

# PROBLEMS ASSOCIATED WITH PARTICULAR FOODS

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Problems associated with foods are many and varied. The four examples discussed in this chapter are typical of the interaction of the host-agent-environment relationship between man and his food. It is clear that constituents of foods normally considered as nutrients (and others not contributing to nutrition of the host but believed to be ordinarily inert, harmless, or capable of being metabolized without causing any harm) can, under certain circumstances arising from inherited or acquired host characteristics, prove deleterious to health or may even cause acute disease. Such a situation emphasizes the need for further studies of the relationship referred to above. These studies can be facilitated in the first instance by a more detailed knowledge of the composition of food, which should extend far beyond its nutrient content and must encompass all constituents, major and minor, including those present in trace amounts.

## DISACCHARIDE INTOLERANCE

Lactose intolerance, as a congenital defect, was reported for the first time in 1959 by Holzel *et al.*,<sup>1</sup> who postulated that it was caused by an

\* Deceased, July 8, 1971.

† Authored section on Toxic Honeys only.

enzyme deficiency. Soon thereafter, congenital sucrose intolerance was also demonstrated.<sup>2</sup> Since then, our knowledge concerning disaccharide absorption has progressed rapidly with an improved understanding of the mechanism involved. Disaccharidase deficiencies with specific defects in the absorption of dietary disaccharides have come to light, and the contributory factors have been elucidated.

Carbohydrates are among the natural constituents of human diets. They range from simple mono- and disaccharides to polysaccharides such as starch and, to a very small extent, glycogen. These polysaccharides are hydrolyzed in the gastrointestinal tract by amylases present in the secretions of the salivary gland and pancreas, during which process disaccharides like maltose and isomaltose are formed. Disaccharidases present in the brush border or the microvilli of the epithelial cells of the small intestine hydrolyze these disaccharides—as well as lactose and sucrose ingested with food—into monosaccharides, which are then absorbed.

Procedures such as heat inactivation, selective inhibition, and gel filtration chromatography have been used to identify several disaccharidases in the intestinal mucosa of man.<sup>3,4</sup> Four maltases, two lactases, and trehalase occur normally. Maltase Ia is identical to isomaltase (oligo-1,6-glucosidase), and maltase Ib is identical to sucrase ( $\beta$ -fructosidase). Maltase II and III ( $\alpha$ -glucosidases) hydrolyze only maltose. One of the two lactases ( $\beta$ -D-galactosidases) hydrolyzes cellobiose in addition to lactose. Trehalase (a specific  $\alpha$ -glucosidase) acts on trehalose, although this nonreducing disaccharide is not a normal constituent of the diet.

Lactose intolerance is due to lactase deficiency, whereas maltose intolerance requires simultaneous deficiency of more than one enzyme.<sup>4</sup> In a deficiency of these enzymes, the digestion of the disaccharides and the absorption of their component monosaccharides are diminished. The unaltered disaccharide remains in the intestinal lumen and causes problems by its continued presence. It may attract water from the tissue to the lumen of the gut, causing osmotic catharsis.<sup>5</sup> In the colon, the undigested lactose is fermented by bacterial enzymes to lactic acid and other short chain acids that raise the osmolarity, lower pH, and interfere with the reabsorption of fluid. The carbon dioxide and hydrogen produced by this fermentation probably contribute to the bloating, frothy diarrhea, and flatulence.

These characteristic stools appear after the ingestion of the disaccharide for which the subject is intolerant.<sup>3,6</sup> Certain observations indicate that acid stools and the presence of low-molecular-weight fatty acids in feces are not necessarily found in disaccharide intolerance in adults.<sup>7</sup> Some individuals who are lactose intolerant do not experience symp-

toms with one, two, or three glasses of milk. Thus it is possible to have a spectrum of symptomatology from no symptoms to that of a severe reaction.

Disaccharide intolerance may occur as a congenital defect in infants and is due to lack of one or more specific disaccharidases. In these cases the enzymic defect is probably inherited. In addition to lactase deficiency, congenital deficiencies of isomaltase and sucrase have been reported.<sup>8,9</sup> However, lactase deficiency is the commonest of all disaccharidase deficiencies.

Lactose intolerance is also frequently manifested later in childhood, during adolescence, or in adulthood.<sup>10</sup> This is far more common than the manifestations of congenital deficiency seen in early infancy. The significance of the late occurrence of lactose intolerance is discussed later.

#### *Methods for Detection*

A history of diarrhea and abdominal pain following the ingestion of certain foods gives an indication of possible intolerance. The offending disaccharide can be identified by noting the effect of its withdrawal from the diet and by feeding tests, during which the onset of symptoms and other signs (e.g., acid stools, presence of reducing sugar in stools, etc.) are noted. However, the tolerance test should not be done when the patient is actually having diarrhea.

The test is accomplished by oral administration of the test sugar in aqueous solution or suspension. The dose for infants and children is 2 g/kg; for adults 50- and 100-g test doses have been used.<sup>4,11</sup> A dose of 50 g sugar per m<sup>2</sup> body surface has also been used for children as well as adults.<sup>6,10</sup> Intolerant subjects experience abdominal pain or diarrhea or both. Abdominal distension may be caused by gas produced through fermentation. The blood sugar curve either is flat or may rise somewhat above the fasting level. The accepted blood sugar rise is usually less than 26 mg/100 ml.<sup>10</sup> Stools collected over several hours will have a low pH (pH 5.5 or less), especially in infants and children, and will contain unaltered disaccharide. The above test should be followed by a tolerance test using the corresponding monosaccharide(s). This helps to differentiate disaccharide intolerance from the general malabsorption syndrome in which the absorption of monosaccharide may also be impaired. In screening large populations, however, additional tests using monosaccharides may be unproductive in revealing lactose intolerance secondary to monosaccharide intolerance.<sup>12</sup>

In recent years peroral biopsy has been utilized to obtain samples of

jejunal mucosa for the determination of enzyme activity. The respective disaccharidase activities are estimated in the mucosal homogenate, which is incubated at 37 °C and pH 5.8 with the appropriate substrates. The results are usually expressed as  $\mu$ moles disaccharide split/min/g wet tissue.

There is considerable variation in the range of activities in the jejunal mucosa from normal individuals, partly because of the variable amounts of epithelial cells and other inactive tissue in the biopsy sample. The results from different laboratories are not always comparable because some authors express them in terms of dry weight of the tissue or per g of protein. However, when expressed as ratios such as sucrase/lactase or maltase/lactase, the results are more comparable. The ratios between the various disaccharidase activities have proved useful in the detection of enzymic defects, first because they vary within comparatively narrow limits in normal individuals, and second because they allow comparison of the findings of different laboratories.<sup>6,13</sup>

Another reason for the lack of comparability of results may lie in the fact that different investigators obtain biopsy samples from different sites in the small intestine. The activities of disaccharidases vary throughout the small intestine. They are low in the proximal parts of the jejunum; they then increase progressively, reaching a peak in adults at about 170 cm (as measured by the length of the tube swallowed) from the teeth and decrease thereafter until minimum values are reached in distal ileum.<sup>13</sup> Although most reported biopsies have been taken in proximal jejunum, there is merit in the suggestion that they should be taken at the ligament of Treitz. Apart from ensuring comparability of results reported from different laboratories, it has also been claimed that values for lactase activity found in specimens taken from this site will correctly diagnose lactase deficiency if it exists. At other sites the values are higher, and the results may be equivocal.<sup>11,13</sup>

Certain caution, however, has to be exercised in interpreting the results of disaccharidase activities, for the biopsy sample may not necessarily be representative of the mucosa of the small intestine. Enzyme assays on biopsy material should preferably be followed by appropriate tolerance tests.

