among these are dimethyl disulfide, a sex attractant in hamster vaginal secretion (17) and a common product of the microbial degradation of organic matter (18,19), and phenylacetic acid and iso-valeric acid, common fermentation productions of amino-acids (20) which have been identified also in the ventral gland of the Mongolian gerbil (21) and in the subauricular gland of the pronghorn (22) respectively. In none of these cases does the possibility of a microbial origin appear to have been investigated, however.

The anal sac constitutes an important fermentative scent source in the carnivores. In the red fox, the two anal sacs form reservoirs of about 1 ml capacity situated laterally to the anus between the internal and the external anal sphincter muscles. Each sac opens to the inner cutaneous anal region through a short duct. Inputs to these reservoirs are the secretions of the glands of the sac walls and desquamated cells from the sac epidermis.

Histology reveals that the fox anal sac tissues resemble those of the dog in containing mainly coiled, tubular, apocrine glands, sebaceous glands being largely confined to the walls of the ducts (23,24). In contrast, the anal sac walls of the cat,

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lion and tiger contain plaques of sebaceous tissue so that these species produce lipid-rich anal sac secretions (25,26), while the anal sac secretions of such mustelids as the striped skunk, mink and polecat differ further in containing substantial quantities of volatile organo-sulfur compounds (27,28). Our observations on mink anal sac tissue using electron microscope techniques suggest that, in this species, these substances probably derive from sulfur-rich granules present in the numerous apocrine glands, which together with sebaceous tissue, make up a glandular complex around the neck of the sac (Albone, Flood, Heap and Zinkevich, in preparation).

A further fact about the anal sac of the red fox (and very probably of other carnivores) is that it supports an abundant microflora (29,30,31). This means that all inputs to the sac from its walls are subjected to the activities of a microbial ecosystem which substantially determines the nature of the secretion finally voided by the fox. Only certain types of micro-organism have been detected in the fox anal sac in substantial numbers. This, together with the degree of uniformity of the aerobic microflora at the genus level observed from fox to fox suggests a structured anal sac micro-ecosystem.

The results presented in Table I reveal that the aerobic sac microflora is dominated by streptococci and *Proteus* spp., while other organisms, such as coliforms and gut lactobacilli are largely absent, even though the sac is sufficiently close to the anus to permit passage of fecal flora into the sac. Staphylococci, common skin micro-organisms in many animals, were not found in the sac. Of 96 anal sac secretion samples from 28 foxes examined, *both Proteus* spp. *and* streptococci were abundant ($> 10^6$ organisms/ml) in 49 samples, and *either Proteus* spp. *or* streptococci were abundant in 43 samples. Only in 4 samples were neither genera abundant. Further, each genus was represented predominantly by one species (*S. faecalis* and *P. mirabilis*). Streptococci were detected in 89 samples (*Proteus* spp in 87) and of these, in 57 samples, streptococci were represented by a single species (80 samples for *Proteus*) and in 31 by two species (7 samples for *Proteus*). There were no obvious correlations in the aerobic microflora at the species level with season, age group, sex or identity of the foxes samples.

The selective nature of the anal sac microenvironment was revealed by the failure of a number of attempts to establish *Escherichia coli* isolated from fox feces as a resident species in an anal sac. For example, *E.coli* injected into a fox anal sac to give an initial population of $8 \times 10^9$ organisms/ml were reduced to 12% of this level in 2 days, to 3% in 4 days and were undetectable after 15 days, while streptococci and *Proteus* spp. in the same sample maintained populations in the ranges $10^8$-$10^{10}$ and $10^6$-$10^9$ organisms/ml respectively. Similarly, a population of *E.coli* ($1.8 \times 10^6$ organisms in 50 µl) incubated anaerobically *in vitro* in 250 µl unsterilized anal sac secretion was eliminated after

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24 hours.

This effect is also reflected by a comparison of anal sac and fecal microflora. Of 18 fecal samples taken from 9 foxes, streptococci were detected in 17 and were abundant ($> 10^6$ organisms/ml) in 12, while Proteus spp. were observed in only 7 samples and were abundant in none and, conversely, E.coli were observed in 14 samples and were abundant in 14. (Compare Table I).

Table I. Occurrence of aerobic bacteria (facultative anaerobes) in red fox anal sac secretions (29,30,31)

| Organism | 96 Samples from 28 foxes examined | |
|---|---|---|
| | Detected (no.samples) | Abundant[a] (no. samples) |
| Streptococcus faecalis | 83 | 60 |
| Streptococcus faecium | 22 | 6 |
| Streptococcus uberis | 9 | 1 |
| Streptococcus mitis | 8 | 2 |
| Proteus vulgaris | 4 | 1 |
| Proteus mirabilis | 81 | 71 |
| Proteus rettgeri | 9 | 2 |
| Escherichia coli | 26 | 1 |
| Klebsiella spp./other coliforms | 13 | 0 |
| Serratia spp. | 5 | 0 |
| Micrococcus spp. | 5 | 0 |
| Staphylococcus spp. | 10 | 0 |
| Neisseria spp. | 1 | 0 |
| Bacillus spp. | 13 | 0 |
| Diplococcus spp. | 6 | 0 |
| Lactobacillus spp. | 6[b] | 0[b] |

(a) $> 10^6$ organisms/ml

(b) Lactobacilli were sought in 37 samples from 15 foxes only. Lactobacilli were only detected in foxes less than 3 months old (4 out of 6 foxes in this age group)

Fox anal sac secretion samples were also examined anaerobically using pre-reduced, anaerobically sterilized media (32), taking rigid precautions to avoid exposure to the air. Anaerobes were classified by obtaining API 20A biochemical profiles (Analytab Products Inc.) and employing single linkage cluster analysis (33) to group similar organisms with selected API 20A reference species (31). Some 127 different anaerobic isolates from 66 secretions from 18 foxes yielded predominantly clostridia (Table II, column A). An examination of 107 further isolates from 37 secretions from 6 foxes, collected under the most rigorous anaerobic conditions and employing a specially designed container to exclude air and maintain a carbon dioxide environment around

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the hind-quarters of the sedated fox during sample collection, revealed the presence of other, more oxygen-sensitive anaerobes (Table II, column B). Anaerobic population determinations are subject to error because of the uncertainty of percentage recoverability in primary culture. From microscopic counts, strict anaerobic populations were estimated to be comparable with those of aerobes, frequently in the range of $10^9$-$10^{10}$ organisms/ml secretion.

Table II. Occurrence of strict anaerobes in red fox anal sac secretions (31). Isolates clustering at similarity coefficient 0.75 on the basis of API 20A biochemical profiles, morphology and Gram stain reaction (see text)

| Reference organism | A[(a)] (no.isolates) | B[(b)] (no.isolates) |
|---|---|---|
| Clostridium perfringens | 50 | 37 |
| Clostridium ramosum | 16 | 0 |
| Clostridium sporogenes | 27 | 4 |
| Clostridium bifermentans | 21 | 1 |
| Eubacterium lentum | 13 | 4 |
| Bifidobacterium eriksonii | 0 | 2 |
| Fusobacterium nucleatum | 0 | 6 |
| Fusobacterium negrogenes | 0 | 17 |
| Bacteroides fragilis fragilis | 0 | 20 |
| Peptostreptococcus anaerobius | 0 | 7 |
| unclustered | 0 | 9[(c)] |

(a) From 66 secretions from 18 foxes, employing anaerobic techniques.

(b) From 37 secretions from 6 foxes, employing anaerobic techniques including an air-free sampling environment, see text.

(c) Clustering at lower similarity with Bifidobacterium eriksonii (6) and Peptostreptococcus anaerobius (3).

Preliminary observations indicate that the fox anal sac may generate highly reducing conditions, with redox potentials down to -400mV. A redox potential of -200mV has been recorded in the cecum of the mouse (34). In the anal sac, it appears that facultative anaerobic organisms, such as streptococci and Proteus spp. create and maintain an anaerobic environment in which strict anaerobes (frequently active odor producers) can grow.

In accord with this, chemical studies so far undertaken on the anal sac secretions of the red fox and other carnivores indicate that major low molecular weight components present are generally commonly encountered products of microbial activity. Volatile fatty acids are major constituents of the anal sac secretions of the red fox and the lion (29,35), the coyote (36),

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the domestic dog (36), and the mink (37). They are also present in the anal pocket secretion of the Indian mongoose (38) and in the perineal gland secretion of the guinea pig (39). In the cases of the red fox and of the Indian mongoose, evidence has been advanced to confirm their microbial origin. These same acids occur in a variety of other mammalian scent sources, including the vagina of primates, where a microbial origin has also been substantiated and a communicatory role discussed (40), as well as in the vaginal fluids of other species, such as mink (41). Thus, in the case of fox anal sac secretion, an inoculum of fresh anal sac secretion (100 µl) collected anaerobically and incubated anaerobically in pre-reduced Robertson's cooked meat medium (10 ml) at 37°C for 48 hours yielded volatile fatty acids at concentrations (acetic acid, 170 mM; total $C_3$-$C_6$ acids, 95 mM, by gas chromatography) comparable with, or in excess of, those encountered in anal sac secretions. 152 strictly anaerobic bacterial isolates from 78 anal sac secretions from 19 foxes were examined for volatile fatty acid production under similar conditions. Anaerobes of all genera produced some or all the volatile fatty acids noted in anal sac secretion, certain clostridia and eubacteria being the most active producers, although great variations in production were noted between different isolates from the same genus.

In the sac itself, microbial production of volatile fatty acids was confirmed by irrigating the sac with 1% aqueous sodium hypochlorite (containing 16.5% sodium chloride), followed with physiological saline and then by filling the sac with antibiotic (10% ampicillin/0.5% tetracyclin in physiological saline). Neither bacteria nor volatile fatty acids were detected in the sac for in excess of 3 days subsequently.

Other anal sac constituents which are commonly encountered products of microbial activity include trimethylamine, noted in the anal sac secretions of the red fox (42), coyote and domestic dog (36), and the aromatic acids, phenylacetic acid and 3-phenylpropionic acid (and related phenolic acids), together with the diamines putrescine and cadaverine as well as ammonia in the anal sac secretions of the red fox and the lion (25,29,35). Indole has also been noted. The lower molecular weight lipids of lion anal sac secretion include many substances expected as hydrolysis products of sebaceous lipids (25). Red fox anal sac secretion also exhibits an anomalous free amino-acid composition with 5-aminovaleric acid predominating (43). The possibility that the sulfur-containing volatiles present in mustelid anal sac secretions are of microbial origin is at present under investigation in our laboratories.

## Fermentative Scent Sources: II) Environmental; Carrion

Carrion, which may be detected at least in part by odor cues, possesses considerable attraction for carnivores, whether as a

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food source or as a site for scent marking. Microbial putrefaction arises in the carcass of a dead animal largely as the result of bacterial penetration of the gut after residual tissue antimicrobial activity has been lost and when anoxic conditions have been established as the result of continued tissue metabolism in the absence of oxygen. Studies in this area are few and have been undertaken principally in the context of forensic science. A valuable review of this work is provided by Corry (44), who reports that, although many different anaerobes reside in the intestine, only a few groups have been implicated so far as major colonizers of corpses during putrefaction. The most important of these is Clostridium perfringens, a vigorous saccharolytic, lipolytic and proteolytic organism which is also commonly resident in the fox anal sac. In addition, volatiles of the type identified in anal sac secretions are commonly produced when gut microorganisms are incubated anaerobically in protein-rich media. Thus, the low molecular weight metabolites produced by clostridia include 5-aminovaleric acid, the volatile fatty acids and the amines identified in anal sac secretions (45,46,47).

Putrefied animal matter has formed the basis for coyote attractants of possible value in pest control programs. Thus, a putrefied fish formulation has been used as a coyote lure and, more recently, attention has been directed to a fermented aqueous suspension of chicken whole-egg powder, developed initially as an attractant for flies (48). The odor components of this material have been subjected to detailed chemical analysis by Bullard et al. (49) and are reported to include volatile fatty acids (77% total; 13 acids identified), bases (13% total, mainly trimethylamine, 9 amines identified), and headspace volatiles, including esters, aldehydes, ketones, alcohols, alkyl aromatics, terpenes and sulfur compounds (10% total, 76 compounds identified). Based on these data, a synthetic mixture, "synthetic fermented egg" has been formulated, composed largely of a mixture of ten volatile fatty acids (81%), together with a diverse range of amines and other compounds (50). This mixture was found to be as attractive to coyotes as the fermented preparation itself. The volatile fatty acid component alone was found to exhibit substantial coyote attractancy also (50,51).

As a further example of the attractancy of microbial products an unfortunate case is quoted in the medical literature (52) of a woman suffering from a skin condition which, as the result of secondary infection, emitted obnoxious odors which caused her to be molested by dogs. In a different context, Amoore and Forrester (53) quoted Linnaeus' observation that the domestic dog is attracted by the odor of the plant Chenopodium vulvaria, the leaves of which are rich (400 ppm) in trimethylamine.

## Mammalian and Environmental Fermentative Scent Sources Compared

Even on the basis of present limited knowledge, chemical and

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microbiological similarities are emerging for the red fox between a mammalian scent source, the anal sac, and an important environmental scent source, carrion. This is not to say that these scents are not distinguishable to the fox. Fundamental differences remain to the extent that the anal sac fermentation is controlled and limited by the living mammal. The observation of structure in the anal sac micro-ecosystem seems to suggest the operation of selective pressures and so to imply the existence of specific biological functions of the anal sac of adaptive value for the fox.

Superimposed on the general attractancy which fermentative scent sources exert for canids, the mammal can convey more specific information to the degree that the anal sac scent source responds to the physiological and social circumstances of the mammal. Although very little research has yet been conducted on any such scent source in this context, two suggestive examples of such processes in other species have been documented. Thus, through hormonal regulation of substrate availability, the rhesus monkey is able to regulate the production of volatile compounds by its vaginal microflora so that microbial scent production from that source reflects the reproductive state of the monkey (16). And, in the rat, maternal pheromone, the scent which attracts the pups to the mother, arises from the action of bacteria on food in the cecum. This is regulated by high prolactin levels which stimulate the lactating rat to high food and water consumption and lead to the production and emission of excess cecotrophe, thus providing the chemical signal (54).

The possibility of mammalian fermentative scent sources acquiring individual or group recognition value has also been discussed in relation to the red fox and the Indian mongoose, although the experimental evidence at present available in support of this occurring in these species is not strong (5,35,55).

The signalling function(s) of anal sac secretion in the life of the fox in its natural environment remain(s) unclear, reflecting the paucity of information which exists on the chemical ecology of wild mammalian species. As with urine, it probably conveys a variety of messages in a variety of circumstances (56). Preliminary field observations (4) indicate that the secretion may be deposited with feces or that it may be emitted without defecation when the fox is alarmed. Anal sac evacuation has been observed at territorial boundaries following eviction of an intruder. However, an exhaustive analysis of the signalling role of this secretion has yet to be undertaken. Our laboratory studies on the behavioral response of the red fox to anal sac secretion have, like those of Doty and Dunbar with beagle anal sac secretion (57), proved of limited value. This is not unexpected when the gross nature of the responses assessed (frequency and duration of investigation) in artificial surroundings are compared with the complexity of the relationship of a wild mammal to its natural environment. Ideally, one would investigate the

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detailed effect of chemical signals on the entire behavioral repertoire of wild animals. Field studies on the significance of scent marks in the life of wild mammals remain few. Notable examples are those of Peters and Mech on the wolf (58) and of Henry on the red fox (56).

## Behavioral studies

Basing our methods on those employed by Linhart and Knowlton (59) using fermented egg product as a coyote attractant, we have studied the response of the fox to fermented products related in some measure to anal sac secretion. A detailed account of this work will be published shortly (Macdonald, Hough, Blizard and Perry, in preparation).

Fermented egg product is produced by exposing an aqueous suspension of chicken whole-egg powder to the air at room temperature for 7 to 14 days. The nutrient is colonized by micro-organisms from the air, and these bring about fermentation. As well as being uncontrolled, this method of inoculation excludes strict anaerobes which are among the most effective odor producers. In our initial experiments, we have compared the attractancy of a fox tissue extract (FE), incubated anaerobically with an inoculum of fresh, anaerobically collected anal sac secretion,(FEI), with (a) a fox tissue extract control, not so inoculated and incubated (FEC); (b) red fox (♀♀) urine; (c) distilled water.

FEI possesses the advantages of being readily prepared in bulk for field testing while mirroring, probably more closely than other readily available fermentations, features of fox anal sac secretions. The fox tissue extract medium was prepared by simmering fresh red fox meat (1.2 kg hind leg muscle/liver) with water for one hour, draining the extract, adjusting its pH to 8.2 and filtering. To 1000 ml of the resulting liquid, 20 ml cysteine/sulfide solution (1.5 g L-cysteine hydrochloride/1.5 g $Na_2S.9H_2O$ in 100 ml aqueous solution) were added followed by 1 g L-methionine, 5 g NaCl and 10 g Difco Bacto-peptone to yield the final fox tissue extract medium. Incubations were conducted at $37^{\circ}$ for 60 hours.

Anaerobic incubations similarly prepared from other media using anal sac inocula, were also tested. These media were Robertson's meat broth (RM), casein acid hydrolysate medium (CHY) and an egg yolk medium (EY) (fresh egg yolk/glucose/Difco brain-heart infusion broth; 5/2/100 w/w/w). After incubation, all fermentations were stopped by the addition of excess solid sodium chloride, and maintained at $4^{\circ}C$ until required for testing.

Tests were conducted in a small deciduous woodland where the movements of many of the resident foxes had earlier been established by radio-tracking (4). Probably at least eight adult foxes (2 male, 6 female) were using the experimental area nightly. From direct observations and field signs, the most heavily used

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fox paths through the woodland were identified and these were used in the subsequent trials.

Vegetation was cleared from two circular areas (60 cm diameter) on opposite sides of, and adjoining, the selected fox path. This process was repeated at intervals of 5 m until six pairs of such circles were prepared. In each case, leaf litter was removed and the top soil finely sifted until it formed a sufficiently deep layer to reliably detect individual footprints. In addition, further sifted areas were prepared spanning the path between each circle pair.

In the late afternoon of the first experimental day (April), a small cane bearing a circular filter paper (4.25 cm) was positioned in the center of each earthen circle, standing at a height of some 15 cm and an aliquot (1 ml) of a given odorant was dropped on to the filter paper. On each subsequent day at a similar time the earthen circles were examined for footprints, the canes were replaced by cleaned canes bearing fresh filter paper and a new odorant, and the soil carefully sifted again. The distribution of odorant along the path (spacial density and location on visually conspicuous objects) resembled the distribution of scent (urine) marks commonly produced by a fox in this environment. Each experiment compared four odorants in each of the six possible binary combinations, one combination for each circle pair. Odorants were thus tested in pairs, randomizing the sequence of presentation of each pair along the path from night to night. The experiment was continued until every paired combination of odors had been exposed to foxes for 10 nights, discounting nights when an absence of tracks on the sifted sections between the circles revealed that no foxes had followed that path that night. Thus, the six possible pairs of four odorants were compared 10 times. A discussion of the statistical analysis of paired comparisons of this type has been given by Brown (60).

In the first experiment, subsequently repeated in triplicate with similar results, fox tissue extract incubation (FEI), fox tissue extract control (FEC), fox urine and water were compared. FEI was found to elicit a significantly greater response than either fox urine or the two controls. This is of considerable interest as qualitative observations on fox behavior have already indicated the great attractancy of alien fox urine (4).

This result was indicated in two ways. First, a simple nominal ranking based on the number of nights odorants were visited over the entire experimental sequence indicated that FEI was visited on significantly more nights than FEC or water, and that urine was visited on significantly more nights than water. Thus, in a typical experimental sequence, FEI was visited on 21 nights; urine, 16; FEC, 11; water, 7 (maximum score, 30). These differences were not random ($\chi^2$, 8.05; df, 3; $p < 0.05$).

In order to rank the attractancy on an ordinal scale, a scaling factor based on the footprint count within 30 cm of an odorant was employed. Scaled mean scores were obtained for each

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odorant on each night using a matrix of difference scores for each odor (60). Overall scaled mean scores for each odorant (the means of the nightly scaled mean scores, taken over the ten nights) are given in Table III. Randomized block analysis of variance showed that in spite of variations between individual nights, differences between scores were statistically significant.

Table III. Attractancy to odorants (see text)

| Experiment | Overall scaled mean scores for odorant | | | | Randomized block analysis of variance | | |
|---|---|---|---|---|---|---|---|
| | FEI | urine | FEC | water | MS | error | F[a] |
| Red fox; footprint number | 1.55 | 0.38 | -0.6 | -1.3 | 15.41 | 1.73 | 8.91 |
| Ferret; frequency | 0.53 | 1.35 | -1.05 | -0.83 | 12.94 | 1.39 | 9.28 |
| Ferret; total duration (sec) | 3.45 | 14.75 | -9.15 | -9.05 | 1316.97 | 38.23 | 34.45 |

(a) $F[E_{\alpha}]_{3,27} = 3.51$ ($p = 0.01$)

The analysis of the significance of differences between pairs of scaled mean scores presents serious statistical problems which have been overcome using Brown's method (60). For a full discussion, see Macdonald, Hough, Blizard and Perry, in preparation. The results allow the following preference rank to be concluded

FEI > fox urine > FEC = water

The measure of attractancy used was very crude and, in order to begin to clarify the species significance of these results, we examined the responses of a ferret, Mustela furo, to these substances. Odorants were presented to a male ferret in pairs (randomized position and order during 60 (10 minute) trials. Aliquots (1 ml) of each odorant were presented on two blocks covered with filter paper. Trials were continued until ten replicates of each of the six binary combinations of the four odorants had been performed. Duration and frequency of odorant investigation were noted.

The ferret showed considerable interest in the blocks bearing FEI and fox urine, relative to those bearing FEC and water, repeatedly visiting these blocks during each trial and slithering across them, sometimes urinating and probably also leaving glandular secretions. The overall scaled mean scores of both the variables measured are given in Table III. The results of analyses of variance of both these measures indicates highly significant differences between these scores ($p < 0.001$).

