ENZYMES

Enzyme Systems of the White Potato

SIGMUND SCHWIMMER
Western Regional Research Laboratory, Albany, Calif.

Survey of the literature on enzymes of white potatoes discloses that this tuber and its constituent enzymes are useful in the field of enzyme chemistry as a source material for large scale preparative purposes, for the elucidation of the mechanisms of starch transformation in plants, and as an analytical tool contributing to knowledge of the phosphate linkages in coenzymes and proteins. Positive evidence exists for the presence of enzymes involved in (and for the operation of) the biologically ubiquitous glycolytic, tricarboxylic, and cytochrome terminal oxidative systems in this tuber. Studies of the relationship of these systems and other enzymes to the problems of enzymatic browning of raw potatoes via phenol oxidase on the one hand, and to the nonenzymatic browning of processed products due to accumulation of reducing sugars on the other, are still in a formative but promising stage. Only meager knowledge is available concerning the enzymic mechanisms involved in the transformation of sucrose and nitrogenous constituents of the potato.

THE CHEMICAL COMPOSITION OF THE WHITE POTATO tuber (*Solanum tuberosum* L.) and changes in its composition as related to variety, culture, harvest, storage, and industrial processing have received detailed attention in numerous studies (*24*, *80*). Yet the level of accumulation of any particular substance in the potato, as in any living organism, can in principle be traced back to the resultant action of the enzymes and enzyme systems catalyzing its synthesis and decomposition. Very few enzyme studies have been directed to the solution of problems of the potato grower and processor. Some members of the enzyme systems that exist in potatoes have been studied in varying detail, but most of the enzyme reactions that are known to occur in other biological sources have received only a rather cursory examination in white potatoes.

As a source material for the enzymologist, potatoes have several advantages. They are available in large quantities, and the easily expressed juice is rich in several enzymes. Stable, partially purified preparations are readily obtainable. On the other hand, the great variability in enzyme content due to variety and storage makes careful selection of the correct lot of potatoes at the right time important. Furthermore, attempts to obtain completely pure crystalline preparations of the enzymes have succeeded so far in only two instances (*15*, *30*). In some cases the apparent enzyme activity of the intact potato tuber is not detectable in the expressed juice.

Negative Findings

Most enzyme studies are designed to test the presence in appreciable quantities of an enzyme in tissues. Only when experiments are devised to detect feeble enzyme action in the presence of natural inhibitors or destructive factors, and are applied to different varieties under various storage conditions, can one accept a negative finding with confidence.

The following survey cannot, of course, review the entire literature, but it discusses in detail those aspects of the enzyme chemistry of the potato that have been fairly well worked out. Furthermore, it illustrates each type of enzyme action and relates knowledge thus far obtained to current enzymological and biochemical interests. Wherever available, data on the effect of variety and storage conditions on the enzyme under consideration are mentioned.

For the most part, this review does not include those aspects of potato physiology which are not related to some specific enzyme or enzyme system. For background material on enzyme systems, a general reference is mentioned frequently (*19*).

Starch-Transforming Enzymes

In the field of metabolism of the potato, our knowledge of enzymes involved in transformations of starch is most extensive. A summary of the

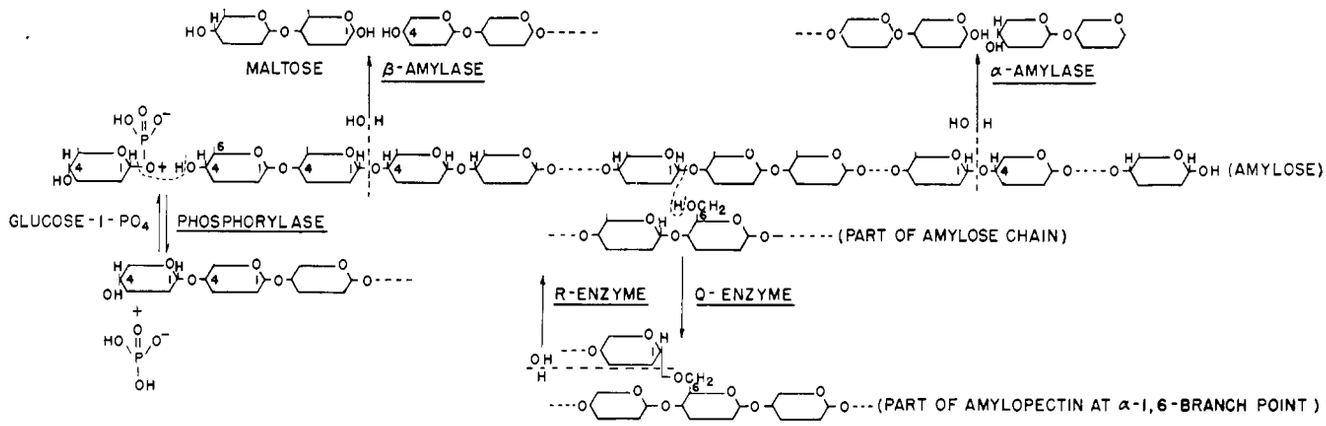


Figure 1. Transformation of starch components amylose and amylopectin by enzymes of potato tuber

known enzymic mechanisms through 1952 is depicted in Figure 1. Potato starch consists of two components: amylose, the long straight-chain polymer of anhydroglucose units averaging 1000 to 4000 units long, depending upon the method of preparation (*22, 48, 79*), linked together by oxygen between the C-1 position and C-4 position of two consecutive units (α-1,4-glucosidic linkage); and amylopectin, the so-called branched-chain component, in which the α-1,4-glucosidic chain is branched by means of a linkage between the C-6 position of the glucose of one chain and the C-1 position of the glucose of another chain.

Amylose is synthesized in the potato tuber by means of the enzyme phosphorylase according to the reaction

$$n \text{ Glucose-1-phosphate} \rightleftarrows \underset{\text{amylose}}{(\text{glucose})_n} + n \text{ phosphate}$$

This reversible reaction comes to an equilibrium, depending upon pH, because glucose-1-phosphoric acid is a stronger acid than phosphoric acid. At pH 7 the equilibrium is such that the ratio of inorganic phosphate to glucose-1-phosphate is 3.1: at pH 5 it is 10.8 (*36, 74, 101*). The acid phosphate ion ($H_2PO_4^-$) is the specific ionic species which enters into this reaction (*101*).