#### *Disaccharidase Deficiency in Infancy*

Congenital disaccharide intolerance is manifested as soon as a disaccharide for which intolerance exists or its precursor is found in the food given to infants. Intolerance to breast milk may be seen after breast feeding starts and is often ascribed to allergy, although it could easily be due to lactose, of which human milk contains 6–7 g/100 ml. If the lac-

tase deficiency is not severe, feeding cow's milk and making up calories by adding sugar other than lactose restores the health of the infant. Lactase deficiency of a congenital nature often leads to a rapid downhill course on the part of the newborn, and the deficiency has to be suspected and diagnosed quickly to ensure the survival of the infant. Cow's milk contains only about 4% lactose, and in adapting it for the infant, lactose concentration is still further reduced.

Inherited sucrose intolerance becomes apparent when the food of the infant contains added sucrose. Since the enzymes that split sucrose and isomaltose are often deficient simultaneously, the infant with sucrose intolerance will also be intolerant to dextrin or starch, both of which give rise to isomaltose during digestion.<sup>6,14</sup> In this type of disaccharide intolerance, the child either recovers spontaneously as he gets older, or he learns to live with the condition by avoiding foods containing sucrose. Furthermore, since isomaltase activity is not as depressed as the sucrase—and since the amount of isomaltose formed is relatively small compared to maltose in the digestion of starch and dextrin—isomaltose intolerance does not cause much inconvenience or affect the individual's health even when starchy foods are eaten. In contrast, inherited lactase deficiency persists into adult life.<sup>3,6</sup>

#### *Lactase Deficiency in Children, Adolescents, and Adults*

Disaccharidase deficiencies have been known to occur in children, adolescents, and adults. Lactase deficiency is the one most commonly encountered and may occur by itself as an isolated deficiency.<sup>15</sup> Several studies have been published<sup>10,18</sup> on lactose intolerance and lactase deficiency in population groups. Most of them involve adolescents and adults and cover an age span from less than 20 yr to over 60 yr of age.

In the United States a high prevalence of lactose intolerance has been found among blacks; the reported figures vary between 70 and 95%.<sup>10,16,17</sup> On the other hand, the incidence in American whites varies between 6 and 10%.<sup>10,11</sup> The subjects in some studies were healthy volunteers or inpatients of hospitals; in both cases, those investigated were free from diseases of the gastrointestinal tract and associated organs. The microscopic appearance of intestinal mucosa in almost all these subjects has been reported to be normal. In an investigation on healthy children, 14 out of 20 black children and 2 out of 20 white children had a family history of milk intolerance. Of these, 7 black and 2 white children showed intolerance to an oral dose of lactose given at the level of 50 g/m<sup>2</sup> body surface.<sup>10</sup> Although the authors did not determine lactase activity, they postulated that intestinal lac-

tase activity decreases with age in blacks and that this may be responsible for the higher prevalence of lactose intolerance observed in the adult black population.

In Uganda lactase deficiency was found among Baganda children and those of neighboring Bantu tribes, whereas subjects with Hamitic ancestry showed higher levels of lactase activity.<sup>18</sup>

A higher prevalence of lactose intolerance and lactase deficiency than that in American whites has been reported from Britain and in Greek Cypriots, but this may be because the latter studies were done in patients having abdominal pain, diarrhea, or bone disease or megaloblastic anemia.<sup>19,20</sup> Observations on a limited number of healthy Asians suggest a high prevalence of lactose intolerance and lactase deficiency among them.<sup>21,22,23</sup> In an investigation in India on 192 infants and children (up to 5 yr of age) suffering from chronic or recurrent diarrhea, 49 children gave a flat curve after a lactose tolerance test. This suggests that in over 20% of the cases, lactase deficiency may have been the cause for the diarrhea.<sup>24</sup> All these 49 children improved clinically on withdrawal of milk from their food. It is possible, however, that in these cases lactase deficiency may have resulted from chronic and recurrent diarrhea and could have been a contributory factor to lactose intolerance. In Cairo, Egypt, isolated lactase deficiency was found in 5 out of 10 healthy subjects and in 9 out of 13 anemic patients with schistosome and/or hookworm infection with or without pellagra.<sup>25</sup> In biopsy specimens from lactase-deficient patients, sucrase and maltase activities were within the reported normal limits. In most of them (including specimens from healthy controls) the intestinal mucosa showed gross and microscopic abnormalities that ranged from mild to severe.

One can conclude, therefore, that lactose intolerance and presumably lactase deficiency may be far more widespread among apparently healthy subjects than has been realized. However, further studies on healthy population groups are needed to get a correct idea of its worldwide prevalence.

There is some question whether lactose intolerance and lactase deficiency found in adolescents and adults is inherited or acquired. Limited evidence on familial incidence and the different racial distribution are arguments in favor of their being inherited. It has been shown that the incidence of lactase deficiency differs widely in healthy subjects of different racial groups who live under the same conditions, who are comparable in socioeconomic status, and who have no history of malnutrition or a major gastrointestinal (GI) disease. On the other hand, certain observations indicate that intestinal lactase is more drastically affected in disorders of the gastrointestinal tract than in other disorders. Further-

more, there is the suggestion that lactase activity decreases with age. In some cases this decrease may reach a point where lactose intolerance manifests itself. These considerations provide a likely reason to consider lactase deficiency in adolescents and adults as an acquired deficiency.

#### *Secondary Lactase Deficiency*

This is associated with diseases of the small intestine, among which can be mentioned infective diarrheas, celiac disease, and tropical malabsorption syndrome, including sprue.<sup>6,26,27,28</sup> In these conditions the gross and microscopic appearance of the intestinal mucosa is abnormal and shows moderate to severe degrees of damage.<sup>29</sup> Second, disaccharidase activities other than lactase are also depressed, and other absorptive defects are found as well. On treatment and recovery from these conditions, absorption defects are rectified, and activities of disaccharidases other than lactase return to normal. The deficiency of lactase, however, may persist.<sup>28</sup>

Diarrhea—infective as well as noninfective—is very often associated with protein-calorie malnutrition (kwashiorkor and marasmus). Some observers have found that it can be exacerbated by treatment of the basic condition with skim milk. Lately it has been demonstrated that lactase deficiency is responsible for milk intolerance in kwashiorkor.<sup>30</sup> Other disaccharidases are also adversely affected but much less so than lactase. Lactose intolerance is believed to be a transient phenomenon in protein-calorie malnutrition. However, recent findings have shown that although lactose absorption improved on recovery, lactase activity of the mucosa did not return to normal, in one case even up to 1 yr on a good protein diet.<sup>31</sup> Thus a lactase deficiency due to malnutrition in infancy and early childhood could become an acquired characteristic.

The finding, admittedly based on limited studies, that lactase deficiency is common among Asians and Africans has led some to question the advisability and wisdom of donating skim milk as food aid to developing countries for supplementary feeding of infants and children.<sup>23</sup> This seems an unfortunate conclusion based on insufficient evidence.<sup>32</sup> Even assuming that lactase deficiency were widespread, proof is still wanting that the reconstituted milk served to infants and children as supplementary food has not been tolerated. It is possible that lactase deficiency may be relative and not absolute. Observations in the United States on healthy subjects have shown that most lactase-deficient subjects are able to tolerate small quantities of milk; several individuals had not had a history of milk intolerance in infancy and childhood. In view of this and the fact that the supplementary milk that any single indi-

vidual receives is not likely to be more than 10–12 fluid oz per day, the chances that it will cause health problems or will be wasted are extremely remote.

