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Changing sugar distribution for increased soybean yields

By John Thorne

Few plants produce seeds of sufficient quantity and nutritional quality to make them important food crops. Those that do arose from man's knowing or unknowing repeated selection, among almost similar plants, for those with the largest edible portion at harvest. The resulting domesticated crops grow faster and produce more seed than the wild types from which they developed. However, despite the best efforts of plant breeders in recent years, there is considerable room for improvement in the production of seeds by these plants.

The general approach, using fertilizer, pest control, and other conventional methods, is to change the way the crop is grown—become better farmers. But even the world's best farmer is limited by the plant itself. Thus, to increase yields more, we must change the plant and how it produces seed. However, we must first understand the processes that limit seed production.

One way is to increase photosynthesis in the leaves and thus increase the supply of the sugars that are available to make seeds. Several scientists at the Station are investigating genetic and chemical alteration of photosynthesis. Another way, which I am trying, is to direct more of the available sugar into seed growth. Growing conditions and other factors often cause crops to commit excessive amounts of sugars to the leaves, stems, and roots at the expense of seeds. My approach involves changing the distribution and metabolism of these sugars so that seed production can be increased. To understand my approach, it is necessary to understand how sugars are transported from the leaves where photosynthesis produces them to the seeds where we want them.

The sugar-transporting system (phloem) must be alive to function. This is unlike the water-transporting system (xylem) in plants, which is functional only as dead, hollow tubes. Leaf phloem cells, although they are alive, are nearly hollow. Adjacent companion cells are necessary to initiate and maintain sugar transport. The companion cells and nearby phloem cells form a functioning phloem complex. A long series of phloem cells joined end to end form sieve tubes. The companion cells load sugars into sieve tubes prior to transport out of the leaf.

The loading of sugar in the leaf phloem is an active process that requires energy. It also is sensitive to temperature and oxygen, and is specific for certain sugars. The loading concentrates sugars within the companion cells. Once the sugars have been loaded, water enters the phloem from surrounding areas by osmosis to create the pressure that forces the sugar solution out of the

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leaf and down the stem through the connecting phloem elements of the sieve tubes.

Less is known about unloading. When the sugar solution reaches an area of utilization, such as a young leaf, growing root, or developing fruit, sugar and water are unloaded from the phloem. This produces a region of sugar concentration and pressure that is lower than that in the leaf. It is this "downhill" pressure gradient that
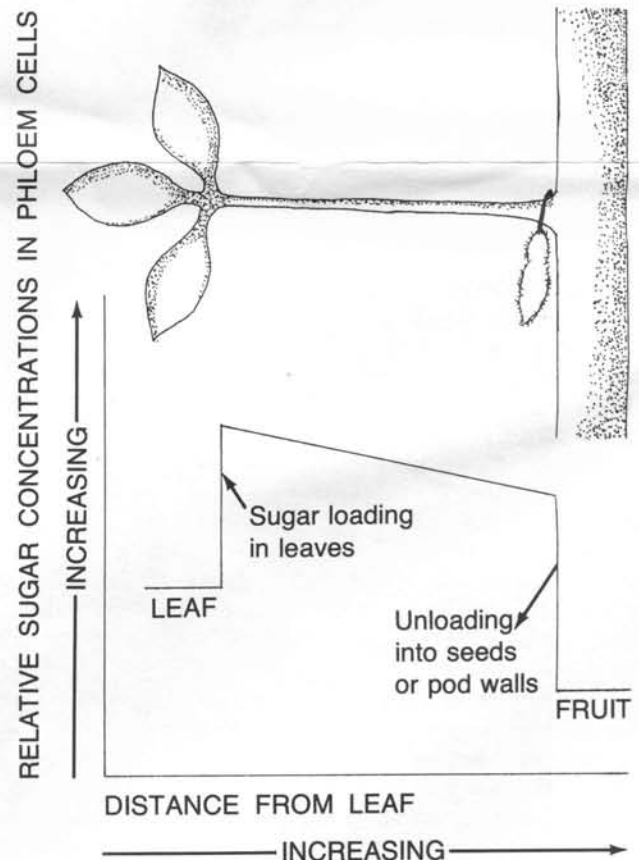

Figure 1. Sugar concentration gradient created by loading of the phloem in the leaf and unloading of sugars from the pholem in the seeds or pod walls.

moves sugar from the leaf. I am learning how this pressure gradient controls sugar distribution within the soybean plant.