Thus, as with the field trials with the fox, fox urine and FEI were preferred to FEC and water, but the ranking was different.

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An analysis as previously gives the following preference rank based on frequency of investigation.

Fox urine > FEI > FEC = water

Although these findings are preliminary and further studies are currently in progress, they do indicate that FEI is very attractive to foxes (although they do not indicate the functional significance of this attraction) and that this attraction arises as the result of anaerobic incubation. The difference in ranking noted in the experiment with the ferret, if confirmed by more extensive testing, would indicate that the attractancy has a degree of species specificity and this, in turn, would open the way to further investigations of both academic and applied importance on the biological functions of scent signals. In this context, it is of interest to note that similar preliminary field studies using anaerobic incubation and unincubated controls of the other media (RM, CHY, EY) have revealed only weak fox attractancy compared with the fox tissue extract incubation. Is this because the fox tissue extract incubation possesses some feature in common with incubations occurring in the foxes' own anal sac? These phenomena require further confirmation and clarification. However, laboratory tests so far undertaken with four adult male foxes using an olfactometer to measure frequency and duration of visits to odor ports are broadly in accord with these field trials (Macdonald, Hough, Blizard and Perry, in preparation).

## Literature Cited

1. Pederson,C.S., "Microbiology of Food Fermentations," AVI Publishing Company, Inc., Westport, Conn., 1971.
2. Margalith,P. and Schwartz,Y., ADV. APPL. MICROBIOL. (1970) 12, 35-88.
3. Webb,A.D. and Muller,C.J., ADV. APPL. MICROBIOL. (1972) 15, 75-146.
4. Macdonald,D.W., D.Phil. Thesis, Oxford University, U.K. 1977.
5. Albone,E.S., Gosden,P.E. and Ware,G.C. "Chemical Signals in Vertebrates," eds. D.Müller-Schwarze and M.M.Mozell, pp.35-43, Plenum, New York, 1977.
6. Mykytowycz,R., "Communication by Chemical Signals," eds. J.W.Johnston, D.G.Moulton and A.Turk. pp.327-360, Appleton-Century-Crofts, New York, 1970.
7. Johnston,R.E. BEHAV. BIOL. (1974) 12, 111-117.
8. Müller-Schwarze,D. ANIM. BEHAV. (1971) 19, 141-152.
9. Berüter,J., Beauchamp,G.K. and Muetterties,E.L. BIOCHEM. BIOPHYS. RES. COMMUN. (1973) 53, 264-271.
10. Vandenbergh,J.G., Finlayson,J.S., Dobrogosz,W.J., Dills,S.S. and Kost,T.A. BIOL. REPROD. (1976) 15, 260-265.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

11. Müller-Schwarze,D. and Mozell,M.M. (eds.) "Chemical Signals in Vertebrates," Plenum, New York, 1977.
12. Albone,E.S. CHEMISTRY IN BRITAIN (1977) 13, 92-96, 99, 112.
13. Baer,G.M., "The Natural History of Rabies," vol.2, ed. G.M.Baer, pp.261-266, Academic Press, New York, 1975.
14. Ewer,R.F., "The Carnivores," Weidenfeld and Nicholson, London, 1973.
15. Schaffer,J., "Die Hautdrüsenorgane der Säugetiere," Urban und Schwarzenberg, Berlin and Vienna, 1940.
16. Michael,R.P., Bonsall,R.W. and Zumpe,D. VITAMINS AND HORMONES, (1976) 34, 137-186.
17. Singer,A.G., Agosta,W.C., O'Connell,R.J., Pfaffmann,C., Bowen,D.V. and Field,F.H. SCIENCE (1976) 191, 948-950.
18. Miller,A., Scanlan,R.A., Lee,J.S. and Libbey,L.M. APPL. MICROBIOL. (1973) 26, 18-21.
19. Rasmussen,R.A. TELLUS (1974) 26, 254-260.
20. Hungate,R.E., "The Rumen and its Microbes," Academic Press, New York and London, 1966.
21. Thiessen,D.D., Regnier,F.E., Rice,M., Goodwin,M., Isaacks,N., and Lawson,N. SCIENCE (1974) 184, 83-85
22. Müller-Schwarze,D., Müller-Schwarze,C., Singer,A.G. and Silverstein,R.M. SCIENCE (1974) 183, 860-862.
23. Montagna,W. and Parks,H.F. ANAT. REC. (1948) 100, 297-315.
24. Spannhof,I. FORMA ET FUNCTIO (1969) 1, 26-45.
25. Albone,E.S. and Grönneberg,T.O. J. LIPID RES. (1977) 18, 474-479.
26. Greer,M.B. and Calhoun,M.L. AMER. J. VET. RES. (1966) 27, 773-781.
27. Andersen,K.K. and Bernstein,D.T. J. CHEM. ECOL. (1975) 1, 493-499.
28. Schildknecht,H., Wilz,I., Enzmann,F., Grund,N. and Ziegler,M. ANGEW. CHEM. INT. ED. ENGL. (1976) 15, 242-243.
29. Albone,E.S., Eglinton,G., Walker,J.M. and Ware,G.C. LIFE SCI. (1974) 14, 387-400.
30. Gosden,P.E. and Ware,G.C. J. APPL. BACT. (1976) 41, 271-275.
31. Gosden,P.E., Ph.D. Thesis. Bristol University, U.K. 1977.
32. Gosden,P.E. and Ware, G.C., J. APPL. BACT. (1977) 42, 77-79.
33. Sneath,P.H.A. and Sokal,R.R., "Numerical Taxonomy," W.H.Freeman & Co., San Francisco, 1973.
34. Celesk,R.A., Asano,T. and Wagner,M. PROC. SOC. EXP. BIOL. MED. (1976) 151, 260-263.
35. Albone,E.S. and Perry,G.C. J. CHEM. ECOL. (1976) 2, 101-111.
36. Preti,G., Muetterties,E.L., Furman,J.M., Kennelly,J.J. and Johns,B.E. J. CHEM. ECOL. (1976) 2, 177-186.
37. Sokolov,V.E., Chikil'din,B.S. and Zinkevich,E.P. DOKL. AKAD. NAUK. SSSR. (1975) 220, 220-222.
38. Gorman,M.L., Nedwell,D.B. and Smith,R.M. J. ZOOL. (1974) 172, 389-399.
39. Berüter,J., Beauchamp,G.K. and Muetterties,E.L. PHYSIOL. ZOOL. (1974) 47, 130-136.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

40. Michael,R.P. and Bonsall,R.W., "Chemical Signals in Vertebrates," eds. D.Müller-Schwarze and M.M.Mozell, pp. 251-271, Plenum, New York, 1977.
41. Sokolov,V.E. and Khorlina,I.M. DOKL. AKAD. NAUK. SSSR. (1976) 228, 225-227.
42. Albone,E.S. and Fox,M.W. NATURE (1971) 233, 569-570.
43. Albone,E.S., Robins,S.P. and Patel,D. COMP. BIOCHEM. PHYSIOL. (1976) 55B, 483-486.
44. Corry,J.E.L. J. APPL. BACT. in press
45. Brooks,J.B. and Moore,W.E.C. CAN. J. MICROBIOL. (1969) 15, 1433-1447.
46. Moss,C.W., Howell,R.T., Farshy,D.C., Dowell,V.R. and Brooks,J.B. CAN. J. MICROBIOL. (1970) 16, 421-425.
47. Mead,G.C. J. GEN. MICROBIOL. (1971) 67, 47-56.
48. Hwang,Y-S., Mulla,M.S. and Axelrod,H. J. AGRIC. FOOD CHEM. (1976) 24, 164-169.
49. Bullard,R.W., Leiker,T.J., Peterson,J.E. and Kilburn,S.R. J. AGRIC. FOOD CHEM. in press.
50. Bullard,R.W., Shumake,S.A., Campbell,D.L. and Turkowski,F.J. J. AGRIC. FOOD CHEM. in press.
51. Shumake,S.A., "Chemical Signals in Vertebrates," eds. D.Müller-Schwarze and M.M.Mozell, pp.357-376, Plenum, New York 1977.
52. Liddell,K. POSTGRAD. MED. J. (1976) 52, 136-138.
53. Amoore,J.E. and Forrester,L.J. J. CHEM. ECOL. (1976) 2, 49-56.
54. Leon,M, PHYSIOL. BEHAV. (1974) 13, 441-453.
55. Gorman,M.L. ANIM. BEHAV. (1976) 24, 141-145.
56. Henry,J.D. BEHAVIOUR (1977) 61, 82-106.
57. Doty,R.L. and Dunbar,I. PHYSIOL. BEHAV. (1974) 12, 825-833.
58. Peters,R.P. and Mech,L.D. AMER. SCIENTIST (1975) 63, 628-637.
59. Linhart,S.B. and Knowlton,F.F. WILDL. SOC. BULL. (1975) 3, 119-124.
60. Brown,R.E. J. COMP. PSYCH. PHYSIOL. in press
61. Macdonald,D.W. pp.90-116 and Lloyd,H.G. pp.117-133 in "Rabies; the facts." ed.C.Kaplan, Oxford University Press/Corgi Books, U.K. 1977.

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# 6

# Biochemical and Physiological Aspects of Animal Behavior: Taste and Smell

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Considerable data has accumulated on the behavior of animals to specific chemicals. The introduction of certain chemicals into the animals area will produce a predictable behavior response such as reproduction, feeding, alarm, attraction, repellence or defense. Taste and smell by animals will be the two physiological detection routes considered in this paper. An excellent review on sensory reception covers the literature up to the early seventies as Vol. 17 of the series Molecular Biology Biochemistry Biophysics (1). The editor Vinnikov and co-editors Kleinzeller, Springer and Wittman has covered the reported research on cytology, molecular mechanisms and evolution of vision, taste, smell, hearing and gravity reception. Many classical molecular biology, biochemistry and biophysical experiments are reviewed (1) (Table I).

As stated earlier, taste, chemicals that initiate a gustatory response, and smell, chemicals that function as odorant molecules have been extensively studied, first with arthropods and more recently with vertebrates. Since the development of very efficient separation techniques and high sensitive detection systems, we are no longer limited to taste panels and threshold odor detection by humans. Table I shows some examples of the behavior response as shown by certain species for a specific chemical.

This very limited list does not include migrations, rhythms and many primary secretory processes initiated by a physical or chemical stimulus. Certain migrations are initiated by chemical stimuli. Rhythms of organisms may be initiated by physical changes but secondary chemical processes are definitely involved. Glandular secretions such as salivation and many others result from chemical odorant molecules or gustatory stimuli.

## Taste

Vinnikov (1) credits Lomonosov (2) with distinguishing seven different taste sensations -- "1. acid, as in vinegar; 2. caustic, as in grain alcohol; 3. sweet, as in honey; 4. bitter, as in tar;

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Table I

Species Response to Gustatory and Olfactory Chemical Stimuli

| Response or Condition | Species | Chemicals or Substance |
|---|---|---|
| Feeding | Deer, Antelope, Cattle<br>Herbivores | Essential oils<br>Tannins |
| Reproduction | Deer<br>Rodents | Muskone<br>Certain grass odors (food supply) |
| Alarm | Deer, Antelope | Predator body odor (low molecular weight organic acids) |
| Territorial markers | Deer, Coyote, Wolf | Gland secretions |
| Defense | Skunk, Civet cat | Gland Secretions (skunk oil) |
| Attractants | Insects<br>Cats | Plant volatiles (essential oils)<br>Nepetalactone (catnip) |
| Repellants | Insects<br>Humans | Plant volatiles<br>Cadaverine, putrescine, $H_2S$ (amines) |
| Death | Mammals | Amines as above |

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5. salty, as in salt; 6. pungent, as in wild radish; 7. sour, as in unripe fruit --". With vertebrates acid, salt, sweet and bitter can be perceived with specific chemcials. Table II shows the classification of gustatory substances as made by most researchers.

Table II

Gustatory Sensitivity of Vertebrates and Some Invertebrates

| Commonly Accepted | Lomonosov Ref. 2 | Other Researchers*† |
|---|---|---|
| Sweet | Sweet (sugar) | Cooling (menthol) |
| Sour | Sour (acid) | Burning (aminoethanol) |
| Salt | Salt (salt) | Biting (vanillin)† |
| Bitter | Bitter (quinine, alkaloids) | |
| | Caustic (ethyl alcohol) | |
| | Pungent (wild radish) | |

*Personal Communication
†Reference 7

The structural organization of the gustatory receptor cells and their location in mammals is well described in Vinnikov's review. Farbman's (3) diagram shows the stages of development of the taste bud while Murray (4) shows taste buds are oval (or bulbous) in shape and their lengths and widths are described. In the 1960's researchers isolated a "sweet-sensitive" protein from taste receptor cells located in the posterior portion of porcine and bovine tongue, Dastoli and Price (5). The interaction of the sweet sensitive protein with fructose shows a straight line association to form a complex (5). Vinnikov (1) suggests more research by modern techniques is needed on the sweet-sensitive protein as the reported molecular weight of 152,000 (6) seems to be well established. It is this author's views that this may be a subunit protein if one considers the state of research on the acetylcholine receptor. Affinity chromatography may be useful in future research on receptor proteins even though homogeneity is well established for the sweet-sensitive protein. A bitter-sensitive protein has been isolated and studied by Dastoli et al. (6). This protein was from the posterior taste buds of the porcine tongue and the interaction characteristics with four bitter compounds was made.

A large number of compounds in the sweet, salt, sour and bitter class are listed by Vinnikov (1). The effects of the addition or natural presence of certain components in plants has been shown to determine feeding stimulus. Hedin et al. (7)

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recently compiled the feeding activity of flavonoid compounds (7) and many other organics for the boll weevil. Formulation of a stimulant feeding mixture was made and no single component was effective but 52 of 286 compounds bioassayed elicited substantial feeding activity. Hedin et al. have also tabulated compound, flavor and odor in their review (7).

Very recently work by Berger et al. (8) shows phenolic plant substances, naturally occurring cinnamic acid and vinylphenols inhibit reproduction in Microtus montanus. This same group of workers (9) have triggered a reproductive response in the same organism by placing fresh green wheat grass in a non-breeding population. This could be an olfactory response as well as gustatory.

McMurphy (10) observed "young forage is more palatable, more digestible, greater in percent crude protein, greater in digestible energy and consumed in greater quantities than older forages." This author would suggest this is true for most herbivores. In Oklahoma there is "Native" and "Introduced" grass species. The chemical composition of the native grasses in Central Oklahoma has been compiled by Waller et al. (11) for a period from 1947 to 1962 and provides a basis for further study on gustatory response.

An approach using modern separation techniques on grass preference by cattle is shown in Tables V through VIII. Table III describes a gas liquid column, detector, conditions and instrument used to separate and detect the compounds of Oklahoma native and introduced grasses. The preference was determined by hand clipping portions and placing the grasses in cattle dry feed lots. The grasses were at a peak lush period so taste and odor were involved.

Tables IV, V, and VI show the grass, essential oil yield (by distillation and extraction) preference by cattle and the number of compounds observed. Taste could not be separated from odor in this limited study but we do see a "low preference" for grasses high in volatile essential oils. One can speculate the nightshade plant was not consumed due to toxic compounds present. This also suggests overgrazing of pastures will give the less desirable plants an advantage.

Vinnikov (1) concludes the taste of salts depends on the cation and anion, sour or acid stimulus of the hydrogen ion, sweet and bitter on intramolecular shifts of protein receptors and gustatory substance complexes. He approaches the problem from a molecular basis of both substance and receptor. Exact behavior to taste has covered mostly feeding and reproduction in this paper and much more field and laboratory research is needed on specific animal species.

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TABLE III
PRIMARY ODORS AND EXAMPLES

| Primary Odor | Chemical Example | Substance |
|---|---|---|
| CAMPHORACEOUS | CAMPHOR | MOTH REPELLANT |
| MUSKY | PENTADECANOLACTONE | ANGELICA ROOT OIL |
| FLORAL | PHENYLETHYL METHYL ETHYL CARBINOL | ROSES |
| PEPPERMINTY | METHONE | MINT CANDY |
| ETHERAL | ETHYLENE DICHLORIDE | DRY CLEANING FLUID |
| PUNGENT | FORMIC ACID | VINEGAR |
| PUTRID | BUTYLMERCAPTAN | BAD EGG |

TABLE IV
ODOR SENSATION
Primary Odors

| Amoore et al. via Vinnikov | Hedin et al. | |
|---|---|---|
| Camphoraceous | Camphoraceous | (1,8-Cineole) |
| Pungent | Pungent | (Benzylamine) |
| Ethereal | Aromatic | (Styreneglycol) |
| Floral | Floral | (Ferulic acid) |
| Minty | Peppermint | (Methone) |
| Musky | Musky | (Cyclopentadecanone) |
| Putrid | Putrid | ($H_2S$ or Nicotine) |
| | Sweaty | (Alpha-ketobutyric acid) |

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TABLE V

COLUMN DESCRIPTION

Column 4% OV-17 on GC-Q; 24' X 1/4", Glass
Injector temp. 180° C.
Detector temp. 260° C. ($H_2$ Flame)
Column temp. 120° C. to 200° C. at 1° C./min.
Helium flow, 43 ml/min (gauge setting 70)
Instrument, Barber-Coleman, Series 5000
Sample size 3 µl of concentrated ether soln.

TABLE VI

OIL YIELD AND COMPOUNDS OBSERVED WITH SELECTED GRASSES

| Common Name | Preference | Oil Isolated mg/kg | Compounds Observed |
|---|---|---|---|
| Marestail | Low | 2297 | 55 |
| Prairie Threeawn | Low | 67 | 64 |
| Louisiana Sagewort | Low | 2730 | 51 |
| Silver Leaf Nightshade | Low | 99 | 54 |
| Piper Sudangrass | High | 30 | 63 |
| Sweet Sudangrass | Mod | 39 | 59 |
| T. E. Haygrazer | Low | 35 | 55 |
| Broomweed | Very Low | 3851 | 44 |

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TABLE VII

ESSENTIAL OIL YIELD AND COMPOUNDS OBSERVED WITH SELECTED GRASSES

| Sample Description | Preference | Oil Isolated mg / kg | Compounds Observed |
|---|---|---|---|
| Big Bluestem | High | 17 25 | 58 64 |
| Switch grass | High | 33 48 | 59 69 |
| Little Bluestem | High | 65 71 | 58 62 |
| Weeping lovegrass | Low | 81 | 55 |
| Johnson grass | Very High | 181 | 70 |
| Prairie Threeawn | Low | 67 | 64 |
| Western Ragweed | (Fair to) Low | 1524 | 56 |
| Caucasian Bluestem | "Medium" | 37 | 60 |

TABLE VIII

ESSENTIAL OIL YIELD AND COMPOUNDS OBSERVED WITH SELECTED GRASSES

| Sample Description | Preference | Oil Isoloted mg / kg | Compounds Observed |
|---|---|---|---|
| S-Blend | Low | 178 | 63 |
| M-Blend | Low | 277 | 69 |
| LL-Blend | High | 375 | 59 |
| L-Blend | High | 189 | 62 |
| I-Blend | No Data | 938 | 52 |

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Smell

Again Vinnikov (1) presents a review on this subject at the molecular level. Function of the olfactory organ is suggested as a simple diffusion of odorant molecules -- "wafted around by air currents" -- as they are volatile. Interaction with receptors in the olfactory region transmits an impulse to the central nervous system.

One important point on smell is that just as with taste molecules of different structures, often totally different molecules, have the same or different smell. An example of rose odor in reference (1) sites the work of Wright (12) where rosetone, phenylethanol, geraniol and pelargol are compounds with very different structures but the same smell. He also attributes camphor smell to camphor, chloroethane and ethyl-tert-butyl ether (12). Other research of this type would be odorant molecules of similar structure with a different olfactory response. In 1929 Braun (13) studied a series of ketones where the carbonyl moved from carbon two through carbon six of an eleven carbon ketone. The odor ranged from rue to fruity for dipentyl ketone. These observations do not directly relate to behavior as tabulated in Table I of this paper but are important on structure and smell. Table III covers a list of primary odors with examples (1,7). The listing is current but animal behavior is still not covered. Amoore (14) also lists primary odors and chemical examples with substance tabulated in Table IV. Amoore *et al*. article presents the concept of olfactory receptor sites into which molecules must fit to give the odor response. A molecule could fit one or more site(s) to show a variable odor combination (14).

Essential oils or volatile organics of plants has already been shown to affect feeding, choice of food and reproduction. There is no question that organic volatiles are repellents, attractants, alarm, defense and other behavior stimuli for animals. The word "pheremone" indicates a chemical that elicits a specific animal response through olfactory stimulus (14, 15). The feline attractant, nepetalactone, has been thoroughly studied as to structure, occurrence and metabolism by Waller and co-workers (16). The biological active component is a bicyclic monoterpene lactone found in *Nepeta cataria* with the common name catnip (17).

An alarm substance has been found by the same group for ants (18). The iridolactones could be classed as defense chemicals for this society insect and is structurally related to catnip. Another ant uses 6-methyl-5-hepten-2-one. Field observers are well aware of alarm substances from predators that are airborne organics. Low molecular weight organic acids (sweaty), aldehydes, ketones, alcohols and amines could serve.

Many attractants have been reported for insects and would be difficult to distinguish from territorial markers (muskone-musk

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deer and civetone for civet cats). Reproduction, attractants and territorial markers become one in these cases. It is also of interest that the putrid amines of dead animals become feeding attractants for bear, raccoons, opossum and perhaps buzzards. Carrion beetles are attracted to dead animals.

Again Vinnikov (1) has thoroughly reviewed the physiological structure of the olfactory system in mammals and arthropods. In conclusion we must say the olfactory system ranks above gustatory stimuli in animal behavior. This author will close with apologies to the many researchers whose reports were not included in this brief review. Other participants in this symposium will cover much of this work.