## FOODS AND FLATUS

Flatulence is a common complaint even among healthy individuals and is one of the most common causes of abdominal discomfort. Flatulence is also associated with such bowel disorders as dyspepsia, constipation, and diarrhea. This discussion is concerned with the mechanism of flatus and with the factors contributing to its formation in health.

Flatus has been associated in the minds of most people with certain types of food. Consumption of beans (of any kind) and other grain legumes is considered to be conducive to intestinal gas formation. A large variety of other foods has also been incriminated from time to time.<sup>33</sup> Among these may be mentioned such tubers as potatoes, sweet potatoes, and radishes; such other vegetables as cabbage, cauliflower, cucumbers, lettuce, onions, and pumpkins; and such fruits as apples, oranges, strawberries, and tomatoes. Eggs and milk have been included in the list of flatulent foods. Fatty foods and those cooked in fat are also believed to contribute to gas formation.

This association between certain foods and flatulence has been linked with their coarseness and/or their relative indigestibility. This concept was not wrong, although the immediate cause of gas formation was not known. In certain cultures, the capacity to give rise to gas was considered a desirable attribute of some foods, among which grain legumes—including beans and pulses—held the pride of place.

With the discovery of microorganisms, the understanding of microbial action on pure compounds and food components, and the knowledge that some microbes normally inhabit the GI tract, a rational approach to the study of flatulence became possible. Interest in intestinal gas was centered chiefly on its clinical aspects during the first half of this century. Little attention was devoted to the elucidation of the relationship between food and flatus until after the second World War. Flatulence associated with high-altitude flying called for investigation. It was, however, the need arising from man's attempt to conquer space and the possibility of astronauts having to spend days or months in a relatively small enclosed space that provided the powerful impetus setting intensive studies in motion.

Normally, a small amount of gas is found in the lumen of the alimentary tract. This originates from three sources: swallowed air, diffusion

of gas into the lumen from across the epithelial border, and gas formed within the lumen by the intestinal flora acting on food residues.<sup>33,34</sup> Gases are also absorbed into the blood from the intestine and are excreted with the expired gases. In addition, the passage of CO<sub>2</sub> across the intestinal barrier is mediated by the enzyme carbonic anhydrase. Nitrogen is absorbed slowly and may cause distress. The quantity of gas found at any time will be the result of the relative contributions from the three sources mentioned above and the gaseous exchange between blood and intestine. However, normally 70% of GI gas is swallowed air. The proportion of gas produced by fermentation and that arising through diffusion from blood vary, with the former predominating.

## Methods

Several methods have been used for the study of gas formation. The simplest is that of placing a flatus tube in position up the rectum and collecting the gas passed. This enables one to study the rate and composition of the gas produced in the colon. Other methods have been devised to study the rate of gas formation and its composition at different sites in the GI tract. Among these are: (1) intragastric administration of a known volume of oxygen and collection of flatus as it emerges per rectum through an indwelling catheter; (2) intubation with a specially designed poly-lumen tube for the withdrawal of gas specimens at predetermined sites and intervals; and (3) an analysis of expired gases, which give information on the contribution of bacterial fermentation to the composition of intestinal gas. The role of intestinal microorganisms has been studied by culturing fresh feces or dejecta from established ileostomies and colostomies followed by fermentation of food constituents or pure compounds and analysis of the gases produced.

## Gastrointestinal Flora

The composition and habitat of the microbial population varies in the alimentary tract.<sup>35,36</sup> The stomach itself is believed to be sterile. Any organisms found there are believed to have originated in the mouth and the pharynx. The upper reaches of the small intestine have also been found to be bacteriologically sterile in about 70% of the subjects studied. Microorganisms occur in appreciable numbers in the terminal ileum and include aerobes and anaerobes, with the former predominating. The colon is the normal habitat of a large variety of microorganisms. The non-spore-forming anaerobes predominate, although enterobacteria of the coli-aerogenes group are also found in large numbers.

The composition of the intestinal flora varies from one person to another and depends to a large extent on the habitual diet of the individual. The total identification of GI flora is difficult, for a sample taken from the intestinal tract may not be fully representative. Attempts have been made to identify the organisms by culturing fresh feces on the assumption that these organisms represent GI flora. They probably represent the colonic flora.<sup>37,38</sup> In a recent National Aeronautics and Space Administration-sponsored study, 16 types of anaerobes have been partially characterized and identified as normally occurring in healthy subjects living on a "typical American" diet. The organisms varied in numbers between 1 and 100 billion per g feces. Non-spore-forming anaerobes predominated in fecal bacterial population over the aerobes by a factor 1,000:1.

Aerobic organisms such as the coliforms produce  $\text{CO}_2$  and  $\text{H}_2$ , and the predominating anaerobes produce  $\text{H}_2$  and  $\text{CH}_4$ . Some of the latter also produce  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ , indole, skatole, and butyric and valeric acids, products that are among those responsible for the malodor of flatus. It is thus clear that the composition of GI flora—which in turn depends upon the type of diet and the substrate thus provided—will determine the composition of the flatus. Although microbial action starts in the small intestine, the major contribution to the flatus results from action in the colon, where food residues stay for a long time and the microbial population is comparatively large and varied.

#### *Amount and Composition of Gastrointestinal Gas*

Gas normally present in the GI tract does not exceed about 200 ml. However, between 400 and 650 ml of flatus is expelled per day, which is composed of up to 50% of  $\text{N}_2$  from swallowed air, about 12–14% of  $\text{O}_2$ , and a variable proportion of  $\text{CO}_2$ ,  $\text{H}_2$ , and  $\text{CH}_4$  as products of fermentation. Between 1,000 and 1,500 ml of gas per day can normally leave the body without causing discomfort.<sup>37</sup> Products of bacterial fermentation may cause spasm by irritating the intestinal mucosa and thus give rise to abdominal distension and discomfort.<sup>34</sup>

Different observers have given varying values for the concentrations of  $\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{H}_2$  in flatus. These variations probably reflect differences in the techniques of flatus collection, the time of flatus collection in relation to meals, the composition of intestinal flora, the substrate available for bacterial action, and the variability among individuals in the capacity to form gases like  $\text{H}_2$  and  $\text{CH}_4$ .

The amount and composition of GI gas is related to meals and is influenced by microbial action. In observations on nine healthy subjects,

using intestinal intubation with a polyluminal tube, it was found that in a fasting state the production of  $\text{H}_2$  was negligible.<sup>39</sup> Within 15 min of the introduction of 60 ml of a 10% solution of lactose,  $\text{H}_2$  production increased and reached a peak at 1 h. Most  $\text{H}_2$  was produced in the cecum and colon and much less in the small intestine. Capacity to produce methane varied in different subjects. In five subjects, no  $\text{CH}_4$  could be detected, but in four others 0.55 ml of  $\text{CH}_4$ /min was produced; this was not influenced by lactose. That some individuals are methane producers and others not has been repeatedly documented. In some people,  $\text{CH}_4$  may always be present in breath; in others it may largely appear in flatus.