My first experiments showed that sugars moved out of a leaf faster if increased photosynthesis produced higher sugar concentrations in the leaves. On the other hand, increased use of sugar by an organ (i.e. seeds) also stimulated greater transport. This suggested that the size and activity (competitiveness) of sugar-using organs might determine the share of the available sugar they get. In other words, the more efficient such an organ is in depleting the sugar concentration in nearby phloem, the steeper the concentration and pressure gradient and the greater the transport of additional sugar from the leaf. This was the basis for my next experiments.

Field experiments with an early and late soybean variety have helped me learn how seeds and pods compete with other plant parts for sugars. During one growing season, I measured growth of various plant parts and related development to sugar concentrations in these parts. I selected Norman because, in Connecticut, it matures and dies weeks before the first frost kills Amsoy-71, a full-season variety that I also studied. Although the early variety's potential for seed production in Connecticut is considerably less than that of the full-season variety, the way the early variety grows does much to maximize the production of seeds during its brief growing season. It germinates quickly and produces its leaves fast, setting the stage for earlier flowering and pod development than in the full-season variety.

An unusual characteristic of the early variety is that its leaves begin to die before the seeds are fully grown. This is surprising because phloem transport of the sugars necessary for seed growth requires that the leaf end of the system have a higher concentration than the seed end. Thus, it appears that seed growth should stop when leaf photosynthesis stops. However, as shown in Figure 2, the seeds of the early variety continue to grow steadily for almost as long as the full-season variety. These results indicate that not all of the sugars used in the early variety's seed growth are coming directly from the leaves.

Chemical analysis showed that pod walls of the early variety, and those of the full-season variety to a lesser extent, temporarily store leaf sugar in the form of starch and different sugars and export it later as the seeds develop. The overall effect of this storage is that the sugar concentration of the phloem leading into the fruit (pods and enclosed seeds) is probably lowered. This allows the fruit to compete more effectively with the rest of the plant for leaf sugar. In the early variety, this occurs even during early seed development when the small seeds require only minimal amounts of sugar and thus cannot compete well. These storage sugars probably end up in the seeds, and may serve as an important source of sugar late in the season when the leaves yellow and die. In fact, they may account for up to 13% of

the seed yield in the early variety, but less than 2% of the seed yield of the full-season variety. This alternate sugar supply may also allow the early variety to maintain a constant rate of growth on cold or cloudy days that prevent normal leaf photosynthesis.

Regardless of whether the sugars used by the developing seeds come directly from the leaves, as in the full-season variety, or from temporary storage, as in the early variety, the seeds grow at about the same rate. This indicates that the seed growth rate is perhaps limited by processes within the seed. I am experimenting with ways to increase the number, rate of growth, and final size of soybean seeds. Plant breeders may be able to apply my findings in their attempts to develop higher yielding plants.

