CARBOHYDRATE METABOLISM IN DEVELOPING POTATOES

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Introduction

In this essay we consider the extent to which we know the sequence and organization of the pathways responsible for the immediate metabolism of the sugar that is delivered to the developing tubers of potato plants. The topic is important because such knowledge is essential if we are to make rational attempts to alter the composition and behavior of this crop. In order to take advantage of the ability of molecular biologists to transform potato plants we must be able to identify which genes to transform to produce a given effect; that is, we must discover how metabolism of the tuber is controlled. The initial requirement for the study of control is to establish the relevant pathways and their organization.

The first point to grasp about the investigation of potato tuber metabolism is that it is exceedingly difficult. The problems of measuring enzymes and substrates, difficult in plants in general (2), are even more so in potatoes. The causes of this interactability are not clearly established, but are almost certainly due to the release and formation of inhibitory phenolic substances during the preparation of extracts, and the persistence in such extracts of robust and active phosphatases. Whatever the causes, the effects can be spectacularly misleading. For example, attempts to compare the maximum catalytic activities of glucose-6-phosphate dehydrogenase and phosphofructokinase in mature tubers showed that only 10% of the dehydrogenase but as much as 70% of the kinase were lost during extraction (38). Taken at their face value activities in such extracts would give a totally erroneous view of the tuber's capacity to catalyze these two reactions. Similar pitfalls occur when measuring substrates. A method that gives accurate values for the amount of fructose 6-phosphate in tubers led to complete loss of fructose-1,6-bisphosphate (11). These, and other observations (2), make it mandatory to authenticate estimates of enzyme activity and substrate content with recovery experiments designed to measure the extent to which pure enzyme or substrate added to tuber tissue survives extraction and assay. The design of such experiments has been discussed in detail (2, 3). Data presented in this essay meet these requirements. The difficulties of working with potatoes mean that many of the key observations, on which our understanding of carbohydrate metabolism in non-

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photosynthetic cells of higher plants in general is based, have not been made for potatoes. We emphasize this distinction throughout the essay.

The Metabolism of Tubers Begins with Sucrose

In general the nonphotosynthetic cells of higher plants obtain most of their carbon for biosynthesis, and their energy, as sucrose delivered via the translocation stream (5). This is almost certainly true for potatoes, (see previous article by Dwelle). Oparka and Prior (36) exposed leaves of potato plants to $^{14}CO_2$ and analyzed the distribution of label in microcores taken from the perimedulla of the tubers, in exudates from cut stolons, and in solutions placed in wells cut into tubers. In all instances over 85% of the label in the water-soluble compounds was in sucrose. It is highly likely that this sucrose arrives in the tubers via the plasmadesmata. First, Oparka and Prior (37) have demonstrated the existence of an extensive symplasmic pathway in tubers. Second, the effects of varying solute concentration on sucrose efflux in wells cut into tubers strongly suggest that most of the sucrose moved via the symplasm (36). Even if there is movement from phloem to tuber cells via the free space, it probably occurs as sucrose taken into the cells via a sucrose-proton co-transport system (7). Tuber tissue has been shown to take up sucrose readily and to do so without hydrolyzing it (16). Thus, regardless of whether transport is symplasmic or apoplastic, the tuber cells almost certainly receive their carbon and energy as sucrose delivered into their cytosol.

Partitioning of Sucrose Delivered to the Tuber

On arrival in the tuber sucrose is partitioned between starch, structural polysaccharide, storage as sucrose or hexose, and entry into the respiratory pathways to provide ATP, reducing power, and carbon skeletons for synthesis (mainly amino acids, organic acids and lipids). We cannot find a detailed quantitative balance sheet of this partitioning. Data from Oparka (35), and Mares and Marschner (29) show that 50 to 70% of the sucrose carbon goes to starch, and no more than about 5 to 10% to structural polysaccharides. The remainder is divided between respiration and storage. Thus the major fates of the incoming sucrose are conversion to starch, entry into respiration, and storage; their relative magnitude will be determined in the first instance by the relative activities of proteins that can react with sucrose in the cytosol. These are probably represented by alkaline invertase, sucrose synthase, and tonoplast proteins responsible for transport of sucrose from cytosol to vacuole.