Incubation of dejecta collected from established ileostomies and colostomies under anaerobic conditions using different substrates showed that whereas  $\text{CO}_2$  and  $\text{H}_2$  were formed in both,  $\text{CH}_4$  was formed only by the colonic dejecta.<sup>40</sup> It seems therefore that  $\text{CH}_4$  results from the bacterial action in the colon.

In another series of investigations in which GI gas formation was studied by analysis of expired air, the following observations were made.<sup>41,42</sup> During fasting, the expired air had 4–7 ppm  $\text{H}_2$ . After a bland meal consisting of egg albumin, sucrose, dextrimaltose, corn starch, corn oil, shortening, and salt mixture, the concentration of  $\text{H}_2$  in expired air rose to 25–30 ppm within 1 h. Flatus formed at the rate of 1–4 ml/30 min during fasting; flatus formation then increased to 11–20 ml/30 min after the meal and was associated with the peak in  $\text{H}_2$  in the expired air.

#### *Effect of Different Foods on Flatus*

A meal of 100 g cooked dried white beans at lunch increased flatus formation, beginning at 5 h after lunch and lasting through 7 hours<sup>41</sup>; 130–170 ml per 30 min of flatus was recorded at the peak. Individual variations in flatus formation were recorded in response to the meals of beans. The authors concluded that large amounts of undigested material can cause flatulence. They found that consumption of 150 g of raw potato starch caused an increase in breath  $\text{H}_2$  for over 15 h (amounting to 40 ml/h), whereas raw wheat starch gave a normal breath pattern with an  $\text{H}_2$  concentration of 3–7 ppm.

The effect of bean diet on flatus formation and the potency of beans in producing large amounts of gas in the GI tract have been confirmed in other studies.<sup>43,44</sup> Healthy volunteers were fed a bland non-gas-forming basal diet containing the following foods: whole wheat bread, potatoes, lean beef, cottage cheese, butter, skim milk, banana, pear, pineapple, coffee, and cream. The diet provided 2,625 kcal/day and contained

246 g carbohydrate, 88 g fat, 152 g protein, and 7 g crude fiber. Flatus was collected by flatus tube for 2 h after lunch and after dinner on the fourth and seventh day on the diet. The volume of flatus was measured and an aliquot analyzed.

After 7 days on the basal diet, a variable proportion of it was replaced by commercial food preparations, such as pork and beans, green lima beans, and Boston baked beans. Each of these diets was fed for 7 days. The carbohydrate, fat, protein, and calorie contents of the diets were adjusted and were approximately comparable to those of the basal diet. Gas collections were made as in the basal period. The results of these studies are given in Table I.

TABLE 1 Influence of Beans on Flatus Formation<sup>a</sup>

Diet	Average Volume of Flatus (ml/h)	Average Composition of Gas (%)				
		CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	H <sub>2</sub>	CH <sub>4</sub>
Basal	15	8.1	3.6	61.2	19.8	7.3
Pork and beans, 57%	176	51.4	1.3	19.1	11.0	17.2
Pork and beans, 27%	66	23.1	1.1	37.7	24.2	13.9
Green lima beans, 28%	127	37.7	0.8	20.2	27.0	14.3
Boston baked beans, 51%	168	49.1	1.1	19.4	8.0	22.4
Basal	14	—	—	—	—	—
Pork and baked beans, 37%	83	—	—	—	—	—
Pork and baked beans, 37% + Mexaform	17	—	—	—	—	—

<sup>a</sup> Adapted from F. R. Steggerda and J. G. Dimmick<sup>43</sup> and F. R. Steggerda.<sup>44</sup>

It was clear that beans were powerful producers of gas in the GI tract. Individual variations in the quantity and composition of the gas produced were again observed. The most notable change in the composition of gas was in CO<sub>2</sub>, which was three to six times higher on bean diets than on the basal diet. The form of the bean product fed (i.e., bean flour or homogenates of the above-mentioned commercial preparations) made little difference in gas production. Green lima beans at 27% of the diet produced only 25% less flatus than Boston baked beans fed at the level of 51%. Thus, the beans differed in their capacity to produce flatus. When an antibacterial preparation (Mexaform) was administered to the subjects, flatus production was suppressed, thus showing the influence of bacterial action on flatus production. Antispasmodics and antiprotozoal compounds, however, were found to be without any influence. The authors conclude on the basis of supporting animal experiments that the clostridial group of anaerobes was responsible for flatus formation.<sup>45</sup>

A careful search for a compound or compounds in beans responsible for flatulence led the authors through various stages of fractionation and testing. They concluded that oligosaccharides (such as raffinose and stachyose) are largely responsible for the flatulent properties of beans.

Raffinose is a trisaccharide consisting of galactose, glucose and fructose residues and is *O*- $\alpha$ -D-galactopyranosyl-(1 $\rightarrow$ 6)-*O*- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-fructofuranoside. Stachyose is a tetrasaccharide also composed of galactose, glucose, and fructose residues and is *O*- $\alpha$ -D-galactopyranosyl-(1 $\rightarrow$ 6)-*O*- $\alpha$ -D-galactopyranosyl-(1 $\rightarrow$ 6)-*O*- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-fructofuranoside. The intestinal tract does not produce enzymes capable of splitting these oligosaccharides; they are acted upon by some anaerobic microorganisms giving rise to gaseous products. The capacity of stachyose and raffinose to produce gas when fed with a bland, non-gas-producing diet has been confirmed independently.<sup>41</sup>

Another reason for flatulence may be intolerance to food, exemplified best by milk intolerance. Several investigations have shown that milk intolerance of varying degrees exists in certain otherwise healthy people. It is intolerance to lactose that commonly is responsible for milk intolerance, and this is due to a deficiency of lactase in the intestinal mucosa. The basic cause of flatulence in lactose intolerance is identical to that discussed earlier. The undigested lactose forms a good substrate for the intestinal microorganisms for the production of gas and of other symptoms described in the earlier section of this chapter. Hence, lactose in such persons behaves in a manner similar to disaccharides in normal persons and becomes a flatus-producing factor.

It would thus appear that the older belief that less-digestible or indigestible foods cause flatulence has now received scientific support. The compounds in beans responsible for excess flatus formation have been identified. Further investigations are necessary, for there still exists the possibility that there may be other unidentified substances in food that might interfere with the absorption of intestinal gas, causing its accumulation in the digestive tract and passage as flatus. Furthermore, in addition to the type of the diet, other factors such as stress (including emotional stress) may cause flatulence in healthy individuals. Thus, flatulence in health may be associated with certain types of food, but it would be unwise to ascribe it to this single factor alone.

#### FAVISM

This topic has been the subject of an excellent review by Mager, Razin, and Hershko.<sup>46</sup>

Favism is the term used to describe a hemolytic syndrome in susceptible individuals following the ingestion of seeds or inhalation of the pollen from *Vicia faba*, commonly known as broad bean, horse bean, or fava bean. The hemolysis varies in intensity and is accompanied by hemoglobinuria and jaundice in the more severe cases. In the majority of cases, the onset of the favic crisis is usually 5–24 h after ingestion of the beans. However, symptoms of hemolysis have been reported within a few minutes after pollen inhalation.

The disease is usually self-limiting in adults; the acute stage lasts 24–48 h followed by complete spontaneous recovery. Repeated attacks may occur on renewed exposure. There is a sex difference in favism in which males appear to be more susceptible than females. Male:female ratios between 2.7 and 21.3 have been reported from different countries. Children appear to be more susceptible to favism and often experience severe hemolytic reactions. The most susceptible age group is that between 1 and 5 yr of age, but cases of favism have been reported in nurslings and infants under 1 yr. In early 1900<sup>47</sup> a fatality rate of 6.8% was reported for children under 6 yr of age. With the advent of blood-transfusion therapy, however, the mortality rates have been greatly reduced.

