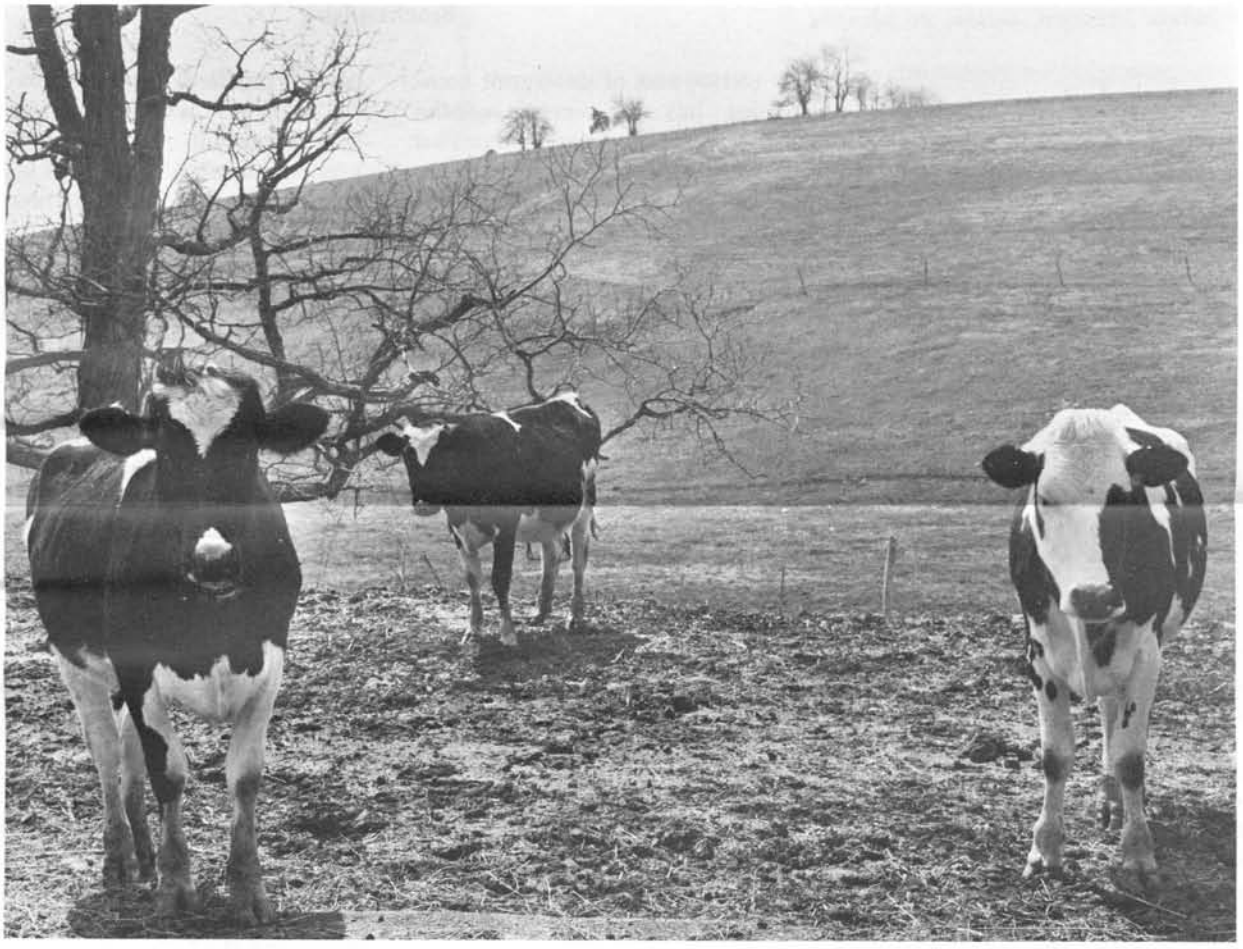
SPRING 1969

Frontiers of Plant Science



Farm nutrient budgets and water pollution . . . *page 4*

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

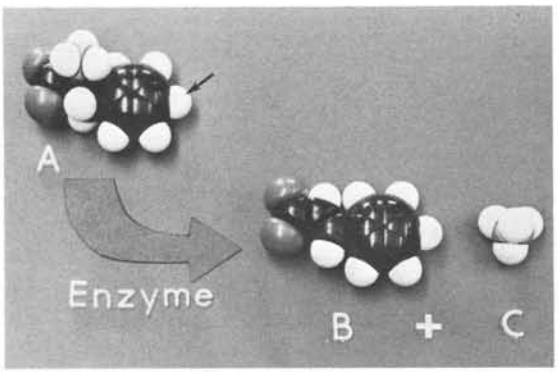


Figure 1. Molecular models. The enzyme splits a molecule of *A* into molecules *B* and *C*. Each bump on the models represents a different atom: carbon, hydrogen, oxygen, or nitrogen.

