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WHY FRUITS ROT, SEEDS MOLD, AND MEAT SPOILS

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One of the most unappreciated classes of interspecific competition is that between microbes and large organisms over three prominent resources: fleshy animal-dispersed fruits, seeds, and newly dead animals. It is my hypothesis that microbes are often under strong selection to render seeds, fresh fruit, or carcasses as objectionable or unusable to larger organisms as is possible in the shortest period of time. The microbes should play this evolutionary and ecological game with toxins, antibiotics, and substrate degradation. Here, I dwell on the microbial side of the game because there is relevant information in the medical, veterinary, and agricultural literature. There is likewise selective pressure for the larger organism to evolve or learn to (a) ignore or mask objectionable flavors and odors that are false warnings and (b) detoxify or otherwise avoid the chemical defenses of the microbes. I largely ignore this half of the story owing to the unavailability of relevant information.

The stakes in these competitive contests are high. The microbes may lose both life and resource, and the large organism may do the same by tackling too fierce a microbe. The weapons of the microbes are those classically associated with interactions among them, but I suspect that these weapons have wider applicability and are selective responses to a wider array of challenges than just those produced by other microbes.

FRUIT

The entire adaptive function of a ripe fleshy fruit is to get the seeds moved to particular places (and, on occasion, to directly or indirectly protect the seeds). At the level of the physiology of the individual fruit, this is generally done by engineering nutrients and secondary compounds in the fruit such that it is a desirable food item to a certain subset of the animal community. These nutrients and secondary compounds are also useful resources to a large number of bacteria and fungi. These microbes may be effectively excluded from the green fruit by the fruit's chemical defenses (just as leaves are protected by toxic secondary compounds). However, once the fruit is ripe, many of the plant's defenses are mutually exclusive with a fruit attractive to dispersal agents. When the fruit wall is broken by abrasion, falling, claw marks, tooth trials, partial consumption, or active entry by a microbe, the bacteria and fungi have entered a race with

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the dispersal agents. "The increased susceptibility of ripe fruit to decay may result from increased liability to mechanical injury, but the more usual situation appears to be that the ripening process involves physiological changes in the host tissues which makes them a more suitable substrate for rapid development of the pathogen" (Eckert and Sommer 1967; also see Tomkins [1951] for similar sentiments). The bacterium or fungus may even be in the green fruit but lie dormant until it is ripe (e.g., Samish et al. 1961; Hollis 1951; Baker 1938; Baker and Wardlaw 1937).

If the dispersal agent eats the fruit after spoilage begins, there are several possible outcomes. The microbes may simply lose their resource and be able to make the trip safely through the gut of the animal. They may even be defecated in quite a different but suitable habitat. On the other hand, the gut digestive system may well be directly lethal to the microorganism population and/or result in its deposition in a lethal habitat.

A microorganism may render a fruit inedible to a large animal in three primary and overlapping ways:

Nutrient Alteration

It is commonplace for a vertebrate-dispersed fruit to be rich in some particular nutrient, such as a sugar, lipid, or organic acid. The microbe has the physiological task of rapidly converting these nutrients to a form inedible, unwanted, or toxic to at least some vertebrates. Selection should favor conversions to items later of use to the microbe and it should favor a microbial biochemistry that can live on the conversion products most easily generated. It is common knowledge that rotted fruits are often off flavor or otherwise objectionable, but nutritionists' analyses stop at that point, apparently because no one cares what nutrients are in a rotten apple; it is to be thrown away. Score one for the microbe. A hint exists in the literature. The fungus that causes brown rot of peaches in the orchard first converts the sweet sucrose to the much less sweet glucose and, apparently, avoids the accumulation of the very sweet fructose formed at the same time. I suspect that it avoids accumulation of fructose by degrading it as it is formed. It makes this conversion long before it uses most of the carbohydrate (Hawkins 1915). Soft rot of apples (*Penicillium expansum*) produces a "characteristic moldy taste which is decidedly unpleasant" (Hesler and Whetzel 1917). It is very difficult to know if a metabolic nutrient conversion has occurred merely to feed the microbe that is responsible for the change or, additionally, to protect its food. However, we will never know if we continue blindly to assume that the former is the case. Microbes may also be destroying the secondary attractant odors and flavors of fruits to this end, but I have been unable to locate an examination of this idea.

It may be that it is easier and quicker to render a fruit toxic or otherwise objectionable with a few molecules than rapidly to convert all the edibles to less desirable molecules. The likelihood of the evolution of such poisoning of a fruit depends greatly on the kinds of edibles in the fruit, the degree of control of the microbe over the fruit, the degree to which it is competing with other

microbes, the voracity of the animals, etc. It should be noted that the race is run in a field where the frugivorous animal is commonly confronted with a glut of ripe fruits and, therefore, can afford to be choosy.

Toxicant Production

Many bacteria and fungi give sour, bitter, "moldy," or otherwise "objectionable" flavors to a ripe fruit. From just the 800 or so varieties of cheeses, over 100 flavor and odor metabolites have been chemically identified (Kristoffersen 1973), and this is probably a very small fraction of the total. What is a toxicant is very dosage dependent, and it is impossible at this stage of the art to determine which are toxicants, warning cues, competitive weapons, mimics, and true waste products. However, in the context of this discussion, when a fungus in a ripe peach produces large quantities of oxalic acid (Hawkins 1915; Jefferson and Harrison 1971) or yeasts in ripe fruit convert sugar to ethanol, they are not only rendering the medium bacteriostatic but they are rendering the fruit generally objectionable to vertebrates. The methanol content of ripe apples is 3.93 mg per 100 g fresh tissue; apple tissue rotted by the fungus *Sclerotinia fructigena* contains 73 mg methanol per 100 g of tissue (Byrde et al. 1973). When Mrak and Phaff (1948) comment that "decomposing fresh fruits have been one of the most common sources for isolation of yeasts," they are really saying that yeasts are specialists at eating ripe fruits. As specialists, I expect them to be sufficiently fine tuned to produce chemicals for direct protection as well as for competition with other microbes.

