Toxic Substances in Plants and the Food Habits of Early Man

Abstract. *The widespread occurrence of toxic substances in plants must have greatly restricted their usefulness as food for primitive man. The development of cooking of plant products is suggested to have been a major evolutionary advance, making a major increase in the vegetable materials palatable to man; this technical advantage apparently occurred only in the most recent 2 percent of the anthropological record.*

The extent to which early man utilized vegetable foods as contrasted with meat is relevant to the evolution of his social as well as his physiological characteristics (*1*). Whether the main time of his evolutionary development was spent as a hunter and meat-eater or as a gatherer of fruits and vegetables should be reflected in the characteristics of modern man. In discussions of this subject (*2*), there has not been an awareness of the widespread occurrence of toxic chemicals in plant materials and the restrictive effects that these chemicals might have had on man's use of vegetable foods. We suggest that the use of fire for cooking overcame many of the chemical limitations on his food supply and increased in a major way the vegetable foods which he could utilize.

Since plants are entirely unable to flee from their predators, it is probable that they have been under continuous evolutionary pressure to solve their survival problems by chemical means. Plants accumulate many secondary substances—chemicals that do not participate in the basic metabolism of the plant (*3–5*). Among these are many chemicals that serve to repel or discourage the use of the plant by insects, microorganisms, nematodes, grazing animals, and man.

The wide range of chemical materials that are toxic or poisonous has been reviewed in several places (*4*), and their influences on animal utilization have been reviewed by Whittaker and Feeny (*5*). A rough outline of the toxic secondary substances in plants according to their types of physiological action might include the following: (i) Most common among toxic substances are the enzyme inhibitors, including protease inhibitors which are toxic ingredients in many legumes and cereals and ubiquitous through the plant kingdom (*6*). The favism substances in legumes (*7*), cholinesterase inhibitors in the tomato family (*8*), and cyanogens (*9*) and some toxic alkaloids (*4*) are widespread examples of enzyme inhibitors. (ii) Physiological irritants might be the next most common category. These include the saponins, which form foams in the gut and occur in approximately 80 families of plants (*10*); the hemagglutinins, which disrupt the function of red blood cells and occur commonly in legumes and euphorbes (*11*); the lathyrogens, which disrupt collagen structure and occur in peas (*12*); the irritant oils, including many cyanogenic compounds that occur most commonly in the cabbage family (*9*), but also in many other food plants including manioc (*13*); and raphides, crystals that may serve as irritants in the mouth or gut, such as the oxalate raphides in beet and rhubarb (*14*). (iii) The third general category might be the allergens, which are usually proteinaceous and are widespread throughout the plant kingdom (*15*). (iv) Fourth might be the compounds that alter hormone systems; estrogens are common in a wide variety of plants, among which those of the yam family could be cited as a principal source (*16*); goiterogens are another hormone-altering type of toxin and occur in many members of the beet family and in legumes (*17*). (v) Lesser in toxicity are the vitamin antagonists such as protoaneomonin (*18*) and amino acid antagonists such as mimosine (*19*).

Animals have evolved various types of adaptations to the toxic substances in plants. One common type of accommodation has been the development of enzymes that are capable of metabolizing the chemicals. Some species of caterpillars, for example, possess oxidases in their gut that are capable of metabolizing the repellent terpenoids such as pyrethrin from chrysanthemums (*20*); the cabbage butterfly is capable of metabolizing the mustard oils from the cabbage family (*5*). A second type of adaptation is the ability of the browsing animal to sequester the chemical. The monarch butterfly, for example, accumulates a cardiac glycoside from the *Asclepias* species which the larvae eat, and the sequestered chemical serves as a deterrent to attacks by butterfly-eating birds (*21*); males of the genus *Bombus* can accumulate farnesol from the oils of flowers and later excrete the same material as a sex attractant (*22*). We are suggesting that man has developed a third major type of adaptation which serves to overcome the repellent qualities of secondary chemicals in potential foods. Cooking of plant products is, of course, a cultural rather than a physiological adaptation.

The removal of toxic substances from plant parts by cooking may occur through three principal means: (i) Protein toxins are disarmed by heat denaturation; this applies especially to the common enzyme inhibitors, which are often protein or polypeptide substances. (ii) Cooking may disarm toxic substances through accelerated oxidations at the elevated temperatures; the cyanogens, which are oxidized to harmless products during cooking, are examples of this type of removal. (iii) Dilution effects account for the disarming of other toxic substances; raphide crystals may be dissolved, or toxic substances may simply be diluted by the water medium which then may be discarded.

In view of the widespread occurrence of toxic substances in plants and the removal of many of these substances by cooking, it becomes relevant to examine the archeological record to find when man began using fire for cooking and how this use of fire may have prepared the way for the agricultural revolution. The subject of the use of fire by early man has been reviewed by Oakley (*23*), and subsequent archeological events, while filling in further details, have left the general history much as he described it.