## Literature Cited

(1) Vinnikov, Ya. V., Molecular Biology Biochemistry and Biophysics, Vol. 17. Sensory Reception, Springer-Verlag, New York, 1974 Chapters V and VI.
(2) Lomonosov, --via reference (1) 1752-1757 Lecture notes.
(3) Farbman, A. I., Developm. Biol. 11, 110 (1965a).
(4) Murray, R. G. and Murray, A., Jour. of Ultrastructure Res. 19, 337 (1967).
(5) Dasteli, F. R. and Priec, S. Science 154, 905 (1966).
(6) Dastoli, F. R., Lopickes, D., Price, S., Biochemistry 7, 1160 (1968).
(7) Hedin, P. A., Miles, L. R., Thompson, A. C. and Minyard, J. P. Jour. Ag. and Food Chem. 16, 505-513 (1968).
(8) Berger, P. J., Sanders, E. H., Gardner, P. D., and Negus, N. C., Science 195, 575-577 (1977).
(9) Negus, N. C. and Berger, P. J., Science 196, 1230-1231 (1977).
(10) McMurphey, W. E. "The Grasses and Grasslands of Oklahoma", Annals of the Oklahoma Academy of Science, Editors J. R. Estes and R. J. Tyrl, Publ. Robert Noble Res. Fdn., Ardmore, Okla., 1976.
(11) Waller, G. R., Morrison, R. D. and Nelson, A. B. "Chemical Composition of Native Grasses in Central Oklahoma from 1947 to 1962", Bulletin B-697, Oklahoma Agriculture Expt. Sta., Stillwater, Okla., 1976.
(12) Wright, R. H., "The Science of Smell", Allen & Unwin, London 1964.
(13) Braun, J. V., Kroper, H., Wienhaus, H., Ber. Dtsch. Chem. Ges. 62, 2880 (1929).
(14) Amoore, J. E., Johnston, J. W., and Rubin, M., "The Stero-chemical Theory of Odor", Scientific American, Feb. 1964, W. H. Freeman and Co., San Francisco.
(15) Wilson, E. O., "Pheromones", Scientific American, May 1963, W. H. Freeman and Co., San Francisco.
(16) Waller, G. R., Price, G. H., and Mitchell, E. D., Science 164, 1281-1282 (1969).
(17) Regnier, F. E., Eisenbraun, E. J. and Waller, G. R., Phyto-

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

chemistry 6, 1271 (1967).
(18) McGurk, D. J., Frost, J., Waller, G. R., Eisenbraun, E. J., Vick, K., Drew, W. A. and Young, J., J. Insect Physiol. 14, 841-845 (1968).

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# 7

# Flavor Chemistry of Carnivore Taste Systems

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## The Carnivores

The animals classified as carnivores constitute a diverse group of mammals including such animals as weasels, foxes, hyaenas, bears, leopards and pandas. Living and extinct species of carnivores are often grouped into two superfamilies on the basis of distinctions in skulls and teeth, with one superfamily possessing a short jaw and the other a long jaw. The carnivores with the short jaw often have retractable claws which are used to grasp the prey (1), whereas the long jawed carnivores grasp prey with the teeth. Examples of species from these two carnivoral superfamilies are the cat and the dog (Figure 1).

The primary distinguishing feature of all carnivores is the possession of enlarged canines and modified cheek teeth known as carnassials (Fig. 1). The canines are used for holding or crushing, and puncturing prey; the carnassial teeth for slicing the carcass into bite sized pieces for ingestion.

Although usually considered primarily in terms of their meat eating propensities, the existing species of carnivores include only a small number of species deriving sustenance totally from the devouring of other animals. The majority of the carnivores are omnivorous in their diet, consuming plant as well as animal foods (2). The giant pandas, though possessing carnassial teeth, are actually herbivorous in their diet, and therefore are non-carnivorous carnivores. Among the most carnivorous of the land carnivores are the felids, and even they consume small quantities of plant foods, either intentionally or accidently. Grass is a common feature in felid feces. Small herbivorous animals are eaten entirely, and herbivore intestines and their contents may be relished by the felids (3). More typical of the majority of the carnivores are the caenids, for whom plant materials may form a large part of the diet. Coyotes, for instance, may exist mainly through the ingestion of plant foods at certain times of year (4). The major constituents of the natural diet of the dog and cat would be vertebrates, invertebrates and plants (Figure 2). Food



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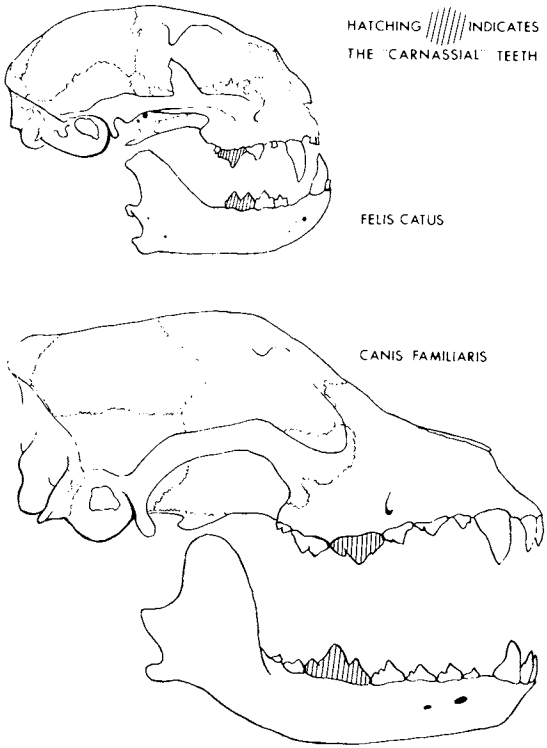


*Figure 1. The skulls of the cat and dog with the shearing teeth, the carnassials, indicated*

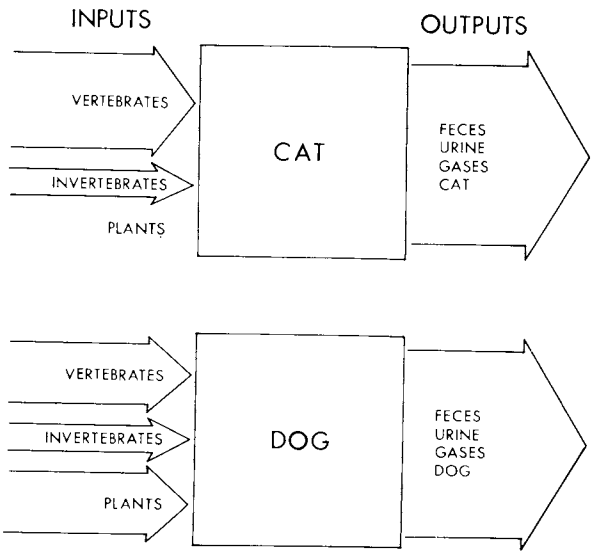


*Figure 2. Diagram of some of the major inputs and outputs of typical carnivores in a natural nutritional ecosystem*

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selection is both within and among these major groups of foodstuffs.

## Food Selection and Chemical Senses

Food selection by carnivores is a complicated process involving a complex of pre-established behavior patterns together with a long training period in the local conditions of prey capture. The felids, for instance, typically remain with the mother until subadults, when their hunting skills are established. In the selection of appropriate foodstuffs the carnivore is aided and directed by the chemical characteristics of the food. Vertebrates, invertebrates, and plants vary markedly in their chemical composition, which is important in diet selection. There exists wide variation in the taste of bird eggs and bird flesh as determined by human and animal palatability judgements (5, 6).

In the oral and nasal cavities of the carnivore are a variety of chemical sensory systems. The chemical sensory systems in the nasal cavities measure primarily volatile compounds, whereas those in the oral cavities are responsive mainly to water soluble compounds. Structurally these sensory systems can be anatomically subdivided into different sensory neural parts; such as a receptor for the measurement of the chemical stimulus, a peripheral neural encoding and transmission section, and a complex central nervous system for further neural processing. At least two different types of chemoresponsive sensory receptors are found in the oral cavities: free nerve endings and taste buds. Free nerve endings are distributed throughout the oral cavity and are supplied by peripheral sensory neurons in the trigeminal ganglion. Taste buds may be located on specialized papillae on the tongue or scattered on the soft palate, tonsils, uvula, epiglottis, larynx and upper part of the esophagus. Taste buds are innervated by peripheral sensory neurons in the geniculate ganglion of the facial nerve, the petrosal ganglion of the glossopharyngeal nerve and the jugular ganglion of the vagus nerve. Neurons in these ganglia send information from the taste buds to the central nervous system (Figure 3).

## Neurophysiology of Fungiform Papillae Taste Systems

The fungiform papillae taste systems consist of taste bud receptors distributed on the fungiform papillae which are located on the anterior surface of the tongue and the peripheral sensory neurons in the geniculate ganglion of the facial nerve. These neurons collect sensory information relevant to the chemical nature of food substances in the mouth. This chemosensory information is encoded into a series of pulses and transmitted to the central nervous system over nerve fibers that stretch from the tongue to the central nervous system (Figure 3). These pulse trains can be measured electrically with microelectrodes in the

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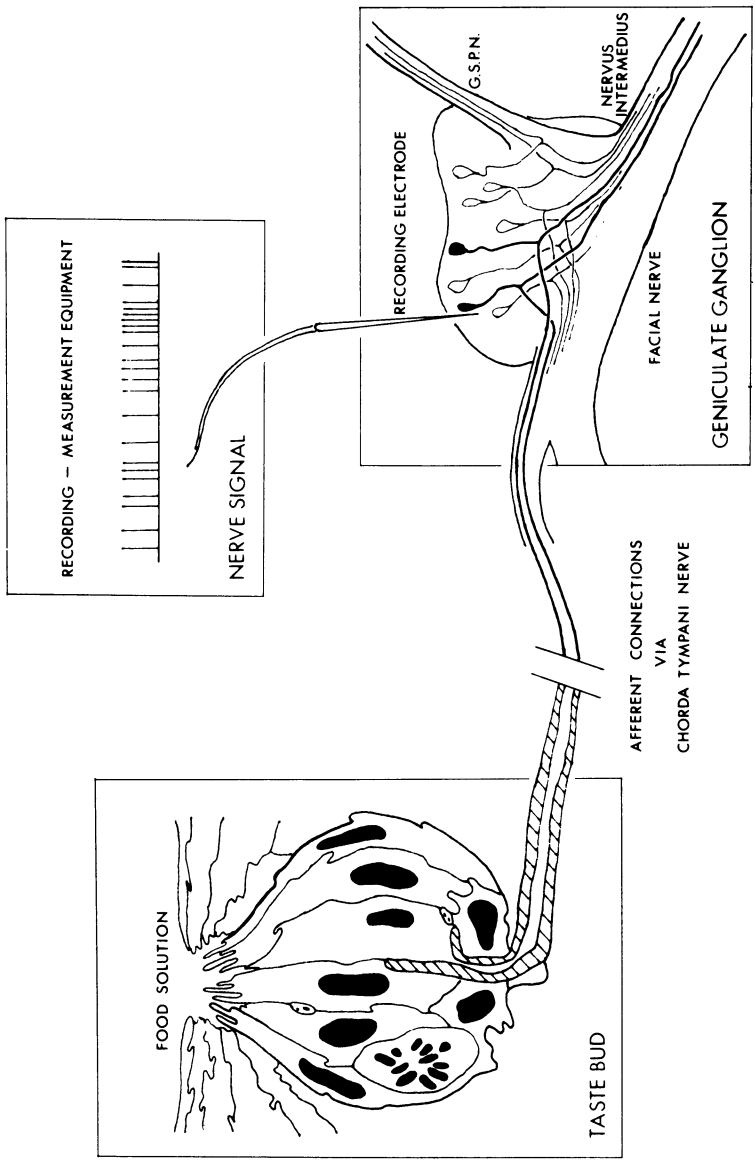


*Figure 3. Diagram of some of the sensory structures involved in oral chemoreception. Pulses being sent from the periphery to the central nervous system are recorded to determine nature of effective stimuli.*

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ganglion (7) and these measures can be used to determine types of active compounds. Studies on these neural systems have determined that the carnivore performs a precise chemical analysis of certain aspects of its prey.

The electrical pulse activity of geniculate ganglion neurons has been studied (8, 9) in both the cat and the dog by recording discharges when the tongue was stimulated with various chemical solutions. In both the cat and the dog these chemosensory neurons have been divided into a number of functional neural groups (9, 10) on the basis of a variety of physiological measures (Table I). Since these different neural groups respond to different types of chemical compounds, the types of effective compounds will be described for each neural group. The cat fungiform papillae taste system has been most intensively studied and therefore it will be described first and then compared to that of the dog.

## Cat Fungiform Papillae Taste Systems

Three different chemoresponsive neural groups have been distinguished in the cat geniculate ganglion on the basis of conduction velocity measures, spontaneous activity measures and their response to chemical solutions (Figure 4 and Table I). The most useful chemical response measure has been that to a series of discriminatory solutions (the "test series") tested on each neural unit by application to the area of the tongue known to be innervated by the unit. On the basis of the differential response of each unit to the test series and other neurophysiological measures over 90% of the chemoresponsive tongue units can be classified into three categories, which have been labeled group I, II and III (Figure 5). These groups have proved valuable for the determination of active chemical stimuli and for the comparison of species although they do not represent total neural population variability since unclassified units exist, as does undetermined within-group variability.

Cat geniculate ganglion group I neurons preferentially innervate receptors on fungiform papillae on the rear and sides of the tongue with fibers of large diameter. They display low spontaneous activity rates and are responsive to distilled water and inorganic acids. In a study testing a variety of organic compounds, it was found that group I units were discharged by compounds with undissociated carboxylic and phosphoric acid groups (11). A few nitrogen compounds were also effective. In all cases the response was pH dependent in that the maximum discharge rate was elicited when the solution pH was at or below the pKa of the active chemical group. The compounds most active in the pH region 5.0 to 7.0 proved to be compounds with a heterocyclic nitrogen ring component (e.g. imidazole ring). Only the proton donor form of the molecule appeared to stimulate.

Group II units preferentially innervate fungiform chemoreceptors on the front and sides of the tongue with medium size

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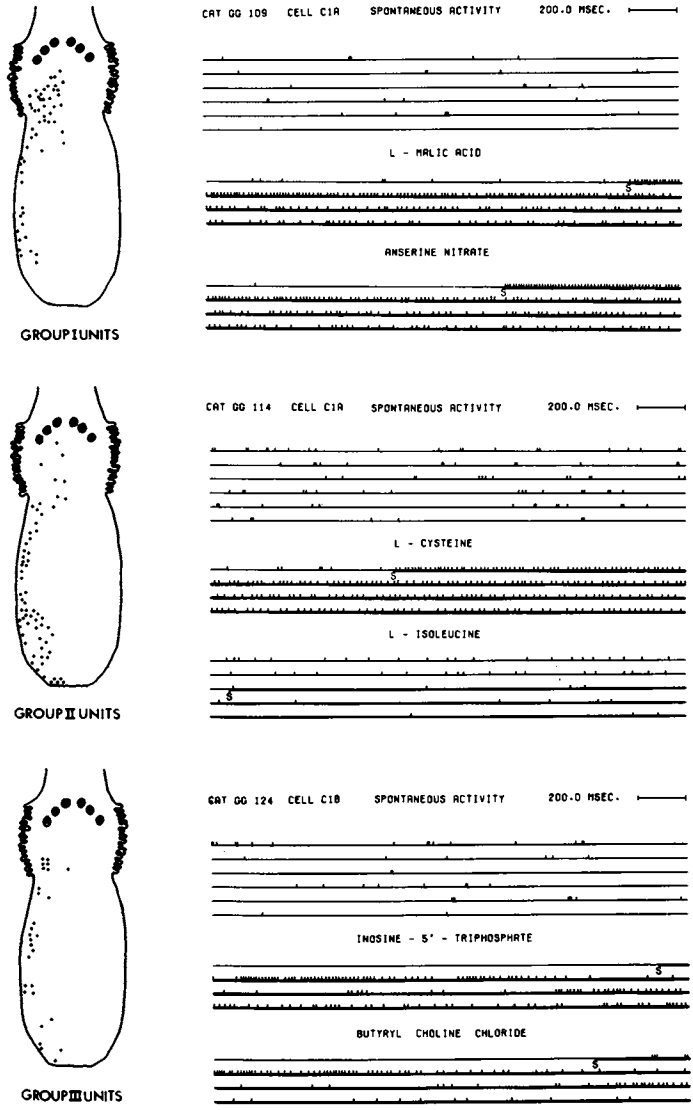


*Figure 4. Examples of projection zones and discharge patterns of cat geniculate ganglion chemoresponsive units. The locations of the fungiform papillae projection zones for each neural group are indicated on the schematic tongue, where each dot represents the locus projection of a single unit. On the right are examples of spontaneous and evoked discharge patterns of group I, II, and III units. S indicates approximate time of stimulus application.*

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Table I: Chemosensory Neural Groups Distinguished in the Geniculate Ganglion of the Dog and Cat.

CAT: Units distinguished on the basis of response to chemicals, electrical stimulation and spontaneous activity measures.

| | |
|---|---|
| Group I Units: | Low spontaneous activity, short latency to electrical stimulation. Respond to malic acid, ATP, ITP, etc. |
| Group II Units: | High spontaneous activity rates, medium latency to electrical stimulation. Respond to L-proline, L-cysteine and di- and triphosphate nucleotides. Inhibited by L-tryptophan and L-isoleucine. Group most responsive to inorganic salts. |
| Group III Units: | Low spontaneous activity rates, long latencies to electrical stimulation. Subdivided into two groups on basis of chemical response. Subgroup IIIA respond to nucleotides; IIIB to various oxygen compounds. |

DOG: Units distinguished by factor analysis of responses to chemical stimulation.

| | |
|---|---|
| Group A Units: | Respond to L-proline, L-cysteine, ITP, ATP, IDP, and NaCl |
| Group B Units: | Respond to L-malic acid, quinine hydrochloride, HCl and ATP. |
| Group C Units: | Respond primarily to nucleotides. |
| Group D Units: | Respond to butyryl choline chloride, phytic acid and quinine hydrochloride. |

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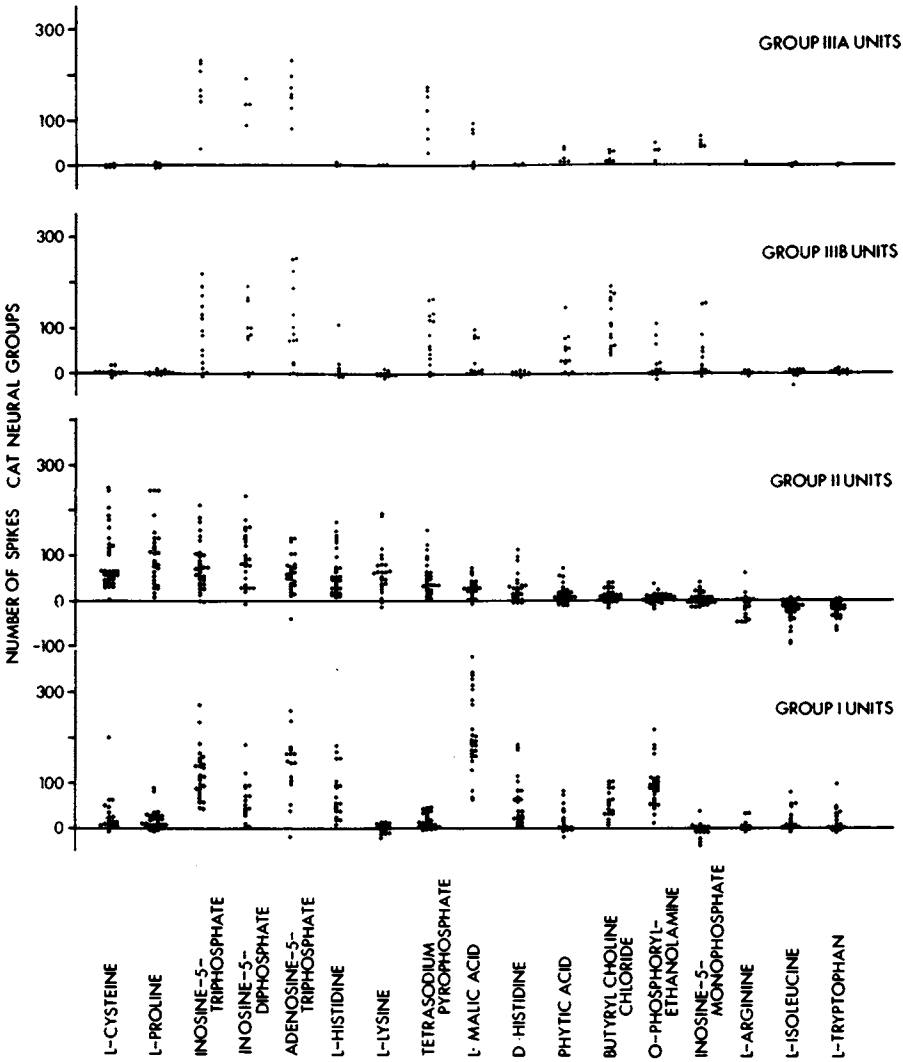


*Figure 5. Responses of cat geniculate ganglion chemoresponsive neurons to the test series chemical solutions (50mM in distilled water). Values represent the number of spikes during the 10-sec stimulus period minus the spikes in the preceeding 10-sec period.*

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fibers. Spontaneous activity rates are often high. A survey (12) of a variety of chemical substances disclosed that the most effective excitatory stimuli were certain amino acids (L-proline, L-cysteine, L-lysine, L-histidine, etc.), di- and triphosphate nucleotides and certain other substances (usually containing nitrogen). In testing compounds related to proline and histidine it was discovered that the heterocyclic ring components pyrrolidine and imidazole were as stimulatory as the parent amino acids. To further specify the properties of excitatory stimuli many simple heterocyclic stimuli were tested. The most stimulatory proved to be four to six member ring nonaromatic (except for imidazole) nitrogen compounds. Inhibition, or a decrease in spontaneous activity was also common with group II units. Common inhibitors are L-tryptophan, L-isoleucine, pyrrole and azacyclooctane.