#### *Geographic Distribution*

Favism occurs principally in the countries of the Mediterranean littoral.<sup>48-50</sup> In certain of these countries—Egypt, Greece, and Italy—reports of favism go back to antiquity. It would appear that the fava bean was grown and eaten in ancient Egypt, since it was found in the tombs of pharaohs dating from the Fifth and Sixth Dynasties, over 2000 years BC. Fava beans have also been mentioned in the ancient papyri. Priests were prohibited from eating these beans, but in ancient Greece fava beans were considered to be appropriate food for athletes in order to improve their athletic performance.

The highest incidence of favism has been reported in recent times from Sardinia, with five cases of favism per year per 1,000 population. The island of Sicily and Calabria on the mainland in South Italy are other areas where the incidence of favism is fairly high. Statistics from countries bordering on the Eastern Mediterranean are lacking. The disease, however, has been reported from the Greek mainland and islands and from Turkey, Cyprus, Lebanon, and Egypt.

Outside the Mediterranean basin favism has been reported from other Middle Eastern countries, such as Iraq and Iran, and from Bulgaria. Recent reports indicate that in Iran it is far more widespread than had been

realized. In 1965 an incidence of favism varying from 2 to 9 per 10,000 population was reported from three provinces of Iran.<sup>49,51</sup> It has also been reported from Szechwan province in western China, and a large number of cases occurred in 1956–1958 in Kwantung province in southern China.<sup>52,53</sup> The disease is observed only sporadically in other European countries and the United States.

Fava bean is cultivated and used as human food in almost all countries of the world. Although it is a well-known and common food, no exact consumption figures are available. According to the 1959/1960 Food and Agriculture Organization Food Balance Sheet, daily consumption of the beans is fairly large in Egypt, with 29 g per capita of various grain legumes available for human consumption. Fava beans formed nearly two thirds of this supply, which would provide about 19 g fava bean per capita per day.<sup>54</sup> Figures for fava beans available in Egypt for human consumption in 1965 were almost identical with the above estimate. It is noteworthy that in spite of such a high daily consumption, cases of favism reported from Egypt are so few as to find little mention in Egyptian medical literature.

Fresh beans are eaten raw or cooked with the skin. Dried beans are usually soaked overnight in water and cooked in several ways, mostly without removing the skin. Beans are commonly consumed by older children and adults. However, fresh beans with or without the skin may be cooked soft, mashed, and fed as supplementary foods to infants during the weaning period.

Favism is most often associated with the consumption of fresh beans with or without skin and cooked or uncooked. Dried beans are also implicated to a lesser extent.

#### *Seasonal Incidence*

Seasonal variation in the incidence of favism in endemic areas is well documented. A characteristic peak incidence is associated with the harvesting of the bean. The harvesting season varies from country to country according to local climatic conditions, and the exact time of the highest incidence of the disease varies accordingly. Most cases of favism appear in the spring and early summer when fresh harvested beans are available. A second peak of incidence has been observed in Egypt in the autumn. This peak is associated with the consumption of dried beans that have been stored underground under anaerobic conditions for 6 months after harvest. This practice is peculiar to a certain locality in Egypt.<sup>55</sup> When these beans are taken out from storage, they are white

or green and outwardly resemble fresh harvested fava beans, whereas beans stored above ground in sacks become brown, probably due to exposure to light and oxygen.

#### *Mechanism of Toxic Action*

Different, usually mixed, strains of *Vicia faba* are cultivated in different countries. It has not been possible so far to associate favism with any particular strain or variety of the bean.

The onset of hemolytic attack on exposure to pollen of the flowering plant has been reported several times in the older literature. Authentic evidence of pollen-causing favism has not appeared in recent publications.

Various early hypotheses to explain the association between fava bean and favism were advanced. The disease was ascribed to infection, food allergy, autoimmune sensitivity, or "toxicity" of the bean. Most of these are now mainly of historical interest. In 1956 Crosby, in reviewing the situation in Sardinia, suggested that hemolysis caused by fava bean resembled that caused by primaquine in certain individuals with an intrinsic abnormality in erythrocytes.<sup>56</sup> The inherent defect leading to drug sensitivity was found to consist of a low concentration and relative instability of reduced glutathione (G-S-H) in erythrocytes, and of a deficiency of the enzyme glucose-6-phosphate dehydrogenase (G-6-PD).<sup>57</sup> In erythrocytes deficient in G-6-PD, the total glutathione content is reduced; however, the glutathione (G-S-S-G) levels are higher and G-S-H levels lower than in normal erythrocytes.<sup>58</sup> It has since been found that all persons with a history of favism have G-6-PD deficiency and that they show the same characteristics of a low level of G-S-H in erythrocytes and an instability of G-S-H toward acetyl phenylhydrazine similar to that found in drug-sensitive G-6-PD-deficient individuals.

G-6-PD deficiency is inherited as a sex-linked trait transmitted by an incompletely dominant gene located on the x chromosome. Two genotypes of individuals can be predicted for males and three for females. Genetic mosaicism may explain the wide variations in enzyme activity in different subjects. Thus, the basic defect that is a prerequisite for susceptibility to favism is inherited.

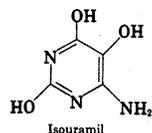
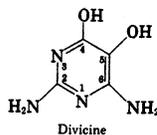
G-6-PD deficiency occurs with varying degrees of prevalence in different parts of the world, and different variants of G-6-PD have been described, a deficiency of any of which can result in drug sensitivity.<sup>59</sup> Not all G-6-PD-deficient subjects are susceptible to favism, although most of them may be susceptible to drug-induced hemolysis. Among the G-6-PD variants, the Mediterranean one is characterized by the unusually low

enzyme activity in erythrocytes of G-6-PD-deficient subjects (less than 5% of the normal) and in addition a low Michaelis constant for glucose-6-phosphate (G-6-P).<sup>60</sup> These characteristics may partly explain the enhanced susceptibility to favism in the Mediterranean type of G-6-PD deficiency. Even then, the reported prevalence of favism shows no relation to that of G-6-PD deficiency in these same countries. For example, in certain agricultural areas of Greece where fava bean is cultivated and consumed, about 20% of the population may be G-6-PD deficient, but cases of favism are comparatively rare in spite of frequent opportunities of exposure to the bean.<sup>61</sup>

The search for toxic factors has thus far yielded information on some active principles in fava bean that may be responsible for hemolysis. Crude or semipurified extracts of fresh young fava beans, on incubation with G-6-PD-deficient erythrocytes and glucose, were found to cause a significant decrease in G-S-H as compared to that in normal erythrocytes.<sup>62,63</sup> Extracts of the pollen or pistils of fava flowers had the same effect. This discriminatory effect of fava bean extracts on erythrocyte G-S-H has been utilized to isolate and identify the compounds in fava bean responsible for favism. Certain similarities between the action of primaquine and fava juice or extract on erythrocytes in G-6-PD deficiency have been demonstrated.<sup>64</sup> They both cause a fall in G-S-H, an increase in osmotic fragility, and a decrease of *in vivo* survival of treated red blood cells from G-6-PD-deficient individuals on transfusion. However, unlike primaquine, fava juice did not cause a loss of potassium from erythrocytes of susceptible subjects.