Ethanol should perhaps be singled out from the other smaller molecules for special mention. The major role it plays in man's life (e.g., Richter 1953) has obscured the relatively obscure role it plays in nature. The vast majority of yeasts either make other defensive compounds or combine the ethanol with other nasty compounds; spoilage of beer and wine by wild yeasts (Walker and Ayres 1970) should be viewed as the natural state of affairs. Ethanol is a small molecule which is easily degraded by the vertebrate liver via alcohol dehydrogenase (Majchrowicz 1975), and thus dosages are very important. It should be viewed as just one of the multitude of small chemicals that a yeast alone can liberate to render its particular fruit less likely to be eaten. Field naturalists know that birds and mammals occasionally get drunk from ripe fruits and that their fitness declines through greater ease of capture, but there has never been a systematic investigation of this phenomenon. The contemporary level of sophistication of analysis is at the level of "alcoholic intoxication is not uncommon, especially in budgerigars. They very readily learn to sit on the rim of a beverage glass and imbibe freely of the contents. Intoxication occurs frequently at parties where the pet bird is encouraged in the practice. Clinical symptoms are depression, the tendency to sit quietly with closed eyes, regurgitation, and loose droppings. Unconsciousness may result in severe cases. As with humans, time is the healer" (Altman 1969). What is often forgotten is that it may well be other fermentation products in the fruit that make a wild animal act "drunk," "high," "tipsy," "on a trip," etc. In the wild, the vast

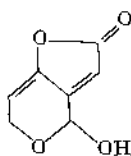


FIG. 1a.—Patulin

majority of fermenting fruits are never touched by vertebrates except when they are starving or the content of secondary compounds is not high enough to mask the sugars or other nutrients present.

Of a stronger nature than ethanol are complex compounds such as patulin (fig. 1a).¹ This compound is produced by *Penicillium expansum*, the major cause of storage rot in apples (Singh 1967; Campbell 1970; Hesler and Whetzel 1917), and by *Aspergillus* and *Gymnoascus* (Mayer and Legator 1969). It is of concern to the cider industry (Rosen and Pareles 1974) as it is extremely toxic to animals (Broom et al. 1944; Campbell 1970; Mayer and Legator 1969; Singh 1967). It is also very toxic to bacteria, yeasts, fungi, and protozoa and, of course, is undoubtedly selected for in *Penicillium* competition with other microbes (but see the following section on antibiotics).

"A convenient definition of food spoilage is that a food is spoiled when a consumer refuses it as food" (Kuehn and Gunderson 1962). Unfortunately, I can locate no studies at all of the abilities or interests of frugivorous animals in discriminating between spoiled and intact ripe fruits. The medical, microbial, and nutrient literature has many descriptions of spoilage organisms altering fruits (e.g., Walker and Ayres 1970; Minz 1946; Ingram 1958; Elazari-Volcani 1946; Mrak and Phaff 1948; Kuehn and Gunderson 1962; Fawcett 1926; Lowings 1956; White and Fabian 1953; Beneke et al. 1954) but no words on what happens to us or other animals if we eat the spoiled fruits. We have a huge cultural tradition reflected in advice to parrot growers: "Foods often become dangerously moldy if not given frequent attention. . . . Most molds cause a general unthriftiness and in some cases diarrhea" (Bates and Busenbark 1963). Hemorrhagic enteritis in parrots and mynahs may occur from eating "spoiled food" (Stone 1967). Spoiled fruits are probably very poisonous in many ways (and see later sections), but the specifics are unknown. Whatever the cause, an awful lot of fruit "goes to rot" under fruiting trees in natural vegetation. I doubt that simple satiation of dispersal agents is the only reason that this fruit is not eaten by vertebrates.

One may argue that in metabolizing the fruit content, the microorganisms have no choice but to produce "waste products" that are objectionable to animals. Does it not seem odd that virtually all of these are initially treated as objectionable by humans and other animals? "Objectionable" is certainly not an intrinsic trait of any chemical. It is significant that the yeast (*Candida*

¹ Figures 1a-1g are examples of the complex molecules produced by fungi and which may function in protection against large animals (see Bamberg et al. [1969] for more).

guilliermondii) and bacteria (*Serratia plymuthica*) specific to and carried by the fig wasp grow inside the developing (and unattractive to animals) fig without causing it to "rot," while the fungi, yeasts, and bacteria that get into the ripe fig after the fig wasp has left cause it to "rot" and "sour" in a few days (Miller and Phaff 1962; Davey and Smith 1933; Hansen 1929). *Sphaerotheca humuli*, a powdery mildew that does not normally live in fruit, does not lead to unsightly decomposition of strawberries, while *Mucor piriformis* and *Botrytis cinerea* lead to major rotting and discoloring of attacked strawberries (Lowings 1956; also see Beneke et al. [1954] and White and Fabian [1953] for a similar species contrast).

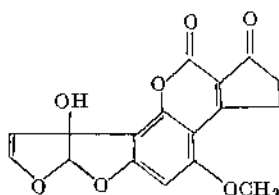
Antibiotic Production

The natural substrate of *Penicillium* is not old slices of bread. It is customary to view antibiotic production by fungi, yeasts, and bacteria as adaptive in their competitive roles with each other (Gause 1960; Alexander 1971a, 1971b; Uroma and Virtanen 1949; Garrett 1970; Ingram 1958; Welsch 1964; Clark et al. 1964; Ayres et al. 1964; Last and Price 1969; Gottlieb and Shaw 1970). However, nowhere have I been able to locate a discussion of how antibiotics may render a fruit or other food uninteresting to animals. At the least, it is recognized that bacterial pigments may protect bacteria from predation by small predators like amoebae (Groscop and Brent 1964).