Potato juice is a rich source of phosphorylase. Although fairly extensive studies have been made on the purification of this enzyme, only recently has it been possible to purify it to the extent that it will crystallize (*15*). The previous lack of success was in part due to instability of the enzyme to the usual reagents used in purifying enzymes (*4, 30, 32, 88, 107*). On the other hand, it is stable to reagents that are usually regarded as general enzyme inactivators (mercury salts, molybdate, fluoride) (*1*). When some of these "inactivators" are added to a potato phosphorylase preparation, thus destroying other starch-transforming enzymes, an amylose, resembling the naturally occurring potato amylose more closely than other phosphorylase-synthesized amyloses obtained heretofore, can be obtained from glucose-1-phosphate (*6*).

The phosphorylase reaction will not proceed unless a small amount of "primer" carbohydrate is present. This carbohydrate may consist of amylose, amylopectin, or any other noncyclic member of the amylose series which contains in its molecule at least two consecutive α-1,4-glucosidic linkages (*29, 42, 107*). The synthesis apparently proceeds by the transfer of glucosyl units from glucose-1-phosphate to the nonreducing end of the primer molecules (*7*) (see Figure 1).

The phosphorylase content of potatoes is not affected by storage at various temperatures (*4*). An inhibitor of phosphorylase action is said to develop in potatoes stored at room temperatures, but this inhibitor does not appear in potatoes stored at a lower temperature (0° C.). The enzyme is competitively inhibited by Schardinger dextrin (*32*), certain sulfonamide derivatives (*71*), and the synthetic plant growth substance 2,4-dichlorophenoxyacetic acid (2,4-D) (*56*).

The amylopectin moiety of potato starch is synthesized by an enzyme which has been termed "Q-enzyme" (phosphorylase being the "P-enzyme"). Its action is similar to that of phosphorylase in that the enzyme effects a transfer of a radical from one molecule to another molecule (see Figure 1). In this case the Q-enzyme effects the transfer of one portion of an amylose chain to another complete amylose chain—i.e., an α-1,4-glucose linkage is opened and replaced by an α-1,6-glucosidic linkage (transglucosylation) (*39, 73, 74*).

Amylopectin can be synthesized from glucose-1-phosphate by the combined action of phosphorylase (P-enzyme) and Q-enzyme (*23*). By variation of the proportion of the two enzymes, amylopectins with varying degrees of branching can be obtained. This action is not reversible (*74*). Q-enzyme has been prepared as a pure crystalline protein from potatoes (*30*).

An enzyme exists in potatoes which can reconvert amylopectin to short amylose chains by a "debranching" mechanism (*43, 74*). This debranching factor or "R-enzyme" specifically catalyzes the hydrolysis of the α-1,6-glucosidic linkage and thus differs from the above-mentioned synthesizing enzymes, phosphorylase and Q-enzyme, in not being a transferring agent.

Recently an enzyme capable of reversibly disproportionating members of the amylose series from amylotriaose (trisaccharide) to amyloheptaose (heptasaccharide) into blue staining amylose, lower oligosaccharides, and glucose has been found in potatoes (*75*). This "D-enzyme" acts by means of transglucosylation but cannot utilize maltose.

Potatoes also possess relatively small but significant amounts of amylase enzymes which catalyze the hydrolysis of the α-1,4-glucosidic linkages of both amylose and amylopectin. Both β-amylase (*4*) and α-amylase types of action (*6*) of potatoes have been described. The β-amylase hydrolyzes maltose from the nonreducing end of the starch molecule, whereas α-amylase can hydrolyze an α-1,4-glucosidic linkage located in the interior of the molecule. The amylolytic action of potatoes is weak, probably amounting to only about 1% of the activity of rich sources like sweet potatoes or barley malt.

Interest in amylases in potatoes recently has been mainly confined to methods of eliminating them as complicating factors from preparations of other starch-transforming enzymes (*4, 6, 32, 107*). Considerable quantities of starch-hydrolyzing enzyme do develop in the potato at the initiation of sprouting (*110*). Storage of potatoes at various temperatures for short periods of time does not seem to change the amylase content (*4*).

Sucrose-Transforming Enzymes

In contrast to available knowledge of the starch-transforming enzymes in potatoes, very little is known about the immediate reactions which sucrose undergoes in potatoes. The enzyme

that catalyzes the hydrolysis of sucrose to glucose and fructose (invertase) is apparently absent in the dormant tuber but appears upon sprouting (*61*).

It has long been known that changes in sucrose content of potatoes readily occur in response to changes in temperature (*69*) and moisture content (*109*), increasing at low temperatures and low moisture contents concomitantly with a decrease of starch content. Yet attempts to demonstrate the conversion of starch to sucrose in potato extracts have thus far failed.

The little that is known about the enzymic synthesis of sucrose in potatoes has been obtained through work with thin slices of living potato tissue (disks) as the source of the requisite enzyme system (*4*, *61*, *64*). These disks convert sugars such as glucose and fructose into sucrose, but the steps of this synthesis are almost completely unknown. The synthesis takes place faster in slices from tubers stored at low temperatures than in tubers from room-temperature storage. Furthermore, the rate of synthesis is said to be greater at lower temperature. This effect, which is opposite to that of most enzyme reactions, may be due to shifting of equilibrium toward synthesis rather than to an increase in rate. Synthesis is linked with respiration of the potato and formation of high-energy phosphate bonds, as it will not occur in the absence of oxygen and is inhibited by various substances which, when added to tissues other than potato, inhibit both respiration and phosphorylation of glucose by adenosine triphosphate (hexokinase reaction). Furthermore, the quantitative relationship between respiration rate of the tuber and its sucrose content describes a curve that is similar to the curve for rate of an enzyme reaction plotted against initial concentrations of substrate upon which the enzyme acts (*9*).

Enzymes That Hydrolyze Phosphate Esters

Potatoes are a rich and convenient source of enzymes that hydrolyze a variety of phosphate esters. So far at least five distinct phosphatases have been characterized.