Enzymes Control Cell Chemistry

Kenneth R. Hanson

Biochemistry

Why Study Enzymes?

There is a firm scientific tradition that both the opening of new frontiers to the imagination and the discipline of such exploration are good in themselves. That which is good, however, is not necessarily appropriate. Our world is faced with food and population problems which cannot be ignored, and it is reasonable to demand a utilitarian answer to this question. In studying enzymes the biochemist is assembling information which can make practical developments possible. The rational development of drugs, fungicides, and artificial hormones depends specifically upon a detailed knowledge of all aspects of the chemistry of living cells. Experimenters searching for more productive and more disease-resistant varieties of plants find information on cell chemistry of increasing relevance to their investigations. In general, information about enzyme action is an integral part of both agriculture and medicine. Thus the study of enzymes is essential because human survival continues to depend on man's ability to manipulate his biological environment.

THE ENTERPRISE of detergent companies has in recent months made the name "enzyme" as familiar to the washing public as virus is to the sneezing public. We have, however, lived from diaperhood with a slippery succession of miracle substances. Few people perhaps are aware that this time they have added a legitimate biochemical term to their vocabulary. Enzymes are present in all tissues. They determine the chemistry of the living cell, and one of the main activities of biochemists is to isolate and define particular enzymes. This quest is a slow and laborious process. What sort of intricate molecules does the biochemist ultimately reveal?

WHAT DO ENZYMES DO?

Enzymes act in living cells as specific *catalysts*. Catalysts are substances which accelerate chemical reactions by participating in the sequence of steps that lead from reactants to products. Very few reactions take place in a living organism at a significant rate without being catalyzed by enzymes, and a given enzyme usually catalyzes only one specific reaction. Enzymes are much better at catalyzing reactions than are the compounds the chemist prepares in the laboratory—so much better that the elucidation of the details of enzyme action is one of the most challenging scientific problems facing both biochemists and chemists.

Let us consider a specific example. Dr. Evelyn Havir and I have recently purified from potato tubers an enzyme which catalyzes the transformation of the compound L-phenylalanine, which we shall call compound *A*, into two new compounds, cinnamic acid and ammonia (*B* and *C*). The transformation, of course, takes place molecule by molecule.

Three-dimensional models of molecules of *A*, *B*, and *C* are shown in Figure 1. In solution a molecule of *A* collides with the giant enzyme molecule and a new unstable molecule is formed. A series of changes rapidly takes place and finally *B* and *C* are released from the enzyme. The enzyme molecule is then available to react with a new molecule of *A* and the cycle is repeated. Solutions of *A* at room temperature are indefinitely stable if no enzyme is added.

In plant tissues the product *B* is transformed with the aid of other enzymes into a series of compounds. In many tissues the final product is lignin, a major structural material of plants; in other tissues, red, yellow, and blue flower pigments are formed; in others, compounds accumulate which are believed to protect plants against infection.

WHAT SORT OF MOLECULE IS AN ENZYME?

All enzymes are *proteins* and some enzymes have additional small nonprotein components. Protein molecules consist of a large number of related small molecules, termed *amino acids*, joined in a head-to-tail

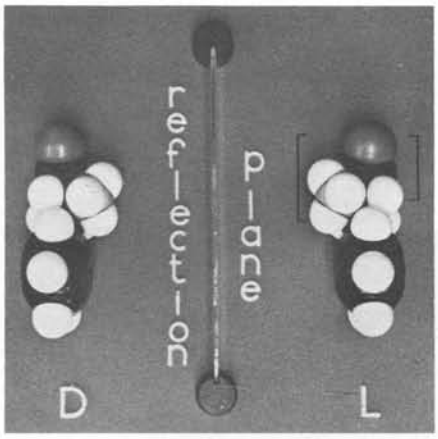


Figure 2. A molecule of *A* can exist in two forms which are mirror images of each other. Only the L-form is found in nature.

fashion to form one or more chains. There are about 20 different amino acids which can occur in these chains, and the sequence in a given chain, which may consist of 100 or more amino acids, is a unique characteristic of a particular protein.