Alkaline invertase has been characterized from soybean nodules and sycamore cells, but not from potatoes. This enzyme catalyses an irreversible hydrolysis of sucrose for which it has a *K*m of 10-65 mM (5). Alkaline invertase has been demonstrated in developing tubers of three potato cul-

tivars at three different stages of development (31). The intracellular location of alkaline invertase in potatoes has not been established; experiments with soybean cells strongly suggest that this enzyme is confined to the cytosol (28).

Sucrose synthase catalyzes the readily reversible reaction.

$$\text{Sucrose} + \text{UDP} \rightleftharpoons \text{UDPglucose} + \text{fructose} \qquad \Delta G' = -4.2 \text{ kJ}$$

The *K*m for sucrose is high, 20-50 mM. Sucrose synthase has been partially purified and characterized from developing tubers (41), and is readily demonstrable in extracts of developing tubers (31). The intracellular location of sucrose synthase in potatoes has not been established. Careful fractionation of soybean cells (28), castor bean endosperm (34), and tubers of Jerusalem artichoke (24) leave little doubt that this enzyme is almost, if not entirely, confined to the cytosol.

The location of sucrose in potato tubers has not been demonstrated. By analogy with other storage tissues such as beetroot (26), it is highly likely that most of the sucrose is in the vacuole, with a relatively small amount in the cytosol. The way in which sucrose is moved into the vacuole is not known for potatoes, and we have only a hazy idea for plants in general. Such information that is available indicates that transport into the vacuole may be via a translocator, possibly a H^+/sucrose antiport with an apparent *K*m of 12-25 mM (18). Within the vacuole is the only other enzyme in higher plants capable of metabolizing sucrose. This is acid invertase, which catalyzes sucrose hydrolysis at pH 4 to 5, is present in developing tubers (31), and has been extensively purified from mature tubers and shown to be a glycoprotein (1). In beetroot this enzyme is almost entirely confined to the vacuole (26). In some tissues some activity is associated with the cell wall. No activity could be found in cell-wall preparations from developing tubers (31), thus it is probable that acid invertase in such tubers is confined to the vacuole.

On arrival in the cytosol sucrose may be metabolized by alkaline invertase and sucrose synthase, or moved into the vacuole where it may be stored as sucrose or hydrolyzed by vacuolar acid invertase to give hexose that may remain in the vacuole or possibly return to the cytosol. Two pieces of evidence collectively suggest that the major fate of newly arrived sucrose in the tuber is conversion to fructose and UDPglucose by sucrose synthase. First, the sugar content of developing tubers is low, Mares and Marschner (29) quote 0.4 to 1.0% of the fresh weight; the values for starch were 7 to 11%. This means that relatively little of the incoming sucrose is stored as sugar. Second, estimates of the maximum catalytic activities of the two invertases and sucrose synthase during the development of three varieties of tuber strongly suggest that the invertases are not present in sufficient activity to mediate more than a small fraction of sucrose breakdown *in vivo*

(31). It is difficult to measure the latter, but the rate of starch accumulation can be determined. As much of the sucrose is converted to starch, the rate of starch accumulation may be taken as a minimal estimate of sucrose breakdown. The data in Table 1 show that the observed activities for each invertase are well below the rate of starch accumulation, whilst the activities for sucrose synthase exceed the rate of starch accumulation.

Our estimates of invertase (Table 1) are complicated by the fact that potatoes synthesize a protein of about 17 000 *Mr* that is a non-competitive inhibitor of acid invertase (9, 39). If this inhibitor is present in significant quantity in developing tubers, and it is important to appreciate that there is no evidence that it is, then the values for acid invertase in Table 1 may be underestimates. However, if, as the work of Pressey (40) indicates, synthesis of the inhibitor is a means of varying the maximum catalytic activity of acid invertase, then it is likely that *in vivo* the inhibitor will combine with the enzyme as the inhibitor is made. Thus the invertase activity in carefully prepared extracts is likely to reflect the actual capacity *in vivo*. Even if this is not so, a significant role for acid invertase in the immediate metabolism of the incoming sucrose is unlikely as the sucrose would have to escape the attentions of the cytosolic sucrose synthase in order to reach the acid invertase in the vacuole. The available evidence suggests that the inhibitor does not react with the alkaline invertase, but this needs further investigation. The inhibitor is specific in that it does not inhibit invertase from either yeast or *Neurospore* (40). Further, the inhibitor had no detectable effect on invertase activity at pH 7.0 in unfractionated extracts of tubers (40).