Antibiotics added to animal feed often make for healthier livestock (Coates and Kon 1956; Quinn 1956; Stokstad 1956), but there are cases where the opposite is true (Gentles and La Touche 1969). Feeding aureomycin to parrots (*Loriculus galgulus*) apparently killed them by lowering their resistance to *Candida albicans*, a fungus that thrives on rotting fruit (Buckley 1965; Tollefson 1969). Aureomycin, oxytetracycline, and streptomycin can kill guinea pigs by disrupting their gut microflora community structure (Roine et al. 1955). It is very incomplete to ask what antibiotics do to a domestic animal feeding on highly edible and nontoxic feeds; the story may be very different if one overloads a wild ruminant's microflora with antibiotics at the same time that the microflora is required to deal with secondary compounds in the plants and other toxins produced by microbes in the rotten fruit. To conclude this section, I note that while simple organic acids and alcohols produced by yeasts are not generally called antibiotics (Carmo-Sousa 1969; Walker and Ayres 1970), from an ecological viewpoint they surely are.

Insects

Fruit-eating and even seed-eating insects that live in ripe fruit or even return to it frequently should be subject to the same selection to produce anti-animal fruit traits as are the microbes. Tephritid fruit flies carry yeasts with them and these yeasts greatly hasten the decomposition of the fruit as well as provide food for the larvae in the fruit (Narayanan and Batra 1960; Christenson and Foote 1960). A similar situation exists with *Drosophila* (e.g., Camargo and

FIG. 1b.—Aflatoxin M₁

Phaff 1957). The soured figs with *Drosophila* larvae in them (Miller and Phaff 1962) are probably less attractive to frugivorous vertebrates than ripe intact fruits. The fruit-piercing tropical moths (e.g., Norris 1936) introduce microbes through the puncture and result in fruit rejection by humans and, I suspect, by certain dispersal agents; this should maximize the amount of fruit available to the moths. Not only might an insect in a fruit associate with those microbes that compete least and yet maximally lower its chances of being eaten, but it may do things directly such as mine through the fruit in a manner so as to speed decomposition and discoloration of the fruit and perhaps even physiologically cause the plant to discard the fruit. Ripe fruits of *Cassia grandis* rapidly become unattractive to dispersal agents once moth larvae have eaten the sweet pulp around the seeds (Janzen 1971a). The plant has a stake in this as well, and the system may be furthered by a physiological decision by the plant to abort rather than pour more resources into an ill-fated fruit (see Minz [1946] and Hesler and Whetzel [1917] for examples of abortion of fungus-infected fruits). The insect may also gall the fruit so as to induce it to produce secondary compounds unattractive to vertebrates, such as tannins. It is noteworthy in this context that insect galls on foliage often have a nutrient content much higher than that of the surrounding foliage (e.g., Bronner 1970). The gall former does not want to be eaten by a herbivore, and the insect manipulates the physiology of the plant such that the plant deposits incredibly high concentrations of tannins and other compounds in the gall (e.g., Howes 1953), which, I hypothesize, protect the insect and its food.

SEEDS

A number of seeds, with grains and pulses being most prominent, lack a fleshy fruit but nevertheless have been gathered and stored by small rodents and man for a very long time. Our contemporary stored food pests have most likely evolved in these seed caches. A fungus that discovers such a cache, which is not difficult because fungal spores are literally everywhere, can quickly establish a potent claim to the seeds if there is sufficient moisture to grow there. Aflatoxins (fig. 1b), produced by *Aspergillus* spp., are the most famous of the toxins produced by fungi on seeds. They are well known primarily because of their mammalian and avian lethality and their ability to produce liver lesions and cancers (Hartung 1971; Ciegler 1975; Brook and White 1966; Mirocha and Christensen 1974; Bamberg et al. 1969; See et al. 1969; Davis and Diener 1970;

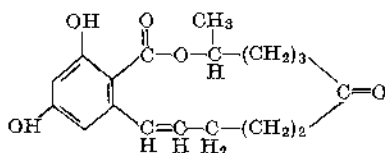


FIG. 1c.—Zearalenone

Hesseltine et al. 1970; Semenuik et al. 1970; Hiscocks 1965; Allcroft 1965; Enomoto and Saito 1972; Nesbit et al. 1962; Wogan and Newberne 1967; Schoental 1967; Masri et al. 1974; Campbell and Stoloff 1974; Shank et al. 1972; Anderson et al. 1975; Wilson and Hayes 1973). "Aflatoxin B₁ appears to be the most potent hepatocarcinogen known"; 15 ppb in rat diets produced a high incidence of liver tumors (Miller 1973). Ergotism, produced by alkaloids from *Claviceps purpurea* on field grains, is another conspicuous human disease produced by spoilage fungi (Caporael 1976; Wilson and Hayes 1973). However, complex toxic metabolites are produced by some species of virtually all genera of fungi that live on field and stored grains. Ten years ago, Majunder et al. (1965) listed no less than 64 for *Aspergillus* and 97 for *Penicillium*, and lesser numbers for *Alternaria*, *Fusarium*, *Curvularia*, *Epicoccum*, *Stemphylium*, *Mucor*, *Gibberella*, *Phoma*, *Trichoderma*, *Rhizopus*, *Absidia*, and *Cephalosporium*.

The pharmacology of these metabolites makes it evident why livestock are hesitant to eat moldy feed.

