combination of subsurface phototrophic production and phagotrophic release, Langmuir circulations (*24*), extraction and transport through bubble action during water turbulence (*25*), and the attraction of the hydrophobic groups to the sea-air interface.

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26. Supported in part by the Biological Oceanography Program of the National Science Foundation under grants DES74-01537-A01 and OCE74-01537-A02. The technical assistance of J. Hannon, B. McBurney, and S. Sieburth, as well as the help of the crew and officers of R.V. *Trident* during TR-170, is gratefully acknowledged.

7 July 1976; revised 15 September 1976

Bioenergetic Considerations in Cereal Breeding for Protein Improvement

Abstract. *The bioenergetic implications of changing cereal grain protein concentrations and amino acid compositions by plant breeding are examined. It is shown that increased inputs of carbon assimilates and nitrogen are necessary when increasing protein concentration in cereal grains while maintaining high yields. Also, energetic requirements for obtaining endosperm proteins with a high lysine content in genotypes of maize and barley are slightly higher than in normal lysine stocks. The implications for plant breeding are discussed.*

In plant breeding programs throughout the world considerable emphasis is being placed on improving the nutritional quality of cereals by increasing the protein concentration and altering the protein composition in grains to ameliorate the balance of lysine and other limiting amino acids. A major problem in such programs is combining high grain yield with increased or nutritionally better grain protein. Numerous observations indicate that the grain protein concentration is inversely correlated with yield in cereals (*1*). The underlying basis for this relationship has not been clearly enunciated. The fact that the gross energy (gram-calories per gram) in the dry matter of high-protein grains is higher than in low-protein grains (*2*), and that this energy differential has to be provided by the plant producing the grain, appears to have been neglected. To better understand the situation, an examination of the energetics of grain production is essential.

Recently, Sinclair and de Wit (*3*) considered seed biomass yield and the nitrogen requirement in 24 different crops with seed protein concentrations varying from 8 to 38 percent. Their study included cereals, pulses, and oilseeds. From their calculations, it can be inferred that in any species simultaneous increases in grain protein concentration and grain yield are incompatible from energetic considerations. To achieve both increments there is competition not only for the available carbon skeletons but also for the energy derived from photosynthates. The synthesis of more protein or more carbohydrate in the grain (or more grain) requires the availability of additional photosynthates to developing grains. Furthermore, an increment in nitrogen input is needed to produce additional protein. Alternatively, more efficient utilization of assimilates toward grain production would achieve the same result. In this report we examine, using the rationale of Sinclair and de Wit (*3*), the requirements for additional photosynthate and nitrogen likely to be associated with improvement in protein quantity or quality of cereals. The implications for breeding programs are discussed. Our purpose is to direct the attention of plant breeders and others to these considerations, which in the long term may align breeding expectations with the realities of the problems in breeding for altered chemical composition of grain. We wish to emphasize that these considerations would apply when the true genetic potential of the plant for energy conversion is expressed. This obtains only under favorable growing environments with the essential supplementary inputs.

Table 1. Energetic cost of increasing grain protein concentration in bread wheat.

Component	Amount (g/100g)	PV*	Equivalent glucose required† (g)
Standard cultivar			
Carbohydrate	82	0.83	98.80
Protein	14	0.40	35.00
Lipid	2	0.33	6.06
Minerals	2		
		Total	139.86
Cultivar with 1 percent more grain protein			
Carbohydrate	81	0.83	97.59
Protein	15	0.40	37.50
Lipid	2	0.33	6.06
Minerals	2		
		Total	141.15‡

*Production value (PV) is calculated as (weight of end product)/(weight of substrate required for carbon skeletons and energy production). †Equivalent glucose units are calculated as (amount of component)/PV, where the amount is expressed as grams per 100 g of grain biomass. ‡Thus, the differential glucose requirement is 141.15 − 139.86 = 1.29, or 0.92 percent greater for a cultivar with increased protein.

Our calculations are based largely on the analysis of Penning de Vries *et al.* (*4*) who, after extensive examination of biochemical pathways and the energy requirements of the component reactions, concluded that in plants, under aerobic conditions, 1 g of glucose can be used to produce 0.83 g of carbohydrates, or alternatively 0.40 g of proteins (assuming nitrate to be the nitrogen source) or 0.33 g of lipids. The derivation of these values and the assumptions made have been discussed (*4*). We realize that the biochemical pathways on which these calcula-

tions are based are derived largely from microorganisms and assume that the pathways in crop plants do not differ significantly.