L-Phenylalanine, besides being the compound *A* of our $A \rightarrow B + C$ enzyme, is one of the amino acids found in proteins. The molecules of phenylalanine shown in Figure 2 and conventionally distinguished by the letters L and D are related as the left hand is to the right hand, i.e. they are mirror images of each other. No amount of twisting will change the left hand into the right hand or change the handedness (or *chirality* as chemists term it) of these molecules. The chirality is associated with the bracketed portion of the molecule and all the amino acids in proteins contain the bracketed arrangement of atoms shown for the L-molecule. The amino acids differ in the unbracketed portion which is known as the side chain. For most amino acids the side chain is smaller than that of phenylalanine. In a protein the bracketed portions are joined head-to-tail so that the backbone of the flexible amino acid chain consists of 3-dimensionally-regular repeating units. Chains from different proteins thus differ in their length and in side chain sequence.

Everything else about the structure of enzyme molecules (considered as proteins) can be regarded as the solution to a 3-dimensional jigsaw puzzle. Great strength can be achieved by combining individually weak components. (The thread of a nylon stocking is easily broken, but the stocking can be employed as a rope.) Thus when an amino acid chain is folded and packed upon itself, a stable molecule can result with a unique structure of optimum stability even though the forces that keep it folded are weak. The repetition of the same chiral unit along the backbone chain permits certain sections to form right-handed helical coils. (A standard corkscrew or the thread on a wood screw is in the form of a right-handed helix.) The weak interactions between the side chains stabilize other sections and pack together the coiled regions. A few strong chemical bonds may occur between particular side chains. These give additional stability to the folded molecule, but not all proteins have such bonds.

Many enzyme molecules are now known to be formed by the association of several chemically identical protein units. Again the individual packing forces bringing about association are weak, but the structures produced have great stability. If we introduce into our 3-dimensional jigsaw puzzle the requirement that each subunit be identical and chiral, then all the geometrical possibilities for such associations may be explored. Two subunits may bind to each other to form a molecule which has the same symmetry as a 2-bladed propeller. Symmetry theory predicts that enzyme molecules composed of 4, 6, 8, 10, etc., identical subunits can exist in the form of rings and this prediction seems to be borne out in a number of cases (Figure 3).

(continued on page 7)