Moldy [with *Penicillium rubrum*] field corn was shelled and fed to 5 pigs, each weighing approximately 40 pounds. Two died within 4 days, showing typical manifestations of the field toxicosis. The remaining three pigs refused to eat the moldy corn and subsequently were sacrificed; necropsy indicated starvation. . . . Two additional pigs were force-fed by stomach tube, milled moldy field corn. One perished in 3 days, and the other in 8 days; necropsy findings were indicative of the acute field toxicoses. . . . A 280 pound calf was force-fed daily 6 pounds of milled moldy field corn. The animal developed depression, weakness, and excessive salivation and became bloated. In addition, the animal ran short distances, would twist and turn, and work its mouth vigorously during the exertion. The animal perished on the third day during one of the seizures. [Forgacs 1965]

The minimum lethal dose of corn infected by *P. rubrum* was less than 8 ounces for a 55-lb pig, with death ensuing within 8 h. The supernatant from 3–4.5 kg of milled moldy corn kills a horse in from 34 h to 5 days (Forgacs 1965). An estrogenic metabolite produced by *Fusarium graminearum* (zearalenone, fig. 1c) resulted in "sows which had consumed moldy feed developed enlarged, tense, and elevated vulvae, enlarged mammary glands, and, in more severe cases, prolapse of the vagina and rectum" (Mirocha et al. 1967). When fed to rats, unpolished rice inoculated with *Penicillium citreo-viride* produced paralysis and death in 8 days when it was 100% of the diet; at 10% of the diet, it killed half the rats in 1 mo (Tsunoda 1970). This fungus produced citreoviridin (fig. 1d). *Claviceps fusiformis* growing on millet seed produces clavine alkaloids that cause loss of lactation in mice and pigs and loss of litters in mice when added to

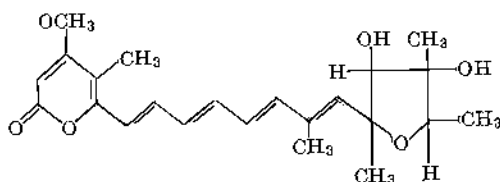


FIG. 1d.—Citreoviridin

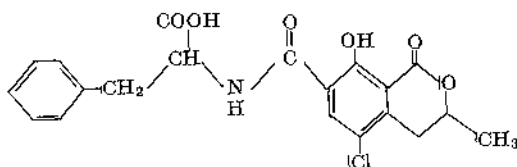


FIG. 1e.—Ochratoxin A

the diet (Mantle 1968). Ochratoxin (fig. 1e), produced by *Aspergillus* spp. and *Penicillium viridicatum*, causes abortions in rats with a single oral dose of 12.5 mg/kg body weight (Mirocha and Christensen 1974). Two hundred and forty-seven cultures of 63 *Aspergillus* species from 10 taxonomic groups were tested on chicks and mice, with 50% of the diet being moldy wheat or soybeans; in 32 cultures, one or both diets killed 50% or more of the chicks, and in 42 cultures, one or both diets killed 50% or more of the mice. Of the "nontoxic" cultures, 52 stunted the growth of one or both animals (Semenuik et al. 1970). (Other examples may be found in Wilson et al. 1968; Giegler 1975; Doupnik and Sobers 1968; Doupnik and Peckham 1970; Peckham et al. 1971; Sobers and Doupnik 1972; Cole et al. 1973; Scott and Somers 1969; Yates et al. 1969; Brook and White 1966; Miyaki 1970; Jaffe 1973; Miller 1973; Kingsbury 1964; Bamburg et al. 1969; Carlton and Tuite 1970; Tsunoda 1970; Wilson and Hayes 1973; and Van Veen 1973.) From a 1976 unidentified Costa Rican newspaper: "More than 29,000 tons of contaminated corn were finally incinerated this week, after a long and hopeless battle to use it productively. The corn was first purchased in 1974 by the National Production Council (CNP), and upon discovery that it contained dangerous toxins, alternate uses other than sale to the public were sought. After last attempts to re-sell it to another country, the decision to burn the corn was finally reached several months ago." It appears that, given adequate conditions for fungal growth, the fungi are winning the battle for the grain.

In the context of whether aflatoxin production may be adaptive merely in intermicrobial chemical warfare, it is particularly interesting that "in general, most bacteria, fungi, and algae tested are relatively insensitive to the substance"

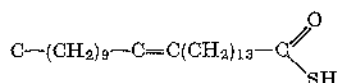


FIG. 1f.—Cladosporin

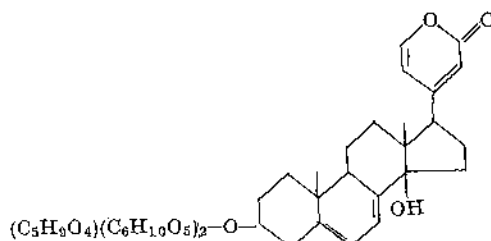


FIG. 1g.—Fusariogenin

(Kiawa et al. 1969). On the other hand, other complex fungal metabolites are toxic to both big and little organisms (e.g., Scott and Somers 1969).

Numerous authors have stressed that the moisture content of stored grain has to be greater than about 15% for fungi to grow on it (e.g., Tsunoda 1970; Davis and Diener 1970; Hansen et al. 1973; Mirocha and Christensen 1974; Christensen 1965; also see Ingram [1958] for a similar stress on fungi in fruit). During the Second World War, grains left standing in the fields over the moist winter were harvested with disastrous results for both livestock and humans. They were poisoned by cladosporin and fusariogenin (fig. 1f, g) produced by seven species of grain-inhabiting *Fusarium*; the toxins were still on the grain 7 yr later (Joffe 1965; Seitz et al. 1975). Peanuts left in the ground in Nigeria more than 1 mo after normal harvesting time have a high risk of being contaminated with aflatoxins (McDonald and Harkness 1967; Porter and Garren 1968). High moisture content is expected in seed caches made by mammals. These observations perhaps also shed some light on why it is that tropical rodents generally do not cache hoard seeds (Janzen 1971b); further, at least four of those that do make seed caches also reject moldy seeds (Rickart and Robertson 1977). If stored at high humidity, coffee seeds, cacao seeds, oil palm nuts, cycad nuts, and peanuts are all superb substrates for aflatoxin-producing *Aspergillus flavus* (Hiscocks 1965; Hansen et al. 1973; Wilson and Hayes 1973). It should be added that spores of *A. flavus* can be isolated from practically anywhere in the world, and Hiscocks (1965) found that 75% of 43 strains tested made aflatoxins. It should be nearly impossible for a rodent to make a fungus-spore-free seed cache. While results are ambiguous, it is possible that the behavior of rainforest rodents of urinating heavily on seed caches