In the small-brained hominid, *Australopithecus*, there is conclusive evidence that meat-eating and systematic tool-making go back 2.6 million years, although in the Omo River valley in northwestern Kenya the record may go back still further (*24*). R. Leakey's excavations in 1969 and 1970 at Lake Rudolf (*25*) have revealed 23 hominid specimens together with stone implements (*26*) and associated fauna, including a dozen species of Bovidae (*27*). These artifacts are almost a million years older than the elder Leakey's famous discoveries at the bottom of Olduvai Gorge (*28*). Yet in none of the carefully investigated East African sites, from Omo in the north to Olduvai, Tanzania, in the south, has any trace of fire been found in association with the autralopithecines (*23*). Neither is there such a trace at the South African sites, despite Dart's (*29*) unsubstantiated claim of fire at the Makapan cave (*23*).

The earliest known evidence for the

use of fire occurs in relics from 300,000 to 350,000 years ago, and only in cold Eurasia. At three locations, all associated with the Mindel glaciation, hearths appear; one is a site at Bouches-du-Rhône in France; another, associated with Verteszollos man, is near Budapest; and a third, known as Locality 13, is the oldest site in the Choukoutien caves near Peking. Oakley has made the reasonable suggestion that while man at this date and perhaps earlier used fire, he could not make it but collected it from natural sources (*23*). So sporadic was its use for hundreds of thousands of years to come that it seems highly unlikely that man could have been dependent on it for his survival.

Even in the Mousterian culture of Neanderthal man, while hearths appear frequently at most sites, they appear not at all at others. In his excavation of the High Cave at Tangier, typically Mousterian, Coon found no evidence for fire (*30*). Throughout all the African continent no evidence for the continual use of fire appears until Kalambo Falls, Zambia, 56,000 years ago. A bit later, in the same Final Acheulean period, hearths appear at two more African sites and then almost simultaneously at a series of sites in Syria, Israel, and southwestern Asia (*23*). In the Jabrud caves in Syria, for example, there are layers of deposits going back into much earlier Paleolithic times without evidence of fire. Then at level 14 (about 40,000 years ago) hearths appear so decisively that nearly half of all artifacts found have been distorted by heat (*31*). Shortly thereafter, Cro-Magnon man, making universal use of fire, displaced Neanderthal man in Europe.

Level 14 is, then, a benchmark; the sudden spread of hearths that occurred about 40,000 years ago indicates that man had added fire-making to his cultural heritage. Oakely (*23*) has suggested that the invention of making fire may have been that of striking flint against iron pyrites, producing incendiary sparks.

Whatever the method, the universal use of controlled fire made cooking possible, and we infer that this opened up to man a greatly expanded food supply. It was an evolutionary step necessarily preceding the cultivation of such cereals as wheat, barley, rice, and maize, all of which require cooking. In recalling that the agricultural revolution began 10,000 years ago, we must not forget that man survived for 98 percent of the proven 2.6 million years of his history without the regular use of fire. That the rapid expansion of modern *Homo sapiens* coincides with the development of cooking is consistent with our proposal that cooking brought a major evolutionary advantage to man.

Evolution has involved a long series of innovations which provide advantages to selected species in the survival battle. A plant species might accumulate secondary chemicals which would deprive animals of ready use of the plant's products, and such a chemical defense would permit that plant species to experience an "evolutionary radiation" (*32*). Some animal species may subsequently develop a means of adapting to this obstacle, such as the formation of an enzyme which could dispose of the chemical repellent, and would thus achieve its own advantage and perhaps experience an "evolutionary radiation" of its own. The successive adaptations by plants and by their animal predators sometimes become very complex (*5, 20, 21*). The utilization of fire for cooking might be considered one of the most impressive adaptations in the evolutionary sequence since it greatly extended man's food supplies by removing or destroying toxic secondary chemicals.

The point can be made that many plant materials are entirely free of toxic substances and form excellent foodstuffs. In fact, many types of ripe fruits contain secondary chemicals which serve as attractants, encouraging their utilization (and dispersal) by animals. Except in the tropics and subtropics, however, edible fruits are available only on a seasonal basis and cannot constitute a regular source of food. Such was the situation facing true man in glacial Europe. While the chimpanzee may support himself by the fruits of his tropical forests, in the course of australopithecine evolution in Africa there is no site indicating the presence or nearness of forests at the time of occupation. From the time of Dart's original discovery of *Australopithecus africanus* (*29*), throughout almost half a century of discovery, the conclusion has become universally accepted that the scene of hominid evolution was the savannah, varying only between steppe and bush (*33*).

The point of our suggestion is not to deny the utilization of fresh fruits in season, nor even to stress their limited availability to evolving man, but rather to point out the large extension of foodstuffs available after cooking had been developed. Studies of hunter-gatherers in various parts of the world today have shown that the extent to which they utilize vegetable foods ranges from about 60 to 80 percent in the case of tropical tribes to almost none in the case of Eskimo tribes. Even in the case of the tropical tribes, for which a great diversity of vegetable materials is available (84 plant species in the case of the !Kung hunters in Bechuana), a large proportion of the species used for food are cooked before being eaten (*2*). The extent of vegetable utilization by hunter-gatherers observed in the world today (all of whom have cooking skills) may be very much greater than in the hominid tribes of Pliocene and Pleistocene time.