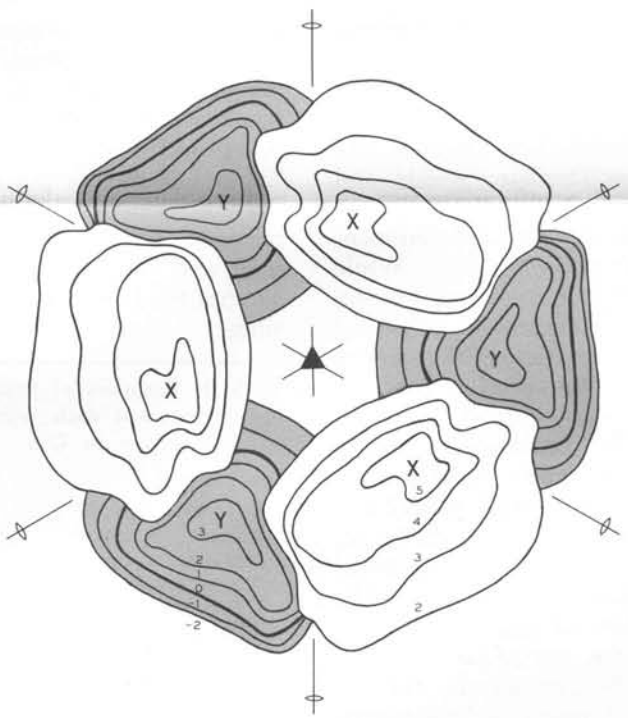
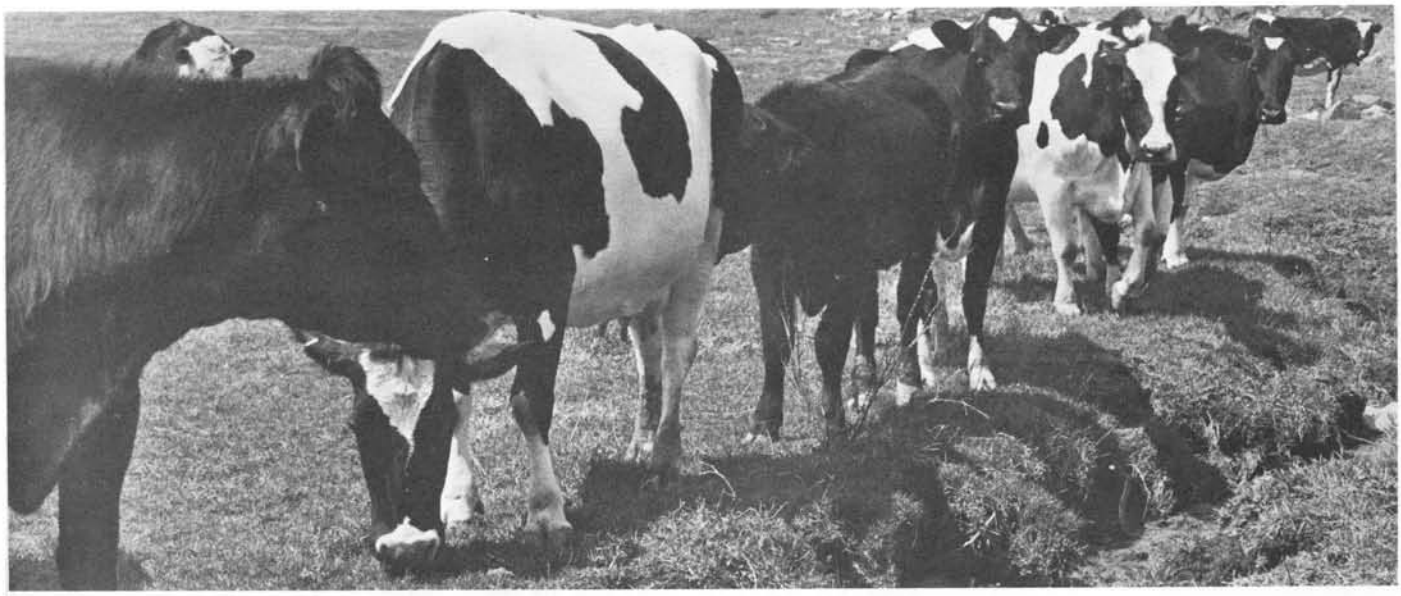


Figure 3. Contour map to illustrate the ring-association of 6 identical protein subunits. The shaded subunits marked Y are the X-subunits seen from the opposite side. The surface of a protein molecule is much more irregular than this drawing indicates. Three axes of 2-fold symmetry (shown as lines) lie in the plane defined by the zero contour. Rotation of the molecule by 180° about any of these axes leaves the appearance of the molecule unchanged. The reader may easily verify that rotation of the drawing by 120° about the point marked by a triangle (equivalent to rotation of the molecule about a 3-fold axis perpendicular to the plane of the ring) also leaves the molecule unchanged.



Farm Nutrient Budgets and Water Pollution

Charles R. Frink
Soils and Climatology

THE ACCUMULATION of nutrients in our lakes and streams is a naturally occurring process with the fancy title of eutrophication. The consequences of this nutrient enrichment are familiar: weeds and algae become increasingly abundant, the lakes slowly fill with sediment and gradually become bogs. While it appears difficult to reverse or even slow nature's inexorable march, it is extremely pertinent to inquire whether we are accelerating the procession.

Some of the sources of nitrogen which are frequently mentioned as the cause of increased eutrophication in Connecticut are shown in Table 1. These are only approximations, and moreover, they ignore the vast quantities of nitrogen already present in the environment. However, they do indicate the relative magnitude of man's various contributions to nutrient enrichment.

Looking more closely at Table 1, one sees that not all these nutrient sources can be clearly separated. For example, some of the nitrogen oxides contained in industrial fumes and automobile exhausts surely return to the earth in rainfall and fertilize lawns, vegetable gardens, and cornfields. A portion of this nitrogen then must appear in human as well as animal wastes. The nitrogen in the agricultural fertilizer is taken up by crops and again appears in various waste products. The dairy and poultry feed shown in Table 1 is usually not considered a nutrient source, but some of the nitrogen in the feed appears in human and animal wastes as surely as does the fertilizer applied to corn silage.