(Rickart and Robertson 1977) may be an attempt in this direction. The urine soaked nests and middens of packrats (Wells and Jorgensen 1964) may have a similar selective basis.

Extratropical rodents, such as chipmunks (*Tamias* and *Eutamias*), which store large caches of seeds for winter use (e.g., Howell 1929), probably engineer their burrow systems so as to avoid mold. Drainage properties of the soil, depth of the cache (and thus its temperature), and the kind of seed should all be important to the rodent. Howell (1929) reported a case where a seed cache found in midsummer, left over from the previous winter, contained moldy corn; I suspect it would never have been eaten by the chipmunk. It may even be that the act of storing large amounts of fat rather than seeds may have part of its adaptive significance in the inability of fungi to get at the food reserves.

While I expect the aflatoxins and other fungal metabolites made on seeds to be very toxic to granivores, I also expect to find resistant animals among granivores. It is interesting that mice are extremely resistant (but not immune) to aflatoxins compared to other animals (Schoental 1967; Wilson and Hayes 1973). Mice are also very resistant to sterigmatocystin produced by *Aspergillus* on rice; on the other hand, they are very susceptible to luteoskyrin produced by *Penicillium islandicum* on moldy rice and rats much less so (Enomoto and Saito 1972). Corn that was moldy with *Penicillium viridicatum* produced a high death loss and liver lesions in mice, yet some in each sample group were apparently unaffected (Carlton and Tuite 1970). This kind of variance could easily be acted on by ordinary selection in climates where mice had frequent access to stores of moldy grain. Of the few birds tested, quail are the least susceptible to aflatoxins (Mirocha and Christensen 1974); this is not surprising since they surely eat infected grain seeds during the winter months. Less explicably, sheep are much more resistant to aflatoxins than are cattle (Wilson and Hayes 1973; Enomoto and Saito 1972; Schoental 1967); sheep pass the toxins in such quantities that their feces make a lethal additive to duck feed (Allcroft 1965).

As mentioned before, livestock may be very leery of moldy grain. Allcroft (1965) noted that "pigs given a choice of feed containing toxic and nontoxic meal showed a marked preference for the nontoxic feed, even though they had not previously received toxic meal." Four species of cloud forest rodents from southern Mexico ate and hoarded fresh peanuts but emphatically rejected moldy ones (Rickart and Robertson 1977). However, when purified aflatoxin was added to an artificial diet, there was no rejection of the feed by rats (Wogan and Newberne 1967). Since some of the poisoning effects are reversible, animals may also learn to avoid moldy grain (e.g., clavine alkaloid toxicity to mice and pigs; Mantle 1968). In view of the toxins, antibiotics, and diseases that can be obtained by eating moldy foodstuffs, I would not find it surprising if their rejection by animals is genetically programmed. It is perhaps relevant in this context that visually orienting birds, such as quail, pheasants, and chickens, reject oddly colored seeds (Kalmbach 1943). Many molds and fungi discolor the seeds they occupy to red, yellow, black, etc. (Miyaki 1970; Tsunoda 1970). Brown fruits were rejected by birds in colored fruit choice tests much more frequently than expected on the basis of their relative abundance in the tests (Turcek 1963).

When a weevil larva or adult exits from a seed in which it has matured, there are often pieces of uneaten seed contents. These bits of high quality food are fed on by many species of fungi, among which are a number that produce (in this microhabitat) metabolites that are very toxic to chicks (Wells and Payne 1975, 1976). This may be why insect-damaged forest tree nuts are generally rejected by squirrels and other vertebrates (unpublished observations), a rejection that obviously favors the fungus. Damaged almond fruits and seeds are colonized by aflatoxin-producing *Aspergillus* (Phillips et al. 1976), and this should reduce their attractiveness to vertebrates. Even intact pecan nuts can become heavily infested with aflatoxin-producing fungi (Doupnik and Bell 1971); squirrels reject moldy but otherwise intact pecans (unpublished observations).

It need not be the microorganisms themselves that produce the toxic compounds. During fermentation (spoilage) of sweet clover plants in silage, a defensive secondary compound of the plant, coumarin, is oxidized by the microorganisms to 4-hydroxycoumarin and thence coupled to make 3,3'-methylenebis (4-hydroxycoumarin). This compound is the causative agent of "hemorrhagic sweet clover disease," which greatly removes or inactivates prothrombin in livestock blood (Stahmann et al. 1941). However, there is no evidence that the complex molecules in figure 1 are anything but synthesized directly by the fungi.

The fungi may also depend on the animal to activate the compound they produce. Apparently, aflatoxin B₁ requires activation to aflatoxin Q₁ by liver microsomal enzymes before it is toxic (Masri et al. 1974). Botulinum toxin, a bacterial product, is a complex of an inactive protein and a very toxic but unstable smaller protein; the animal has to split this complex molecule in order to be poisoned (Schantz and Sugiyama 1974). I suspect that the three sugar molecules on fusariogenin (fig. 1g) have to be stripped off by the animal before that compound is toxic. However, the toxic compounds excreted by microorganisms are generally not excreted as glycosides or in some other inactive bound form. This makes them quite distinctive from the majority of secondary compounds produced as defenses by higher plants, but, then again, higher plants, for the most part, keep their defensive compounds to themselves rather than release them into the environment.