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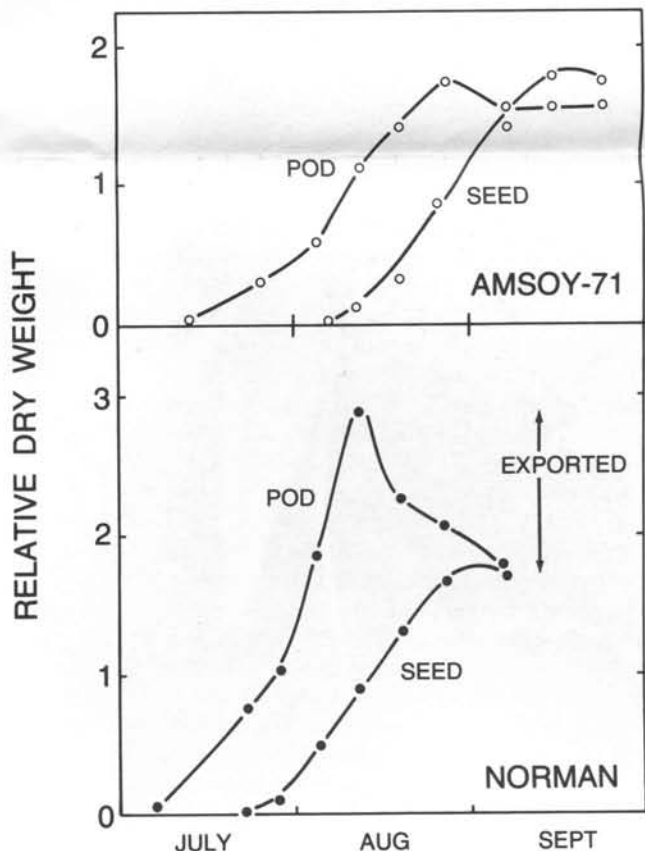
Figure 2. Seasonal patterns of growth and dry weight accumulation in soybean pods and seeds. Sugars make up a large percentage of the materials exported from the pod walls.

Egg laying of tree cricket leads to canker on red maple

By Gordon S. Taylor and Robert E. B. Moore

Each fall, the flaming red and yellow colors of eastern red maples (*Acer rubrum* L.) tell that winter is coming. Many homeowners would like to see these colors in their yards, but the small leaves and many seeds of the native red maple make the species unpopular with nurserymen who prefer to grow sugar and Norway maples and to use them for ornamental plantings.

However, several selections of *Acer rubrum* have been developed that overcome the objections of the nurserymen. Such names as: "Red Sunset", "October Glory", and "Autumn Flame" suggest their brilliant, long-lasting color. These trees, which are more compact than the native varieties, are propagated in western

Figure 1. The narrow-winged tree cricket, *Oecanthus angustipennis Fitch*, on a small red maple branch.

The first sign of the disease was a running, wet spot on the bark.

nurseries by grafting them to roots of wild maple. They are then shipped to Connecticut to be grown to market size.

In May 1977, hundreds of grafted red maples growing in a Hartford County nursery were killed by cankers that girdle the bark. The first sign of the disease was a running, wet spot on the bark. The tissue beneath was soft and leaked a liquid when pressed. Even trees that were not girdled had many cankers on the bark. The Experiment Station was asked to find the cause of the disease.

The first step in our detective work eliminated two innocent fungi. We knew of a maple disease called "bleeding canker" that is caused by a fungus of the genus *Phytophthora*. Usually this disease occurs near the base of trees and causes a lesion with no definite shape. Isolations from the diseased trees did not yield *Phytophthora*. Further, the cankers we found were scattered well up the trunk and onto lower branches and had a definite oval or lens shape. Therefore, we were convinced that the disease in the Connecticut nursery was not bleeding canker.

Another disease called "annual canker", caused by a fungus of the genus *Fusarium*, has cankers that have a lens shape and that occur all along the trunk. Although the first report noted briefly that a similar canker was found on red maples, the species reported most affected was sugar maple. We did not find cankers on sugar maples growing adjacent to diseased red maples, nor could we isolate *Fusarium* from the diseased tissue. Instead, we consistently found a fungus called *Cryptosporiopsis*. We found also that branches of red maple, but not sugar maple, became diseased when inoculated in the laboratory with the *Cryptosporiopsis* fungus that we had isolated from the Connecticut cankers. We concluded that the disease of our maples was not "annual canker", but was a new disease of red maples.