Table 1. Estimates of nitrogen added to the environment each year from various sources in Connecticut

Source	*Tons*
Industrial smoke	44,000
Automobile exhaust	38,000
Domestic wastes	16,500
Animal wastes	13,500
Dairy and Poultry feed	13,000
Agricultural fertilizer	4,600
Non-agricultural fertilizer	2,800

This rather crude nitrogen budget shows that we lack many measurements necessary to draw up a precise balance sheet for the state. However, precise data are available for about 350 dairy farms in the Northeast, permitting us to derive a dairy farm nutrient budget.

The basic assumption required is that the highly specialized dairy farms in the Northeast are operating solely as machines for conversion of dairy feed and fertilizer into milk. Considerable re-use occurs on the farm as nutrients pass through the cow, are applied to the field, taken up by the crop, and returned to the cow. Since no machine is perfect, some of the nutrients are lost and either accumulate in the fields or leave the farm by various paths.

A budget of the three major nutrient elements, nitrogen, phosphorus, and potassium for a typical dairy farm in Connecticut is shown in Table 2. Additional items in the nitrogen budget include the atmospheric nitrogen fixed by legumes and bacteria, as well as a small amount in rainfall, with losses by volatilization to the atmosphere offsetting most of this input. A small correction for all three elements is also shown in the form of meat leaving the farm.

Although these calculations show that a substantial loss of nutrients occurs during cycling on the farm, they do not reveal the ultimate fate of these nutrients. Much of the phosphorus and potassium is undoubtedly fixed in the soil in forms unavailable to plants and thus remains on the farm. Nitrogen, however, is not readily retained by soils and it appears likely that much of the calculated net loss will leave the farm and eventually appear as nitrate in ground water.

The reasonableness of this assumption can be determined by comparing predicted with observed concentrations of nitrate in ground water. In the milkshed of Windham and Tolland counties, about 15% of the area is in cropland and pasture, while 75% is wooded and the remaining 10% may be classed as rural. If all the cropland is assumed occupied by dairy farms, a simple rainfall-dilution calculation predicts a maximum potential nitrate concentration in the water of 3 parts per million (ppm) expressed as nitrogen. Analyses of the ground water in the Shetucket and Quinebaug river basins by the U.S. Geological Survey revealed a maximum concentration of 14 ppm, and a median from 0.2 ppm to 1.9 ppm depending on the nature of the bedrock. Thus, the estimated maximum of 3 ppm seems reasonable. Although this concentration is well below the level of 10 ppm set by the U.S. Public Health Service for drinking water, it is high enough to contribute significantly to weed and algal growth.

What can be done about this assist we are giving our lakes along the downhill road of eutrophication? Some answers are evident from an analysis of the efficiency of nitrogen conversion on dairy farms in other states in the Northeast. Nutrient budgets comparable to those in Table 2 were calculated for dairy farms in five other states, and the results expressed as nitrogen lost per acre of farm. These losses for these five states and Connecticut were then related to farm size (Figure 1). Clearly, the intensity of land use is a strong determinant in the loss of nitrogen to streams. As the available land per cow is reduced, the movement of nitrogen out from the farm increases sharply.

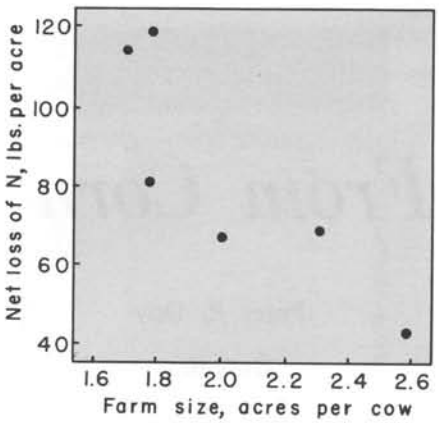


Figure 1
Estimated annual losses of nitrogen in pounds of N per acre from dairy farms in six states in the Northeast. As the available land per cow is reduced, the potential movement out from the farm increases sharply.

It appears that one could considerably reduce this nitrogen discharge to streams by increasing the cropland area, thus allowing plants to soak up more efficiently the nutrients applied. Corn hybrids could be selected for their efficiency in scavenging nitrogen from the soil, and dairy cows could be bred for greater efficiency in conversion of nitrogen in the feed to protein in the milk.