In the meantime, Lin and Ling claimed that vicine, a nucleoside occurring in fava bean, was responsible for hemolysis and hence for favism.<sup>65,66</sup> They found that vicine had a growth-retarding effect in rats and caused a mild hemoglobinuria when administered to dogs by stomach tube at a dosage level of 0.2 g/kg. Furthermore, vicine inhibited the activity of G-6-PD *in vitro*. Vicine was isolated in 1870 from *Vicia sativa* seeds, and another related compound, convicine, was later isolated from the same source. Vicine and convicine were also found to occur in *Vicia faba*. Vicine is 2,6-diamino-4,5-dihydropyrimidine-5- $\beta$ -D-glucopyranoside,<sup>67</sup> and convicine has been assigned the formula of 2,4,5-trihydroxy-6-aminopyrimidine-5- $\beta$ -D-glucopyranoside.<sup>68</sup> Both of these glucosides are hydrolyzed by emulsin, a  $\beta$ -glucosidase. Mild acid hydrolysis of the glucosides yields the aglycones divicine and isouramil, respectively. It is possible that the aglycones are released in the GI tract by the action of  $\beta$ -glucosidase prior to their absorption.

Both the aglycones, divicine and isouramil, caused a decrease in G-S-H when incubated with human erythrocytes suspended in phosphate buf-



fer saline at pH 7.4.<sup>69</sup> The presence of glucose in the incubating medium abolished this effect on erythrocytes from normal subjects but not on those from G-6-PD-deficient individuals. These effects were about 20–30 times greater than those of acetylphenylhydrazine (APH) and ascorbic acid. There was no stoichiometric relationship between the amounts of aglycone used and the extent to which G-S-H was decreased. This suggested a catalytic mode of action. Furthermore, whereas APH required the presence of hemoglobin to cause a decrease in G-S-H, the aglycones exert this effect on G-S-H in the absence of hemoglobin. The activity of the aglycones is attributed to the presence of a carbonyl conjugated amino-enol system analogous to the endiol in ascorbic acid. The glucosidic linkage at position 5 in the pyrimidine ring disturbs this system and renders the parent glucosides completely inactive.<sup>46</sup>

The presence of another active compound in fava beans has been demonstrated. It is 3,4-dihydroxy-L-phenylalanine or L-DOPA.<sup>70</sup> (See Chapter 7.) It also has the capacity to cause a fall in G-S-H in erythrocytes obtained from G-6-PD-deficient subjects. L-DOPA occurs in fava bean in the free state and also as a glucoside having the formula  $\beta$ -[3-( $\beta$ -D-glucopyranosyloxy)-4-hydroxyphenyl]-L-alanine. Both compounds are found in the dormant and germinating cotyledons of fava bean.<sup>71</sup> They also occur in garden peas (*Pisum sativum*). However, consumption of peas is rarely associated with the acute hemolytic attacks that occur in favism. Later investigations showed that L-DOPA alone had no effect on G-S-H levels in erythrocytes from G-6-PD-deficient subjects, whereas dopaquinone, formed by the action of tyrosinase on DOPA, did cause a lowering of G-S-H. A synergistic action between divicine and isouramil on the one hand, and L-DOPA on the other, in causing a rapid decrease in G-S-H in G-6-PD-deficient erythrocytes has been described.<sup>72</sup> The suggestion that the active hemolytic principle in fava bean is dopaquinone, produced from the L-DOPA contained in the bean, has to be viewed in this context.<sup>73</sup>

Thus, the presence of more than one active compound responsible for the rapid fall in G-S-H in G-6-PD-deficient erythrocytes has been demonstrated in fava beans. There is a strong probability that they are responsible for the hemolysis that is characteristic of favism, although the ac-

tual mechanism of hemolysis has not been elucidated. Additional aspects of favism remain to be clarified. First, that only some of the known G-6-PD-deficient subjects are susceptible to favism needs to be explained. If the active compounds in the bean are responsible for hemolysis, their occurrence in fava pollen remains to be demonstrated, and attacks of favism on inhalation of fava pollen need to be authenticated, for these have been attributed to "cryptoconsumption" of raw fava bean while walking through the fields of ripening fava. Finally, some correlation has to be established between the amounts of fava bean consumed, the form in which it is consumed, and the severity of favism in susceptible individuals. It is possible that, apart from genetic factors in man and the concentration of the active compounds in the bean, other variables (such as the effect of cooking, digestion, absorption and metabolic disposal) may determine the susceptibility to and severity of the toxic effects of fava bean.

#### TOXIC HONEYS\*

##### Introduction

Honey is a plant product that is gathered, modified, concentrated, and stored by honeybees for their use. Considering the variety of plants that have toxic components, it should not be surprising that materials toxic to humans may occasionally be found in honey. Such occurrences are relatively rare for several reasons: The bees themselves may be poisoned before storing the honey<sup>74</sup>; and the beekeeping industry sees that honey from known toxic flora in their area does not reach the market.

Toxic honeys have been known since antiquity, the oldest record being the description by Xenophon of the mass poisoning of the expedition of Cyrus in 401 BC (the retreat of the ten thousand) near Trebizonde in Asia Minor. It is presumed that the honey was produced from the nectar of *Rhododendron ponticum*; many more occurrences have been recorded in this area of the world. Nearly 2,300 yr later, Plugge isolated a compound he called andromedotoxin from Trebizonde honey.<sup>75</sup> Honey from Ericaceae (*Rhododendron*, *Azalea*, *Andromeda* and *Kalmia* spp.) is implicated in the greatest number of reports.<sup>75,78,79,85,90,96</sup> The beekeeping literature contains detailed descriptions of the experiences of those consuming honey from various members of Ericaceae. A physician described intoxication by mountain laurel honey (*K. latifolia*) as follows<sup>87</sup>:

\* Literature reviewed through October 1971.

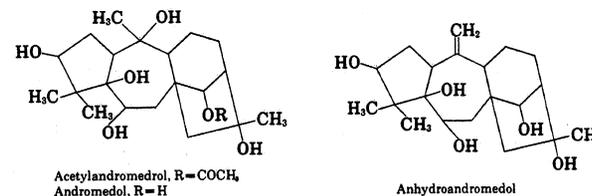
Symptoms of poisoning—These have varied in severity from a mere tingling in the skin to almost death. No two persons reacted exactly alike, yet they did enough so as to be recognized from a common causative agent. Shortly after eating, within a few minutes to two hours, the person felt a tingling and numbness in the extremities and lost consciousness, sometimes but momentarily and others for several hours. The pulse weakened to imperceptibility and went down to 50 or even 30. The face turned ghastly blue so characteristic of a heart attack but without anginal pain. A cold sweat appeared. These symptoms lasted from a few minutes to four or five hours. Usually there was no nausea or other gastro-intestinal symptoms. Recovery was complete within from a half to 12 hours, usually 6 to 8, except for a numbness or a tingling in the skin of the extremities.

A differing description is found in the review by Howes,<sup>85</sup> who reports a Civil War surgeon's description of the effects on soldiers of presumably the same honey:

It has a highly poisonous effect, being an extremely distressing narcotic, varying in its effects in proportions to the quantity eaten. . . . Some time after eating a queerish sensation of tingling all over, indistinct vision, caused by dilation of the pupils, with an empty, dizzy feeling about the head and a horrible nausea which would not relieve itself by vomiting. The first case or two I saw were entirely overpowered by it, and their appearance was exactly as if they were dead drunk . . . the enervation of all the voluntary muscles was completely destroyed. The usual remedies for narcotics partially restored them in a few hours, but the effects did not completely wear off for two or three days, and I was assured that fatal consequences have been known to follow a too free indulgence.