Considerable quantities of "acid" phosphatase are present in potatoes. This type of enzyme is usually found in plant extracts. It can hydrolyze a variety of mono-orthophosphate esters such as the phosphate esters of phenolic compounds, glycerol phosphate, etc. It acts best in the relatively acid pH range (pH 5 to 6) (*40*). Several partially purified preparations have been made (*41*, *86*). It is inhibited by a variety of substances including fluoride, molybdate, and arsenate (*40*, *41*, *63*). On the other hand, magnesium salts and magnesium complexing agents (magnesium is required for the activity of other phosphatases) are without effect when phenolphthalein phosphate is used as a substrate (*40*). Hydrolysis of glycerol phosphate has been reported to be accelerated by magnesium ions (*30*). The acid-phosphatase content of ethylene chlorohydrin–treated potato tubers is decreased, presumably owing to the formation of a specific dialyzable inhibitor (*35*).

Potato phosphatase has been used as an analytical tool (*92*) and also to characterize the phosphate linkage of certain phosphoproteins (*76*, *77*). In the latter study it was found that potato phosphatase acts like intestinal phosphatase in hydrolyzing off both phosphate groups of ovalbumin at the same time, whereas prostate phosphatases split off one phosphate group at a time.

The phosphate esters of glucose and fructose are hydrolyzed by an enzyme in potato juice whose pH optimum is 8. It is inhibited by molybdate (*6*). Storage of potato tubers at various temperatures does not affect the content of this phosphatase and its action at 0° C. is negligible (*4*).

A third type of phosphatase action (apyrase) in potato juice is very specific for the hydrolysis of two of the three phosphate linkages in adenosine triphosphate (ATP). There is much interest in this enzyme (or possibly system of enzymes) because of the central significance of adenosine triphosphate in the metabolism of all biological organisms (*19*). Apyrase is said to have a role in stabilizing the resting state in potato tubers (*50*).

The apyrase of potato exists in insoluble and soluble forms (*53*). The former has been extensively purified but is not as specific in its action on phosphate esters as is the insoluble form, which can hydrolyze only adenosine triphosphate. The enzyme acts best at pH 6 to 7, and is stable to pressures as high as 4000 atmospheres (*44*). Potato apyrase, when adsorbed on the muscle protein myosin, cannot be separated therefrom by repeated washings; thus it resembles the apyrase of muscle tissue (*47*). New potatoes (variety not mentioned) obtained in May and June consistently contained more apyrase than stored potatoes purchased in October and November (*53*). It has been reported that above 7° C. potato apyrase preparations split off two inorganic phosphate groups from adenosine triphosphate, whereas below 7° only one phosphate group was split off (*57*). It may not be too far-fetched to relate this observation to a similar critical temperature for the well-known starch-sucrose shift in potatoes. The possibility that the second phosphate is split off owing to the presence of myokinase (or, more correctly, adenylatekinase) (ATP + AMP = 2ADP) was tested and no evidence for the presence of myokinase in potatoes could be found (*52*). Although the apyrase of muscle tissue is specifically activated by calcium ions, potato apyrase preparations have been reported as being activated (*54*, *57*) and not activated (*99*) by calcium salts. These and other discrepancies [such as the relative values of the rate constants of the two hydrolyzable phosphate linkages of adenosine triphosphate (*57*)] raise the question as to the enzymic homogeneity and identity of different apyrase preparations as prepared by different investigators. Potato apyrase preparations have been used to prepare pure adenosine monophosphate from adenosine triphosphate (*98*).

Another rather highly specialized phosphatase in potatoes is limited in its action to the hydrolysis of the pyrophosphate bond in certain coenzymes (*38*, *49*, *72*) such as diphosphopyridine nucleotide, flavine-adenine-dinucleotide,

Figure 2. Evidence of enzymes of glycolysis in potato tuber

Numbers denote appropriate references demonstrating described enzyme action. *a* represents reactions not demonstrated as yet for the potato.

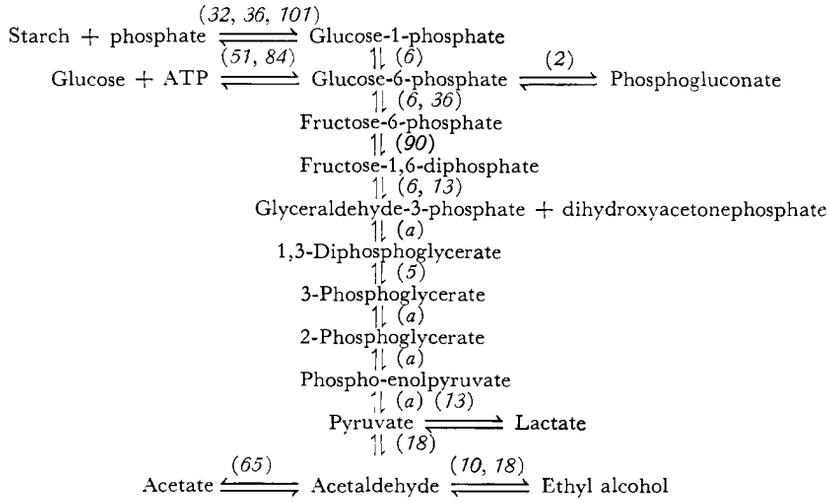


Table I. Glycolytic Cycle as a Source of Possible Intermediates for Synthesis of Important Potato Constituents

Glycolytic Intermediates	Enzymes	Potato Constituents	References
Glucose-1-phosphate	Phosphorylase, Q-enzyme	Starch	(*39*, *74*)
Glucose-6-phosphate	TPN dehydrogenase	Ribose (in nucleic acids), heptuloses	(*2*)
Hexose phosphates	Phosphatases	Glucose, fructose	(*4*, *6*)
Hexose phosphates	?	Sucrose	(*4*, *61*, *64*)
Pyruvic acid	Carboxylases, dehydrogenases	Ethyl alcohol, acetaldehyde, organic acids	(*4*, *13*, *18*)
3-Glyceraldehyde phosphates	Aldolase + ?	Ascorbic acid	(*89*)

cocarboxylase, coenzyme A, and adenosine triphosphate. This coenzyme or nucleotide pyrophosphatase has played an important role in the elucidation of the chemical structure of these coenzymes. The coenzyme pyrophosphatase content of eleven varieties of freshly harvested Maine potatoes varied as much as eightfold, Chippewa containing the least and Irish Cobbler the most (*49*). After 6 weeks of storage at 3° C. the contents of all the varieties had increased. White Rose rose to the highest level, the enzyme activity increasing over fourfold.

