Radioactive Sugars

synthesized for studies in the metabolism of plants and animals

W. Z. Hassid

Carbohydrates, essential foods in human nutrition, are manufactured by green plants. All other living creatures are completely dependent upon plants, because they alone have the power to make sugars from the simple materials of carbon dioxide and water. This is accomplished by a process called photosynthesis.

As a result of photosynthesis, gaseous carbon dioxide combines with hydrogen from water in the presence of light and the green pigment—chlorophyll—in the living cell to form sugar and oxygen. Thus far scientists have not been able to imitate this process in the laboratory.

The sugars manufactured by plants serve as potential stores of energy. In living cells they combine with oxygen and are oxidized, liberating energy that corresponds to the energy from the combustion of heating materials in factories. In the process of oxidation about 100 kilocalories of heat are released for each 10 grams of carbon they contain.

The energy liberated in the respiration—oxidation—process is used for practically all the metabolic processes of the plant. Respiration provides the energy required by the plant for the synthesis of numerous organic compounds, such as proteins, fats, vitamins, etc.

Complex Carbohydrates

Glucose—corn sugar or dextrose, and fructose—fruit sugar or levulose, are the so-called monosaccharides, or simple sugars, which are readily formed in the process of photosynthesis, and serve as building units or blocks for the formation of the more complex carbohydrates. The disaccharide sucrose which is ordinary sugar consists of a molecule of the monosaccharide, glucose and the monosaccharide, fructose, formed through the elimination of a molucule of water, which leaves the two monosaccharides being held by an oxygen bridge. Another disaccharide, maltose, is similarly formed from two glucose units joined by an oxygen atom. In its molecule, raffinose contains glucose, fructose, and galactose. Polysaccharides such as starch, glycogen, or cellulose, consist of many hundreds of glucose units.

The complex sugars as well as the polysaccharides may be built of the same monosaccharide unit, or in some cases, of two or more different monosaccharides. The starch molecules in turn combine to form starch granules and the cellulose molecules form cellulose fibers. The polysaccharides—cellulose, hemi-cellulose, and others—provide the plant with a structural frame, giving the plant rigidity. Cellulose, xylan, and hemi-cellulose usually make up the bulk of the plant.

Sugar Formation

One phase of the work of the Division of Plant Nutrition is the study of how complex sugars are formed—synthesized—from simpler building units, and also how these complex sugars, after being formed, are broken down—degraded—through a reverse process. For example, by what mechanism do glucose and fructose condense to form sucrose, which accumulates in large quantities in many plants; or how is glucose condensed to form the giant starch or cellulose molecule.

Enzymes

Synthesis as well as degradation of organic compounds in nature is carried out by the aid of enzymes. The enzymes are large complex protein molecules which are present in all living cells. One of the best-known examples of an enzyme is invertase which breaks down sucrose to glucose and fructose. Enzymes are responsible for the fermentation of sugar to carbon dioxide and alcohol.

In connection with the study of the process of sucrose formation, a search is being made to find an enzyme which could combine glucose and fructose to form the disaccharide, sucrose.

Several years ago a research worker in the Department of Bacteriology succeeded in making an enzyme preparation from the bacterium *Pseudomonas saccharophila* which could break down sucrose in the presence of inorganic phosphate, forming glucose-phosphate and fructose. It was found later that this process could be reversed—glucose-phosphate could be combined with fructose to form sucrose, splitting off inorganic phosphate in the reaction. Thus by means of an enzyme elaborated by bacteria, sugar was synthesized in the laboratory for the first time.

The discovery of a method by which crystalline sugar can be synthesized enzymatically is significant for its scientific implications. Since the functioning of a living cell depends on the sum total of a great multitude of enzymatic reactions taking place within it, the understanding of the processes involved is of primary importance.

The knowledge of how sucrose is formed may lead to an understanding of conditions whereby greater yields of sugar can be obtained from sugar beets and sugar cane. Since it is known that inorganic phosphate is involved in the mechanism of sucrose formation, higher sugar yields may be concerned in part with phosphate fertilization, although many other factors are undoubtedly involved.

The same enzyme which forms sucrose from glucose-phosphate and fructose also will combine sucrose with a number of simple sugars other than fructose, forming new disaccharides. Four new crystalline sugars were synthesized in the laboratory by the aid of this enzyme.

It is generally accepted that enzymes are specific in their action with regard to substrates—a certain enzyme will perform only one specific reaction. For example, the enzyme amylase or diastase is capable of breaking down only starch to maltose and dextrins. Another enzyme, invertase, will split sucrose to glucose and fructose, but will not attack any other complex sugar. In the case of sucrose phosphorylase, apparently there is an enzyme which is characterized by its versatility. It is capable of synthesizing several disaccharides from various monosaccharides and also of reversing the reaction—breaking the complex sugars down to their constituent monosaccharides.

From these studies much has been learned about the mechanism of the reactions involved in the synthesis of disaccharides. It was found that the enzyme—sucrose phosphorylase—is capable of synthesizing sucrose and other disaccharides without phosphate taking place in the reaction. The phosphate in glucose-phosphate can be substituted by other monosaccharides.

It is possible that many of the plant carbohydrates, such as inulin or pectins, are synthesized by a similar mechanism, which does not require phosphate.

Radioactive Sugars

Radioactive glucose, fructose, sucrose, and starch can be prepared by exposing various plants to radioactive carbon dioxide in the presence of light and allowing them to photosynthesize for certain periods of time. The radioactive carbohydrates may then be isolated and sepa-

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rated. They are now being used in the study of the mechanism of carbohydrate formation and breakdown in plants.