TABLE 1.—*Maximum catalytic activities of enzymes of sugar metabolism and rate of starch accumulation in developing tubers of Pentland Javelin potatoes.*

Enzyme	Reference	Enzyme activity (nmol per min/g fresh wt.) in tubers of fresh wt.[1]		
		1g	10g	20g
Sucrose synthase	31[2]	340±10	220±30	220±20
Alkaline inverase		12± 4	7± 1	8± 1
Acid invertase		2± 1	2± 1	3± 1
Hexokinase		78±10	43± 5	43± 5
PFK (PP_i)		810±40	710±70	860±70
Phosphofructokinase		88±11	78±10	75± 7
UDPglucose pyrophosphorylase			31,400±1,300	
UDPglucose phosphorylase	14		None detected	
Fructose-1,6-bisphosphatase	13		None detected	
Starch accumulation over developmental period studied	31[2]		47	

[1]Values are means±s.e.m. of estimates from 3-5 tubers.
[2]Similar data were obtained for 2 other cultivars, King Edward and Maris Bard.

We suggest that the initial metabolism of sucrose in developing tubers proceeds via sucrose synthase, with alkaline invertase making a smaller contribution.

Studies of a range of plants have shown that they contain specific fructokinases and hexokinases with a preference for glucose. At least one of each type is located in the cytosol (5). The ability of extracts of developing tubers to phosphorylate fructose and glucose is appreciable (Table 1). We suggest that the fructose formed by sucrose synthase, and any hexose formed by alkaline invertase, are promptly converted to hexose 6-phosphates by these kinases in the cytosol.

Some of the UDPglucose formed by sucrose synthase is probably used directly for the synthesis of cell-wall polysaccharides. As only a small fraction of the incoming sucrose is used in cell wall synthesis, the bulk of the UDPglucose formed from this sucrose must be converted to hexose monophosphates for further metabolism. A general failure to confirm the presence of appreciable activity of UDPglucose phosphorylase in plants makes it unlikely that this is the route from UDPglucose to hexose monophosphate (5, 44). In addition, there is now considerable evidence, given in detail in (5, 7), for the following pathway.

$$\text{Sucrose} + \text{UDP} \xrightarrow[\textit{Sucrose synthase}]{} \text{Fructose} + \text{UDPglucose}$$

$$\text{Fructose} + \text{UTP} \xrightarrow[\textit{Fructokinase}]{} \text{Fru-6-}P + \text{UDP}$$

$$\text{UDPglucose} + \text{PP}_i \xrightarrow[\textit{UDPglucose pyrophosphorylase}]{} \text{Glc-1-}P + \text{UTP}$$

$$\text{Fru-1,6-}P_2 + \text{P}_i \xrightarrow[\textit{PFK(PP}_i\textit{)}]{} \text{Fru-6-}P + \text{PP}_i$$

The essence of this scheme is that re-cycling of some of the fructose-1,6-bisphosphate, formed in glycolysis, to fructose 6-phosphate by pyrophosphate : fructose 6-phosphate 1-phosphtransferase [PFK(PP_i)] generates enough PP_i to maintain the UDPglucose pyrophosphorylase step close to equilibrium. The whole sequence is confined to the cytoplasm. The following is direct evidence that this sequence operates in developing tubers. Each enzyme of the scheme has been demonstrated to present at adequate activity at three stages of tuber development in three different cultivars (Table 1). Developing tubers contain sufficient Fru-2,6-P_2 to allow PFK(PP_i) to function. The content of UDPglucose falls when the supply of sucrose is cut off (31). Finally, there is now direct evidence of substrate cycling between Fru-1,6-P_2 and Fru-6-P in developing tubers (17). The missing link in the evidence from tubers is that the requisite concentration of PP_i has yet to be demonstrated. This is almost certainly a technical problem caused

by persistence of pyrophosphatase in extracts made with $HClO_4$; a problem that can probably be solved by using the procedure of Weiner *et al.* (45).