Potatoes also contain an enzyme capable of hydrolyzing inorganic pyrophosphate (*70*). Its properties are very similar to the pyrophosphatases of animal tissues, in that the enzymes from both sources act optimally above pH 7 and are activated by magnesium ions.

The Glycolytic Cycle

Many, if not most, living organisms possess in common a complicated mechanism for the breakdown of sugars (*19*). This glycolytic scheme, depicted in Figure 2, consists of a series of chemical reactions catalyzed by some 12 well characterized enzymes. Many of these reactions have been shown to occur in the potato tuber, although few have been subjected to more than a cursory examination. Thus, as far as has been investigated, potatoes break down sugars at least in part via the glycolytic cycle. Whether this is the principal pathway of metabolism of the sugars is not known. Obviously, the scheme must be modified to include the transformations of sucrose. Furthermore, there exists in many tissues, including potatoes (*2*), the so-called "oxidative shunt" whereby glucose-6-phosphate is oxidatively converted eventually to phosphate esters of ribose and heptulose. The ribose may then be incorporated into nucleic acids or may re-enter the glycolytic cycle via triosephosphate, although the experimental data for such interconversions in potato are not yet available. While yeasts are known to break down the sugars to ethyl alcohol and muscle tissue to lactic acid, potatoes contain enzymes that can form both of these substances; and both have been found in potatoes (*10*). The enzyme pyruvate carboxylase, which breaks down pyruvate to acetaldehyde, has been variously reported as being absent (*103*) and present (*18*) in potato. Negative findings should be subjected to appropriately designed experiments before they can be accepted with confidence. Whereas most plant hexokinases are only partially bound to the cell mitochondria, that of potatoes is completely bound to the cellular particles. The insoluble plant hexokinase can also phosphorylate fructose (*84*). The glycolytic cycle may thus be a source of intermediates which can be utilized enzymatically for the direct synthesis of a variety of substances found in potato tubers (Table I).

Of the known sugars and sugar phosphates reported to be present in relatively large amounts in the potato tuber (*4*), glucose, fructose, fructose-6-phosphate, and glucose-6-phosphate are known to undergo the nonenzymatic Maillard reaction with amino acids to form brown-colored polymers (*87*), which may be responsible for the discoloration or browning in many foods, including processed potatoes (*80*). The known enzymatic reactions in the potato which could lead to the formation, interconversion, and disappearance of these hexoses and their phosphates are shown in Figure 3.

A valuable review on recent evidence for glycolytic enzymes in plant tissue is available (*94*).

Metabolism of Acids and Tricarboxylic Acid Cycle

Whereas glycolysis breaks down sugars in the absence of air to ethyl alcohol and lactic acid (in potatoes), another ubiquitous mechanism for the further degradation of metabolites is the tricarboxylic acid cycle (*19*), a short version of which is shown in Figure 4. The operation of this cycle generates the useful energy required for the many physiological processes required by the living cells, such as synthesis of other compounds, selective transfer of electrolytes across membranes, and complete oxidation of metabolites.

Figure 4 indicates that almost all of the enzymes required for the operation of this cycle have been detected in potatoes. Failure to detect isocitric dehydrogenase (*2*) may be due to the presence of coenzyme-destroying factors in potato juice (*38*, *49*). Other coenzyme-requiring enzymes of this cycle can be demonstrated (*2*, *28*, *102*) but seem to be present in quantities lower than those present in other vegetables. Recent evidence points strongly to the operation of the tricarboxylic acid cycle in the intact potato tuber (*12*). When potatoes that have been stored in nitrogen (so that glycolytic but not respiratory processes can occur) are exposed to air, the accumulated lactic acid (an end product of glycolysis) rapidly decreases. This decrease is associated with an initial outburst in carbon dioxide evolution and a concomitant rapid initial accumulation of pyruvic and α-ketoglutaric acids, members of the tricarboxylic acid cycle. The carbon dioxide apparently originates from the decarboxylation of these and possibly other cycle intermediates. Earlier observations on the evolution of carbon dioxide associated with a decrease of citric acid in ethylene chlorohydrin treated potatoes are also in accord with operation of this cycle in potato (*34*, *66*).

Potato juice is a fairly good source of an enzyme which oxidizes long-chain unsaturated fatty acids (lipoxidase), which may play a role in the development of rancidity (*95*, *96*). Potatoes also contain enzymes that act on lecithin by splitting off the fatty acids (lecithinase) and splitting the linkage between phosphate and choline (phospholipase) (*26*).

Terminal Oxidation

The transfer of hydrogen (or electrons) from the intermediates of tricarboxylic acid cycle to oxygen is usually conceived as being mediated by the "terminal oxidase" system. In most living tissues this system consists of a series of heme-protein pigments, including the cytochromes and cytochrome oxidase (*19*). Because potatoes contain substantial amounts of naturally occurring phenols and an enzyme which can oxidize these phenols, it was at first assumed that this enzyme was the only one responsible for the terminal oxidation in the potato tissue (*8*, *20*, *21*). [Adequacy of scalding of potatoes prior to processing has been tested by measuring the net effect of this enzyme acting on its natural substrates in potato juice (*104*, *105*).]

This enzyme, which has been variously named phenol oxidase, polyphenol oxidase, catecholase, and tyrosinase, has

been purified to a considerable extent and shown to be a copper-containing protein (*55*) which is inhibited by many aromatic compounds, including sulfonamides (*16*), and destroyed by ionizing radiation (*97*). Potato catecholase has less affinity for its substrate and for competitive inhibitors thereof than does mushroom catecholase (*106*).

Most of the enzyme seems to be present in the potato in a soluble form, although a small but appreciable part seem to be associated with insoluble intracellular material (*10, 91*). More than half of the total copper present in potato is bound to the tyrosinase (*100*). This oxidase has been used for oxidation studies of some physiologically important phenols (*16, 17, 89*), such as the phenolic compounds in poison ivy responsible for its toxic effect. This is probably the basis for the proposed therapeutic effect of applying sliced raw potato to the area of contact with poison ivy. The blackening of a freshly cut potato and the occurrence of blackening after boiling of some potatoes are caused by phenol oxidase action on the naturally occurring phenols, especially the amino acid tyrosine (*82*). The product of oxidation of tyrosine by phenol oxidase undergoes a series of degradative and polymeric changes to give first a red (hallochrome) and finally a black (melanin) pigment (*19*). While this melanin formation is a physiological process in such tissues as animal skin, it occurs in the potatoes only under nonphysiological or pathological conditions.