Although individual trees in a clump of wild red maples had multiple cankers, they were rarely girdled as were the trees in the nursery. Some wild trees had no cankers, which suggested that red maples varied in susceptibility to the disease. Thus, it was possible that the named clones of nursery trees selected for color and form were unwittingly selected for susceptibility to this disease as well.
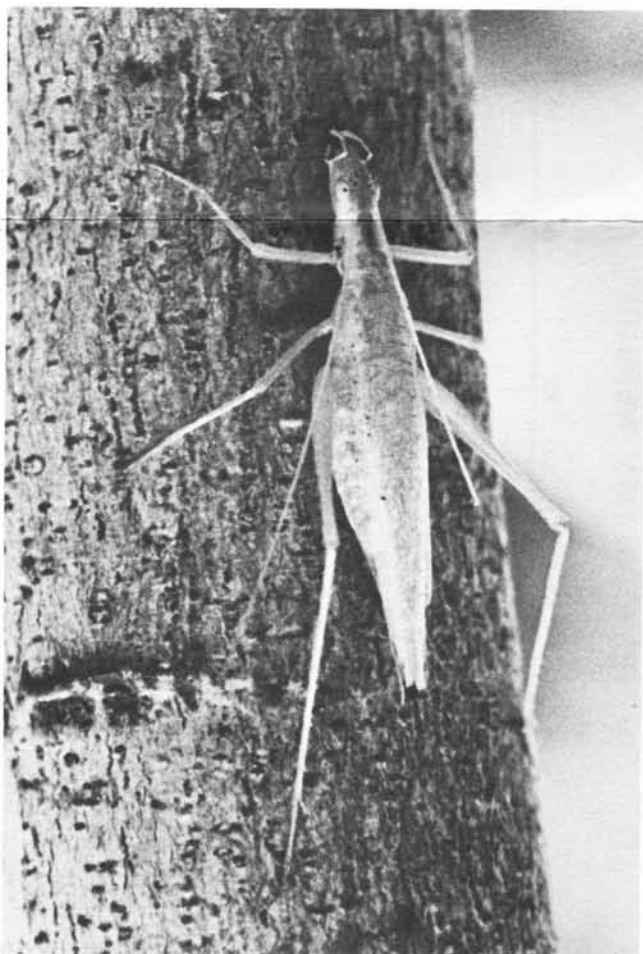
The break came in late August, 1977. While examining trees, we noted many tiny holes in the bark about 1 mm in diameter; some were oozing what appeared to be sap. When we looked into these holes we found two yellowish, sausage-shaped eggs arranged in a "V" parallel to the cambium half in the bark and half in the new wood. Since the eggs were like those of a tree cricket, we looked for adult tree crickets. We found some by shaking trees. The female tree crickets we collected laid eggs on red maple branches in the laboratory exactly as we had found on the nursery trees. Markings on the antennae identified the insect as the narrow-winged tree cricket (*Oecanthus angustipennis* Fitch). This species is common in Connecticut and the Eastern United States. On hot summer nights it trills a background to the katydid chorus.

We then found wounds made by this tree cricket as it laid eggs in red maples throughout the affected nursery and in wild red maples nearby. In most cases, if eggs were deposited the previous year, a tiny bump had formed. If we cut the area open, we could find the remains of the hatched egg. The tree cricket nymphs had emerged through the hole made during egg laying.

Many wounds made by the cricket did not cause a canker, but 90% of the cankers we observed were associated with an egg laying wound. This suggests two possible controls: eliminate the tree cricket or control the fungus. A third control would come from finding trees that resisted canker formation.

Tree crickets and cankers are not a new combination. In 1914, a bulletin of the New York Agricultural Experiment Station reported a canker on old apple trees that developed around holes made by the narrow-winged tree cricket. In 1978, we found this type of canker on unsprayed apple trees in Connecticut. The fungus causing the canker was not the *Cryptosporiopsis* we found on maples. The absence of such cankers in sprayed orchards suggests that control of the tree cricket would solve the nurserymen's problem with red maples.

We still need to learn if the fungus is carried by the insect when eggs are laid or if the fungus merely enters the wound later. In depositing her eggs, the female tree cricket chews the bark surface and then drills a hole with her two-sided ovipositor, which works like an electric knife. This hole is about 1 mm in diameter. Generally, two eggs are deposited in the hole, usually at the cambium layer. After the eggs are laid, the female plugs the hole with chewed bark. Thus, any *Cryptosporiopsis* spores or mycelium on the bark could be inoculated into the freshly made wound.