Numerous workers have noted that toxin (as well as antibiotic) production is not related to some anthropocentric concept of "the best growth conditions." "A maximal growth of *A. flavus* at certain temperatures is not necessarily accompanied by good yields of aflatoxin" (Enomoto and Saito 1972). This is roughly similar to noting that the fattest horses do not make the best race horses. If I try to imagine what is the evolutionary history of toxin-producing seed-eating fungi, it is clear that there would be some environmental circumstances where there is no necessity to produce toxins, while there would be others where it is best to slow the hyphal growth rates at the expense of toxin production.

Virtually all the pharmacological work with secondary compounds from spoilage fungi has been with mammals, and for compelling reasons; "most types [of fungi] that are hazardous to human health are storage fungi" (Enomoto

and Saito 1972). However, granary insects and other seed eaters are also competing directly with spoilage fungi for seeds. Aflatoxin-producing fungi can establish themselves in unharvested insect-damaged field corn as early as the late milk stage (Anderson et al. 1975). Aflatoxins added to food of *Heliothis virescens* are lethal (Godauskas et al. 1967). This moth larva is a sister species to *H. zea*, the corn ear worm. *Aspergillus flavus* in cotton bolls and seeds produces aflatoxins (Marsh, Simpson, Ferretti, Campbell et al. 1969; Marsh, Simpson, Ferretti, Merola et al. 1969), and I wonder if this could deter one of the cotton boll worms (*H. virescens*) from eating moldy bolls. Aflatoxins also show high oral toxicity to *Drosophila* sp. and *Musca domestica* and lower the reproductive output of these flies and of mosquitoes (Matsumura and Knight 1967). *Aspergillus ochraceus* makes an insecticide (L-prolyl-L-leucine anhydride; Takahashi et al. 1974). Insects may also produce secondary compounds to protect seed stores. *Tribolium* beetles, which naturally inhabit relatively unused caches of broken grains, are well known to "foul" their microhabitat by producing bitter quinones (Ghent 1963). Generally interpreted as being important in intra- or interspecific competition among the beetles, these compounds could also be serving to deter rodents and fungi that would eat both the grain stores and the beetles.

The interaction of stored grain, stored grain mites, and stored grain beetles should probably be reevaluated in the light of toxin production by the fungi. There are some interesting hints of what might be found. In a number of choice tests of many species of stored grain mites for their preferences of grain fungi to feed and reproduce on, *Aspergillus* spp., *Penicillium* spp., and *Streptomyces griseus* appear consistently at or near the bottoms of the host lists (Sinha 1964, 1966). For the rusty grain beetle (*Cryptolestes ferrugineus*), wheat infected with four species of *Aspergillus*, one of *Penicillium*, and *S. griseus* was unacceptable as breeding substrates, while these beetles prefer to feed and oviposit on grain infected with other fungi rather than clean grain (Loschiavo and Sinha 1966; Sinha 1965); none of the preferred fungi are infamous as producers of metabolites toxic to animals, though some may do so. Associated with this, *S. griseus*, *Aspergillus* spp., and *Penicillium* spp. tended to be the most abundant fungal species in multispecies competitive communities (Sinha and Wallace 1965); the majority of the mite species however "probably invade the deteriorating grain to feed and reproduce not on the dominant microflora of the seed but on some secondary species of fungi which happened to have flourished in a restricted niche" (Sinha 1964). Why?

Potent direct toxins are not the only thing produced by grain-spoilage fungi. When *Aspergillus niger* is moved from a nitrogen-rich medium to a nitrogen-free medium (as in comparing legume seeds with grains or fruits), it produces a large amount of oxalate after a 6–8-h lag time (Jefferson and Harrison 1971). Inoculation of wheat, oats, and Bermuda grass with a number of fungi produced enough oxalate to bind all the calcium in the diet; "mice developed signs of severe malnutrition and died within a few days after being placed on a diet of moldy feed containing excess oxalate. . . Rabbits and guinea pigs fed moldy food not containing excess oxalate lost much weight but at no time had overt

signs of acute oxalate poisoning'' (Wilson and Wilson 1961). When *Aspergillus*, *Penicillium*, and *Paecilomyces* were cultured on cacao beans, the free fatty acid content of the beans rose from 1.4% to 28%–62% (Hansen et al. 1973); such a change serves not only to warn a rodent that there are potentially toxic microorganisms in the beans, but the free fatty acids themselves may be toxic in such high concentrations. Unsaturated fatty acids from beer and from baker's and fodder yeast are bactericidal and bacteriostatic (Uroma and Virtanen 1949) and may be toxic by virtue of disruption of the gut flora. *Pseudomonas cocovenans* produces a fatty acid, bongkreik acid, on coconut press cake infected with *Rhizopus oryzae*; this fatty acid is very toxic to the fungus and to humans (Van Veen 1973).

As would be expected of a widely distributed organism with a variety of substrates, the toxin-producing microorganisms may only produce defensive compounds on certain substrates and in certain environments. *Pseudomonas cocovenans* produces bongkreik acid only on coconut press cake, not on soybean and peanut press cake (Van Veen 1973). If several kinds of toxins are made by a fungus, different ones are sometimes made on different substrates (Wilson and Hayes 1973). *Alternaria* and *Cladosporium* in black raspberry fruits do not make pectinases or lead to visible decomposition of the raspberry but in other media make pectinases (White and Fabian 1953); I interpret this to mean that it is maladaptive for the fungus to structurally decompose the fruit. When *A. flavus* attacks birds as a parasite (Ainsworth and Austwick 1959), it probably does not produce aflatoxin. While this may, of course, be due to different building blocks being available in different substrates, it may also reflect the external threats to the fungus on different natural substrates. Unfortunately, biologists have made no progress in understanding the quasi-natural environments in which spoilage microorganisms have evolved.