Group III units innervate chemoreceptors on fungiform papillae located primarily on the sides of the tongue (10). Latency measures to electrical stimulation of their receptive fields are greater than 14 msec, indicating that the fibers are of extremely small diameter. The spontaneous activity from these neurons is of low rate and often extremely bursty. Discharge to chemical stimulation of the tongue may start as long as one second after stimulation, and spike patterns are often quite irregular (Figure 4). Less is known about the chemistry of group III units than of the other two unit groups. On the basis of their response to chemical stimulation group III units have been tentatively subdivided into two subgroups: those largely responsive to nucleotides (IIIA) and those responsive to nucleotides and other compounds, especially butyryl choline chloride (IIIB). Units responsive to nucleotides may show responses to concentrations less than 0.1 mM. Units responsive to butyryl choline chloride have recently been found to respond to a variety of other compounds such as vanillin, 5-hydroxypentenal, methyl maltol, and certain lactones. The common feature to these compounds seems to be a carbonyl group associated with a long hydrocarbon chain. The nature of group IIIB stimuli is currently being investigated.

## Comparison of Cat and Dog Fungiform Papillae Taste Systems

The chemoresponsive neurons of the dog geniculate ganglion have been divided into four separate functional classes on the basis of factor analysis of their responses to the test series and to other chemical stimuli (9). Two of these unit classes seem quite similar to two groups distinguished in the cat, with dog class A similar to cat group II, class B with group I. Class D units seem similar to group IIIB units, but only a few dog class D units have been investigated. Dog unit class C seems to consist in part of units similar to cat unclassified units and in part of units similar to cat group IIIA units. Because of the

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paucity of information on class C units and their general unresponsiveness, they are not further discussed in this report.

The responses of dog class B units to the test series are virtually identical to those of cat group I units (Figure 6). Malic acid is by far the best stimulus for both species; and the responses to other test series compounds are proportionally similar. The major differences are the smaller response shown by the dog units to butyryl choline chloride and a larger response to L-cysteine. L-histidine is the test series amino acid most excitatory to both species. Other active stimuli for both species are HCl, quinine HCl, and citric acid.

Dog class A units are similar in many of their properties to cat group II units. In a comparison of responses to test stimuli, the most active compounds in the cat proved to be those most excitatory in the dog, namely the amino acids L-proline and L-cysteine (Figure 7). L-tryptophan, the most effective cat group II inhibitor, was also the strongest dog class A inhibitor. Significant differences exist between the two species, however. Fewer compounds seem to inhibit dog class A units. Dog class A units are also much less responsive to nucleotides than are cat group II units.

In addition to the test series chemicals, a variety of amino acids (Figure 7) and nitrogen heterocycles (Figure 8) were tested on the dog class A units. As with the test series many amino acids elicited similar responses from the two species, but the response of the dog units was less than that of the cat units to amino acids with carboxyl and hydroxyl groups on the side chains. L-histidine is also less stimulating in the dog. As with the test series, fewer compounds inhibited in the dog; in fact, L-phenylalanine and L-tyrosine were excitatory in the dog. On the other hand, a comparison of the responses to simple nitrogen heterocycles reveals remarkable similarities between the two species, even though the chemicals were tested in distilled water solution for the dog and in a neutral saline solution for the cat. A relationship between nitrogen heterocycle pKa values, ring size, and neural response is seen in both species (Figure 8). Heterocycles with pKa values less than 7.0 are inactive or inhibitory in both species.

As in the cat group II units, dog class A units were those most responsive to NaCl. Unlike cat units, however, dog class A units also discharged to sugars, with fructose and sucrose being the most excitatory sugars (Figure 9). Other dog unit groups were unresponsive to sugar solutions. The response to sugars, even in group A units, was much less than the response shown to an equivalent concentration of L-proline (Figure 9).

Dog class D units are similar in part in their response properties to cat group IIIB units. The most effective test stimulus for both species is butyryl choline chloride (Figure 10). The other test series chemicals stimulate both species to approximately the same degree, although compounds with phosphate

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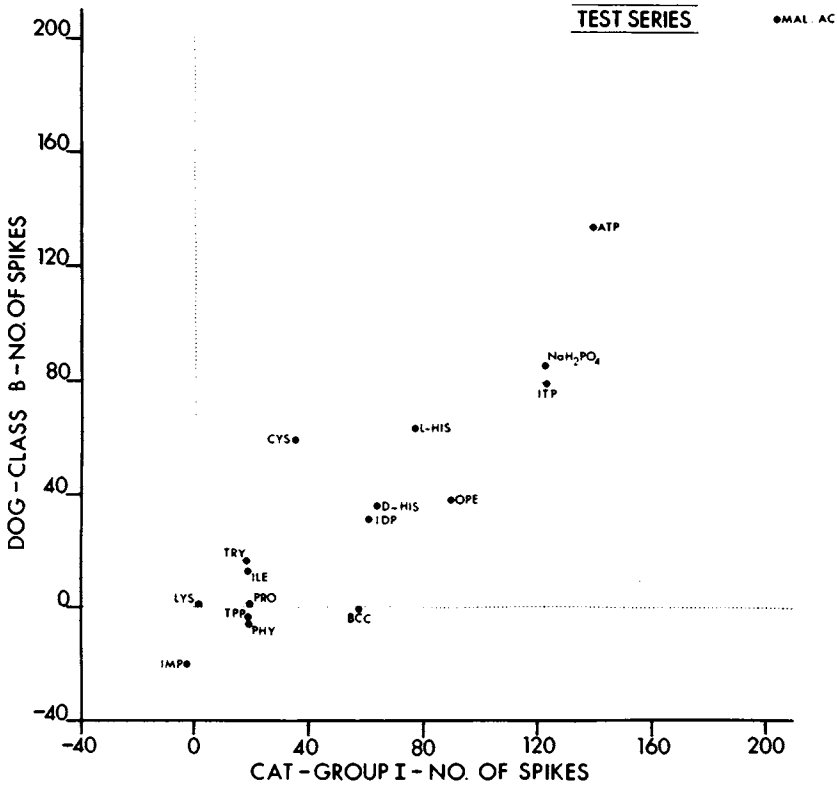


*Figure 6. Comparison of neural responses of cat group I units to test series stimuli with those of dog class B units. Nonstandard abbreviations used are malic acid—Mal. AC.; tetrasodium pyrophosphate—T.P.P.; O-phosphoryl ethanolamine—PE; butyryl choline chloride—B.C.C., and phytic acid—Phy.*

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*Figure 7. (left) Comparison of neural responses of cat group II units to test series stimuli with those of dog class A units. (right) Comparison of neural responses of cat group II units and dog class A units to L-amino acids.*

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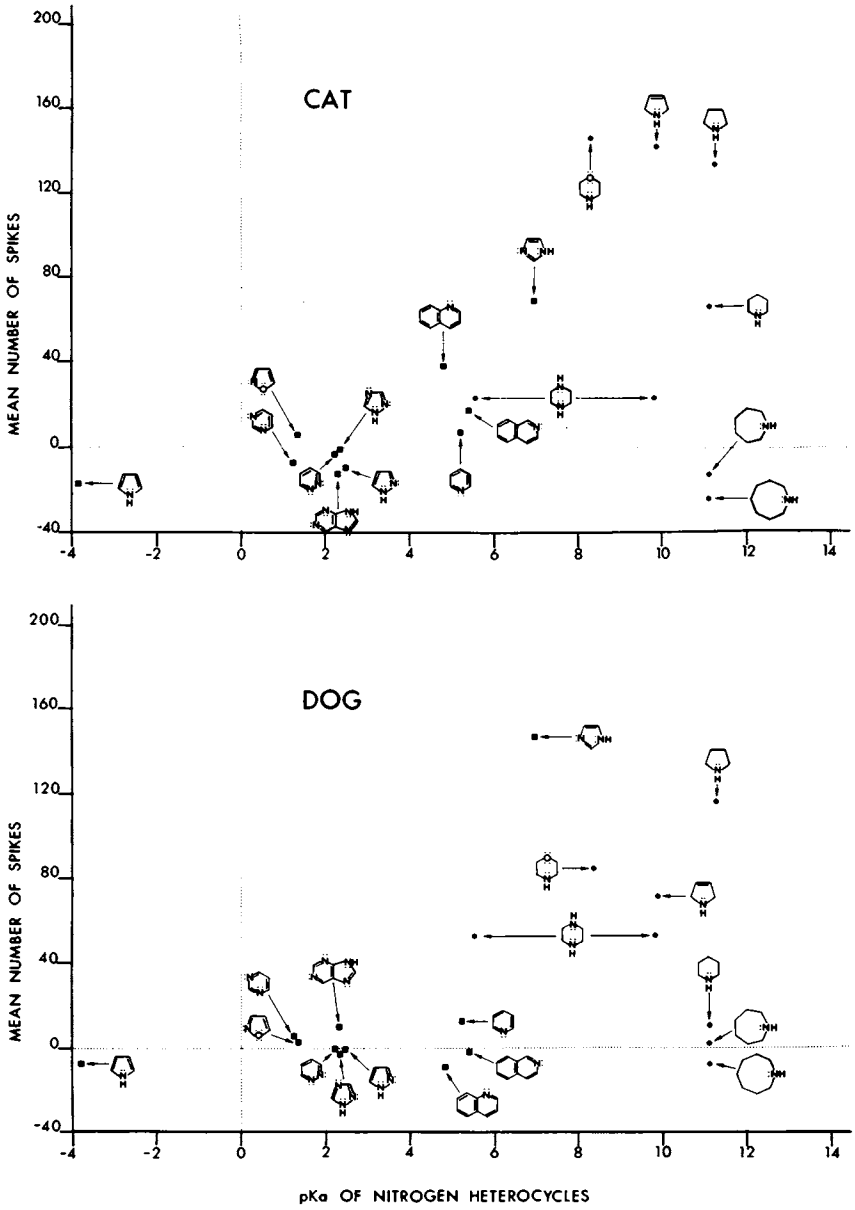


*Figure 8. Neural respones elicited by heterocyclic compounds. Compounds dissolved in distilled water for testing on dog and in 50mM saline, neutralized, for the cat.*

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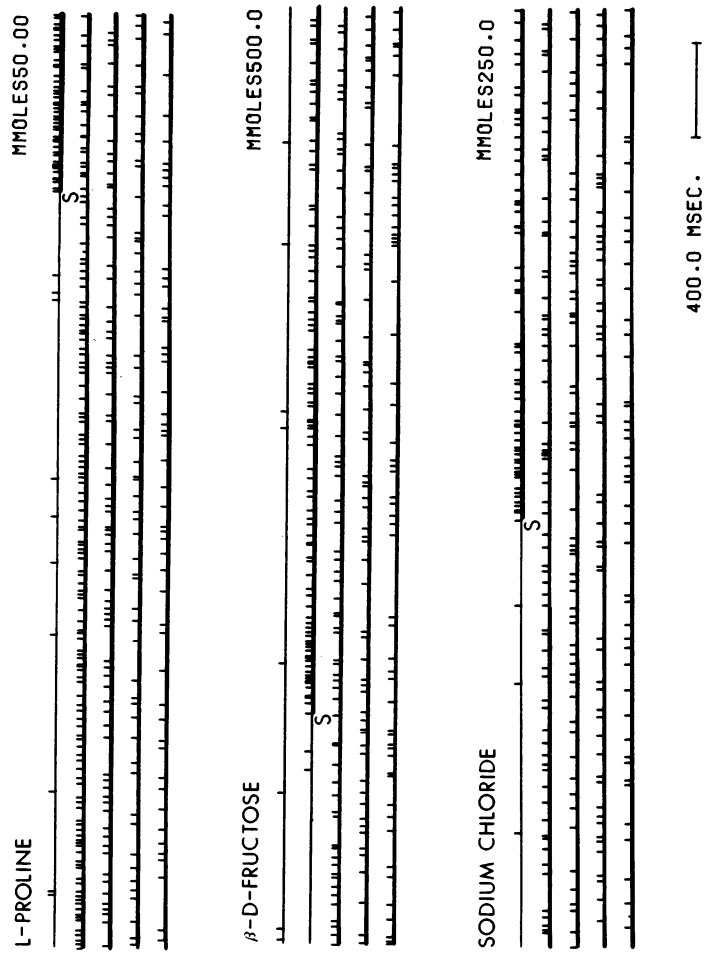


*Figure 9. Computer printout of spike discharges from a dog class A unit to NaCl, fructose, and L-proline. S indicates application of stimulus solution.*

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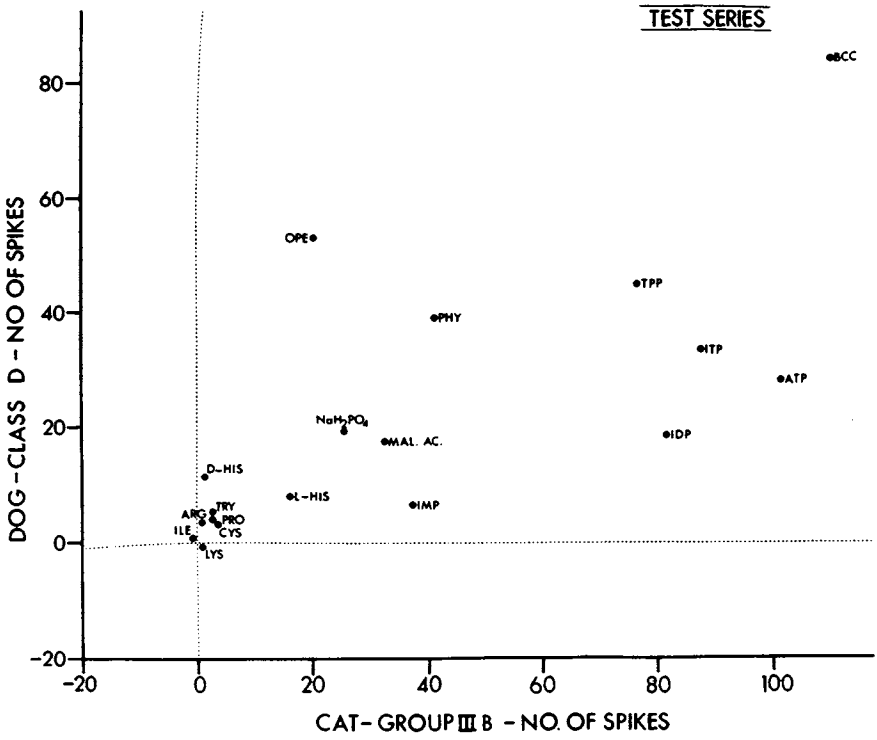


*Figure 10. Comparison of cat group IIIB and dog class D unit responses to the test series*

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groups are less stimulatory in the dog. As with the cat, certain oxygen compounds are quite effective stimuli for dog class D units. Some of the most active stimuli for dog class D units are furaneol, ethyl maltol, and methyl maltol. Furaneol, a potent dog group D stimulus has been relatively inactive in the cat. The chemical stimulus dimensions of both unit groups have been inadequately investigated, but both dog class D and cat group IIIB units are less responsive to nitrogen compounds than the other unit groups.

## Flavor Chemistry

By flavor chemistry is meant the chemistry of the total complement of sensory responses elicited by a food or a food component. In human sensory research, the sensory responses are psychophysical sensations, whereas in neurophysiological studies the responses are measured from neurons. The flavor chemistry of the geniculate ganglion fungiform papillae taste systems would then consist of a description of the neural responses to foods and to the types of compounds present in food. The neural responses of both the dog and the cat have been examined with respect to the excitability of many of the compounds found in vertebrate tissues. The cat has been tested with more compounds than the dog, but the results will also apply in large part to the dog, since the two species are so similar with respect to the majority of the compounds considered.

Cat taste units are responsive to water extracts of various animal tissues such as chicken, beef, and pork liver (8). To examine the underlying chemical substrate of this food response, many of compounds found in animal tissues were tested on the different neural groups. The results of these studies are partially summarized in Table II, which contains the responses of the different neural groups to compounds commonly found in relatively high concentration in vertebrate muscle tissues.

The monovalent inorganic salts NaCl and KCl excite few neurons in 50 mM concentration, although group I spontaneous discharges are inhibited. In addition group II unit discharge to amino acids is potentiated by KCl and NaCl. At higher concentrations these salts stimulate group II units. The salts $CaCl_2$ and $NaH_2PO_4$ have proved to be among the most excitatory inorganic compounds tested on group II units.

Amino acids selectively stimulate unit groups I and II. Only L-histidine and, to a lesser extent, taurine stimulate group I units, whereas group II units respond to a variety of amino acids. Among the most potent group II stimuli discovered are L-proline and L-cysteine. Other amino acids stimulating group II units to a lesser extent are L-lysine, L-histidine, L-alanine and L-ornithine. The amino acids L-tryptophan, L-isoleucine and L-phenylalanine tend to inhibit group II unit discharge.

The dipeptides carnosine (beta-alanyl-L-histidine) and

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Table II: Simplified Summary of Cat Taste Neuron Responses to Compounds Commonly Found in Flesh Foods, Solutions 50 mM, pH 6-7.

| | Group I Units | Group II Units | Group III Units |
|---|---|---|---|
| **Inorganic Salts** | | | |
| NaCl, KCl | - | potentiate | |
| $CaCl_2$ | | ++ | |
| $NaH_2PO_4$ | + | + | + |
| **Amino Acids** | | | |
| L-Proline | | +++ | |
| L-Cysteine | | +++ | |
| L-Lysine | | ++ | |
| L-Histidine | ++ | ++ | |
| L-Tryptophan | | - | |
| L-Taurine | + | + | |
| **Peptides** | | | |
| Anserine | ++ | | |
| Carnosine | ++ | ++ | |
| **Nucleotides** | | | |
| Di- and Triphosphate | + | +++ | +++ |
| Monophosphate | | | ++ |
| Bases | | - | |
| **Other** | | | |
| Thiamin Compounds | ++ | ++ | |
| Pyridine Compounds | + to ++ | | |
| Imidazole Compounds | ++ | ++ | |
| Oxygen Compounds | | | +++ |
| Creatine, Creatinine | | - | |
| Polyamines | | - | |

+: excite
-: inhibit

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anserine (beta-alanyl-methyl-L-histidine), of widespread occurrence in vertebrate tissues, are often potent stimuli for group I units. Only carnosine stimulates group II units since N-methylation of a heterocyclic ring nitrogen typically eliminates group II response.

The di- and triphosphate nucleotides are active stimuli for all neural groups, although at pH 7.0 the response from group I units is minimal since the phosphate group is largely dissociated. The di- and tri-phosphate nucleotide salts are among the most potent stimuli for group II units and some group III units. Only group III units are responsive to any degree to monophosphate nucleotide solutions. The heterocyclic nucleotide bases are in general inactive except for inhibition of group II units.

A variety of other compounds found in vertebrate tissues also affect taste neurons. Thiamin compounds, especially thiamin pyrophosphate, are active stimuli for both group I and group II units. Compounds with a pyridine ring such as nicotinic acid and desmosine will often stimulate group I units, as will compounds with an imidazole ring such as 1-methyl imidazole. The major factor limiting the effectiveness of many of these compounds is the presence of a dissociated carboxyl group.

Various heterocyclic compounds other than those described above will also stimulate cat taste neurons. Group I units will be activated by compounds with thiazolidine, quinoline or isoquinoline rings. Group II units would be activated by compounds with pyrrolidine, pyrroline, piperidine and piperazine rings. The heterocyclic rings active on either group can be separated in part by the pKa values of the nitrogen groups.

A variety of carbonyl compounds, some derived from lipids, are present in animal tissues. Other oxygen compounds present in low concentration may include various oxygen heterocycles and lactones. Some of these compounds stimulate cat group IIIB units. Since the chemistry of group III units is inadequately understood, only the vague term "oxygen compounds" is used to describe these stimuli.

Some compounds present in vertebrate tissues are relatively inactive on cat taste systems. Various organic acids such as lactic, pyruvic and levulinic are inactive in the proton acceptor form in which they commonly occur. Various polyamines (spermine, putrescine, etc.) are of widespread occurrence but tend to inhibit group II neurons. Sugars (glucose, fructose, etc.) are inactive in the cat. The compounds creatine and creatinine, of common occurrence in vertebrate tissues, excite no neurons and inhibit group II units.

In a few cases, mixtures of different compounds were studied. The salts NaCl and KCl have been found to potentiate the response of group II units to $NaH_2PO_4$ and to L-proline and L-cysteine. This potentiation even occurs when the NaCl solution by itself is below threshold. The response of group I neurons to many solutions is attenuated by adding NaCl to the solution. In

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a similar fashion mixing L-tryptophan with L-proline will inhibit the response of a group II unit to L-proline alone.

The response of dog unit groups to the compounds listed in Table II would in general be quite similar to that illustrated for the cat. The major difference would be the reduced response of dog class A and class D units to nucleotides. Dog class D units would also probably discharge to somewhat different oxygen compounds than cat group IIIB units.

## Theoretical Considerations

Implicit in the designation of distinct functional sensory neural subsets of chemoresponsive units is that each functional unit group is selectively responsive to a distinct chemical signal or signals. These chemical signals can be represented in terms of the types of compounds stimulating, common chemical factors among the stimulants, or as a general property of the aqueous solutions of these compounds. In practice, signal description proceeds from the specific to the general, depending in part on the stage of the investigation and in part on the understanding of the chemical factors involved.

At least seven distinct chemical input signals are suggested from neurophysiological studies on the carnivore system (Table III). Those signals can be characterized to different degrees of generality. Cat group III input signals are described primarily in terms of the unique chemical compounds that stimulate, whereas somewhat more general statements can be utilized in typifying group I and group II input signals. Group II excitatory and inhibitory nitrogen heterocycles, for instance, can be characterized in terms of pKa values and ring size (Figure 8). The depiction of group I excitants goes even further since all of these compounds can be characterized as Brønsted acids, i.e. in terms of their behavior in solution. These chemical signal descriptions must in all cases be considered preliminary and inadequate. In the consideration of group II stimuli for instance, little consideration is given to inorganic salts.