A series of reactions has recently been proposed to account for the formation of melanin under these or similar conditions (*14*). According to this proposal, reserves of malic and possibly isocitric acid, intermediates in the tricarboxylic acid cycle, become depleted, thus preventing the transfer of hydrogen from these metabolites to oxidized glutathione through the mediation of glutathione reductase (*2*) and the coenzyme triphosphopyridine nucleotide (TPN). The glutathione under normal conditions prevents ascorbic acid from being oxidized to dehydroascorbic acid and is depleted prior to ascorbic acid in potatoes held in pure oxygen (*11*). The absence of ascorbic acid then results in the irreversible oxidation of naturally occurring phenol and formation of melanin. Ascorbic acid delays the formation of color by a phenoloxidase-substrate system. In this connection, potatoes are apparently devoid of a specific ascorbic acid oxidase (*46, 78*), although ascorbic acid may be oxidized via phenol oxidase.

The theory that the naturally occurring phenols and phenol oxidase constitute a system associated with terminal oxidative processes was based on observations that compounds (cyanide, sulfide, 8-quinolinol) which complex with copper, the coenzyme for potato phenol oxidase, as well as competitive inhibitors of catecholase, also lowered the oxygen uptake (respiration) of potato tissue (*8, 13*). Phenols such as catechol and chlorogenic acid, a polyphenol found in potatoes (*45*), accelerated oxygen uptake beyond the stoichiometric amount added (*20, 21, 83*). It was suggested that this oxidase system is linked to the pyridine nucleotide dehydrogenase systems (which are operative in the glycolytic and tricarboxylic acid cycle) and to catalase (*13, 55*).

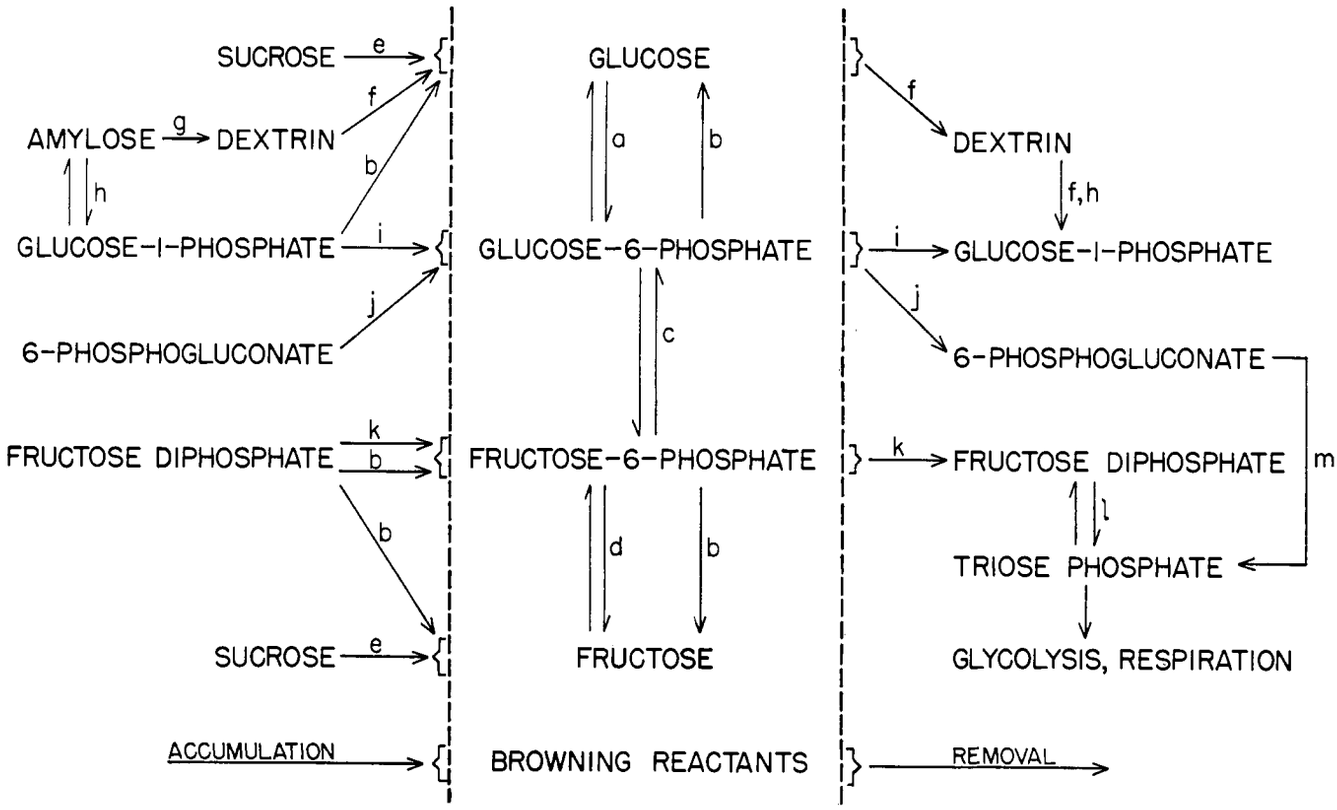
More recent work has indicated, however, that the phenol oxidase system may not account for the entire terminal oxidation system of the potato. The evidence indicates that there exists in potatoes a cell-bound cytochrome oxidase system (*13, 31, 59*), which is involved in the terminal oxidation of many biological tissues. This enzyme is present in sufficient quantity to account for all the oxygen uptake (respiration). Furthermore, the use of catechol as a substrate for endogenous respiration is questionable, since catechol acts as a cell poison (*85*).