The archeological record tells us that it was late in the course of man's development when he learned the value of fire, and much later when he learned to create and control fire, making possible his subsequent dependence on cooked vegetable foods.

Some archeologists have suggested that the importance of vegetable foods used by early man may have been grossly underestimated because vegetable materials would not persist in archeological remains (*34*). Recognition of the widespread occurrence of toxic substances might make the opposite argument more tenable; if one makes comparisons with present-day hunter-gatherer tribes, all of which possess cooking skills, the importance of vegetable foods to evolving man may have been overestimated.

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23 October 1971; revised 13 March 1972

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Trophic Regulation of Acetylcholine Sensitivity of Muscle: Effect of Electrical Stimulation

Abstract. *Denervation of skeletal muscle results in a spread of acetylcholine sensitivity over the entire surface membrane. Electrical stimulation, programmed to mimic the normal activity pattern, was applied continuously to the denervated rat diaphragm in vivo. After 4 days, the acetylcholine sensitivity was far less in the stimulated diaphragms than in denervated controls. Muscle activity may account for "neurotrophic" regulation of the acetylcholine sensitivity.*

Denervation of skeletal muscle increases its sensitivity to acetylcholine (ACh) (*1*). In a normally innervated muscle fiber, only the motor endplate region is highly sensitive to ACh; after section of the motor nerve, the sensitivity of the remainder of the surface membrane increases more than one-thousandfold until it equals that of the endplate (*2*).

The mechanism by which the motor nerve regulates the ACh sensitivity of the muscle membrane is not known. It has been generally held that activity or usage of muscle could not account for this and other "trophic" influences of the motor nerve (*3*). However, evidence has appeared suggesting that usage of muscle might be responsible for neurotrophic regulation of chemosensitivity (*4–7*). We have studied the effect of direct electrical stimulation of denervated mammalian muscle on chemosensitivity to ACh. To optimize the effectiveness of the stimulation, we used programmed stimulus patterns designed to mimic the normal action of the nerve; these patterns were applied continuously throughout the period of denervation. In this way, we tested the possible "trophic" effect of depolarization and contraction of muscle on one well-defined denervation phenomenon, ACh sensitivity.

Sprague-Dawley rats weighing 100 to 150 g were used. Denervation of the left hemidiaphragm was carried out when the animal was under chloral hydrate anesthesia (0.4 g/kg, intraperitoneally). The phrenic nerve was snared with a sterile crochet hook inserted through a left thoracic incision, and was severed as close as possible to the diaphragmatic surface. In the control rats, no further procedure was carried out until the testing of ACh sensitivity. In the experimental animals, the insertion of wire electrodes for stimulation was done aseptically. A midline incision was made from the midabdomen to the xiphisternum and was carried laterally along the left costal margin. Flexible multistrand stainless steel wire, stripped of its Teflon insulation for 1 to 1.5 cm and knotted at the tip to prevent slipping, was sutured to the abdominal surface of the diaphragm just below the costal margin, or at the level of the entry of the phrenic nerve. A second wire lead was sutured to the diaphragmatic tendon (Fig. 1). The wires were led out through the animal's flank and the surgical incision was closed. The rat's limbs and tail were fastened to a Lucite board with masking tape, and the head was restrained with a U clamp. Food and water were placed within easy reach, and the animals were encouraged to eat and drink.

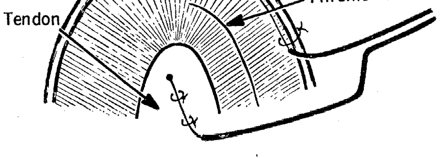

Fig. 1. Drawing that shows placement of stimulating electrodes in rat diaphragm. In four rats (not illustrated), the outer electrode was fixed near the site of the phrenic nerve (*X.*, position of the xiphisternum; *N.*, nerve).

Electrical stimulation was provided by a Grass SD5 or S48 stimulator through a capacitance-coupled stimulus isolation unit. The stimulus variables were chosen to produce vigorous contractions of the diaphragm at a rate slightly slower than the normal respiratory rate. This was achieved by delivering trains of 6 shocks each, 72 trains per minute. The stimulus was 7 to 9 volts (monitored by oscilloscope) and its duration was 0.25 to 0.3 msec.

At the end of each experiment (91 to 99 hours), the rat was anesthetized with chloral hydrate, and the abdominal incision was reopened. The diaphragm was observed while it was stimulated, and in all instances but one responded with vigorous contractions. (In the exceptional case, the diaphragm responded weakly, presumably because of poor contact between the electrodes and the muscle fibers.) The diaphragm was then removed, and the full-length segment that had been in contact with the electrodes was mounted, thoracic surface upward, on a silicone rubber platform. The preparation was bathed in Trowell's T-8 medium (*8*) and equilibrated with 5 percent CO_2 and 95 percent O_2.

To avoid any possible effect of fibrosis around the electrode wires, we used the thoracic surface of the diaphragm for sensitivity testing, whereas the electrodes had been applied to the abdominal surface. Fibrosis was not notable in the stimulated diaphragms.

The ACh sensitivity of fibers was tested in each diaphragm by a method similar to that used by others (*9*). Trans-