The representation of input signals in terms of solution chemistry is most desirable, since the active molecular species is known to be a supramolecular complex formed by the interaction of the solute and water (13, 14, 15). Compounds such as inorganic salts, acids, sugars, and amines are known to strongly interact with water molecules (16, 17, 18). The solute is often assumed to act through a water bridge or to have its properties modified by the interaction of water molecules. The limiting factor in the description of the stimulus in terms of solution chemistry is the inadequate understanding of the general structural and functional properties of solutions.

The most general description of a carnivore taste signal has been achieved for the cat group I (dog class B) input signal. It has been shown that all of the group I stimuli can be described

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Table III: Major Chemical Stimulus Factors of Importance In Carnivore Fungiform Papillae Taste Systems

| Excitatory Factors | Example of Stimulus | Carnivore Neural Group |
|---|---|---|
| Nitrogen Factor | L-proline, L-cysteine, pyrrolidine, morpholine | Cat II, Dog A |
| Brønsted Acid | malic acid, L-histidine, pyridine | Cat I, Dog B |
| Oxygen Factor | butyryl choline chloride, furaneol (dog), 5-hydroxypentenal | Cat IIIB, Dog D. May be different in two species |
| Nucleotide Factor | ATP, ITP, ADP, IMP, etc. | Cat IIIA |
| Unknown Factor | ATP, ITP | Cat II, Dog A Different in the two species |
| Inhibitory Factors | | |
| Nitrogen Factor | L-tryptophan, pyrrole | Cat II, Dog A |
| Brønsted Base Factor (?) | IMP, tetrasodium pyrophosphate | Cat I, Dog B |

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as Brønsted acids (e.g., carboxylic acids, phosphoric acids, and the protonated bases pyridine and imidazole). These compounds are known to possess the characteristic of rapid proton transfer in water (19). One suggested mechanism for this phenomenon is that a solute-water circuit is formed in such a way that a proton added to one part of the circuit facilitates the transfer of a proton elsewhere in the molecular circuit (Figure 11).

## Human Comparisons

The taste signals identified for the carnivore in Table III can in part be related to human taste signals (Table IV). This comparison can be made largely on the basis of the similarities in chemical stimuli active in the two species. Especially valuable in cross species comparison, have been the L-amino acids which in part selectively elicit different sensations just as they selectively activate different neural groups (Figure 12). Cat group I and dog class B unit stimuli are quite similar to the stimuli that elicit human sour sensations, such as carboxylic and phosphoric acids. Among the amino acid solutions, aspartic acid solutions and even histidine solutions elicit a human sensation with a sour component (11).

Some of the compounds eliciting a sweet sensation in the human stimulate group II (dog class A) units and those that inhibit often taste bad or bitter (12). In both the cat and dog the amino acids with a sweet component (20, 21) tend to stimulate (e.g. L-proline, L-lysine, L-alanine, L-glycine and, probably, L-cysteine). Other compounds which often taste sweet are NaCl and KCl, which stimulate both cat group II and dog class A units, and sugar, which stimulates dog class A units. Inhibitory compounds in both species include the strongly bitter amino acid L-tryptophan and quinine.

Cat group III stimuli include nucleotides, phosphate compounds, various carbonyl compounds, phenolic compounds, oxygen heterocycles and lactones. Many of these compounds are common food substances and food additives. The human taste sensations elicited by these compounds are of an indistinct variety and may be described with the terms pleasing, sweetish, creamy, etc. (22, 23). Nucleotides elicit a sensation which the Japanese term "umami," or deliciousness (24). It is quite probable that small fiber systems similar to those seen in the cat are present in the human.

A variety of compounds have been reported to be important in meat flavors (25, 33). The most prominent naturally occurring compounds are amino acids, nucleotides and other phosphate compounds, and inorganic salts. Of lesser importance are various sugars and organic acids. Especially prominent are nitrogen-sulfur compounds such as cysteine and thiamin. Various other compounds that have been reported to be of importance in meat flavor include the peptides anserine, carnosine and glutathione.

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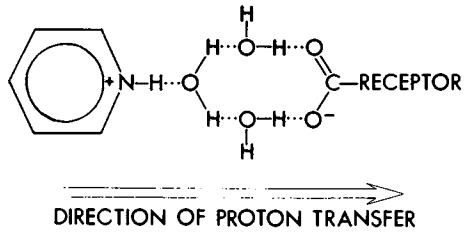


*Figure 11. Possible molecular circuitry associated with receptor activation by a cat group I (dog class B) stimulant*

Table IV: Some Suggested Relationships Between Humans and Carnivores Based on Similarities Among Chemical Stimuli

| Carnivore Taste Signals | Human Sensations |
|---|---|
| **Excitatory Chemical Factors** | |
| Nitrogen Factor (II & A) | Sweet |
| Brønsted Acid Factor (I & B) | Sour |
| Oxygen Factor (IIIB & D) | (pleasant, creamy, sweetish?) |
| Nucleotide Factor (IIIA) | (Umami?) |
| Unknown Factor (II & A) | ? |
| **Inhibitory Chemical Factors** | |
| Nitrogen Factor (II & A) | Bitter |
| Brønsted Base Factor (I & B) | ? |

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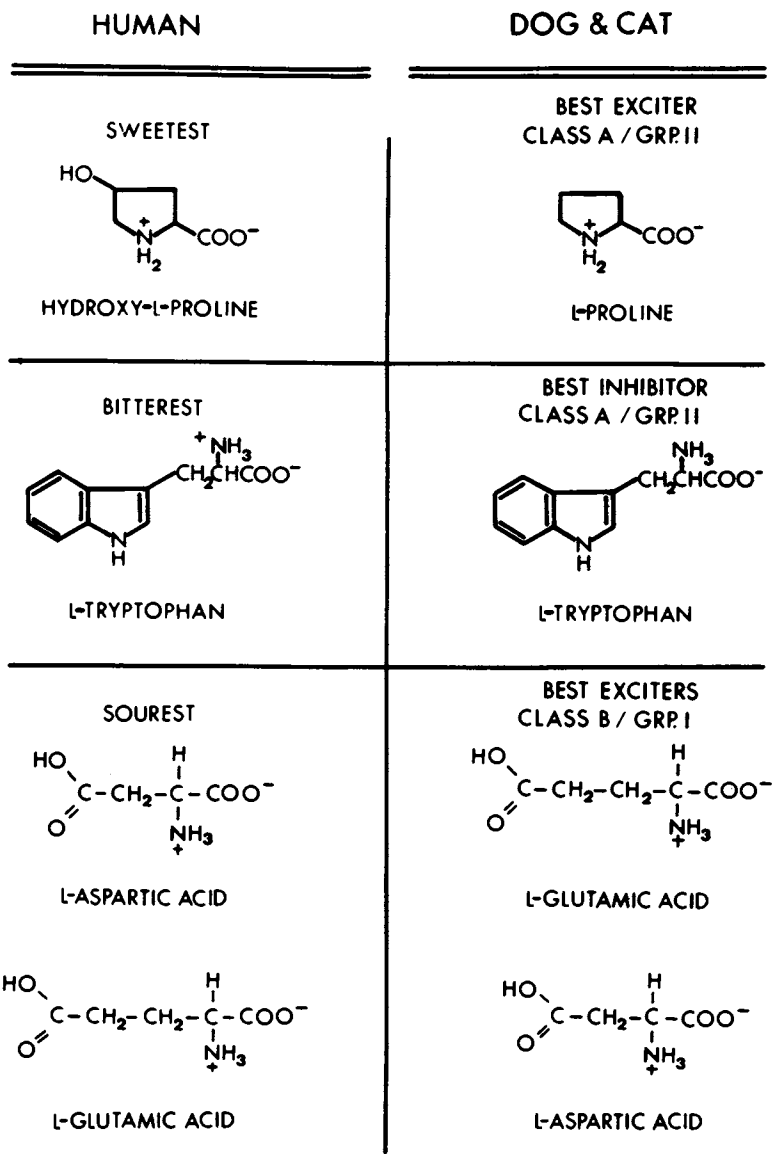


*Figure 12. Taste responses of carnivore and man to some amino acids. Human data obtained from Ref. 20.*

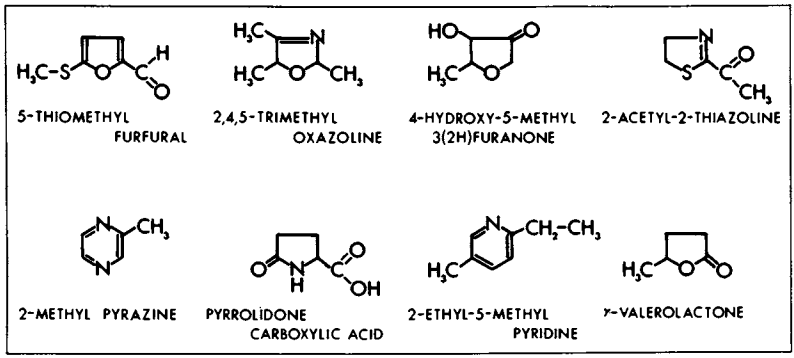


*Figure 13. Some heterocyclic compounds associated with meat flavor in humans*

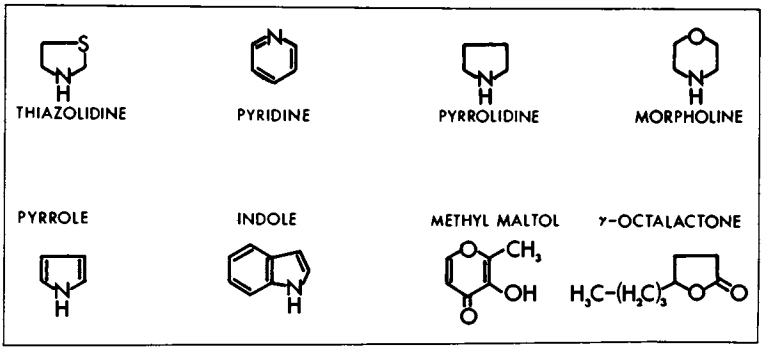


*Figure 14. Some of the most effective heterocyclic stimuli in the carnivore. Pyrrole and indole are inhibitory.*

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Off-flavor or bitterness is usually reported for compounds such as creatine, creatinine and nucleotide bases. A mixture of several different types of compounds has been reported necessary to reconstruct meat flavors. This chemical complexity suggests that different sensory systems are being activated. Many of these compounds appear to exert their effects through human geniculate ganglion taste systems; others obviously stimulate other oral chemoresponsive systems.

Raw flesh is assumed by all but the Japanese to have little flavor. True "meat" flavor is assumed to develop following cooking. When flesh is cooked, various chemical changes occur, many of which result in the formation of flavor products. Particularly important are flavor compounds produced through the pyrolysis of nitrogen and nitrogen sulfur compounds, largely amino acids and through their interaction with other compounds, such as sugars and carbonyls. Also prominent are various compounds derived from reactions with lipids. Many of these heat formed compounds have been reported to be heterocyclic compounds, some of which are illustrated in Figure 13. These heterocyclic compounds are usually assumed to function as volatiles, affecting the nasal chemoreceptor systems.

The close structural similarity between these compounds and many of those active on the carnivore taste system (Figure 14) argues for their being potent taste stimuli as well. Prominent stimuli for both human primate as well as carnivore contain 5 and 6 member heterocyclic rings. The major difference between the two groups of compounds is the preponderance of aromatic compounds in the odors (Figure 13).

Literature Cited

1. Gonyea, W., Ashworth, R., J. Morphol. (1975) 145: 229-238.
2. Ewer, R. F., "The Carnivores", Cornell University Press, Ithaca, N.Y., 1973.
3. Schaller, G. B., "The Serengeti Lion", The University of Chicago Press, Chicago, 1972.
4. Meinzer, W.P., Eukert, D.N., Flinders, J. T., J. Range Manag. (1975) 28: 22-27.
5. Cott, H. B., Proc. Zool. Soc., London, (1946) 116: 371-524.
6. Cott, H. B., Proc. Zool. Soc., London, (1953) 123: 123-141.
7. Boudreau, J. C., Tsuchitani, C., "Sensory Neurophysiology," Van Nostrand Reinhold Co., N.Y., 1973.
8. Boudreau, J. C., Bradley, B. E., Bierer, P. R., Kruger, S., Tsuchitani, C., Exp. Brain Res. (1971) 13: 461-485.
9. White, T. D., "Neurophysiological Investigation of the Geniculate Ganglion of the Dog", M.S. Thesis, Univ. of Texas at Houston, Graduate School of Biomedical Sci., 1976

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

10. Boudreau, J. C., Alev, N., Brain Research (1973) 54: 157-175.
11. Boudreau, J. C., Nelson, T. E., Chemical Senses and Flavor (1977) 2: 353-337.
12. Boudreau, J. C., Anderson, W., Oravec, J., Chemical Senses and Flavor (1975) 1: 495-517.
13. Franks, F. (ed.), "Water A Comprehensive Treatise", Vol. 1-4, Plenum Press, N.Y., 1972-1975.
14. Franks, F., Phil. Trans. Roy. Soc., Lond. B. (1977) 278: 33-57.
15. Pullman, A., Pullman, B., Quart. Rev. Biophy. (1975) 7: 505-566.
16. Blandamer, M. J., Burgess, J., Chem. Soc. Rev. (1975) 4: 55-75.
17. Jones, F. M. III, Arnett, E. M., Prog. Phys. Org. Chem., (1974) 11: 263-322.
18. Suggett, A., J. Sol. Chem., (1976) 5: 33-46.
19. Caldin, E., Gold, V., "Proton-Transfer Reactions", John Wiley & Sons, N.Y., 1975.
20. Ninomiya, T., Ikeda, S., Yamaguchi, S., Yoshikawa, T., In: "Rep. 7th Sensory Evaluation Symposium," JUSE, 109-123, 1966.
21. Kirimura, J., Shimizu, A., Katsuya, N., J. Agr. Food Chem., (1969) 17: 689-695.
22. Furia, T. E., Bellanca, N., "Fernaroli's Handbook of Flavor Ingredients," Vol. 2, CRC Press, Cleveland, Ohio 1975.
23. Arctander, S. "Perfume and Flavor Chemicals," Vol. I-II, Published by author, Penn., 1971.
24. Yamaguchi, S. In: "Sixth International Symposium on Olfaction and Taste, Abstracts," Paris, 1977.
25. Dwivedi, B. K., Crit. Rev. Food Tech., (1975) 5: 489-538.
26. Hashida, W., Food Trade Rev., (1974) 44: 21-32.
27. Hashimoto, Y., In: "The Technology of Fish Utilization" (R. Kreuzer, ed), 57-61, Fishing News (Books), London, 1965.
28. Herz, K. O., Chang, S. S., Adv. Food Res. (1970) 18: 2-83.
29. Mabrouk, A. F., In: "Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors", 146-183, Am. Ch. Soc., Wash. D. C., 1976.
30. Schutte, L., C.R.C. Crit. Rev. Food Tech., (1974) 4: 457-505.
31. Solms, J., In: "Gustation and Olfaction an International Symposium", 92-110, Academic Press, N.Y., 1971.
32. Wilson, R. A., Agr. Fd. Chem. (1975) 23: 1032-1037.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

33. Wilson, R. A., Katz, I., Flavor Industry (1974) 5: 2-8.
34. Boudreau, J. C., Oravec, J., White, T. D., Madigan, C., Chu, S. P., Arch. Otolaryngol. (1977).

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# 8

# Development of Diets for Food-Producing Animals

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Animal foods such as meat, milk and eggs are used to upgrade the nutritional quality of plant food sources by virtue of their high quality protein, excellent B vitamin content and iron in highly available form (1). The current world's animal population, quantities of food produced and current and projected requirements of animal food sources are summarized in Tables I and II.

## Nutrient Requirements

Diets for food-producing animals performing various physiological functions such as maintenance, growth, lactation and reproduction are formulated to contain specified amounts of nutrients. In the United States, the most widely used standards are those published by various committees of the National Research Council under the auspicies of the National Academy of Sciences. In Great Britain, standards of the Agricultural Research Council are employed (2). Similar bodies in other countries provide recommendations on animal nutrient requirements, but the most widely used standards are those from the United States and Great Britain.

It is important to recognize that recommendations such as those of the National Research Council are specified in terms believed by the committees to be minimum requirements of animals of a given species, age, weight and productive status. No provisions or safety factors are provided to compensate for deviations of specific animal populations or influences of environment, disease, parasitism or other types of stress (2).

The metabolic machinery of all animals must be provided with water, amino acids, energy, minerals and vitamins. In ruminants, utilizable nutrients are provided by a combination of dietary sources plus those synthesized by rumen bacteria and protozoa and until recently little attention has been directed towards dietary supplies of amino acids and B-complex vitamins. However, information accrued during the last decade demonstrates that ruminal outflows of these nutrients is not always sufficient for high rates of productivity (3, 4, 5).

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TABLE I. Number of animals and production[1]

| | Developed countries | Developing countries | Centrally planned countries |
|---|---|---|---|
| Cattle[2] | 285,189 | 660,658 | 202,588 |
| Buffalo | 1 | 507 | 551 |
| Sheep | 351,329 | 432,348 | 258,396 |
| Goats | 15,863 | 307,181 | 70,814 |
| Pigs | 174,305 | 110,137 | 366,104 |
| Chickens | 1,618,783 | 1,690,655 | 2,335,629 |
| Ducks | 11,122 | 78,931 | 49,197 |
| Other | 15,240 | 74,447 | 35,070 |
| Meat[3] | 53.33 | 20.05 | 36.73 |
| Milk | 207.96 | 78.73 | 130.26 |
| Eggs | 11.29 | 3.38 | 7.94 |
| Wool | 1.41 | 0.57 | 0.61 |

1 El-Shazly and Naga (1)
2 Animal numbers in thousands
3 Production in million tons

TABLE II. Meat and milk supplying minimum animal protein requirements for the world population in 1970, 1985 and 1990[1]

| | 1970 | 1985 | 1990 |
|---|---|---|---|
| Minimum animal protein requirements (thousand tons/day) | 105 | 142 | 154 |
| Projected world meat consumption (million tons) | 107 | 168 | 197 |
| Protein consumption from meat/day (thousand tons/day) | 58 | 92 | 108 |
| Projected world milk consumption (million tons) | 389 | 532 | 597 |
| Protein consumption from milk/day (thousand tons/day) | 37 | 51 | 57 |
| Protein consumption from meat and milk/day (thousand tons/day) | 95 | 143 | 165 |

1 El-Shazly and Naga (1)

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Diets for food-producing animals also routinely contain additives which do not supply nutrients. Agents which control disease, increase growth rate and/or improve feed efficiency include antibiotics, antibacterials, anabolics and chemicals which control specific pathways of rumen fermentation. Compounds which improve handling, processing properties and acceptability are also utilized (6).

## Diet Formulation

The objective in formulation is to translate knowledge about nutrients, feed ingredients and animals into diets that will be consumed in sufficient quantities to provide nutrients for maintenance plus production at least cost. Mathematical principles are relatively simple but because of the multitude of manipulations required to provide exact amounts, minimums, maximums, ranges or ratios of nutrients and feed ingredients, computerized linear programming methods are routinely employed. Computer models being developed to describe entire production systems formulate diets based upon sex, age, breed, frame size and initial flesh condition of animals, feed additives and growth stimulants used, and environment; they simulate use, grade and price differentials and make economic projections. Information obtained can be used to select the most profitable type and weight of animal and evaluate the economics of alternative feeding systems (7).

## Feed Ingredients

Animals are often criticized as consumers of food grains which could be consumed directly by humans. However, grains are fed when they are economical sources of nutrients and when prices increase, less grain is fed to livestock and more is sold directly (8). In 1974, 50% of the world's grain production was consumed directly by humans, 40% went into animal feeds and the remaining 10% was used for industrial processes, seed and other purposes (9). Seventy-five percent of the feed grains produced in the United States was fed to livestock but this accounted for only 27% of feed consumed (9) because nutrients were also provided by forages, residual materials from the manufacture of human foods, crop residues, animal wastes and pure chemical forms of nutrients. Ruminants in particular provide a mechanism to utilize nutrients from the 64% of the world's agricultural land suitable only for forage production (10) and the large reservoir of carbon in crop and industrial by-products (11).

The Ralston Purina Company Arkavalley Farm at Conway AR is an excellent example of food production utilizing limited quantities of conventional food grains and protein supplements. During 1974, 1414 milking cows consumed daily 30 kg sorghum silage and 14 kg of the grain mixture in Table III and produced an average of 21 kg milk daily (12). Sixty-nine percent of the ingredients in the

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TABLE III. Composition of grain rations fed to milking cows at Arkavalley Farm during 1974.[1]

| Ingredient | % |
|---|---|
| Corn | 1.04 |
| Milo | 18.78 |
| Rice bran | 20.75 |
| Soybean hulls | 24.14 |
| Wheat middlings | 5.47 |
| Cottonseed meal | 12.46 |
| Soybean meal | 6.02 |
| Brewers grains | 3.48 |
| Urea | .59 |
| Fat | .47 |
| Molasses | 1.92 |
| Vitamins and minerals | 4.88 |

[1]Kertz and Everett (12)

concentrate mixture (i.e. rice bran, soybean hulls, wheat midlings, cottonseed meal, brewers grains, urea, fat and molasses) are not suitable for human consumption or processing costs to make them suitable are prohibitive. Of the remaining 31%, corn, soybean meal, and milo could be converted into food for humans but milo is not used for human food in the United States. With beef cattle, 1 kg of beef protein can be produced with 3 kg or less of feed grain plus oil seed meal protein (8). This is a birth-to-market calculation which takes into account that perhaps 80% of the beef animal's lifetime diet is forage and that about 75% of the beef is produced before the animal goes on a grain diet. This ratio can be narrowed further if some nonprotein nitrogen is used as a protein concentrate extender (10).