Fertilizer and manure handling practices could also be improved, since it is at this point in the cycle where large losses can occur. Rather than considering manure as waste to be disposed of, methods of handling and storage could be developed so that it may be applied to the crop during the growing season and not to bare soils. Similarly, more attention could be given to methods of applying commercial fertilizers to the growing crop, such as summer side-dressing of corn or application in irrigation systems. Also, we can perhaps take better advantage of present cost-accounting systems to insure against fertilization beyond the point of economic return.

Finally, we must realize that current agronomic practices have arisen from economic pressures on the farmer for increasingly efficient crop production. If we are to promote increased efficiency of nutrient recovery and conversion as well, additional economic incentives will be required. Moreover, the other nutrient sources shown in Table 1 must also be examined closely for their contributions to eutrophication.

Table 2. Nutrient budget for a Connecticut dairy farm

Item	*Nitrogen*	*Phosphorus*	*Potassium*
	Pounds per cow per year		
INPUT			
Concentrate	180	40	45
Fertilizer	110	50	90
Fixation	200	–	–
Rainfall	10	–	–
Total	500	90	135
OUTPUT			
Milk	70	10	18
Meat	10	2	2
Volatilization	155	–	–
Total	235	12	20
NET LOSS	265*	78†	115†

* Much of this ultimately reaches waterways.
† Largely fixed in the soil.

New Publications

The publications listed below have been issued by the Station since you last received Frontiers. Address requests for copies to Publications, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, Connecticut 06504.

Soils and Climatology

B696 *Experimental Errors in Derived Thermodynamic Constants.* C. R. Frink and P. E. Waggoner.

Entomology

B699 *Control of Mites and Aphids On and In Bulbs.* John C. Schread.

C230 *Privet Thrips.* John C. Schread.

Christmas Trees

B700 *Chemical Control of Weeds in Christmas Tree Plantings.* J. F. Ahrens, T. R. Flanagan, and M. L. McCormack, Jr.

From The Connecticut Station

New Wealth From Corn Genes

Peter R. Day

Genetics

PROGRESS IN PLANT BREEDING may seem to be painfully slow measured by the week, but measured by the lifetime it is often dramatic. Observations that may seem trivial at the time can turn out to be of great importance later. A good example comes from the corn work directed by the late Donald F. Jones at this Station.

In 1922 the Station received a sample of a white flint variety of corn grown at Hazardville, Connecticut, by a Mr. Olmsted. His daughter still lives in that town today. She tells us that her father's farm is now occupied by houses. Over the years 1922–1930 the Olmsted seed was sown at the Lockwood Farm in Hamden and two mutants (sports) were found which affected the endosperm. This is the nutritive tissue of the kernel surrounding the embryo. One mutant, called "brittle," was described by Paul Mangelsdorf in Station Bulletin 279 published in 1926. Kernels with "brittle" endosperm have a shrunken appearance. The gene responsible was later shown to occur on chromosome 5.

Donald F. Jones at the age of 26, shortly after he began his studies of the genetics of corn at this Station.

The second mutant, discovered by Jones and W. Ralph Singleton, turned out to be even more interesting. Our first record of it is in a 1930 notebook. It was called opaque. Normal seeds of the flint variety are translucent and in fact Dr. Singleton used to classify the seeds in segregating progenies by placing them over a light in a printing frame in a darkroom. Similar to another endosperm mutant already called opaque, the new mutant gene was shown to be on another chromosome so it was given the name *opaque-2*. Several brief accounts of its linkage with other genes on the same chromosome were published in the 1930's by Singleton. But until quite recently the mutant was simply one of a collection of maize mutant genes maintained by us and by other corn geneticists for possible use.

In the early part of this century chemists at the Station were examining the food value of cereal proteins. Osborne and Mendel, working on amino acids in proteins, showed that zein (the alcohol-soluble fraction of the endosperm proteins of corn) contains no detectable lysine or tryptophane and that young rats fed on a diet in which it was their only source of protein would die. Zein accounts for some 50% of the corn endosperm protein. This work established in 1914 the principle that amino acids are essential in our diet.

In 1963 a Station alumnus, Dr. Oliver Nelson, and his colleague Dr. Edwin Mertz at Purdue University, decided to look at some endosperm mutants to see if any might have higher proportions of the two vital amino acids. They discovered that the endosperm of the *opaque-2* mutant contains twice as much lysine and tryptophane as normal corn. (Their work is described in a recent Lockwood lecture by Dr. Nelson in the Centennial Series to be published by this Station later this year.)