At least one author feels that during the rest period respiration is mediated principally by means of the tyrosinase system, whereas the cytochrome oxidase

Figure 3. Reactions catalyzed by enzymes demonstrated to be present in potato

Enzymes could lead to accumulation and removal of hexoses and hexose phosphates (reported to be present in potatoes) which can undergo browning reactions with amino acids. Enzymes responsible for these reactions are:

a. Hexo(gluco)kinase *b.* Alkaline phosphatase *c.* Phosphohexose isomerase *d.* Hexo(fructo)kinase *e.* Invertase *f.* D-enzyme *g.* Amylase *h.* Phosphorylase *i.* Phosphoglucomutase *j.* Glucose phosphate dehydrogenase *k.* Phosphofructokinase *l.* Aldolase *m.* Hexose phosphate "shunt" (not demonstrated in potatoes as yet)



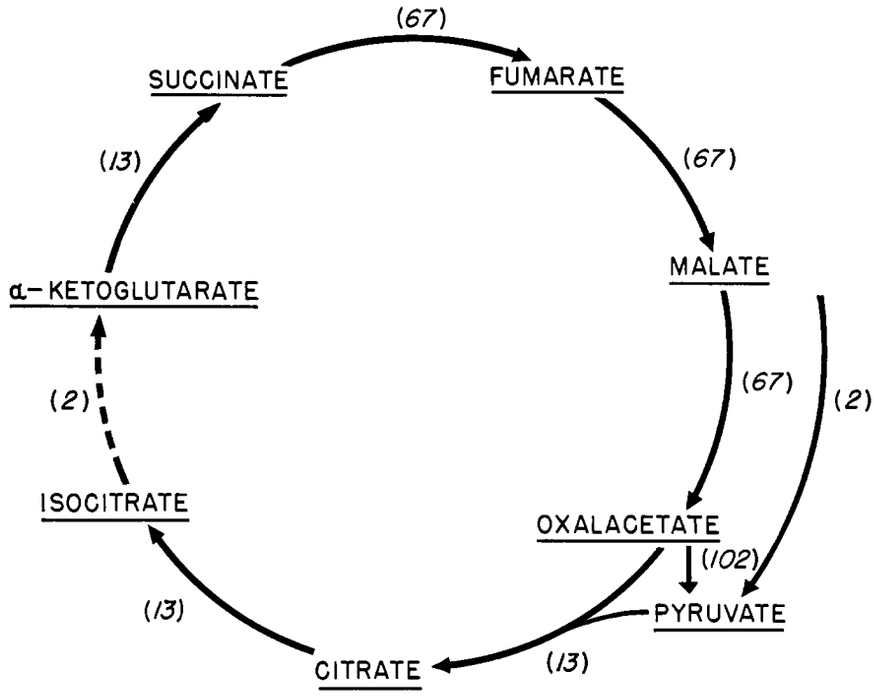


Figure 4. Evidence of enzymes of the tricarboxylic acid cycle (simplified version) in potato tuber

Numbers denote appropriate references demonstrating the described reaction. The dotted arrow indicates that the corresponding enzyme, isocitric dehydrogenase, is reported to be absent in potato juice.

is involved during growth of the tuber (*100*).

There is evidence for the existence of phenol oxidases in potato tissue other than the polyphenol oxidase or tyrosinase under discussion. Thus laccase, which differs from tyrosinase in that it can oxidize hydroquinone, has been detected in potato (*19*). Two specific potato phenol oxidases different from both laccase and tyrosinase have been reported; one will oxidase certain indophenol dyes (*91*) and the specific substrate of the other is scopulitin, a coumarin derivative which accumulates in potato plants suffering from certain virus diseases (*3*).

Little or none of an enzyme known as diaphorase, which has been considered to be a link between the pyridine nucleotide dehydrogenase enzymes and the cytochrome oxidase system, has been detected in potatoes (*60*).

Other oxidative enzymes found in potatoes include peroxidase, which transfers oxygen from hydrogen peroxide to a suitable reducing substance, and catalase, which liberates molecular oxygen from hydrogen peroxide. Tests for peroxidase are used frequently for testing the adequacy of blanching of potatoes (*68*). The catalase content of potatoes decreases rapidly during the first 3 or 4 weeks after harvest (*27*).

Enzymes Involved in Nitrogen Metabolism

Although there is a substantial amount of nitrogen (1%) (*24*) in potatoes, much of which can be found in protein and some 22 amino acids (*25*), very little is known about the enzymes which affect these compounds. Potato juice has the ability to liberate tyrosine from proteins and hence contains some proteolytic activity (*81*). A polypeptide which may be similar to the proteinase inhibitors in lima and soybeans has been obtained from potatoes (*108*). This polypeptide inhibits the action of kallikrein, an enzyme, probably proteolytic (found in snake venom, urine, and pancreas), which converts a fraction of blood serum globulin (kallidinogen) to kallidin (bradykinin), which lowers the blood pressure. This kallikrein inhibitor is found only in freshly harvested potatoes or potatoes that have been stored at low temperatures. Storage at room temperature or higher results in progressive loss of the inhibitor. Potatoes also contain a transaminase (*13, 58*) which catalyzes the following transfer of an α-amino group:

Glutamic acid + oxalacetic acid → ketoglutaric acid + aspartic acid

but not the closely related transaminase:

Glutamic acid + pyruvic acid → ketoglutaric acid + alanine

The widely distributed L-amino acid oxidase has been reported as being absent in potato (*13*).

The free amino acids seem to constitute a reserve source of nitrogen for protein synthesis (*25, 93*). Protein synthesis from the amino acids can be shown to occur in thin potato disks in the presence of oxygen—that is, the energy of respiration is required for the synthesis (*93*). The soluble nitrogen for the synthesis is drawn from a variety of amino acids, which disappear, but the relatively large amounts of glutamine, asparagine, and the corresponding acids (glutamic and aspartic) remain. This is in accord with the recent ideas that protein synthesis may proceed through glutamic acid and glutathione (*37*) (transpeptidation), which is also present in potatoes (*33*). There is an enzyme present in potato which reduces oxidized glutathione (GSSG) in the presence of the reduced coenzyme triphosphopyridine nucleotide (TPNH) (*2*) according to the equation:

$$TPNH + H^+ + GSSG \rightleftarrows 2GSH + TPN^+$$

The relationship of this enzyme to other systems has been discussed. This nucleotide is also the coenzyme for the enzyme nitrate reductase present in relatively small amount in the potato (*28, 62*):