## Voluntary Feed Intake and Production

The maintenance of an energy balance in animals requires that the metabolizable energy available to an animal be similar to the energy demands (or energy loss plus deposition). Feed is the source of metabolizable energy and, therefore, a high correlation usually exists between feed intake and the rate and efficiency of production. What in fact is being regulated or which are the dependent and which are the independent variables of the energy metabolism systems of an animal are not always apparent. Clearly, production of milk or growth rate can be reduced by limiting feed intake, but either can increase feed intake. For any one diet there probably is a maximum feed intake for an animal, under a single set of conditions, due to both physical and metabolic

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factors. Various interrelated factors determine how near this maximum intake an animal will voluntarily consume.

There is a commonly held belief that animals fed ad libitum eat all they "can". But is it implied that there are some physical limitations to eating more, or rather physiological and/or psychological limitations? Several species have been shown to be capable of digesting and metabolizing substantially more feed than that voluntarily eaten. For example, chicks force-fed for 15 days so that their intake was 170% of the control group grew 50% faster and retained nearly 40% more of the feed-derived energy in their carcass than did the controls (13). Therefore, the control chicks were eating at a level that did not approach their physiological capabilities.

It is a common observation that those groups of animals which eat more of a specific ration gain more rapidly and efficiently. As illustrated in Figure 1, growth rate is proportional to the net energy available for growth, while the net energy for maintenance remains constant. Since the end of a growth period is usually established by animals' reaching a certain weight range or finish and not a set number of days, the cost of production is reduced for animals with a greater proportion of net energy available for growth as a result of their greater feed intakes. As shown in Figure 2, the cost of net energy for maintenance decreases (for the period required for a 200 kg gain in a steer) as the proportion of net energy for growth is increased to that for maintenance, which could result from increased feed intake. Therefore, less feed is required for the production of the same quantity of meat from an animal which is eating more and making a greater proportion of the total net energy available for growth on any one day, because the steer needs to be maintained fewer days.

The controller for feeding is subject to modulation by several relatively independent physiological systems as illustrated in Figure 3. Feeding behavior in a wide range of environmental conditions appears to operate quite independently of the body temperature regulatory system. But under heat stress this system can apply considerable pressure to the controller of feeding behavior resulting in reduced intake. Similarly, the regulator for body water balance plays a very passive role except under conditions of dehydration which places restrictions on feed intake. A third modulator of feeding behavior encompasses a wide variety of sensory inputs with many roles. Briefly, external sensory cues are important in the selection or aversion of possible foods. Also, they very often potentiate hunger drives or the inhibition of satiety and thus participate in precipitating slight shifts in motivation to produce meal patterns — feeding-no feeding conditions. The internal condition modulation is frequently the only apparent cause of the hypophagia associated with most pathological states.

The remaining system illustrated in Figure 3, the energy balance regulator, is theoretical, but there is substantial circumstantial evidence that it may exist. This regulatory system is

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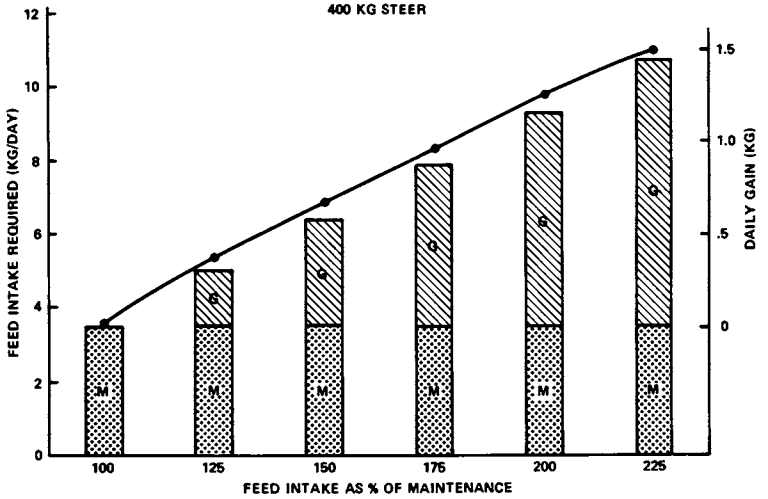


*Figure 1. The dependence of rate of gain on the proportion of the net energy available for growth (G). These relationships assume a 400-kg steer is receiving the same diet but in increasing amounts relative to that required for maintenance (M).*

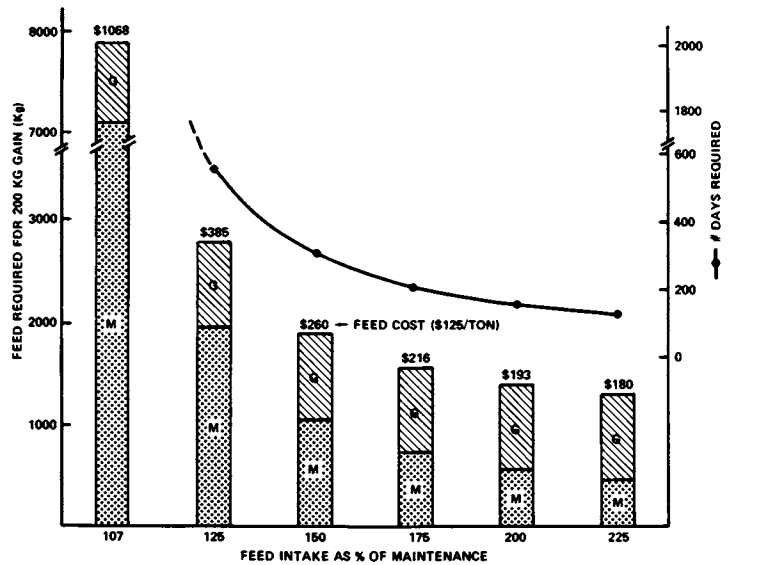


*Figure 2. The cost of growing a steer from 300 to 500 kg when increasing feed intakes relative to maintenance are maintained. The requirement for net energy for the gain (G) of the 200 kg remains constant, but the net energy required for maintenance is reduced as the number of days required for the gain is reduced.*

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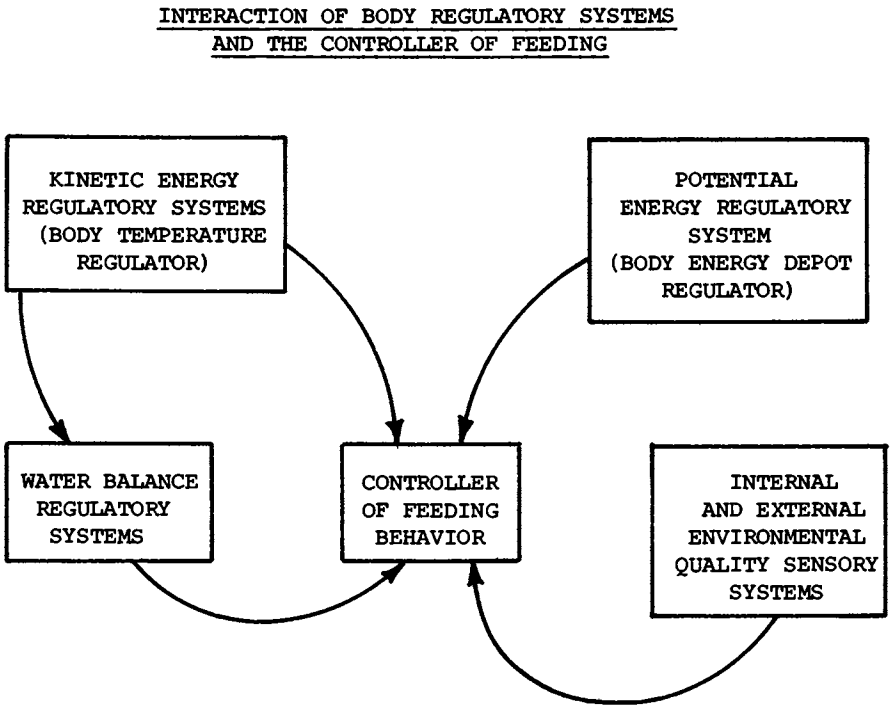


*Figure 3. The dependence of the controller of feeding behavior on several physiological systems. Body temperature and water balance regulatory systems apply little pressure on the controller of feeding behavior except when either reach much imbalanced conditions, e.g., heat stress, water dehydration. The sensory systems are especially important in initiating a meal and in selecting and avoiding food stuffs. There is strong circumstantial evidence for an energy balance regulatory system which modulates feeding behavior* (14).

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thought to be the reason body weight (or body energy content) remains rather constant in a variety of environmental and nutritional conditions. It applies pressure on the controller of feeding behavior so that the summation of the energy derived from the meals per day or per week closely matches the energy demands (15). Therefore, the concept of a relatively independent energy balance regulator requires that there be an interface with the relatively dependent controller for feeding.

## Chemical Senses and Feeding

The chemical senses are composed of stimuli from the external environment which act on specialized cells. They are commonly divided into three classes: 1) olfaction, 2) gustation, and 3) the common chemical sense. Olfaction permits reception of distant airborne volatile substances often at very diluted concentrations while gustation generally requires contact with the chemical stimulant source. Irritants are the main stimulants for the common chemical sense and involve little specificity.

Food-producing animals utilize a sniffing behavior often associated with the apprehension, selection and prehension of feeds. The importance of odor in food selection by sheep was investigated by offering control and bulbectomized sheep food from control containers versus containers that had been adulterated with acetic acid, camphor or iodobenzene (Table IV). Bulbectomized sheep selected approximately equal quantities of food from each container whereas control sheep only consumed about 25% of their 60-minute intake from adulterated containers (31).

Taste is very species dependent and seldom is predictable based on the experiences and preferences of man. While we classify our tastes as sweet, sour, bitter and salty, animal behaviors in relation to taste are better described as evidence of preferences, aversions, or indifference (19). In studies with cattle, sheep and normal and pygmy goats with solutions representative of the four classical tastes, cattle discriminated sweet, salty and sour tastes at lower concentrations than the other species. Cattle, however, showed the poorest discriminating behavior with bitter tastes and also tolerated the bitter taste at the greatest intensity (20).

As suggested above, the chemical senses play several roles in the selection of feed and the control of feed intake. The animal can sometimes be induced to adjust its feeding behavior by changing sensory qualities of its feed. The aversive sensory qualities of certain feeds can be masked to allow economical utilization of the feeds. For example, it has been shown that sheep will eat aversive feeds if a local anesthetic, e.g. carbocaine, has been added to the feed (16). In studies with taste modifiers on responses of pygmy goats to sucrose and quinine hydrochloride (17), gymnemic acid and monosodium glutamate reduced sensitivity to sweet and bitter substances whereas inosinic acid enhanced

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TABLE IV. Percent feed eaten after sixty minutes from treated containers by control and bulbectomized sheep given a choice between treated and control containers.[1]

| Treatment | Control | | Bulbectomized | |
|---|---|---|---|---|
| | No. Sheep | % | No. Sheep | % |
| Acetic acid (Moles/kg feed) | | | | |
| .21 | 12 | 30±3.8 | 6 | 51±3.2* |
| .05 | 8 | 41±6.2 | 5 | 51±5.7* |
| Camphor[2] | 12 | 22±6.1 | 6 | 58±5.6* |
| Iodobenzene | 12 | 25±8.9 | 6 | 63±7.5* |

1 Baldwin, McLaughlin and Baile (31)

2 A gauze saturated with either a 33% camphor solution or iodobenzene was wiped around the inside top of the container to form a 2 to 5 cm ring.

* Significantly different from control sheep at $P<.02$.

sensitivity to the bitter chemical. Feed mixtures containing non-protein nitrogen chemicals are consumed in lesser amounts than those supplemented with protein but the unpalatability of diets containing more than 2% urea has not been overcome in numerous experiments using various flavors, odors, coatings, extrusions, and other physical and processing methods (18). The use of flavors in preferred feeds is of apparent benefit in only specific situations as discussed later.

In Table V are listed some of the common additives used in animal feeds to influence sensory qualities. Unfortunately few have fully substantiated advantages and many of the mixtures are prepared primarily for the benefit of the person feeding the animal and are not necessarily flavors or fragrances preferred by the animal. In addition, both molasses and fat are common dietary ingredients. Both improve diet acceptability, but at least part of the improvement is the result of these feed ingredients reducing dustiness of processed feeds. Molasses is usually considered an aid to acceptability by contributing sugar or sweetness to processed feeds. It is often used as a carrier of otherwise aversive ingredients (e.g. urea). Because molasses is a variable product, a flavor is often added to it to maintain a required standard of acceptance.

Those additives for the young animals have perhaps the best evidence for efficacy (21, 22, 23). The baby pig has been recognized to respond favorably to sugar (22, 24) and to certain fats

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TABLE V. Additives used to flavor feeds[1]

| Name | Classification under food additives amendment | Limitations or restrictions[2,3] |
|---|---|---|
| Aniseed | Spice, seasoning | None |
| Capsicum; red pepper | Spice, seasoning | None |
| Fennel | Spice, seasoning | None |
| Fenugreek seed | Spice, seasoning | None |
| Ginger | Spice, seasoning | None |
| Glycyrrhizin ammoniated | Spice, seasoning | None |
| Monosodium glutamate | Spice, seasoning | None |

1 Muir, Stutz and Smith (6)

2 Generally recognized as safe

3 No quantitative restrictions although use must conform to good manufacturing practices

(25) which can result in earlier creep feed consumption prior to weaning. The pigs that are supplementing their milk consumption from the sow with substantial dry feed endure the stresses of early weaning (e.g. 3 weeks of age) with greater growth and lower mortality rate.

While some additives merely serve as attractants to the feed, another approach recently tried has involved apparent imprinting. The etiology of suckling behavior in neonatal rat pups has been studied extensively (26, 27). The neonatal pup's sensory experiences during the first hours of life are major determinants of cues for suckling (28). Therefore, the cues for suckling can be adjusted in experimental conditions to be quite foreign to those occurring naturally.

Perhaps in a related manner, a substance (Firaner No. 3, Firmenich et Cie, Geneva) added to the feed of sows during lactation was shown to develop better acceptance by the pigs of a feed also containing the substance (29). The pigs that 1) were from sows fed the substance during lactation and 2) also received creep feed with the substance added ate more and grew faster during the post weaning stages than pigs that 1) received none of the substance, 2) received the substance only in the feed, or 3) received only the basal creep feed and suckled sows fed the substance. Some evidence has been generated to indicate that the substance can be transmitted to the pigs via the sow milk when the substance is included in the sow feed (personal communication, F. C. Madsen, Syntex Agribusiness, Inc., Springfield, MO). Furthermore, only those pigs receiving the substance via the milk developed a preference over the basal feed for the creep feed containing the substance.

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Thus there is some evidence that post-weaning stresses can be reduced in pigs by a manipulation of the sensory cues associated with suckling and feeding.

Another group of neonates, dairy calves, under many management systems also are encouraged to initiate consumption of dry feeds at as early an age as possible. Various flavor additives are available to help entice the calves to eat the feeds, perhaps with some success. In a recently reported study a flavor was added to both the milk and dry feed of calves for the first 5 weeks of life (30). These calves ate more and grew faster than calves receiving either no flavor in milk or feed or flavor only in the milk. The authors suggested that this flavor was providing a means for association between the milk and the dry feed thus causing better acceptance of the dry feed, i.e. greater rate of intake.

## Summary

The formulation of rations must first provide the nutrients in an appropriate ratio for the animal to be fed. In many cases when animals are under environmental or physiological stresses, voluntary feed intake limits production and in some instances feed acceptability plays a major role. In these cases the ration formulation processes should include measures for the use of additives to induce appropriate intakes by the animal.

The use of flavors during the transition between diets can often be beneficial and this is especially true when neonates are being changed from milk to a dry diet.

## Literature Cited

(1) Anonymous. Dairy Council Digest. Nat. Dairy Coun. (1976) 47:1.
(2) Church, D. C. Page 132 in "Livestock Feeds and Feeding", D. C. Church (Editor). O and B Books, Corvallis, OR, 1977.
(3) El-Shazly, K. and M. A. Naga. Page 485 in "Proceedings of the World Food Conference of 1976". Iowa State University Press, Ames, 1977.
(4) Chalupa, W. Page 99 in "Reviews in Rural Science II, T. M. Sutherland, J. R. McWilliam and R. A. Leng (Editors). Univ. New England Publ. Unit, Armidale, NSW, Australia, 1976.
(5) Clark, J. H. J. Dairy Sci. (1975) 58:1178.
(6) Muir, L. A., M. W. Stutz and G. E. Smith. Page 27 in "Livestock Feeds and Feeding", D. C. Church (Editor). O. and B Books, Corvallis, OR, 1977.
(7) Fox, D. G. and J. R. Black. Feedstuffs (1977) 48(23):21.
(8) Anderson, W. Feedstuffs (1975) May 12:110
(9) Church, D. C. Page 2 in "Livestock Feeds and Feeding", D. C. Church (Editor). O and B Books, Corvallis, OR, 1977.
(10) Chalupa, W. Proc. Nutr. Soc. (1973) 32:99.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

(11) Heichel, G. H. American Scientist (1975) 64:64.
(12) Kertz, A. F. and J. P. Everett, Jr. J. Dairy Sci. (1976) 59:775.
(13) Nir, I., N. Shapira, Z. Nitzan and Y. Dror. Brit. J. Nutr. (1974) 32:229.
(14) Baile, C. A. Oklahoma Vet. (1975) 27:2.
(15) Baile, C. A. and J. M. Forbes. Physiol. Rev. (1974) 54:160.
(16) Baile, C. A. and F. H. Martin. J. Dairy Sci. (1972) 55:1461.
(17) Mehren, M. J. and D. C. Church. Anim. Prod. (1976) 22:255.
(18) Kertz, A. F. and J. P. Everett. J. Anim. Sci. (1975) 41: 945.
(19) Kare, M. R. Page 1161 in "Duke's Physiology of Domestic Animals", 8th Ed., M. J. Swenson (Editor). Cornell University Press, Ithaca, NY, 1970.
(20) Goatcher, W. D. and D. C. Church. J. Anim. Sci. (1970) 31: 373.
(21) Hagsten, I. B. Confinement (1976) 1(8):21.
(22) Catron, D. V. and L. A. Facto. Distillers Feed Conference (1960) 15:60.
(23) Aldlinger, S. M., V. C. Speer, V. W. Hays and D. V. Catron. J. Anim. Sci. (1959) 18:1350.
(24) Kennedy, J. M. and B. A. Baldwin. Anim. Behav. (1972) 20: 706.
(25) Speer, V. C. Swine Nutrition and Management (1976) 48:25.
(26) Galef, B. G., Jr. and M. M. Clark. J. Comp. Physiol. (1972) 78:220.
(27) Galef, B. G., Jr. and D. F. Sherry. J. Comp. Physiol. (1973) 83:374.
(28) Teicher, M. H. and E. M. Blass. Eastern Psychol. Assoc. 48th Annual Meeting (1977), p. 40.
(29) Campbell, R. G. Animal Prod. (1976) 23:417.
(30) Morrill, J. L. and A. D. Dayton. J. Dairy Sci. (1977) 60: 72.
(31) Baldwin, B. A., C. L. McLaughlin and C. A. Baile. Appl. Anim. Ethol. (1977) 3:151.

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# 9

# Developing Palatable Foods for Domestic Pets

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The development process for Pet Foods is, by its very nature, of greater complexity than the development process for human foods. The family pet typically eats only once a day, although there are exceptions; puppies and extremely geriatric or ill pets might require more frequent smaller feedings. Because this once-a-day feeding represents the pet's total caloric consumption for the day, it is necessary that this food must be nutritionally complete and, in addition, sufficiently palatable so that the pet will consume a large enough quantity to receive an adequate amount of the nutrition built into the food.

A major point of differentiation from the human food development guidelines is the reservoir of ingredients that the Pet Food formulator has available from which to draw. Due to cost considerations, Pet Food researchers are dealing with commodities that are largely outside the human chain, such as oilseed meal, condensed fish solubles, animal by-products, etc. These items, although nutritionally satisfactory, have low palatability indices and must be strenuously upgraded to make them organoleptically acceptable to pets.

This has made Pet Food development a field which is highly technologically oriented and therefore a very capital intense industry.

However, by far the most fascinating aspect of Pet Food development is the consumer dichotomy dilemma. Who is the consumer — the individual who purchases the product, or the pet that literally consumes it? One is concerned with cost, appearance and convenience and the other is concerned with palatability, aroma, texture, etc. It is this dichotomy, satisfying two distinctly different consumers, that makes Pet Food development the intriguing challenge that it is.

## The Development Process

The Development Process typically includes four major stages: Idea Search and Exploratory, Early Development, Advanced

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Development and Test Marketing.

A. Search Stage

In the Idea Search or Idea Generation Stage, the goal is to identify a consumer want (or need) which is significant enough to represent a profitable business. Furthermore, this want or need must also capitalize on the existing strengths of the corporation and tie directly to corporate objectives and strategies.

The idea generation may start with an exploration of consumer needs -- focus groups and creative consultants are sometimes employed to assist in the identification of these needs. Alternatively, it may start with a technical opportunity.

Example: The development of semi-moist dog foods stemmed from the central idea of stabilizing the contents of the typical canned dog food product. Technical considerations thus centered on how to maintain the nutrition, palatability (taste) and cosmetic aspects of retorted/canned pet foods. Constraining the development of semi-moist products was the lack of know-how to control the free water associated with the canned dog foods and achievement of microbiological stability. Thus, the key to successfully achieving the desired goal was to develop a process and formulation system which would keep the water activity (Aw) at levels below that necessary to support microbial growth. The packaging system needed to be one which would protect the product from moisture loss over the desired shelf life while providing ease of opening and low cost.

The search process may also grow out of strategic considerations as a way of encouraging category growth, or it may be prompted by other considerations; for example, the business environment or the desire to more effectively use existing assets.