In a preliminary experiment no cankers developed the following spring from inoculations made in September by placing agar cultures of *Cryptosporiopsis* into holes made by tree crickets. However, typical cankers did develop the following spring when agar cultures of *Cryptosporiopsis* were placed immediately into 1 mm holes we had drilled.

We suspect that no large-scale loss of wild red maples will occur although we found the disease throughout
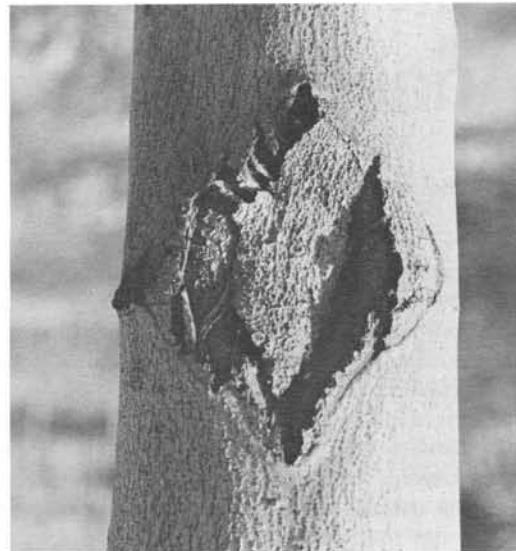

Figure 2. A canker on a red maple. The wound caused by the egg laying is in the middle.

Connecticut. Cankers tend to heal in one or two seasons, leaving a deformed area. Nurserymen may reduce their losses through pest control. Eventually, beautiful selections of red maple may be found that resist cankers or are unpalatable to tree crickets.

Figure 3. Damaged trees growing at a Hartford area nursery. Note the damaged bark on the tree in the foreground.

Salt-free? low in fat? sugar-free? Analysts check claims on labels

By Paul Gough

During 1977, Experiment Station chemists examined 4,413 samples of food products—ranging from apples to yogurt—to ensure that the foods were not adulterated and that label claims were met. The claims range from simple weight declarations to "all natural". The analyses were carried out for the Department of Consumer Protection, which enforces the laws and regulations. June Barzilauskas and I went through these reports to see what kinds of results the analysts got during their tests.

If no special claim is made, hamburger cannot contain more than 30% fat. There were 372 such samples tested during 1977, and 12 were found to exceed the 30% limit set by law. The amount of fat in these samples ranged from 8.5% to 47.9%. However, the analysts found 29.6% fat in one labeled "lean" and 30.1% fat in another labeled "extra lean".

Some stores have begun promising less fat in their hamburger than the 30% maximum permissible under the law. In these cases, the fat content of the hamburger must meet the claim. However, the labels sometimes promise "70%" lean", when anything less would be illegal. One sample even was claimed to have "not *less* than 28 percent fat"; the claim was not met because the sample contained less than 28% fat. Of 13 samples of hamburger that made "not less than . . . lean" claims, two failed to meet the ambiguous claims.

In the cases of specific claims to lower fat contents, 18 of 60 samples failed to meet the standard. In fact, several of these, as shown in Table 1, exceeded the 30% limit for regular hamburger. As shown in the table, all claims for 14% fat or less and 20% fat or less were found to be accurate in the Experiment Station's analytical laboratory. However, the deficiency rate for samples claimed to have 23% or less or 22% or less fat was more than 50%.

Of a total of 158 samples of sausage and sausage products examined by Station analysts, 26 had excessive fat or excess or undeclared water present. Out of 38 samples of swordfish checked for mercury (the maximum permissible is 0.5% parts per million), nine samples were found to exceed the guideline, including one that had 1.2 ppm.