This resembles more closely the classic description by Xenophon in that vomiting was present.

A review of the relationships among the toxic agents isolated from Ericaceae may be found in the paper of Scott *et al.*<sup>104</sup> Of the 12 closely related compounds, 4 have been isolated from honey and chemically identified. Acetylandromedol<sup>111</sup> was isolated in a 0.010% yield from an authenticated sample of honey from *K. latifolia* and identified by paper electrophoresis, mixed melting point, and infrared spectrum. Scott *et al.*<sup>104</sup> isolated three compounds from a honey of unknown source and identified them by TLC with three solvent systems and four chromogenic reagents. These, and their levels of occurrence, were andromedol (7 ppm), anhydroandromedol (3 ppm), and desacetyl pieristoxin B, which, though quantitative data were not obtained, appeared to be present in amounts greater than andromedol. Carey *et al.*<sup>75</sup> isolated acetylandromedol from nectar of *Rhododendron thomsonii* and identified it by melting point, optical rotation, and IR spectrum. Animal toxicity data were obtained by the latter two groups of workers both for the isolated materials and the original nectar or honey. The structures of acetylan-



dromedol, andromedol, and anhydroandromedol<sup>89</sup> are shown above. That of desacetyl pieristoxin B is not known.

#### Andromedotoxin and Related Substances

Moran *et al.*<sup>93</sup> have reported the most extensive study of the pharmacological actions of andromedotoxin. The material they used (melting point 258–260 °C,  $[\alpha]_D^{25} -8.4^\circ$  in ethanol) was isolated from the leaves of *R. maximum* by Wood *et al.*<sup>113</sup>; a later paper from this group<sup>108</sup> named it acetylandromedol. Moran *et al.* concluded:

The rapid intravenous injection of andromedotoxin produces bradycardia, hypotension, and respiratory depression. Atropine prevents the bradycardia, and diminishes the hypotensive component. Vagotomy abolishes the entire response. Three manometer technique studies demonstrate a reflex vasodilation, as well as bradycardia, as contributing to the hypotensive effect. The minimal effective dose in eliciting this reflex is two to three microgm./kgm.

A distinct hypotensive action in vagotomized animals occurs in a dose range of 2 to 20 microgm./kgm., the magnitude of the depressor effect being proportional to the dose. Associated with this response is a blockade of the carotid sinus pressor reflex and the production of postural hypotension. The absence of a peripheral autonomic blocking action, the failure to obtain the response in the spinal cat, and the absence of direct vasodilatation on intra-arterial administration demonstrate a lack of peripheral action. Studies with the three manometer technique indicate a nerve mediated vasodilatation. It is believed that the action is a direct depression of the vasomotor center or a stimulation of receptors in the head and neck with a reflex vasodilatation.

Electrocardiographic changes such as ventricular extrasystoles, A-V nodal and ventricular tachycardia, conduction impairment, and ventricular fibrillation occur with doses of 35 microgm./kgm. and greater.

Andromedotoxin in doses of 40 microgm./kgm. and greater causes a rise in blood pressure due largely to the release of epinephrine from the adrenal medulla, as shown by the absence of the greater part of the effect after adrenergic blocking doses of phentolamine and after adrenalectomy. Because of a transient venous pressure lowering effect, the possibility exists of a positive inotropic action on the heart as a contributory factor in this pressor effect.

A veratrine-like action on the isolated frog sartorius muscle appears in concentrations of 1:250,000, along with a progressive decrease in the twitch height. Unlike veratridine there is no significant initial increase in twitch height, only a delayed relaxation. Andromedotoxin also antagonizes the action of veratridine.

Emesis occurs in unanesthetized dogs upon parenteral administration of andromedotoxin in doses of 7 microgm./kgm. and greater.

Respiratory stimulation or depression occur in vagotomized dogs with doses of 20 microgm./kgm. and greater. The mechanisms of these actions are unknown.

Stimulation of the central nervous system followed by depression occurs with high doses.

The actions of andromedotoxin are of short duration, lasting less than one hour even with toxic doses.

The close similarity in actions of andromedotoxin to the veratrum alkaloids has been discussed.

Nothing is known of the metabolic fate of andromedotoxin.<sup>93</sup> Hardikar<sup>80</sup> has reported that at least one third of the poison is eliminated in toxic form in the urine after subcutaneous administration to animals.

Investigating a British Columbia honey that had caused nausea, blurred vision, shortness of breath, and mild paralysis of hands and arms, Scott *et al.*<sup>104</sup> isolated the three compounds noted above. Aqueous solutions of the isolated materials as well as crystalline standards were injected intraperitoneally into male albino mice (21–44 g). The LD<sub>50</sub> values for the standard toxins were: acetylandromedol (grayanotoxin I), 1.28 (1.11–1.49) mg/kg (95% confidence levels); andromedol (grayantoxin III), 0.908 (0.805–1.03) ng/kg; anhydroandromedol, not toxic at doses up to 4 mg/kg. Clinical symptoms were dyspnea, periodic clonic convulsions, lordosis, paralysis, exophthalmos, and sedation, with death apparently due to respiratory failure; ventricular fibrillation was noted in animals autopsied shortly after cessation of respiration. The toxic honey extract had an LD<sub>50</sub> value equivalent to 34 g honey/kg (17.5–65 g/kg) with similar symptoms.

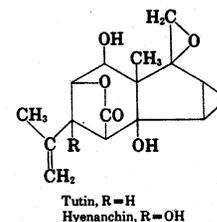
Carey *et al.*<sup>75</sup> examined the toxicity to honeybees and mice and the pharmacology of nectars from 17 *Rhododendron* species and 14 hybrids. Andromedotoxin was isolated from *R. thomsonii* nectar (which itself had an approximate LD<sub>50</sub> for mice of 10 ml/kg). Toxic nectars when injected into cats (0.1–0.5 ml/kg) caused depression of respiration, associated with contractions of the diaphragm, bradycardia, and either a sharp, short-lived fall in blood pressure (often followed by a smaller rise), or a sustained hypertensive effect; similar effects were obtained by injection of 10–40 µg/kg of andromedotoxin. Bradycardia and hypotension were abolished by 1 mg/kg of atropine sulfate; the pressor response by 2 mg/kg of phentolamine. These results are similar to those reported by Moran *et al.*<sup>93</sup> and Hardikar.<sup>80</sup>

Ancient and modern literature reports of human poisoning from Ericaceae honey appear to refer most frequently to areas in Asia Minor, U.S.S.R., eastern and Pacific Northwest United States, and Japan. In areas where production of this honey is a recurring problem, biological tests have been proposed for its detection. Pulewka<sup>102</sup> suggested that the andromedotoxin content of honey could be approximated from its action on the respiration of white mice and guinea pigs; a cramp-like contraction of the diaphragm and glottis is observed similar to the action of aconitine, which is used as a standard. Popova *et al.*<sup>101</sup> proposed the use of pollen analysis and biological testing for this purpose.