Example: Development of soft-moist puppy food for Gaines fulfilled this type of Idea Generation as physical assets having the capability for producing products of this nature existed. Technical activities were undertaken to develop a product meeting the taste, nutrition and cosmetic optimums for puppies and constrained by the fact that it must meet the requirements of the process which existed.

So, where do you start looking for new products? Corporate objectives, mentioned earlier, provide focus for starting. Ideas for new products generate from many sources; i.e., consumers, employees, technology and market analysis. However, one must be careful to remain aware that if the origin of an idea is not the

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result of the extrapolation of a consumer need, but developed from a technological breakthrough, then this breakthrough must be oriented toward an identified consumer need. No product can exist in a market where there is no need or desire for it. It is possible to have all the technological capabilities and still not succeed.

An example of a technical capability that existed which did not fulfill a consumer need was Pizza Sticks; the consumer response to this high technology product turned out to be very poor. A critique as to why this occurred showed that the products taste satisfaction was not as high as the concept led the consumer to believe, for the price asked; thus, non-desirability occurred via the consumer and the product failed as a business venture.

On the other hand, if the consumer need is real, an early assessment must be made of its technical feasibility. If the product can't be developed, no matter how great the consumer need, it is a technological failure.

An example of this type of product would be a dog food which has the consumer attributes of convenience and economy, and has superior taste qualities which dogs prefer. At this point in time the achievement of that goal is a tough challenge.

This completes the formal idea generation process; however, there is an informal one which consists of conversations with technical and non-technical members of the organization and elsewhere. From this process new ideas which have been generated by the formal process can be assessed for vitality.

B. Early Development

The goal during the early development stage is to resolve issues related to the project which are judged to be fundamental to its success or failure. While these are somewhat unique to the specific project, the following areas are typically pursued. From a consumer standpoint, there is a need to precisely define the want or problem to be addressed by the new product and to assess the breadth of appeal to gain confidence that the need is a substantial one.

The initial parameters of the business opportunity are also generally defined in this stage of development to make sure that the magnitude of the consumer response is in line with product cost and capital spending requirements for the business. The focus is toward end product characteristics and not on cost at this juncture.

This will help answer the question -- "Can any product be made that will fulfill the basic consumer/dog promise?" It will also provide the experience needed to revise objectives and performance targets.

A discussion on the actual process used in the formulation stage is essential as it has some very interesting considerations. It is basically similar to that which would be used in the fabrication of a food for human uses; that is, the key factors

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that must be considered are: (1) selection of ingredients, (2) product fabrication or the process of preparations, (3) nutritional guidelines, and (4) palatability or acceptance. In the latter case, this is meant to be acceptance by dogs as opposed to people.

(1) Ingredient Selection. The materials chosen to yield balanced and complete animal ration will include a meat protein source, a vegetable protein source, a ration-balancing protein supplement and other nutritional supplements. Cereal grains or cereal grain by-products such as hominy feed, whole grained corn, wheat mids, Red Dog, etc., are also employed as sources of calories. One or more of these components may be omitted depending upon animal preference and how nutritional requirements have to be balanced out.

The term "meat protein material" refers to a group consisting of meat, meat by-products and meat meal as well as mixtures of these materials. The term "meat" applies to the flesh of cattle, swine, sheep and goats. The term "meat by-products" refers to those non-rendered parts of the carcass of slaughtered animals including mammals, poultry and the like. They may also include constituents included in the term "meat by-products" described in the Definitions of Feed Ingredients published by the Association of American Feed Control Officials.

The term "meat meal" refers to the finely ground, dry rendered residue from animal tissues including dried residues included in the definitions of the Association of American Feed Control Officials.

The term "vegetable protein source or concentrate" applies to oilseeds and legumes, the oil-expressed/extracted meals and protein isolates recovered by acid or alkali digestion and precipation. These materials normally originate from vegetable protein sources such as soybean meal, cotton seed meal, peanuts, peanut meal, etc.

The term "ration-balancing protein supplement" refers to milk products as defined by the American Association of Feed Control Officials and includes such materials as dried buttermilk, dried skim milk, dried whole whey, casein and cheese rind. It also includes yeast (as that term is defined by said Association) and hence refers to such materials as distiller's dried yeast and torula dried yeast.

The term "sugar" as it is employed is any of a number of useful saccharide materials which provide water binding and sweetness impacts. Included in the list of useful sugars are the reducing and non-reducing water-soluble mono-saccharides and the reducing and non-reducing poly-saccharides. In the case of semi-moist, the sugars are of a low molecular weight so as to offer a substantial effect in increasing the osmotic pressure.

Although these materials are key to most Pet Food formulators, there are other key factors of major concern that must be

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considered. Are the ingredients available in quantities large enough to support the size of the business you have projected? What percent of the total market will your formulation utilize for each ingredient? When you enter the market as a purchaser of the selected ingredients, how will your large volume purchases affect the cost of the particular raw material? All these questions have to be addressed and resolved before continuing.

(2) Product Fabrication. Animal foods and particularly dog and cat foods are commonly prepared for the consumer in three forms: the meal-type ration which has a dry more-or-less cereal-like texture and a low moisture content, typically about 10%; the canned-type ration which has a more-or-less meat-like texture and a high moisture content in the neighborhood of 75%; and, the semi-moist-type ration which has a moist meaty appearance and a moisture range of 15 - 35%. Due in large measure to the difference in moisture content, these three forms of animal foods have widely divergent product characteristics, some desirable and some undesirable. Such foods are generally formulated from: (i) meat and/or meat by-products, or (ii) one or more vegetable protein sources as well as combinations of these together with (iii) other nutritional supplements.

Meal-type animal foods, on the one hand, generally have a very high nutritional and caloric value, providing a complete and balanced diet for the animal, and excellent storage characteristics, thus permitting the use of relatively inexpensive packaging techniques. However, the palatability of many dry meal-type animal foods is poor and, in many cases, the animal will not eat them at all in dry form, necessitating the addition of liquids prior to their consumption. Liquid addition often fails to solve the palatability problem since the products become mushy or doughy and are rejected by the animal assuming there are alternative food sources available. Such reconstitution fails to bring forth the inherent initial palatabiltiy factor possessed by meat and meat by-products. Therefore, the desirable nutritional characteristics of this form of animal food may be defeated by its relatively poor palatability. In general, product stabilization against microbiological spoilage is achieved in such products by maintaining the moisture content below the critical level for vegetative growth of such organisms as yeasts, molds, and bacteria.

Canned-type animal foods, on the other hand, are generally received very favorably by animals, apparently due in part to their meat-like texture, consistency and aroma. However, the elevated moisture content of such products necessitates thermal processing in sealed containers to obtain a commercially sterile product, thereby adding considerably to product cost. Furthermore, once such a can is opened, it must be quickly consumed since the product is quite conducive to supporting microbial growth and hence will deteriorate very rapidly unless stored

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under refrigeration.

Semi-moist Pet Foods are generally received more favorably by the dog than are dry, meal-type animal foods. Compared to canned foods, the semi-moist products are sometimes preferred in the case of lower priced maintenance diets, but less preferred than the higher priced (higher meat-type) diets.

These diets generally will have moisture contents less than 35% and greater than 15% with a level of water soluble solids between 15 and 35 percent by weight. Ordinarily, much of the soluble solids are soluble sugars while others are other low molecular weight materials such as propylene glycol, sorbitol, sodium chloride; all of which contribute to the microbiological stability (osmotic effects) and dog palatability.

The fabrication steps required to produce the various types of palatable Pet Foods are quite varied and each step in the fabrication process has significant impact upon the animal acceptance level.

a. Dry Fabrication. Normally, such products are made by blending and mixing the selected ingredients in the proper ratio to meet the nutritional targets. This admixture is then traditionally preconditioned by grinding, sizing and moisture addition just prior to the heating, cooking and extrusion steps. This phase of processing can be accomplished in a variety of equipment types but generally is effected in a continuous cooker/extruder/expansion chamber. Product issuing from the exit of the expansion chamber is then dried to less than about 10% moisture and palatants like animal fats added to increase the level of pet acceptance prior to packaging.

b. Semi-Moist Fabrication. Raw materials meeting the nutritional, stability and acceptance levels desired are selected from the spectrum of ingredients available within the imposed cost constraints and combined in such a manner to form a complete matrix prior to final heat treatment. During cooking, temperatures of 180 - 212°F are achieved to effectively pasteurize the matrix and reduce the mesophilic bacterial population to the point where the stability system can maintain the desired shelf life. Once pasteurized, the cooked product is cooled and formed into the desired product shape for packaging and dog acceptance.

c. Canned Fabrication. Traditionally, the selected materials meeting the nutritional, cost and acceptance levels desired are mixed to the proper moisture level and placed in a container which can be hermetically sealed. The container is then steamed, closed and retorted under conditions which will achieve commercial sterility (shelf life for a period of not less than 90 days). The time and temperature conditions employed to achieve commercial sterility having significant impacts on animal and consumer acceptance levels.

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Once retorted, the product is cooled, labeled and packaged for sale.

(3) Nutritional Guidelines. Nutritional research is normally conducted parallel with the formulation and fabrication development testing using nutritional parameters which are more stringent than most human foods; the reason being, as mentioned earlier, that the diet of the animal will not vary as much as a human's. Therefore, the total nutritional requirements must be present in each feeding/serving provided to the animal. These requirements are clearly described in the National Research Council guidelines for each product -- Canned, Dry or Semi-Moist. They list all the key factors that must be included and suggested levels based on anticipated animal consumption.

In addition to insuring that specific ingredients are contained within a new product, the food must be subjected to a series of rigorous nutritional tests, or meet the nutrient profile established by the NRC. For example, to claim that the product is nutritionally sufficient for adequate growth, one must demonstrate that puppies will develop normally (i.e., within breed standards). The procedures have been dictated by AAFCO and include tests for growth, gestation and lactation and maintenance.

More specifically, to substantiate through testing that a food is nutritionally complete and balanced, the industry must conduct the AAFCO gestation and lactation study as follows:

A sufficient number of pregnant bitches must be employed to insure at least six impregnated females during the study. Feeding of the food to be tested is initiated at the first sign of estrus and continued until the offspring are weaned. Each bitch is to be fed only the test product and water.

The dependent measures include food intake, body weight (bitch and puppies), the sex distribution and the size of the litter, hemoglobin and packed-cell volume, and a routine physical exam conducted by a Veterinarian.

Criteria for success include weight gain during gestation and subsequent return to pre-gestation weight, 75% of the one-day-old pups must be weaned, and the pups must develop normally within the breed standards. Until these data are collected, no claim can be made about a product's adequacy for gestation and lactation.

(4) Palatability or Acceptance. In order for the food to satisfy the dog's nutritional requirements, it must be ingested by the animal. Therefore, intensive acceptance (or "preference") testing of a new product must be conducted to insure adequate palatability. A standard preference testing method (simultaneous presentation of two products) is used to measure the average difference in amount consumed, converted to a dry weight basis.

Early Development is typically concluded with results which confirm consumer appeal of the idea, provide promise that prototypes can achieve consumer expectations and that both of the



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above are in line with business proposition objectives.

C. Advanced Development

The goal in the Advanced Development stage is to bring all the elements together to make the product a commercial success. From a technical aspect there is a shift from product to cost emphasis. The focus is now on maintaining product performance standards within cost targets which make it an acceptable business proposition while developing a commercial process for producing the product on a test market and national scale.

This involves a coordinated effort between Research, Engineering, Operations, Finance and Purchasing to make sure that all aspects of the Development Process are now focused on one goal -- a satisfactory product at an acceptable cost to produce a viable market opportunity.

Product reformulations within the Advanced Development phase is normally a reiterative process similar to that in the Early Development phase. The goal at this juncture, however, is to refine or fine tune the animal acceptance levels by making small changes in texture, flavor, size, shape, etc., as needed to optimize desired effect. Also, and possibly of even greater significance, are the changes brought about to optimize the consumer's preference.

D. Test Marketing

When Advanced Development has been successfully concluded, the new product is readied for Test Market. The goal of this phase is to evaluate the idea in a competitive environment and gain the experience necessary to improve it prior to a national launch. There is the opportunity to gain experience with a small scale process, therefore better defined than national process. There is also the opportunity to revise the product based on consumer feedback. It also may provide insight as to how our competition will react when this new product is entered on a national basis.

If the new product meets or exceeds Test Market objectives, the national expansion may begin. Alternatively, Test Market experience may suggest a need for major changes which may require subsequent Test Marketing. Since Test Marketing is an expensive step in the total process, every effort is made previously to screen out candidates which do not have a high probability of success and make sure the best plans and programs are developed for the new product.

To summarize, the entire process can be described as follows:

> The Search Step attempts to identify an entry point into the market. Early Development provides indication from both a consumer and business standpoint as to whether this entry point really makes sense. Advanced Development

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determines how to best take advantage of this entry point and provide indication as to whether or not it can be an attractive business. Test Marketing provides the competitive experience needed to accurately and fully evaluate the new product. It is both the last stage in the Development Process and the first step toward making the product a commercial reality.

The aforementioned activity can take as little as one year to complete or may take as long as five years depending on the "newness" of the concept, the program priorities, the need for technical breakthroughs, consumer response to the concepts and prototypes developed, and a whole host of other factors, some beyond the control of the Researcher.

This short paper attempts to capture the flavor of the Development Process for an area that is unique in that there is no direct communication with the real consumer to "talk about the product."

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# 10

# Repellents to Protect Crops from Vertebrate Pests: Some Considerations for Their Use and Development

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The concept of employing a non-lethal repellent to control wildlife depredation on crops arose early in agricultural history and has been pursued vigorously ever since. The present popularity of this idea reflects the realization that other methods (i.e. reproductive inhibition, killing) are frequently impractical or socially unacceptable. The purpose of this paper is to examine the concept of repellency with respect to the changes that are occurring in the methods of developing repellents for controlling depredations upon forest and agricultural crops by vertebrate pests.

For the purpose of this discussion I will define a repellent as a compound or combination of compounds that, when added to a food source, acts through the taste system to produce a marked decrease in the utilization of that food by the target species. The action can be primary, where the animal reacts to the taste of the repellent alone, or secondary where the animal uses the taste of the repellent as a cue to later adverse effects. This definition specifically excludes taste-acting additives to packaging materials and olfactory-acting materials that are placed in or around food. That is not to say that materials used in these manners cannot be considered repellents, but rather that the present discussion is restricted to conditions where the animal receives gustatory information directly from the food.

The early literature on repellents has been summarized many times (e.g. 1, 2, 3, 4). The impression left on the reader of these reviews is that no consistently effective chemical repellent has been developed for use against vertebrate pests. The reasons for this are complex, but stem from the fact that we have tended to be anthropomorphic in the most basic assumption of repellent action. Most humans realize that when offered an array of foods, some of which taste bad, we alter our feeding pattern to consume only those that taste good. This basic human experience has been translated directly to wildlife populations. The original assumption in repellent development seemed to be that if a potential food could be made bad-tasting enough, the pest

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species would stop eating it. Thus it appeared that the only problem to be confronted was to find the bad-tasting chemical. Consequently, chemical formulations were mixed with, or applied to, animal food in extensive screening tests. The major outcome of these tests was a long list of chemicals most of which did not repel target species satisfactorily under field conditions. This long list of non-repellents was a direct result of the search for a chemical solution to a biological problem without really attempting to understand the biology of the system. In what should have been a coordinated effort between biologists and chemists, biologists abdicated their responsibility. Dambach and Leedy in 1949 (5) suggested that simple field tests and screening efforts comprised a superficial approach to the problem of developing repellents. They realized that a coordinated effort between several disciplines was necessary before the problem could be understood and solved. Thirty years later this effort has only just begun.

The major short-coming of the past efforts to develop repellents was the basic lack of cognizance that we are dealing with animal behavior and physiology in all their complexity. The use of a repellent implies an attempt to cause a change in behavior. Specifically, a chemical stimulus is being asked to cause an animal to stop feeding upon the most readily accessible, abundant and palatable food. For this to occur the pest species is required to leave the area, or at the very least to make a major change in food habits. The development and discovery of such a chemical out of the vast array of available compounds demands that we study more than chemistry and the final solution involves more than chemistry.

Pharmacologists have long known that empirical screening tests, though most commonly used are relatively unproductive. Much modern effort in seeking drugs for medical purposes has emphasized study of the chemical structure-function relationships and the nature of target tissue-drug interaction. They have realized what the locksmith realizes when he is confronted with a lock without a key. It is more productive to study the lock to identify the kind of key needed to open it than to try every available key. By the same token, knowledge of physiology and behavior of feeding are imperative before consistently effective repellents for vertebrate pests can be developed.

## The Chemical Defenses of Plants

The elaboration of chemicals to control the feeding activities of herbivores is not new. We who have attempted to develop repellents against herbivores for a few hundred years would do well to study a group of organisms that has been developing these defenses for millions of years. That group, of course, is the plants, particularly the angiosperms. The pressure brought to bear on the angiosperms by herbivores may be the major force in

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their evolution (6, 7). In the face of such pressure this group has developed a bewildering array of chemicals (the so-called secondary plant substances) for defense against herbivores. These chemicals whose functions were once the subject of much conjecture are now recognized to be the major determinant of the acceptability of plants to their predators, the herbivores (8, 9, 10, 11, 12, 13).

Chemically, the secondary plant substances fall into five large groupings: phenolics, terpenoids, alkaloids, nitriles, and others including amines, mustard oils, long chain acetylenic alcohols, ketones, etc. Functionally, these secondary substances may inhibit growth, delay reproduction and attainment of sexual maturity, cause weight and fur loss, neurological disorders and shorter life span (including death) of animals consuming even small amounts of them (13).

The ecological relationships and effects of the secondary substances on herbivores have been most frequently studied in invertebrate-plant systems (cf. 14). Only a few studies have addressed this aspect of the plant-mammalian herbivore interaction (cf. 15, 16, 17, 18, 19) and almost none are concerned with birds. It is interesting that the many extensive studies of the food habits of herbivorous animals seldom address the reasons why some plants are utilized little or not at all. The following examples, one for a mammalian herbivore, two for avian herbivores, and a final one for a molluscan herbivore will illustrate how secondary plant substances can affect the utilization of plants by herbivorous species.

Sherbrooke (19) studied the utilization of the seeds of jojoba (*Simmondsia chinesis*), in southern Arizona by four species of heteromyid rodents. Of the species studied, only *Perognathus baileyi* was able to survive on a diet of jojoba seeds. The other three species, after initial sampling of the seeds, refused to eat them and subsequently died of starvation. This rejection Sherbrooke attributed to the presence of 2-cyanomethylenecyclohexyl glucoside (simmondsin) in the seeds. Simmondsin (rat oral LD50 $\geq$ 4 g/Kg, 20) has also been found to inhibit feeding in laboratory rats (21). The results of this study indicated that *P. baileyi* possesses a mechanism for detoxifying simmondsin that the others lacked. It is also interesting to note that earlier work by Rosenzweig and Winakur (22) suggested a direct connection between the distribution of jojoba and that of *P. baileyi*. Thus the jojoba plant completely protects its seeds from three potential predators by employing a toxic principle (simmondsin). This is not a static system however, for one mammal, at least, is equipped with a mechanism to detoxify simmondsin and can utilize jojoba seeds.

Cook et al. (23) examined the relationship between the mourning dove (*Zenaida macruora*) in California and one of its favorite foods, the seeds of the dove weed (*Eremocarpus setigera*). They reported that two types of seeds are produced by this plant:

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mottled seeds that are highly palatable; and grey seeds that are not palatable. They further report that water extracts of grey seeds contain a chemical that is toxic to goldfish. Water extracts of mottled seeds do not contain the toxic principle. This material they assumed, is what affects the palatability of the grey seeds to doves. Figure 1 illustrates the extreme selectivity of mourning doves given a mixture of mottled and grey seeds for a 30 minute feeding trial on each of 5 consecutive days. Field data (23) also demonstrate an increase in proportion of grey seeds (3.8% vs. 30.2%) in two samples collected about 6 weeks apart from a field population of Eremocarpus. The increase was attributed to doves selecting the more palatable mottled seeds.

This unpalatability of grey seeds is accompanied by a reduced viability of the seeds. The polymorphism has a genetic base. Its phenotypic expression is apparently induced by adverse environmental conditions and senescence that cause a shift to the "grey syndrome". Along with reduced seed viability this syndrome produces almost complete immunity to dove predation. Apparently the metabolic cost of producing the unpalatability in grey seeds causes a decrease in viability that is outweighed by the advantage of protection from doves in areas where seed predation is important.

Sherburne (24) studied the feeding patterns of a number of frugivorous birds on several species of fleshy-fruiting plants in New York. He concluded that unripe fruits were actively avoided by birds. The avoidance was hypothesized to result at least in part from the presence of toxic secondary compounds in the unripe fruit and the disappearance or diminished concentration of these same compounds from the ripe fruits. This was directly demonstrated for the fruit of one of the plants he studied (Rhamnus cathartica).

Another polymorphic population, that of the wild ginger (Asarum caudatum)was studied by Cates (25) in western Washington. He noted that populations of this plant are polymorphic for growth rate, seed production, and palatability to a native slug (Ariolimax columbianus). In sites where slugs were abundant the unpalatable form (characterized additionally by fewer seeds and later time of flowering) predominated, whereas the converse was true in those sites where slugs were less common (Figure 2). These data led Cates (25) to hypothesize that in areas where slugs were not abundant those plants that allocated more of their energy into growth and reproduction would be at a competitive advantage. However where slug predation was greater, those plants emphasizing the antiherbivore characteristic (unpalatability) would be selected for.