Starch Synthesis

Although the idea that both ADPglucose and UDPglucose served equally as immediate precursors of starch arose in part from studies of potatoes (27), the present evidence now strongly suggests that only ADPglucose is used. This evidence has recently been reviewed (7) and is now summarized. No UDPglucose-dependent starch synthase has been purified from any plant tissue. Nobody has shown activities of "UDPglucose-dependent" starch synthase in tissue extracts that are comparable to the rates at which starch synthesis occurs *in vivo*. ADPglucose-dependent starch synthase, ADPglucose pyrophophorylase and alkaline pyrophosphatase are present in sufficient amounts to mediate the rates of starch synthesis found *in vivo*, and are confined to the plastid. In contrast UDPglucose pyrophosphorylase and UDPglucose itself are absent from plastids and largely, if not entirely, confined to the cytosol. We suggest that starch synthesis in potato tubers proceeds through the following reactions:

ADPglucose pyrophosphorylase

$$\text{Glc-1-}P + \text{ATP} \rightleftharpoons \text{ADPglucose} + PP_i$$

ADPglucose starch synthase

$$\text{ADPglucose} + \alpha(1\text{-}4)\text{glucan } n \rightarrow \text{ADP} + \alpha(1\text{-}4)\text{glucan } n+1$$

Alkaline inorganic pyrophosphatase

$$PP_i + H_2O \rightarrow P_i + P_i$$

Recent work with mutants of peas confirms that the formation of the $\alpha(1\text{-}6)$ bonds in starch is due to branching enzyme (14, 43). Each of these steps is confined to the plastid. Evidence for this pathway from work with potatoes is restricted to demonstrations that developing tubers contain appreciable activities of ADPglucose pyrophosphorylase and starch synthase, *e.g.* (29). However, the consensus of evidence from tissues as widely different as wheat endosperm, soybean suspension cultures, and *Arum* spadix (7) make it very unlikely that potatoes will prove to be an exception to the pathway proposed.

The intracellular distribution of the enzymes of sucrose breakdown and starch synthesis means that carbon must move from hexose monophosphates in the cytosol to glucose 1-phosphate in the amyloplast. Two hypotheses have been proposed to explain such movement. The C-3 hypothesis holds that the immediate products of sucrose breakdown are converted by glycolysis in the cytosol to triose phosphates, which then enter the plastid via the phosphate translocator; once in the plastids triose phosphates are said to be converted to hexose 6-phosphates via aldolase and

fructose-1,6-bisphosphatase. The alternative, or C-6, hypothesis holds that carbon for starch synthesis enters the amyloplast as hexose monophosphate. These views have been discussed recently (7, 23) and for wheat endosperm, at least, we accepted the C-6 hypothesis and suggested that glucose 1-phosphate entered the amyloplast.

There is now clear evidence against the C-3 hypothesis for developing tubers. First, on this hypothesis movement of carbon from hexose phosphate in the cytosol into starch in the amyloplast would entail the almost complete equilibration of hexose carbons 1, 2, and 3 with carbons 6, 5, 4, respectively. This would result from equilibration of the triose phosphates formed during the conversion of the incoming sucrose to starch. Such equilibration would occur both in the cytosol and in the amyloplast. Hatsfeld and Stitt (17) supplied developing tubers with [1-^{14}C]-, and [6-^{14}C]glucose, and showed that only 13% of C-1 and 8% of C-6 were redistributed during incorporation into starch; that is, there was little equilibration between the two halves of the hexose molecule during starch synthesis. The second count against the C-3 hypothesis for potatoes is the extensive evidence that they lack the plastidic fructose-1,6-bisphosphatase required by this pathway (13). Claims to the contrary (30) may be explained by the ease with which PFK(PP_i) may be taken for fructose bisphosphatase (12, 13). Similarly, claims (30) that amyloplasts from potatoes can convert triose phosphate to starch are not conclusive because the amyloplast preparations used were contaminated with cytosol so that much of the [^{14}C]fructose-1,6-bisphosphate added to generate labelled triose phosphate was probably converted to hexose monophosphate by PFK(PP_i), hexosephosphate isomerase and phosphoglucomutase (13). Thus we suggest that carbon enters the amyloplasts of developing tubers as hexose monophosphate; until physiologically active amyloplasts of tubers can be prepared and studied in detail, we cannot decide precisely which hexose monophosphate crosses the amyloplast envelope.

Carbohydrate Oxidation

Quantitatively, after starch synthesis, the next most important fate of sucrose arriving in a tuber is entry into the pathways of respiration. Although the respiratory metabolism of slices of potatoes has been studied extensively, the difficulties of investigating the metabolism of whole tubers mean that we have relatively little data that have been obtained with tubers as such. It is important to realize that slices differ from intact tubers. The rate of respiration of thin freshly cut slices of tuber is 3 to 5 times that of the intact tuber, and incubation of these slices in aerated water leads to a further 4- to 5-fold increase in respiratory rate. In addition the substrate and pathways of respiration in freshly cut slices differ markedly from those operating in the tuber (19).