Feeding experiments have shown that *opaque-2* maize is superior to normal maize as a source of proteins for animals and man. In fact *opaque-2* is now the subject of a number of breeding programs to increase the useful protein content of maize. The consequences for the protein hungry world may well be invaluable.

Another example of slow but steady progress began with Jones' first experiments in inbreeding corn. The discovery of hybrid vigor put a premium on the systematic development and testing of inbreds. The most successful of the Connecticut inbreds is the line known as C103. First released in May 1945, C103 was one of three inbreds developed by inbreeding and selection from the variety Lancaster Surecrop received from Mr. Noah Hershey, of Parkesburg, Chester County, Pennsylvania, in 1939. The unusual characteristic of this inbred was discovered more or less accidentally by a graduate student who found it difficult to kick its mature stalks over in the fall. It had very strong resistance to stalk breakage by wind and rain (lodging) and breakage by corn borers and foot rots. The stalks had an unusually high sugar content, and the line attracted some attention as a possible sugar cane substitute.

Although the high sugar content was recessive and thus not shown in its single or double cross hybrids, its ability to stand well was shown. As a result of this and its good performance in hybrids, C103 was and still is widely used as a seed corn parent throughout North America and many other parts of the world.

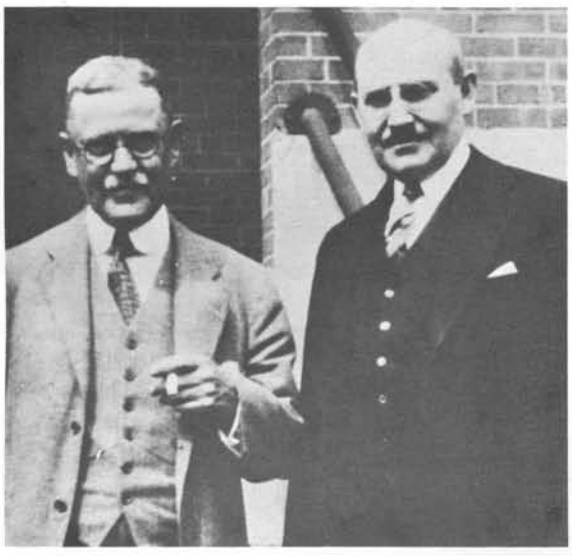
Since 1947 the Station has sent out seed of C103 in response to requests from more than 200 U. S. breeders and others in more than 30 countries around the world. During the intervening years it has played a prominent part in many hybrids. Even some of the newer lines replacing C103 include it in their parentage.

Estimates of the peak use of C103 indicate that 10 years ago more than one quarter of all the corn acreage in the corn belt was planted with hybrids which included this inbred in their parentage. Five years ago one large company alone produced nearly one million pounds of hybrid seed corn involving Connecticut inbreds, largely C103. In 1968 C103, and a related Connecticut inbred C123, together accounted for an average of 10 to 15% of the total production of 19 seed corn producers in the mid-west.

Thus we can see in retrospect what Jones and his colleagues accomplished. He had discovered, before this story begins, a practical way to put hybrid vigor to work in Connecticut cornfields. Then he and his colleagues faced the exciting challenge of systematic development and testing of inbreds useful in manufacturing hybrid varieties. He combed the countryside for kinds of corn that collectively held the genetic resources of the genus. Of the hundreds of inbreds discovered and tested over many years, one (C103) was so valuable it was bred into the corn planted on millions of acres, year after year. And a mutation, or spontaneous genetic change brought about naturally, in a corn variety that once grew on Mr. Olmsted's farm in Hazardville, now enables breeders to improve dramatically the nutritive value of corn. All in all, corn research at this Station has clearly "paid off" in edible (and taxable) wealth on an impressive scale.

Plant Science Day

Plant Science Day, the 57th annual open house of The Connecticut Agricultural Experiment Station, will be held at Lockwood Farm in Hamden on Wednesday, August 13. Station Director James G. Horsfall announced that Dr. Lester Hankin of the Station staff in biochemistry will again serve as general chairman. The event offers Connecticut citizens an opportunity to see plant science research underway at the Station farm and to hear staff scientists describe discoveries and how they are made. In recent years the open house has been called Science at Work.



Thomas B. Osborne, left, and Lafayette B. Mendel, who established the principle that amino acids are essential in our diet.