The foregoing examples lead to several conclusions about the antiherbivore characteristics of plants that hold important implications for the development and use of repellents. They are: (1) Part of the protected plant must be eaten; (2) not all plants have utilized the same secondary substances; (3) not all secondary

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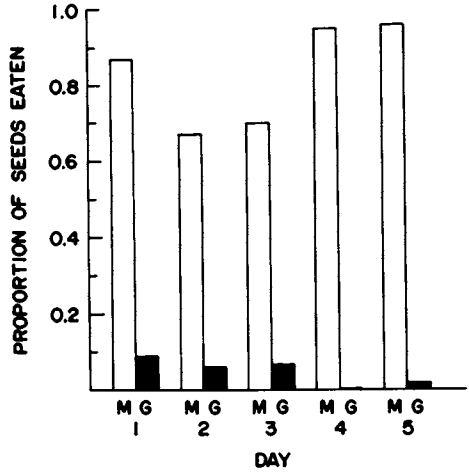


*Figure 1. The responses of mourning doves (6) to mixtures of 30 mottled (M) seeds and 15 grey (G) seeds. Drawn from data presented by Cook et al. (23).*

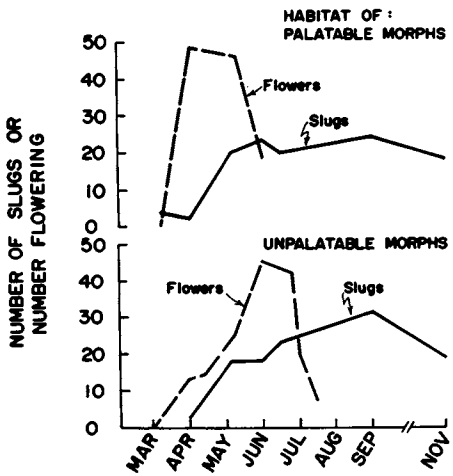


*Figure 2. Relationship between the number of slugs and flowering time of wild ginger (Asarum caudatum) in the habitat of palatable and unpalatable morphs. Redrawn from Cates (25).*

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substances are effective against all herbivores; (4) toxicity or some other adverse physiological effect is an important aspect of each plant-developed repellent; (5) the substances are not necessarily bad tasting upon first contact; and (6) all require a behavioral adjustment on the part of the herbivore.

## Poison Avoidance by Animals

The necessary behavioral response to a repellent has received little attention in the literature of repellency. On the surface it seems obvious that the required response is a cessation of feeding on the protected source. The physiological-psychological mechanism to achieve this result is less obvious. Most species have developed a behavioral mechanism with which to cope with toxic substances in their food. A brief reading of the vast literature concerning the use of toxicants against rats demonstrates this phenomenon. The major problem in the field use of rat poisons is that those animals receiving sublethal doses of the toxicant refuse to consume any more of the poisoned food (26). This behavioral response, a problem in lethal control, is exactly the behavior we are trying to induce with the use of repellents. This adaptive behavior (bait shyness) has been exhaustively studied as conditioned taste aversion by psychologists. Rozin and Kalat (27) have summarized much of the exhaustive literature, principally for the rat, and have described how the rat handles the problem of food selection: "...A rat becoming sick at a garbage dump [where he was poisoned]....may have eaten a few different foods. He knows it was a food that made him sick and can discount any familiar safe foods. With the capability of forming associations over long delays, he is now likely to associate his illness with the last relevant thing or few things he ate over the last few hours....(p. 478)". Thus, the taste of a food that made an animal sick is subsequently avoided-a conditioned aversion is formed.

Though it seems probable that most if not all pest species are capable of learning conditioned aversions in the laboratory, the conditions under which this most readily occurs would not necessarily be present in crop depredation situations. The depredating species is required to form an aversion to a familiar food-aversive agent combination. One might expect the learning of the aversion to be more difficult given the importance of novelty in taste aversion learning (27). It has, however been shown (28)that at least one crop depredating species, the red-winged blackbird (*Agelaius phoeniceus*), is able to accomplish this with relative ease. The possible decrease in response because the treated food has previously been considered as "safe" might be somewhat ameliorated by the fact that the aversive agent (repellent) would not usually be present in one of a vast array of foods. Thus the repellent would be expected to add a novel or unfamiliar taste to the familiar food. A great proportion of crop

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depredations occur in nearly monoculture situations (e.g., corn, rice, large fruit orchards) where, before the onset of the adverse post-ingestional effect, the target species would be expected to have consumed only a very limited number of foods, and possibly only the protected crop with the repellent material added, thereby simplifying the problem of associating the illness with a particular food.

Mammalian species seem to be able to form conditioned aversions most readily to gustatory cues and rely upon other food-related cues (visual, olfactory) to a lesser extent (27). Evidence indicates that at least one bird, the bobwhite quail (Colinus virginianus), may form aversions more readily to visual than to gustatory cues (29). Nevertheless bobwhites, and more than likely other avian species, were able to utilize gustatory stimuli as cues to adverse post-ingestional effects.

The question might be asked whether the reaction to a toxic material in the food is any more effective at altering food habits than the other possible mechanism of repellent action, a simple bad-taste effect. Two lines of evidence suggest an answer to this question. First, and most powerful, is the evidence, discussed above from plant evolution. The plants have emphasized defenses that adversely effect the physiology of their vertebrate predators, they have not frequently used taste stimuli except as cues to toxic events. Second, is an experiment (30) that directly compared the effectiveness of toxic and non-toxic materials at altering the feeding behavior of red-winged blackbirds.

In this experiment several materials were compared in three, two-choice tests where the palatability of the alternative to the treated food was varied from highly palatable through mildly avoided to highly offensive. By measuring the time the birds took to transfer feeding to the alternative it was possible to examine the motivating strength of the various repellent stimuli (Table I). In these tests it was determined that the only materials consistently effective in altering the feeding behavior were those that confronted the bird with a choice between illness for continuing to eat the treated food, and the alternative bad-tasting but toxicologically harmless diet. The data from that experiment supported the very important contention of Alcock (31) who suggested that except as signals to toxic or emetic effects, negative gustatory cues from prey are of little significance in determining food preferences of wild animals.

## Applications of Conditioned Repellency

The idea of using the conditioned aversion approach to the development of chemical repellents is not new. It was listed by Neff and Meanley (1) as one of the possible types of repellency, but was considered in their review as distinct from "true repellency". Tevis (32) after a study using 1080 (sodium fluoroacetate) as a poison on Douglas-fir (Pseudotsuga menziesii) seeds to

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protect them from rodents suggested that one of the advantages of bait shyness as a repellent response was that it created a population of non-seed-eating rodents that continued to be resident in the area. Their continued presence did not allow the great influx of migrants that commonly occurs after lethal control campaigns. The possibility of conditioned aversion as a mode of repellent action has also been raised in passing by others (e.g., 33, 34, 35) but generally was not pursued until recently.

Lately, the concept has received increased attention, possibly because of the failure of other approaches, or because of the increase in our knowledge of the principles of taste aversion learning. Currently conditioned aversion as a repellent response is being used to manipulate the food habits of three diverse depredating groups (wild canines, seed-eating rodents, and fruit and seed-eating birds). It is interesting that in these three cases the conditioned response is the mechanism being exploited, but that in each situation it comes about by an entirely different route.

The first of these cases, while not involving a botanical "crop" in the traditional sense illustrates one route of approach. Coyote (Canis latrans) predation upon sheep and lambs in the Western U.S. is locally severe. With the controversy over destructive measures of coyote control and the general removal of the poisons 1080 and strychnine for use against them, new approaches are being sought to control coyote predation where it occurs. John Garcia (a pioneer in the development of knowledge of conditioned aversion in the field of psychology) and several of his associates, recognizing the ubiquity of the conditioned response, tested this mechanism directly under field and laboratory conditions as a means of controlling coyote predation. In the restricted laboratory environment they (36) discovered that coyotes could learn after one trial with (toxic) lithium chloride - treated meat to avoid the flesh of that prey. They reported that a few trials with treated lamb or rabbit flesh specifically suppressed the attack upon the averted prey. A limited field test of this phenomenon using lithium chloride-treated packets of sheep meat and treated carcasses indicated to them that their treatment had caused a 30-60 percent reduction in sheep killed by coyotes on the 3000 acre sheep ranch where the test was carried out (37). The approach taken by this group involved the recognition of the strength of the conditioned response and employing it to help solve a specific problem. They have verified that feeding can be manipulated by this means in the laboratory and are attempting to establish conditions that will control predation in wild populations.

Endrin[1] was used for a number of years in combination with

1. Use of chemical, trade, or company names does not imply endorsement by the Federal Government.

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tetramethylthiuramdisulfide (TMTD) as a seed treatment to deter rodent feeding on conifer seeds. This formulation (which may act by the conditioned route) is no longer acceptable because of its nonselectivity, high toxicity, and persistence in the environment (38). The search for a replacement for endrin focused upon mestranol {3-methoxy-19-nor-17α-pregna-1,3,5 (10)-trien-20-yn-17-ol, Syntex Corp.}, a synthetic estrogen used as a reproductive inhibitor (39). Mestranol was selected as a candidate repellent because when used as a reproductive inhibitor it produces a conditioned aversion in the target rodents (40, 41). Registration of this material (2% w/w) as a repellent on conifer seeds is pending. We have here a case of a material that was a failure for its intended use being recognized (because of "bait shyness") for another.

Finally, the long screening search for a bird repellent has left us for a moment with a single material, methiocarb. Methiocarb {3,5-dimethyl-4(methylthio)phenol methylcarbamate-Mesurol$^{R}$, Chemagro Division, Baychem Corp.} is now a registered bird repellent for use on corn seed and ripening cherries. Its success was shown to be due in part to the fact that it acts as a conditioning repellent (30). Because of the potential of methiocarb as a broad-spectrum bird repellent it has received much attention aimed both at improving its usefulness and as a model conditioning repellent for studying conditioned aversion in depredating birds (30, 28). Work has indicated that the conditioned response acquired to it by red-winged blackbirds persists in the laboratory for periods up to 16 weeks (Fig. 3). Additionally, this species forms an aversion directly to the methiocarb and can recognize the material on other foods as well as its absence from the original food (Table II). Behavioral observations of common grackles (Quiscalus quiscula) feeding in newly planted corn plots indicate that these birds quickly learn to discriminate between treated and untreated seed (42). Under these field conditions the observed birds consumed a significantly higher proportion of untreated seeds (Table III) while not being repelled from entering the treated area thus allowing them to remain and perform any beneficial activities. Thus research to date with methiocarb indicates that it is effective because it possesses a taste that can be used as a cue to its toxic post-ingestional effects.

In the three examples presented, the conditioned aversion response played the leading role. In the first, it was recognized that the response is probably the most effective at manipulating the food habits of a wild carnivore. In the second, the problem associated with this response ("bait shyness") in getting adequate acceptance of a treated bait when using a reproductive inhibitor led to its use as a repellent. In the third, extensive screening efforts for a bird repellent identified one material, methiocarb,

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Table I. Average number of hours required by male red-winged blackbirds to learn to avoid several repellants.[a] Numbers in parentheses refer to numbers of birds of 18 tested that responded (sucrose octaacetate) or survived (methiocarb, LiCl). NR, no response. From Rogers (30).

| Choices | Compounds | | |
|---|---|---|---|
| | SOA | LiCl | Methiocarb |
| Treated vs. untreated mash | 2.8 (18) | 2.4 (18) | 2.7 (18) |
| Preferred vs. nonpreferred (corn or rice) | 3.2 (18) | 6.6 (13) | 10.0 (12) |
| Mash vs. DMA checkerettes | NR (0) | 9.1 (12) | 11.4 (11) |

[a]Means not underlined by the same line are significantly different from each other by Duncan's New Multiple Range Test ($P < 0.01$).

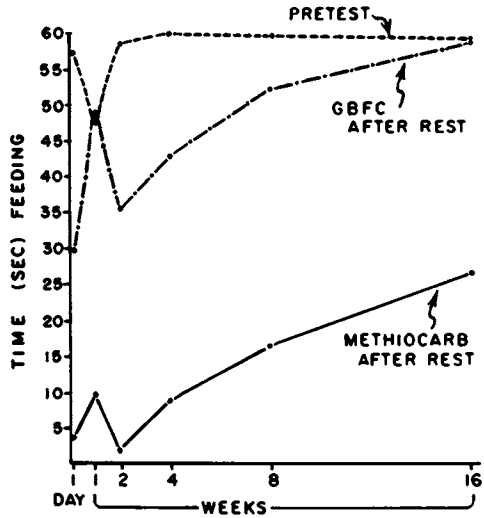


*Figure 3. Feeding responses of six groups of nine male red-winged blackbirds at various intervals after formation of a conditioned aversion to 0.07% methiocarb. Top curve; feeding on untreated food before training. Middle curve; feeding on untreated food after the rest interval (1 day, 1, 2, 4, 8, 16 weeks) before retesting with methiocarb. Bottom curve; feeding on treated food after the rest interval. GBFC refers to the normal diet. From Rogers (28).*

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Table II. Feeding response in a 1-min exposure of eight male red-winged blackbirds to untreated foods and foods treated with 0.07 percent methiocarb. The treatments are arranged in order of presentation from top to bottom (28).

| Treatment | Time spent feeding (sec ± SEM) |
|---|---|
| Pretest untreated GBFC | 57.9 ± 2.1* + |
| First exposure methiocarb in GBFC | 59.5 + 0.5* |
| Methiocarb in GBFC after training | 5.1 ± 3.4 |
| Untreated GBFC | 45.5 ± 7.0 + |
| Untreated rice | 48.5 ± 7.0* + |
| First exposure methiocarb in rice | 17.4 ± 3.9 |

All means not marked with the same symbol are significantly different from each other (Duncan's New Multiple Range test ($P = 0.05$).

GBFC = normal diet.

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Table III. Feeding activities of grackles that entered both the treated and untreated plots in the experimental corn seed planting.[a] Number of birds in parentheses (42).

| | Treated | Untreated | Combined |
|---|---|---|---|
| Seconds/row[b] | 5.6±0.9 | 7.1±1.0 | 6.3±0.8 (47) |
| Total No. seeds eaten | 15 | 59 | 74 (29) |
| Seconds/seed* | 29.2±2.8 | 24.0±7.7 | 28.1±2.7 |
| Total No. seeds dropped | 3 | 7 | 10 |

[a]1975 - 24 untreated and 12 rows of seed treated with methiocarb. 1976 - 18 untreated rows and 18 treated rows.

[b]Means ± SEM.

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that is effective because of its mode of action - the conditioned aversion.

It may be inferred from the preceding discussion that I am advocating the use of naturally occurring plant chemicals as bird repellents. This is an attractive possibility that has been suggested before (cf. 24). However, because of the high, broad spectrum toxicity and chemical lability of many of the plant secondary substances it might not prove a practical approach. Plants, along with developing secondary substances have often developed an efficient delivery system (e.g., vacuoles and vesicles) that isolates the chemical from the environmental conditions that might promote rapid degredation. For this reason the practical aspects of treating a crop might make this approach unfeasible.

In the earlier discussions of the development of chemical defenses against predation by wild ginger (25) and dove weed (23) it will be recalled that whereas these chemicals conferred protection from predation, they also were not without disadvantages to the plants. In both cases plants possessing these defenses were at a competitive disadvantage when predation was absent. Though data are extremely limited, three unpublished tests of methiocarb as a bird repellent (43, 44, 45) indicated that under conditions pertaining in New York, Delaware, and North Carolina in 1975 methiocarb, though reducing the number of corn sprouts damaged by birds, did not increase the sprout population that emerged and survived the period of vulnerability to bird damage. In other words methiocarb reduced bird damage, as measured by observations of missing and damaged sprouts, but also may have carried with it some phytotoxic effects. Thus, it may be that, as with some plants that have developed their own chemical defenses, the addition of a chemical repellent to a crop may only be advantageous under conditions of high levels of herbivore predation.

## Conclusion

Whatever mode(s) of action the ultimate repellents for protection of agricultural crops from vertebrate pests emphasize it would be important to reiterate the lessons learned from the chemical defense of plants against herbivores and to restate them in terms of man-developed repellents. First, it is reasonable to expect at least a low level of damage during the conditioning period of the pest population - 100 percent protection is not a reasonable expectation. Second, because of differences in the crop, a repellent that is effective at protecting one crop will not necessarily protect others - physical characteristics of the crop may dictate differing rates of consumption, or techniques of consumption (e.g., hulling of topically treated seeds by the depredating species). Third, it is very unlikely that any one repellent will be effective against all depredating species--it is unreasonable to expect a panacea because of differences in

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behavior and physiology of the species involved. Fourth, all the presently viable vertebrate repellents (as defined earlier) involve as an important part of their mode of action some adverse effects upon the physiology of the target species - it is likely that future repellents will possess the same components. Fifth, the effective repellents are not necessarily bad-tasting--the pest learns to associate the taste with an adverse physiological effect. Sixth, the use of a repellent demands a behavioral response from the target animal - this response is an alteration in feeding behavior. Seventh, any repellent is likely to be most effective when adequate alternative foods are available. Conditioned aversion is the mode of action that most likely will produce the required response. The response, of course is an alteration of food habits.

As a whole these points should make it obvious that there will be no panacea repellent and only through a coordinated series of studies on all aspects of the repellent - plant-animal complex will appropriate repellents be developed. We must take into account the behavior, physiology, and ecology of the specific problems to understand the nature of the problem. We must understand the problem before effective repellents can be developed.

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Literature Cited

1. Neff, J. A. and B. Meanley. Progress Report No. 1. Denver Wildlife Research Center, Denver, Colorado (1956).
2. Welch, J. F. J. Agr. and Fd. Chem. (1954) 2:142-149.
3. Besser, J. F. and J. F. Welch. Trans. 24th North American Wildl. Conf. (1959) 24: 166-173.
4. Armour, C.J. Forestry Abstracts (1963) 24: xxvii-xxxviii
5. Dambach, C.A. and D.J. Leedy. Trans. 14th N. American Wildl. Conf. (1949) 14: 592-603.
6. Ehrlich, P.R. and P.H. Raven. Evolution (1964) 18: 586-608.
7. Brower, L.P., J.V. Brower, and J.M. Corvino. Proc. Nat. Acad. Sci. (1967) 57: 893-898.
8. Fraenkel, G.S. Science (1959) 129: 1466-1470.
9. Fraenkel, G.S. Entomologica experimentalis et applicata (1969) 12: 473-486.
10. Hsiao, T.H. Entomologica experimentalis et applicata (1969) 12: 777-788.
11. Whittaker, R.H. "The Biochemical Ecology of Higher Plants". In: E. Sondheimer and J.B. Simeone (Eds.). Chemical Ecology. Academic Press, New York, 1970.

12. Whittaker, R.H. and P.P. Feeny. Sci. (1971) 171: 759-770.
13. Freeland, W.J. and D.H. Janzen. Amer. Nat. (1974) 108: 269-289.
14. Dethier, V.G. "Chemical Interactions between Plants and Insects". In: E. Sondheimer and J.B. Simeone (Eds.). Chemical Ecology. Academic Press, New York, 1970.
15. Gilham, M.E. J. Ecol. (1955) 43: 172-206.
16. Jones, D.A. Nature (1962) 193: 1109-1110.
17. Jones, D.A. Genetica (1972) 43: 394-406.
18. Bell, E.A. & D.H. Janzen. Nature (1971) 229: 136-137.
19. Sherbrooke, W.C. Ecol. (1976) 57: 596-602.
20. Elliger, C.A., A.C. Waiss, Jr., and R.E. Lundin. J. Chem. Soc., Perkin Trans. (1973) I: 2209-2212.
21. Booth, A.N., C.A. Elliger and A.C. Waiss, Jr. Life Sci. (1974) 15: 1115-1120.
22. Rosenzweig, M.L. and J. Winakur. Ecol. (1969) 50: 558-572.
23. Cook, A.D., P.R. Atsatt, and C.A. Sinion. Bioscience (1971) 21: 277-281.
24. Sherburne, J.A. Unpubl. Ph.D. dissertation (1972), Cornell University, New York.
25. Cates, R.G. Ecology (1975) 56: 391-400.
26. Rzoska, J. Anim. Behav. (1953) 1: 128-135.
27. Rozin, P. and J.W. Kalat. Psychol. Rev. (1971) 78: 459-486.
28. Rogers, J.G., Jr. Auk (1977): in press.
29. Wilcoxon, H.C., W.B. Dragoin, and P.A. Kral. Sci. (1971) 171: 826-828.
30. Rogers, J.G., Jr. J. Wildl. Manage. (1974) 38: 418-423.
31. Alcock, J. Anim. Behav. (1970) 18: 592-599.
32. Tevis, J., Jr. J. Mammal. (1956) 37: 358-370.
33. Rediske, J.H. and W.H. Lawrence. Forest Sci. (1962) 8: 142-148.
34. Teichner, W.H., R. Warranch, M. Lopiccolo and C. Campbell. U.S. Army Natick Labs (Natick, Mass.) Tech. Report. 70-69PR (1969).
35. Omura, K., S.F. Takagi and O. Harada. Gunma J. Med. Sci. (1961) 10: 217-227.
36. Gustavson, C.R., J. Garcia, W.G. Hankins, and K. Rusiniak. Science (1974) 184: 580-583.
37. Gustavson, C.R., D.J. Kelly, M. Sweeney and J. Garcia. Behav. Biol. (1976) 17: 61-72.
38. Radwan, M.A. Proc. 4th Vert. Pest. Conf. (1970) 4: 77-82.
39. Lindsey, G.D., R.M. Anthony, and J. Evans. Proc. 6th Vert. Pest. Conf. (1974) 6: 272-279.
40. Howard, W.E. and R.E. Marsh. J. Wildl. Manage. (1969) 33: 403-408.
41. Marsh, R.E. and W.E. Howard. J. Wildl. Manage. (1969) 33: 133-138.
42. Rogers, J.G., Jr. and J.T. Linehan. J. Wildl. Manage. (1977) 31: in press.

In Flavor Chemistry of Animal Foods; Bullard, R.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

43. Linehan, J.T., C.R. Ingram and P.W. Lefebvre. Unpubl. report U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Md. (1975) 10 pp.
44. Mitchell, R.T., B. Meanley, R.E. Matteson and C.R. Ingram. Unpubl. report U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Md. (1975), 10 p.
45. Stickley, A.R., Jr. and C.R. Ingram. Unpubl. Report, U.S. Fish and Wildlife Service, Patuxent Wildlife Research Ctr., Laurel, Md. (1975) 11 pp.

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