Radioactive glucose prepared in the laboratory is being used in the Medical School to study the problem of the utilization of glucose by diabetic animals. The problem of how a diabetic animal oxidizes sugar is still obscure.

The discovery of the enzymatic synthesis of sucrose from glucose-phosphate and fructose permits the study of the metabolism of this disaccharide in plants and animals with regard to each monosaccharide constituent. Thus far the behavior of glucose or fructose during the course of metabolism could not be observed since it has been impossible by ordinary methods to distinguish one sugar fragment from the other in their final utilization by plants and animals.

The use of the tracer technique, whereby radioactive carbon isotopes are incorporated into the molecules, permits a direct observation of the course of metabolism of individual compounds.

As sucrose now can be synthesized enzymatically from glucose-phosphate and fructose, it is possible to label each half of the molecule of sucrose at will. Radioactive sucrose with a labeled or tagged glucose molecule can be prepared by combining radioactive glucose-phosphate with inactive fructose by the aid of the bacterial enzyme. Sucrose with a tagged fructose molecule then can be synthetically made from inactive glucose-phosphate and radioactive fructose and the same enzyme.

These two radioactive sugars actually have been synthesized in the laboratory, and it is hoped to learn about the fate of each half of the sucrose molecule, by following their course of metabolism in plants and animals.

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VACCINE

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cine because there is no readable take such as poultrymen check following laryngotracheitis or fowl pox vaccination. Like any living product, live-virus vaccines are subject to deterioration. Because there is no easily readable take, it is difficult to tell whether such loss of effectiveness has or has not occurred.

Poultrymen have been lulled into a false sense of security, thinking that live-virus vaccines provide the final answers to the control of pneumoencephalitis. This is far from true. While much is known about the pathology of the disease, and something about the virus, little is known about the development of the disease in different species of birds, the immune reactions, and the basic source of the infection—that is, where it over-winters. In exploring these fields, improved means of control or possible eradication may be found. Poultrymen should recognize the need for continuing such basic research. The cautious, intelligent use of live-virus PE vaccine does provide an expedient method for reducing the economic losses due to this infection where it exists and is a problem. Such use gives time for needed investigations.

Other Control Measures

Poultrymen would be unwise to consider that live-virus vaccination is the only method to control PE. There are other possibilities. First, use the excellent diagnostic facilities available, promptly and adequately.

Second, sources of new infection should be eliminated; adult or started birds should not be brought to the ranch.

Third, sound sanitation—curtailed visits, free areas for loading and unloading, etc.—should be practiced. Continuous brooding should be avoided.

Fourth, formalin-killed vaccines might be useful.

Lastly, and only until better means are available, in those areas where pneumoencephalitis is known to exist or is an immediate threat, live-virus vaccines may be helpful.

Though it may be assumed that live-virus PE vaccine provides an easy-to-use method of conferring solid immunity to vaccinated birds and probably provides a short time, passive immunity to chicks hatched from vaccinated hens, there are numerous limitations. However, it is not safe or a good immunizing agent for chicks under four weeks of age; it should not be used on laying birds, and birds to be vaccinated should be in good health. Since the vaccine infection spreads, all other birds on the ranch should be immune as a result of previous vaccination or a natural outbreak. The effect of live-virus vaccine on the future of the birds is not known. The product is subject to misuse, and there is no visible take. The stability of the vaccine virulence is not fully established. Most important, live-virus vaccine perpetuates the disease or may introduce the infection into clean areas.

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Published evidence and papers presented at various meetings have been drawn on freely in preparing this report.

NEW PUBLICATIONS

A copy of the publications listed here may be obtained without charge from the local office of the Farm Advisor or by addressing a request to Publications Office, College of Agriculture, University of California, Berkeley 4, California.

SWINE FEEDING EXPERIMENTS, by E. H. Hughes and Hubert Heitman, Jr. Bul. 709, October, 1948.

The daily gain in weight of pigs is important to the swine raiser. This bulletin reports the results of four investigations, which show that (1) cooked lima beans may efficiently be added to the diet of pigs, saving some tankage; (2) potato meal can replace part of the barley in the ration; (3) soybean meal is an excellent plant protein; and (4) a good, cost-reducing practice is to allow hogs to harvest a crop of dwarf milo and cowpeas.

SPRINKLING FOR IRRIGATION, by F. J. Veihmeyer. Cir. 388, November, 1948.

Sprinkling for irrigation has a place in California agriculture, but it is not a cure-all. This circular covers four main questions: The kind of topography, soil, climate, and crops which make the use of sprinklers advisable. The kind of system the farm may need. The cost and the justification for the costs.

INVESTIGATIONS OF THE FLOR SHERRY PROCESS, by W. V. Cruess. Bul. 710, October, 1948.

DONATIONS FOR AGRICULTURAL RESEARCH

Gifts to the University of California for research by the College of Agriculture accepted in October, 1948

BERKELEY

Dr. Gabriele Goidanich and Dr. C. M. Tompkins	A rare early Italian treatise on diseases of cereal grains, 1759
Lederle Laboratories	10 grams special feed supplement
National Research Council — For work in plant microbiology	$500.00
Sugar Research Foundation, Inc. — For food technology research	$1,000.00
Sugar Research Foundation, Inc. — For plant nutrition research	$702.00

RIVERSIDE

California Fertilizer Association — For studies on determination of effects of various nutrient variables on absorption of phosphorus by citrus plants	$2,000.00