MEAT

At the time of death, a piece of meat is generally acceptable to just about any carnivore (assuming behavioral requirements have been met). In warm climates, it is unacceptable to just about all within a few hours to days, except those who specialize in eating meat in the later stages of decomposition. When a bacterial clone finds itself in such a rich source of nutrients, there should be very strong selection for it to do what it can to keep out vertebrates. *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi*, and *Bacillus stearothermophilus* are all toxin-producing bacteria that may be suspected of playing this game. *Clostridium botulinum* may be carrying the game in several directions. By multiplying on soggy grain it kills the animals that would (and do) eat that grain, such as ducks and pheasants (Rosen 1971a) and mice (Schantz and Sugiyama 1974); mice are apparently the most sensitive animal known to the botulin toxins (Schantz and Sugiyama 1974). If they do eat the grain, the anaerobic bacterium then multiplies in their carcasses (and on other chunks of meat—Ando and Karashimada 1970; Nakano and Kodama 1970), and even the fly larvae in the carcasses become sufficiently toxic to kill

ducks and pheasants that eat them (Rosen 1971). It makes me wonder if the diarrhea and vomiting induced by certain bacteria is not a microbe's way of harvesting a large food source.

Such movement of the microbes (and the toxins) up the food chain is probably commonplace and probably explains why many carnivores will not eat putrid carcasses. "There is evidence of oral transmission of avian cholera from diseased carcasses to predators and scavenging birds. It was observed that during one epizootic in which mice (*Microtus montanus*) were infected, 44 short-eared owls (*Asio flammeus*) and 5 marsh hawks (*Circus cyaneus hudsonicus*) died from avian cholera"; their guts contained mouse remains and *Pasteurella multocida* (Rosen 1971b). Predatory cats and dogs may get mouse favus, caused by the fungus *Trichophyton mentagrophytes*, from their prey (Austwick 1968). *Clostridium perfringens* produces so-called alpha toxin leading to gas gangrene and food poisoning in man; "most market meats and poultry are heavily contaminated with *C. perfringens*," and it is "more widely distributed over the surface of the earth than any other pathogenic bacterium" (Nakamura and Schulze 1970).

It is difficult to distinguish between a carnivore rejecting meat because of the toxins produced by the bacterium or to avoid the disease that killed the dead animal. Irvin et al. (1972) had a relevant warning to people working with wild animal cadavers: there is "little danger [of contracting aspergillosis] unless the dead animal is opened to expose fungus in the body cavity." Neither tigers, panthers, nor jackals will eat Indian cattle killed by rinderpest (Morris 1932), and I wonder if the adaptive value of "playing possum" is to mimic an animal sufficiently sick to die immediately of shock upon being captured. Certainly wild birds and small mammals can get *Salmonella* and other bacterial poisoning from what they eat (Davis et al. 1971; Taylor 1968; Ludlum 1954; Bates and Busenbark 1963; Ainsworth and Austwick 1959). How important this is in their biology is unclear; if they are generally very careful about avoiding rotting meat, then it may be very important in limiting their resources yet never manifest itself in conspicuous mortality. Food poisoning is hardly mentioned in Halloran's (1955) thousands of compiled references on animal disease or in McDiarmid's (1969) symposium on diseases in free-living wild animals, but this may also be because only the lethal diseases are noted and most animals are probably sufficiently conservative not to eat lethal quantities of spoiled food.

The microbes in meat are fairly competent at advertising their presence (and animals should be fairly competent at recognizing the advertisement). Amylamine, putrescine, cadaverine, tyramine, and other odoriferous amines are actively generated by decarboxylation of amino acids (Lovenberg 1973). The microbes have a large number of amino acids to modify to warning signals if they are decomposing meat. Further, some of these amines, such as isoamylamine (from leucine), p-hydroxyphenylethylamine (from tyrosine), and phenylethylamine (from phenylalanine), are strong inducers of high blood pressure (e.g., Barger and Walpole 1909); these may be viewed as toxic protectants for the bacteria in their own right. As Lovenberg (1973) put it, "food substances that have been prepared by a fermentation process, or have been exposed to microbial contamination during aging or storage, are likely to contain vasoactive

amines." The same philosophy may be applied to the multitude of short-chain fatty acids, alcohols, aldehydes, ketones, esters, sulphides, and mercaptans made by bacteria on protein-rich substrates (Kristoffersen 1973). Food infected with *C. botulinum* generally has a rancid, butyric acidlike or cheeselike odor (Ando and Karashimada 1970). I should note, however, that man at least has come to recognize even the nonmicrobe-induced decomposition of meat as objectionable; "heme pigments have been found to catalyze oxidation of muscle tissue lipids. This results in a stale or rancid odor and flavor in the meat, sometimes referred to as warmed over flavor. Free radicals produced in oxidizing lipids can oxidize and decompose heme pigments. Meat in which this reaction has occurred is brown in color and likely to be rejected by the consumer" (Greene and Price 1975). Such a human response could, however, be a human-generated case of pseudomimicry. Our revulsion to microbial presence is strong, irrespective of whether taught or learned. "Single cell protein (SCP) has become a widely accepted term for microbial cell material intended for use as food or feed. It has a certain psychological value since any unpleasant connotations with terms such as bacterial, fungal, or microbial proteins are thereby avoided. In SCP feeding tests, a rather common observation seems to be the occurrence of gastrointestinal upsets in unsuspecting humans when the intake is increased above" about 15–25 grams per day (bacteria) or 3–100 grams per day (yeast) (Kihlberg 1972). Why are we so quick to throw out rotten food, long before its actual nutrients are exhausted? Score three points for the microbes.