$$TPNH + H^+ + NO_3^- \rightarrow NO_2^- + TPN^+ + H_2O$$

Literature Cited

(1) Allen, R. J. L., Dept. Sci. Ind. Research (Brit.), *Rept. Food Invest. Board*, **1938,** 196–8 (1939).
(2) Anderson, D. G., Stafford, H. A., Conn, E. E., and Vennesland, B., *Plant Physiol.*, **27,** 675–84 (1952).
(3) Andreae, W. A., *Nature*, **170,** 83–4 (1952).
(4) Arreguin-Lozano, B., and Bonner, J., *Plant Physiol.*, **24,** 720–38 (1949).
(5) Axelrod, B., and Bankurski, R. S., *J. Biol. Chem.*, **204,** 939–48 (1953).
(6) Bailey, J. M., Thomas, G. J., and Whelan, W. J., *Biochem. J.*, **49,** lvi-lvii (1951).
(7) Bailey, J. M., and Whelan, W. J., *Ibid.*, **51,** xxxiii (1952).
(8) Baker, D., and Nelson, J. M., *J. Gen. Physiol.*, **26,** 269–75 (1943).
(9) Barker, J., *Proc. Roy. Soc.* (*London*), **119B,** 453–73 (1935).
(10) Barker, J., and el Saifi, A. L., *Ibid.*, **140B,** 362–403, 508–50 (1952).
(11) Barker, J., and Mapson, L. W., *New Phytologist*, **51,** 90–115 (1952).
(12) Barker, J., and Mapson, L. W., *Proc. Roy. Soc.* (*London*), **141B,** 321–67 (1953).
(13) Barron, E. S. G., Link, G. K., Klein, R. M., and Michel, B. E., *Arch. Biochem.*, **28,** 377–98 (1950).
(14) Bate-Smith, E. C., Dept. Sci. Ind. Research (Brit.), *Rept. Food Invest. Board*, **1952,** 19–20 (1953).
(15) Baum, H., and Gilbert, G. A., *Nature*, **171,** 983 (1953).
(16) Baur, E., and Ruf, H., *Helv. Chim. Acta*, **25,** 523–7 (1942).
(17) Bergstrom, S., Theorell, H., and Westman, A. A., *Arkiv Kemi Mineral. Geol.*, **19B,** No. 6 (1944).