The $^{13}C/^{12}C$ ratios of intact tubers provide clear evidence that their respiratory substrate is carbohydrate (19). Comparative biochemistry, of which there is a great deal (4), very strongly suggests that developing tubers are like other plant tissues studied and oxidize carbohydrate via glycolysis and the oxidative pentose phosphate pathway. The relative activities of the two pathways will vary according to conditions and the state of development, but glycolysts will predominate with the pentose phosphate pathway accounting for, perhaps, 10 to 25% of the total. The latter produces fructose 6-phosphate and glyceraldehyde 3-phosphate; some of the fructose 6-phosphate may be recycled through the pathway but the bulk of the products of the pathway will be converted to phosphoenolpyruvate and pyruvate as shown in Fig. 1. The significance of the pentose phosphate pathway is that it allows the synthesis of $NADPH_2$ required for biosynthesis via a route that is largely independent of the steps used in the synthesis of ATP. Both glycolysis and the pentose phosphate pathway will contribute to the

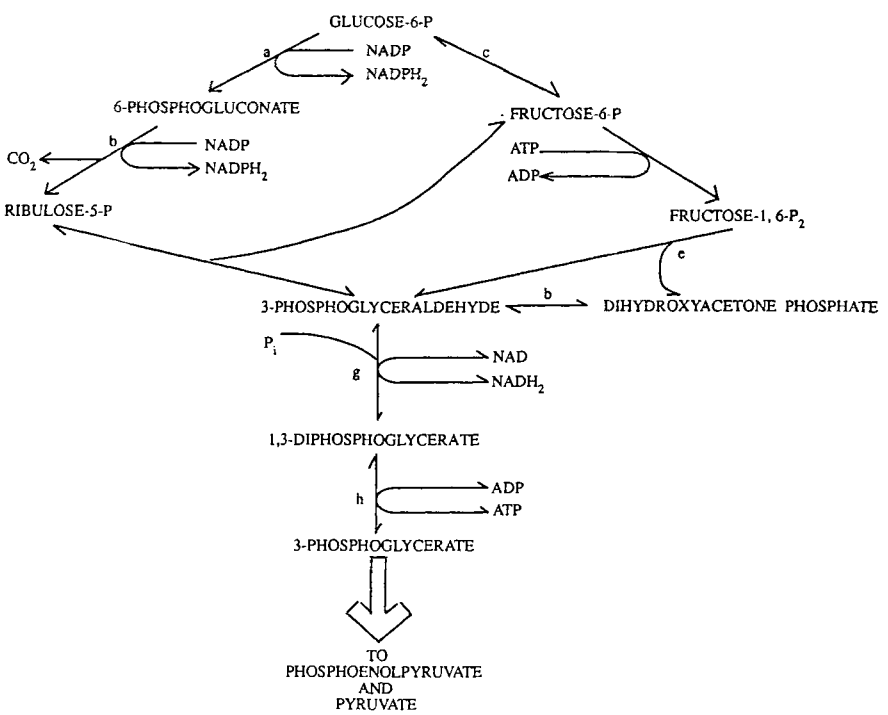

FIG. 1. Scheme to show the relationship between glycolysis and the oxidative pentose phosphate pathway in the cytosol. Letters denote enzymes as follows: a, glucose-6-phosphate dehydrogenase; b, 6-phosphogluconate dehydrogenase; c, glucosephosphate isomerase; d, phosphofructokinase; e, fructose-bisphosphate aldolase; f, triosephosphate isomerase; g, glyceraldehydephosphate dehydrogenase; h, phosphoglycerate kinase.

provision of carbon skeletons needed for biosynthesis. In rapidly growing tubers the respiratory carbon used in biosynthesis is likely to be comparable to that oxidised to CO_2(4).

Apart from comparative biochemistry, there is the following major evidence that the scheme shown in Fig. 1 operates in tubers. First, each of the enzymes of glycolysis and the two key dehydrogenases of the pentose phosphate pathway have been demonstrated in extracts of potato tubers (21, 22). Second, when tubers are made anoxic the products of glycolysis accumulate (8). Third, changes in glycolytic intermediates that occur when the rate of CO_2 production of tubers is varied are entirely consistent with the operation of glycolysis in the intact tuber (11). Finally, both pathways have been shown to mediate the respiration of tuber slices (6).