The bacteria in carcasses are confronted with an interesting compromise situation. On the one hand I would expect selection to favor the generation of toxins that excluded all higher animals; on the other hand, higher animals may be important in both opening up the carcass and dispersing the microorganisms. Payne (1965) found that, even at summer temperatures, pig carcasses mummified if insects were prevented from getting to the carcass; as a class project I repeated the study with large dead dogs in Illinois and got the same result. On the other hand, the antibiotics produced by fungi have a conspicuous, direct, and detrimental effect on *Agria affinis* flesh fly larvae when introduced into their food in vitro; this effect persists even when the larvae are being grown on bacteria-free media (Singh and House 1970a, 1970b, 1970c). The ideal strategy for the fungus in a carcass would be to produce a small dangerous zone around itself, yet not exclude the larger insects that open up the carcass when mining through it.

The microbes and the smaller animals in carcasses are also competing with large scavenging specialists, such as vultures. How these birds handle the incredibly foul things that they eat is unclear, but Kear (1968) noted that "unlike other birds, they whitewash their legs and feet with faecal matter, suggesting the presence of a protective antiseptic in the gut." Some mammals also eat ripe carrion with impunity (Janzen 1976) and in the scavenger-rich tropical habitats, I suspect that many groups of microorganisms and carcass-inhabiting insects survive at a much lower density, if at all, than they do in mid- and northern latitudes where carcasses have the time to generate large populations of small organisms.

DISCUSSION

When a large number of conspecific individuals are jointly contributing to a trait that results in survival of all of them, such as producing a foul-tasting fruit, the system is clearly demonstrating a kind of group selection. In effect, each rotting fruit is a population, or to some degree a clone, which survives on the basis of its group traits. No single bacterium can produce enough antibiotic to influence any large animal (though a single fungus might). Depending on the size of the initial inoculum in the fruit, and the outcome of the inter- and intra-specific competition among the members of the inoculum, the final characteristics of a rotting fruit may well be the results of only a few microbial clones despite the large number of "individuals" present. Even when a number of species are involved in producing the traits of the rotting fruit, they should all be evolutionarily interested in not being eaten and, therefore, all be the product of past "group" selection. However, it should be noted that, in any given rotting fruit, the conspecific microorganisms are likely to represent only a few closely related lineages, and thus "kin selection" might be a more appropriate term, if indeed a term is desirable. A discussion of selection and fitness in microbes is greatly hampered by the lack of discrimination in the literature on their natural history between where they can be found in the wild by isolation techniques, and where they multiply. It is as though weedy plant biology was based largely on examination of seed banks in the soil.

While much of the chemical alteration of fruits and carcasses by microbes (and by certain insects) may be to keep the fruit or meat and the microbe from being eaten, much may also be a consequence of selection for chemical competitive abilities among the microbes. Without experimentation directed at distinguishing between the two processes, there is no way to tell which, if either, is the more important. Here I emphasize the former simply because I have found no evidence of its exploration in the literature. To balance the picture, we badly need experimental feeding tests with wholesome and rotting wild foods with wild animals in various states of health and welfare.

I have emphasized what the microbes do and what the frugivores or carnivores do in response. The plant (not the carcass) may also play an active role. It is conceivable that numerous characteristics of ripe fruit are directed at exclusion of those microbes (or fruit-eating insects) that will render the fruit unacceptable to potential dispersal agents. For example, maturing apples, attacked by *Nectria galligena* apple rot, produce benzoic acid; this stops fungal growth until the apple has been ripe for a long time (Swinburne 1973) and presumably, in nature, been dispersed. Second, certain fruit defenses against small invaders may be incompatible with optimal fruit dispersal. For example, tannin deposition in a ripe fruit, to wall off a boring insect and its associated microbes, may well make the fruit too astringent for a frugivore. Large fruits requiring repeated visits by frugivores for many days after opening may have a particularly difficult time. This may be why there are so very few such fruits.

While my discussion has focused on fruit, seeds, and carcasses, the same argument should apply to the three-way interaction between vertebrates and

microbes and other rich food sources. For example, fungi cause the sweet potato tuber they have invaded to produce high concentrations of toxic furano-sesquiterpenoids (Wilson and Hayes 1973), and moldy sweet potatoes infected with fusarium can be lethal to cattle if eaten (Peckham et al. 1972).

In concluding, I note that there are numerous times when man (and I suspect other animals) holds its nose or puts on spices and takes a bite (cheese, fermented beverages, pickled vegetables, etc.) either because it is known that the particular microorganisms are mimics, man is capriciously immune, or intoxication is desired. This can all go awry when unexpected microorganisms get into the brew, as with *Clostridium botulinum* in Japanese izushi, a fermented fish and vegetable dish (Nakano and Kodama 1970).

SUMMARY

When an animal eats "spoiled" or "rotten" food of any kind, it runs a largely unknown risk (except in the case of grains) of being injured by toxins or microbe-produced antibiotics, getting food with lowered nutrient content, and infecting itself with microbes. Of course, these consequences may all be the product of microbes interacting with each other. However, I suspect that these traits have also been molded by the generally maladaptive event of having yourself and your resources eaten by a large animal. Animals have probably also done their part in evolving fairly accurate means of knowing when a food item contains organisms that do not wish to be eaten and therefore have coevolved sensory input. I would not suggest that the selective pressure for the production of alcohols, free acids, aflatoxins, quereomycin, botulinin, enterotoxin, etc., is solely the repulsion of large animals, but I would suggest that large animals have played a large and virtually unrecognized role in evolution of their production. Fruits rot, seeds mold, and meat spoils because that is the way microbes compete with bigger organisms.

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