(18) Bodnar, J., *Biochem. Z.*, **73,** 193–210 (1916).
(19) Bonner, J., "Plant Biochemistry," New York, Academic Press, 1950.
(20) Boswell, J. G., and Whiting, G. C., *Ann. Botany (N. S.)*, **2,** 847–63 (1938).
(21) Boswell, J. G., and Whiting, G. C., *New Phytologist*, **39,** 241–65 (1940).
(22) Bottle, R. T., Gilbert, G. A., Greenwood, C. T., and Saad, K. N., *Chemistry and Industry*, **1953,** 541–2.
(23) Bourne, E. J., and Peat, S., *J. Chem. Soc.*, **1945,** 877–88.
(24) Brautlecht, C. A., and Getchell, A. S., *Am. Potato J.*, **23,** 531–50 (1951).
(25) Dent, C. E., Stepka, W., and Steward, F. C., *Nature*, **160,** 683 (1947).
(26) Ducet, G., *Biochem. J.*, **51,** 433–42 (1952), thesis, Paris, cited by M. Holden.
(27) Emilsson, B., *Acta Agr. Suenaca*, **3,** 189–282 (1949).
(28) Evans, H. J., and Nason, A., *Plant Physiol.*, **28,** 233–54 (1953).
(29) French, D., and Wild, G. M., *J. Am. Chem. Soc.*, **75,** 4490–2 (1953).
(30) Gilbert, G. A., and Patrick, A. D., *Biochem. J.*, **51,** 181–90 (1952).
(31) Goddard, D. R., and Holden, C., *Arch. Biochem.*, **27,** 41–7 (1950).
(32) Green, D. E., and Stumpf, P. K., *J. Biol. Chem.*, **142,** 355–66 (1942).
(33) Guthrie, J. D., *Contrib. Boyce Thompson Inst.*, **5,** 331–50 (1933).
(34) *Ibid.*, **6,** 247–68 (1934).
(35) *Ibid.*, **9,** 293–7 (1937).
(36) Hanes, C. S., *Proc. Roy. Soc. (London)*, **129B,** 174–208 (1940).
(37) Hanes, C. S., Hird, F. J. R., and Isherwood, F. A., *Nature*, **166,** 288–92 (1950).
(38) Hasse, K., and Specht, M., *Biochem. Z.*, **322,** 502–6 (1952).
(39) Hehre, E. J., *Advances in Enzymol.*, **11,** 297–337 (1951).
(40) Helferich, B., and Stetter, H., *Ann.*, **558,** 234–41 (1947).
(41) *Ibid.*, **560,** 191–201 (1948).
(42) Hidy, P. H., and Day, H. G., *J. Biol. Chem.*, **160,** 273–82 (1945).
(43) Hobson, P. N., and Whelan, W. J., *J. Chem. Soc.*, **1951,** 1451–9.
(44) Ivanov, I. I., and Ivanov, T. I., *Doklady Akad. Nauk, S.S.S.R.*, **77,** 657–60 (1951).
(45) Johnson, G., *Science*, **115,** 675 (1952).
(46) Johnson, S. W., and Zilva, S. S., *Biochem. J.*, **31,** 438–53 (1937).
(47) Kalckar, H. M., *J. Biol. Chem.*, **153,** 355–67 (1944).
(48) Kerr, R. W., and Cleveland, F. C., *J. Am. Chem. Soc.*, **74,** 4036–7 (1952).
(49) Kornberg, A., and Pricer, W. E., Jr., *J. Biol. Chem.*, **182,** 763–78 (1950).
(50) Kotelnikova, A. V., *Doklady Akad. Nauk, S.S.S.R.*, **73,** 945–8 (1951).
(51) *Ibid.*, **78,** 737–40 (1951).
(52) Kresnoski, J. E., Lee, K. H., and Eiler, J. J., Abstracts, 123rd Meeting, Am. Chem. Soc., p. 23C, 1953.
(53) Krishnan, P. S., *Arch. Biochem.*, **20,** 261–71 (1949).
(54) *Ibid.*, pp. 272–83.
(55) Kubowitz, F., *Biochem. Z.*, **292,** 221–9 (1937).
(56) Leary, J. B., and Clagett, C. O., *Proc. N. Dak. Acad. Sci.*, **5,** 47–51 (1951).
(57) Lee, K. H., and Eiler, J. J., *Science*, **114,** 393–5 (1951).
(58) Leonard, M. J. K., and Burris, R. H., *J. Biol. Chem.*, **170,** 701–9 (1947).
(59) Levy, H., and Schade, A. L., *Arch. Biochem.*, **19,** 273–86 (1948).
(60) Lockhart, E. E., *Biochem. J.*, **33,** 613–17 (1939).
(61) McCready, R. R., Ph.D. thesis, University of California, 1944.
(62) Marvalhas, K., *Dusenia*, **2,** 239–96 (1951).
(63) Massart, L., and Vermeyen, K., *Naturwissenschaften*, **30,** 170–1 (1942).
(64) Meeuse, B. J. D., Van der Eijk, A., and Latusan, H. E., *Biochem. et Biophys. Acta*, **8,** 478–9 (1951).
(65) Michlin, D., and Severin, B., *Biochem. Z.*, **237,** 339–42 (1931).
(66) Miller, L. P., Guthrie, J. D., and Denny, F. E., *Contrib. Boyce Thompson Inst.*, **8,** 41–61 (1936).
(67) Millerd, A., *Proc. Linnean Soc. N. S. Wales*, **76,** 123–32 (1951).
(68) Morris, H. J., and Lineweaver, H., *Food Packer*, **26** (1), 40, 42 (1945).
(69) Muller-Thurgau, H., *Landwirtsch. Jahrb.*, **11,** 751–828 (1882).
(70) Naganna, B., *Current Science*, **20,** 101 (1951).
(71) Nakamura, M. J., *J. Agr. Chem. Soc. Japan*, **24,** 5–8 (1950).
(72) Novelli, G. D., *J. Cellular Comp. Physiol.*, **41,** 67–88 (1953).
(73) Nussenbaum, S., and Hassid, W. Z., *J. Biol. Chem.*, **196,** 785–92 (1952).
(74) Peat, S., *Advances in Enzymol.*, **11,** 339–75 (1951).
(75) Peat, S., Whelan, W. J., and Reese, W. R., *Nature*, **172,** 158 (1953).
(76) Perlmann, G. E., *J. Am. Chem. Soc.*, **74,** 6308–9 (1952).
(77) Perlmann, G. E., *J. Gen. Physiol.*, **35,** 711–26 (1952).
(78) Ponting, J. D., unpublished observation.
(79) Potter, A. L., and Hassid, W. Z., *J. Am. Chem. Soc.*, **70,** 3774–7 (1948).
(80) Ross, A. F., *Advances in Food Research*, **1,** 257–90 (1948).
(81) Ross, A. F., and Tottingham, W. E., *J. Agr. Research*, **57,** 433–41 (1936).
(82) Ross, A. F., Tottingham, W. E., and Nagy, R., *Plant Physiol.*, **14,** 549–57 (1939).
(83) Rudkin, G. O., and Nelson, J. M., *J. Am. Chem. Soc.*, **69,** 1470–5 (1947).
(84) Saltman, P., *J. Biol. Chem.*, **200,** 145–54 (1953).
(85) Schade, A., Bergmann, L., and Byer, A., *Arch. Biochem.*, **18,** 85–96 (1948).
(86) Schramm, G., and Flammersfeld, G., *Naturwissenschaften*, **34,** 216–17 (1947).
(87) Schwimmer, S., and Olcott, H. S., *J. Am. Chem. Soc.*, **75,** 4855–6 (1953).
(88) Schwimmer, S., and Pardee, A. B., *Advances in Enzymol.*, **14,** 375–409 (1953).
(89) Sizer, I. W., and Prokesch, C. E., *J. Pharmacol. Exptl. Therap.*, **84,** 363–74 (1945).
(90) Smith, F. G., *Plant Physiol.*, **27,** 736–44 (1952).
(91) Smith, F. G., and Stotz, E., *J. Biol. Chem.*, **179,** 865–80 (1949).
(92) Stetter, H., "Enzymatische Analyse," pp. 14–26, 192, Wernheim, Verlag Chemie, 1951.
(93) Steward, F. C., and Street, H. E., *Ann. Rev. Plant Physiol.*, **3,** 17–34 (1952).
(94) Stumpf, P. K., *Ibid.*, **3,** 17–34 (1952).
(95) Sullmann, H., *Helv. Chim. Acta*, **26,** 2253–63 (1943).
(96) Sumner, R. J., *Ind. Eng. Chem., Anal. Ed.*, **15,** 14–15 (1943).
(97) Sussman, A. S., *Nuclear Science Absts.*, **7,** No. 718, 87 (1953).
(98) Thoai, N., and de Bernard, L., *Compt. rend.*, **232,** 1152–4 (1951).
(99) Thoai, N., Roche, J., and Saltman, P., *Compt. rend. soc. biol.*, **144,** 1588–9 (1950).
(100) Todd, G. W., dissertation, University of Missouri, 1952; *Biol. Abstr.*, **27,** 10859 (1953).
(101) Trevelyan, F., Mann, P. E., and Harrison, J. S., *Arch. Biochem.*, **39,** 419–49 (1952).
(102) Vennesland, B., *J. Biol. Chem.*, **178,** 591–7 (1949).
(103) Vennesland, B., and Felshor, R., *Arch. Biochem.*, **11,** 279–306 (1946).
(104) Wallerstein, J. S., Alba, R. T., and Hale, M. G., *Biochem. et Biophys. Acta*, **1,** 175–89 (1947).
(105) Wallerstein, J. S., Bergmann, L., Alba, R. T., and Schade, A. L., *Food Research*, **12,** 44–54 (1947).
(106) Warner, C., *Australian J. Sci. Research*, **4B,** 554–60 (1951).
(107) Weibull, C., and Tiselius, A., *Arkiv Kemi Mineral. Geol.*, **19,** No. 19 (1945).
(108) Werle, E., and Maier, L., *Biochem. Z.*, **322,** 414–17 (1952).
(109) Wolff, A. J., *Ibid.*, **176,** 225–45 (1926).
(110) Wright, R. C., and Peacock, W. M., *Ice and Cold Storage*, **41,** 212 (1938).

Received for review May 11, 1953. Accepted October 12, 1953.