Several products that are claimed to be sugar- and/or salt-free were tested at the Experiment Station during 1977. Analysts found a "sugar-free" diet soda that contained 11.25% sugar—probably from the honey declared as an ingredient. In another case, they found niacin tablets sold as a "salt, sugar, starch, and color-free" dietary supplement contained 70% sugar (lactose) and a small quantity of starch as filler. The tablets contained the claimed amount of niacin.

The claims range from simple weight declarations to "all natural".

A sample of "salt-free" bread contained 61 mg salt per 100g bread, but was labeled as containing five times less. The analysts commented: "The labelling relative to the sodium content is inaccurate, therefore misleading," and pointed out that the claim that the bread is salt-free is false and misleading. A sugar substitute labeled as "Sodium-free/Lactose-free" was found to contain no lactose, but had 347 ppm of sodium.

"Natural" and "Organically-grown" claims also present difficulties. Canned peaches, listed as "Organically grown (to best of ability)," were found to contain 0.12 ppm parathion. "Organically-grown material should not contain this pesticide, although the amount is insignificant, under ordinary circumstances," stated the report of the analysts. Station analysts found sodium ascorbate and sodium nitrate (preservatives) in a sausage advertised as "all natural", and found undeclared sorbate used as a preservative in "pure, natural, unfiltered" apple juice.

The Station checks calorie claims. One hard candy labeled "low calorie" and "sugar-free" was found to be sugar-free as claimed, but had 386 calories per 100g, which was not a sufficient reduction to warrant the "low calorie" statement. A "diet" soda that was claimed to contain one calorie per 6 fluid oz. had 3.6 calories per 6 oz. In another case, a frozen yogurt product that has a name that implies that it is low in calories had 141 calories per 100 g, whereas another frozen yogurt that made no claim contained 97 calories per 100g.

Table 1. Comparison of results of tests on hamburger samples that were claimed to contain less than 30% fat.

Claimed Percent fat	Number of Samples OK	Number Deficient	Average Percent fat	Range of fat (%)
<14	3	0	13.2	11.8-14.3
<16	2	3	18.7	11.9-25.4
<18	3	1	16.6	10.8-23.0
<20	7	0	18.7	16.0-20.5
<22	2	4*	24.6	20.1-31.1
<23	16	9*	22.1	14.4-31.6
<26	7	3	25.3	23.0-28.3
<28	20	2*	23.6	15.6-32.5

* 1 of <22%, 1 of <23% and 2 of <28% had over 30% fat.

Vitamin D, rats, rickets, and fortified milk

Vitamin D is a fat-soluble vitamin that is needed for the absorption and utilization of calcium and phosphorous, which are essential for normal bone and tooth formation. A deficiency of vitamin D causes rickets, which causes deformed bones and poor teeth. Nutritional studies have shown that 400 USP units of vitamin D daily will protect growing children against rickets.

Food from animals, such as eggs, milk, and butter are the major sources of vitamin D, but these generally provide only about 125 USP units daily. Therefore, for many years, milk has been fortified with vitamin D to supply these necessary amounts of the vitamin, especially to children.

Although milk does not have to be fortified with vitamin D, the General Statutes of Connecticut state that fortified milk must contain 400 USP units of vitamin D per quart. Since 1935, the Experiment Station has been testing fortified milk to ensure that it is fortified with vitamin D as claimed.

Until recently, the only available method was a bioassay that employs young rats fed for 21 days on a vitamin D-free diet that induces rickets. The rats are then fed the samples of the milk being tested. If the milk contains vitamin D, the leg bones, when stained with silver nitrate, show healing of the rickets, and the amount of healing is proportional to the amount of vitamin D in the milk.

The bioassay was costly because of the expense of keeping the animals and the 30 days required to test 12 samples. Since the accuracy of the results depends on the judgment and experience of those examining the bones, it is done on a pass or fail basis.

Using the liquid chromatograph to measure vitamin D in milk

By Susan K. Henderson

For the past two years, Station chemists have been seeking a method for determining vitamin D in milk that would be cheaper, faster, easier, and more accurate than the old method that employed live rats.