Enzymes

(continued from page 3)

Obviously only a small portion of these giant molecules can be involved in a chemical reaction with a molecule the size of a single amino acid. The region of interaction is known as the *active site,* and for the symmetrical enzymes described above one expects to find one active site per identical subunit. The shape of this region is of critical importance. For our $A \rightarrow B + C$ enzyme the molecule of A must be held at the site on the enzyme by weak forces in precisely the correct position before a chemical reaction takes place. Another amino acid (L-tyrosine) present in plants differs from A by having one extra oxygen atom at the position marked by a small arrow in Figure 1. As this second amino acid does not fit the active site of the enzyme no reaction takes place. The evolutionary modification of the protein has led to a molecule folded in exactly the right way to make this discrimination possible.

In addition to the active site having the correct shape, certain reactive groups must be present for catalysis to take place. In some cases the regular groups of the amino acid side chains suffice; in others, compounds with special chemical properties are attached to the protein. The $A \rightarrow B + C$ enzyme contains a reactive group not previously encountered in enzymes. To understand how the enzyme works the reaction between this group and A, and the further steps leading to $B + C$ and free enzyme must be identified.

Biochemists have found that cell chemistry is regulated both with great precision and with a remarkable economy of means. It is of the essence of biochemistry that such economies be sought out and defined.

A Note To Readers

When you move, or if your address on page 8 is incorrect, please notify the editor, giving both your old and new Zip Code numbers.

Our Daily Bread

James G. Horsfall
Director

As a cornfield philosopher and erstwhile farmer, I am continually astonished by the various attitudes of my city friends toward agriculture.

For example, I frequently observe that a man of the cloth, invited to say grace before a meal, fails to thank God for the food he is about to receive. This is like editing the Lord's prayer, deleting "Give us this day our daily bread." After all, bread is cheap at the supermarket. And if the clergyman's supply of grapefruit runs low in February, he is likely to blame the store manager, not the act of God that brought on the freeze in Florida.

On the other hand, we city dwellers, belying our vaunted urbanity, really have a soft spot in our hearts for the country. As long as we don't have to milk the cows at sunup and sundown, we are all for a picturesque herd of cattle grazing the green grass. I suppose we recapitulate in our subconscious an era not more than a century past when most Americans were farmers.

We trust that the minister who forgets to give thanks for his daily bread does not forget the agricultural base of a part of his ethos. One of his titles, "pastor," derives from the same root word as pasture. He looks after the sheep in his pasture.

Current television commercials play on our soft spot for the farm and the ranch: "You can take Salem out of the country, but you can't take the country out of Salem," and "Come to Marlboro Country," the cowhand pleads.

You can see our identification with the land in the words and phrases we use every day. We broadcast the news. A radio and TV station in Hartford proudly displays in its lobby a statue, "The Broadcaster." This statue, the work of Mrs. Frances Wadsworth, depicts a man broadcasting seed over the land —not an announcer at the microphone. And this station televises a program called RFD #3 to urban and urbane Connecticut.

Their use of the word, broadcasting, must extend its roots far back into ancient agriculture. It cannot be recent because farmers have sown seeds in rows ever since Jethro Tull published "The New Horse Hoeing Husbandry" in 1731. Only lawn makers broadcast seeds today.

Our everyday language abounds in words and phrases that reveal our background. I suspect that a little digging would turn up the following phrases even in the *New York Times;* sow the seed, plant an idea, cultivate an acquaintance, thresh out a problem, winnow the chaff from the wheat, seek grass-root opinions, graft on new ideas, and prune out excess verbiage. At times some of us feel that we have a hard row to hoe. Meanwhile, we read of transplanting hearts. "There's a rotten apple in every barrel," we say, but who stores apples in barrels any more?

One of my favorite quotable quotes is, "You can never plow a field by just turning it over in your mind."

These rustic remnants in our language do remind us of our agrarian heritage, our agricultural base. They should remind us, too, that however sophisticated be the urban society we set up, we still must eat, and that the products of agriculture feed us.

I trust that these phrases will remind us, too, that our agriculture must increase its sophistication in parallel with that of industry if we are to continue to have enough wholesome food on our tables. This is the field we continue "to cultivate" here at the Experiment Station. We cannot "farm it out." We must improve the yield of the land and the quality of that yield, that American agriculture may provide our daily bread so that we may continue, if we choose, to edit the Lord's prayer with impunity.

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Vol. 21 No. 2 May 1969 Bruce B. Miner, *Editor*

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