Studies of a sufficient range of plant tissues have established that a distinctive feature of plant respiration is that the pathways of carbohydrate oxidation are compartmented (4). No data are available for tubers but it is safe to assume the following. First, the complete sequence of glycolysis and the pentose phosphate pathway operates in the cytosol as shown in Fig. 1. Second, substantial fractions (15 to 45%) of the total tuber content of many of the enzymes of both pathways are confined to the plastids. The precise extent of this duplication of the pathways in the plastids has been found to vary with the plastid (4). Insufficient plastids have been examined to permit prediction of the situation in developing tubers. However, we must now appreciate that entry of substrate into the respiratory pathways of developing tubers involves the simultaneous operation of two pathways in the cytosol and at least parts of the same two pathways in the plastid. Thus measurements of the total tuber content of the pathway intermediates or enzymes will not necessarily reveal what is taking place in any specific compartment.

Storage as Sugars

As we indicated earlier we know little of the mechanism that results in storage of incoming sucrose either as sucrose or as hexose. In view of the commercial importance of the sugar content of potatoes, this ignorance is particularly reprehensible. The following questions should be addressed. First, does storage only occur when supply exceeds the rate at which the tuber can metabolize the incoming sucrose, or is a fraction of the input always stored? Second, are the sugars stored in the vacuole? The work referred to earlier suggests that this is so for sucrose, but there is little definitive information for glucose and fructose. Third, how do sugars get in and out of the vacuole? There is little to add to what we have said already except to argue that studies with a wide range of tissues other than potatoes have provided compelling evidence that the maximum catalytic activity of vacuolar acid invertase is a primary determinant of the extent to which sucrose is stored in the vacuole (5). There is a widespread inverse relationship be-

tween acid invertase activity and sucrose content. For beetroot we (26) demonstrated such a relationship for isolated vacuoles. Perhaps the simplest explanation is that sucrose arriving in the vacuole is stored there unless it is hydrolyzed by vacuolar acid invertase to hexoses that then move into the cytosol for subsequent metabolism.

Whatever the underlying mechanisms, the amounts of sucrose, glucose and fructose in developing tubers vary widely, within individual tubers, between tubers of the same plant (29, 35), between cultivars and according to growth conditions (10). The factors that affect sugar content are likely to be complex and varied, and to include: the supply of sucrose, the rate and pathway of sucrose breakdown, the relative rates of phosphorylation of glucose and fructose, and the activity of vacuolar acid invertase. As sugars are central to the metabolism of tubers then almost anything that affects metabolism is likely to affect sugar content, and it is hardly surprising that there is so much variation in the latter.

Regulation

The way in which the pathways that we have discussed are regulated is central to our understanding of tuber growth and development, and to any attempt to manipulate these processes in a premeditated way. It is not a very difficult task to genetically engineer potatoes, but it is extremely difficult to determine which genes to engineer. The classic picture of control of metabolism is one in which a minority of strategically placed key "regulatory steps" dominates control of metabolism as a whole (33). If this is so, then redirection of metabolism through genetic engineering may be relatively straightforward. However, evidence to show that this view of control is too simple is gradually accumulating. Kacser has persistently and persuasively argued that control is a property of the pathway as a whole and is shared between each component step so that no one step dominates (20). Further, the extent to which a single step exerts control is seen as varying with the flux through the pathway and the ambient conditions. Studies of the control of pathways as diverse as tryptophane degradation in rat liver (42), gluconeogenesis in rat liver (15), starch synthesis in mutants of *Clivia* (25), and sucrose synthesis in spinach leaves (32), all provide sound experimental proof of Kacser's arguments.

The situation in developing tubers can only be resolved by experiment. The exciting present prospect is that these experiments are now practicable if we use Kacser's formulations in conjunction with transgenic plants, mutants and flux analysis. For example, the contribution that a single step makes to control of the complete pathway is given by the control coefficient,

$$\frac{\partial J}{J} \qquad \frac{\partial V}{V}$$

where J is pathway flux and V is the maximum catalytic activity of the enzyme. If we vary the latter through genetic engineering to produce a range of plants varying in the capacity to catalyze that single step, then measurement of flux will permit us to determine the control coefficient of the enzyme. In this way we will not only be able to obtain a clearer picture of the roles of individual enzymes, but also to estimate their contribution to control. From this information we shall be in a much stronger position to determine whether it is feasible to alter the properties of the potato in a directed and useful way.

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