Sporadic instances of nonfatal human poisoning by honey have occurred in the Bay of Plenty area of New Zealand for about 50 yr. The offending toxic material was traced to honeydew collected by bees from the leaves of *Coriaria arborea* (tree tutu), a plant known to contain a toxic substance, tutin. The honeydew was produced by the passion-vine hopper, *Scolypopa australis*; the flowers do not secrete nectar and the pollen is not toxic.<sup>81,96,97,99,106,107</sup>

#### Tutin and Related Substances

Sutherland and Palmer-Jones<sup>106</sup> isolated from the honey a crystalline material, different from tutin, which they named mellitoxin, noting that it did not account for all of the toxicity. No mellitoxin could be isolated from the plant,<sup>107</sup> but Palmer-Jones and White<sup>98</sup> demonstrated that tutin was converted to mellitoxin by passage through the passion-vine hopper. Structure of the latter was elucidated<sup>84</sup> and shown to be identical with hyenanchin, a hydroxy tutin.



Since that time, 1,000 mi<sup>2</sup> of the area have been closed to beekeeping, and samples from that and other areas are periodically assayed for the toxic properties. Assay until 1955 was by oral dosing of guinea pigs with honey extracts. Since then, more sensitive tests, such as intracerebral injection of the mouse<sup>76,92</sup> and a TLC procedure<sup>83</sup> have been used.

These were compared by Turner and Clinch<sup>110</sup> and found to agree under certain interpretative restrictions. Hodges and White<sup>83</sup> isolated both hyenanchin and tutin from the toxic honey and described a TLC procedure for their detection in honey at levels of 40 and 10  $\mu\text{g}$  in 5 g. A honey known to be toxic was estimated to contain 100  $\mu\text{g}$  tutin and 800  $\mu\text{g}$  hyenanchin in 5 g. Clinch and Turner<sup>77</sup> discussed sample testing between 1962 and 1967 by guinea pigs, mice, and TLC methods. In general, tutin, which is nine times more toxic to mice, is present in much smaller amounts than is hyenanchin. Data are generally reported as the tutin equivalent; about 0.10 mg tutin equivalent per 100 g honey is detectable by the mouse test. Approximate quantities in samples are estimated by determining the LD<sub>50</sub> of toxic honeys, taking the LD<sub>50</sub> for tutin to be 0.01 mg/kg. Results for 40 samples ranged from <0.1 to 6.7 mg/100 g tutin equivalent. A honey responsible for human poisoning assayed at 4.0 mg/100 g, and on the basis that a 1-mg dose of tutin caused human nausea, vomiting, and incapacity for work for 24 h, this amount would be contained in 25 g of such honey.

Intoxication of the human by the tutin plant causes vomiting, giddiness, delirium, great excitement, stupor, coma, and convulsions, and in several cases loss of memory according to Fitchett (in Palmer-Jones<sup>97</sup>). Physicians' descriptions of symptoms in early cases of honey poisoning in New Zealand stated that the poison was deliriant; patients became extremely violent, suffering from giddiness, pains in the abdomen and head, and vomiting, followed by rigidity of the limbs and convulsions, and often coma and loss of memory. No fatalities were seen.<sup>96</sup> Honey that was toxic to man was found on oral ingestion not to affect pigs, sheep, rabbits, mice, rats, or bees. Guinea pigs were quite susceptible, though not as susceptible as are humans, who may be severely affected by a half to one teaspoonful of the toxic honey.<sup>96</sup> The toxicology of tutin, mellitoxin (hyenanchin), and picrotoxin was examined by Palmer-Jones.<sup>97</sup> The honey toxins are closely related to picrotoxin. The toxic dosages were reported as shown in Table 2.

TABLE 2 LD<sub>50</sub> (mg/kg) for Tutin, Hyenanchin, and Picrotoxin

Toxicant	Stomach Tube		Subcutaneous		Intraperitoneal	
	Guinea Pigs	Rats	Guinea Pigs	Rats	Guinea Pigs	Rats
Tutin	1.2 <sup>a</sup>	~20	0.75 <sup>a</sup>	~4	0.7	~5
Hyenanchin	12	~40-90	9	~30	9	~30
Picrotoxin	27	15.5	3	4.5	5.5	~3.5

<sup>a</sup> LD<sub>75</sub>.

Palmer-Jones also examined the antagonism of tutin, hyenanchin, and picrotoxin by barbiturates in guinea pigs. The most satisfactory method of treatment for poisoning by the three toxins was to give the maximum IP dose of pentobarbital sodium (10 mg for guinea pigs) as soon as convulsions commenced, and repeat dosing with 5- or 10-mg amounts when necessary. Two IP injections, each 10 mg of pentobarbital sodium, were sufficient to cause rapid recovery in guinea pigs given lethal doses of the toxic honey by stomach tube.

#### Other Honey Toxins

Hazslinsky<sup>82</sup> described a toxic effect of a Hungarian honey, which he ascribed to belladonna alkaloids from the nightshade, but Örsi-Pál<sup>94</sup> claimed that the source was Egyptian henbane (*Datura metel*) and that the poisoning was by scopolamine, not atropine. Lehrner<sup>91</sup> and Svider-skaya<sup>105</sup> have described honey poisoning ascribed to atropine; in the latter article the source was said to be *Datura stramonium* and *Hyoscyamus niger*.

Several records of honey poisoning in the United States in the nineteenth century are described by Kebler,<sup>90</sup> including an occurrence at Branchville, S.C., where 3 children died of 20 persons affected. Wiley<sup>112</sup> stated that the honey contained gelsemine, and Howes<sup>85</sup> ascribes the honey origin as the yellow jasmine (false jasmine, jessamine, *Gelsemium sempervirens*), a well-known poisonous climbing vine common to southern United States and Mexico, with yellow fragrant flowers in February and March. A report in Pellett's book<sup>100</sup> describes fatal honey-bee poisoning ascribed to this plant. Howes<sup>85</sup> quotes the following course of a case of the Branchville poisoning, presumably from this source: "A boy eleven years old was the first of the family to eat some of the honey. In an hour afterwards the child became giddy and staggered as he walked and could not see. He was affected with general lassitude and slight nausea. In two hours he was seized with convulsions and died." An account of the symptoms of poisoning by *Gelsemium* is given by Osol and Farrar<sup>95</sup> as "dizziness, dimness of vision, dilated pupil, general muscular debility, and unusual prostration, reducing the frequency and force of the pulse and the frequency of respiration." Further details of the course of fatal poisoning are given. Toxicology of the *Gelsemium* alkaloids is also reviewed there but will not be included here, since only one instance of presumed poisoning by honey from *Gelsemium* is recorded.

Juritz<sup>88</sup> discussed so-called Noors honey from several species of South African *Euphorbia*, which produces a strong, burning sensation in the

throat and which may have caused poisoning resembling that from belladonna. Sanna<sup>103</sup> reported that a Sardinian honey with a sour, bitter taste was found to contain the glucoside arbutin, derived from *Arbutus unedo* L.

Bitter honeys are not uncommon; Joachim and Kandiah<sup>86</sup> attributed bitterness in a Ceylon honey to alkaloids from pollen of the Ceara rubber plant. Very little chemical work has been done with bitter honeys; likewise, little information is available on the materials responsible for the strong, sometimes nauseating odors and flavors of certain honeys, such as that from *Melaleuca*,<sup>109</sup> *Agave*,<sup>100</sup> and privet.

Even though some 250 million lb of honey are harvested and consumed annually in the United States, the odds are negligibly small of anyone encountering a toxic honey in commercial channels. Beekeepers are well aware of the availability in their areas of sources of toxic honey and take appropriate action to keep any such material out of the markets.

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