Analysis of vitamin D in milk is a difficult analytical problem. The vitamin exists in a complex mixture and is decomposed by oxygen and light. Milk contains fatty acids, phospholipids, cholesterol, carotenoids, and the fat-soluble vitamins A, D, E, and K. In addition, milk also contains proteins, carbohydrates, and salts. To complicate the analysis further, vitamin D is surrounded by a 3-millionfold excess of fat.

We have now perfected a chemical test that requires only a few steps of sample preparation prior to a simple analysis, using a modern instrument called a liquid chromatograph.

This test is faster and less costly to run than the old method and gives us precise results. Furthermore, in this test, we can distinguish between vitamin D_2 and vitamin D_3.

An important part of our test is the treatment of the milk sample prior to analysis. Alphonse Wickroski has developed a unique treatment that eliminates interfering components from the sample and recovers the vitamin D. He first separates the bulk of the fats by reacting the milk with potassium hydroxide, which converts the fatty acids into water-soluble esters. The

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vitamin D is extracted with an organic solvent and the fats are discarded. He then puts the partially purified sample through a column of aluminum oxide, which absorbs cholesterol and carotenes. The sample is concentrated by evaporation and injected into a liquid chromatograph, which separates a sample with many components into individual components. This is accomplished by using special metal columns packed with a chemical substance that selectively causes some chemicals to be retained on the surface of the column. The extent of retention is also affected by the nature of the liquids that are put through this column.

The chemical interaction of the sample with the column surface and the chosen solvent is the basis of the separation. The solvent is pumped through the column at high pressure, normally 500-2000 psi (pounds per square inch). The high pressure causes fast separation of compounds with high resolution. The liquids passing out of the column are continuously monitored by detecting changes in absorption of ultraviolet light with a wavelength of 254 nanometers. A typical liquid

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Vitamin D in milk (*Continued*)

chromatographic analysis of a milk sample containing both vitamin D_2 and vitamin D_3 is shown in Figure 1.

To test our method we added known amounts of vitamin D_2 and D_3 to unfortified milk. After preparing the sample, we injected it into the liquid chromatograph and measured how much vitamin D was recovered. We recovered 96% of the added vitamin D. We found our precision to be 5%.

Using this new test we have analyzed nearly 100 samples of milk. The results of our tests have been reported to the Connecticut Department of Agriculture. They are responsible for regulating milk quality.

Many private companies as well as state regulatory agencies are interested in our procedure. Private companies in California, Texas, and Oregon are already trying the method. We are now working to extend this method to the analysis of other fat-soluble vitamins.

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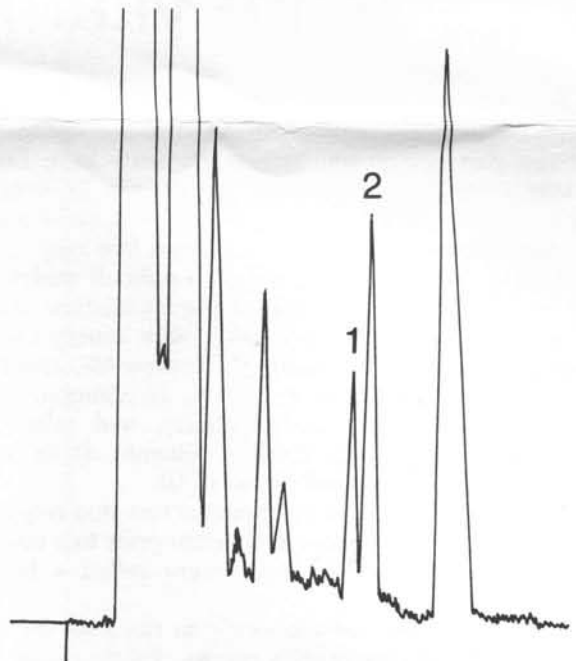
Figure 1. Peaks obtained for a sample of milk with vitamin D added. The peak marked (1) is for vitamin D_2. The peak marked (2) is for vitamin D_3.