Further, the nitrogen source is considered to be nitrate, which can either (i) be reduced to NH_4^+ in roots, where the energy required for reduction is provided by the photosynthetic assimilates transported from the shoot, or (ii) be transported as such to leaves, where nitrate reduction is carried out, at least under high light intensities, by using reduced nicotinamide adenine dinucleotide phosphate (NADPH), thereby circumventing the use of carbon assimilates (*5, 6*). It is recognized that in a photosynthesizing plant there may be a number of processes, including biosynthetic reactions, which are driven by adenosine triphosphate and NADPH derived directly from the light reactions of photosynthesis without utilization of carbon assimilates for energy. At present, the magnitude and kind of contribution from these sources cannot be calculated.

Increase in grain protein concentration. Since carbohydrates are the major constituents of the dry matter of grain, it is assumed that any increase in the protein percentage of the grain will be associated with a proportionate decrease in the carbohydrate percentage. The energetic cost of such an alteration is illustrated (Table 1) by taking the example of a 1 percent increase of protein in a typical bread wheat variety consisting of 14 percent protein, 82 percent carbohydrate, 2 percent lipid, and 2 percent minerals. The assumption is made that the weights of grain and constituents other than protein and carbohydrate remain constant. The net overall increase of photosynthates required to produce grain with a 1 percent increase in protein would be roughly 1 percent. According to our calculations this would apply to other cereals as well. However, in the case of flooded rice, which takes most of its nitrogen in the form of the ammonium ion, the additional photosynthate requirement would be less, since 1 g of glucose yields 0.67 g of protein when NH_4^+ is the nitrogen source.

These additional photosynthate requirements could be met by having a higher rate of photosynthesis or additional leaf area, by extending the period of photosynthetic activity, or by maximizing the mobilization of photosynthetic reserves into the grain. Several of the alternatives themselves require an energy expenditure, but ultimately it is the net available assimilate that is important. The demand for assimilates is known to increase the rate of photosynthesis, as indicated by leaf excision experiments (*7*). If this additional demand is not met, any increase in protein concentration is likely to be associated with depression of grain yield, which could be limited by (i) assimilate production (source), (ii) capacity for assimilate storage (sink), (iii) both assimilate production and capacity for assimilate storage, or (iv) capacity for translocation (*7*). In situation (ii), it would be relatively easy from the plant breeding viewpoint to meet the additional assimilate requirements for increased grain protein concentration without any decrease in yield. However, modern cereal cultivars with very high yields probably have their photosynthetic capacity and sink size closely balanced (*7*). Increasing grain protein concentration in such cultivars may therefore require considerably greater efforts.

Table 2. Standard chemical compositions and nitrogen requirements (milligrams of N per gram of photosynthate) for cereal grains. Assumed standard composition is from Spector (*8*). [Nitrogen requirement is calculated by assuming that protein is 16 percent nitrogen by weight. The last column gives the percentage increase in nitrogen requirement for a 1 percent increase in protein.]

Crop	Assumed standard composition (% dry matter)				Nitrogen requirement (mg/g)		Increase in nitrogen requirement (%)
	Protein	Carbohydrate	Lipid	Ash	With standard protein	With 1% increase in protein	
Wheat	14	82	2	2	16.0	17.0	6
Rice							
NO_3 as N source	8	88	2	2	9.7	10.8	11
NH_4^+ as N source					10.4	11.7	12
Maize	10	84	5	1	11.3	12.3	9
Barley	9	80	1	4	11.5	12.3	10
Sorghum	12	82	4	2	13.6	14.6	7
Oats	13	77	5	5	14.8	15.8	7
Rye	14	82	2	2	16.0	17.0	6

Nitrogen requirement. The additional nitrogen required for seed production with increased protein was calculated by the method used by Sinclair and de Wit (*3*) and is given as percentage increase in Table 2. Taking the typical protein concentration of various cereals (*8*), we calculated the additional nitrogen requirement for increasing the protein concentration 1 percent (Table 2). It is apparent that each 1 percent increase in grain protein would require an additional 6 to 11 percent nitrogen for grain protein stoichiometry alone, depending on the crop variety and the initial protein concentration. This nitrogen can come either by direct uptake from the soil during grain filling or by greater remobilization of nitrogen present before grain filling in leaves and other plant parts. The former would imply an additional fertilizer requirement for the crop and the latter requires internal energy. It has been estimated that "to relocate 11 mg N, 100 mg of dry matter is expected to be lost from the source organ, and the receiving organ would additionally receive a total of 70 mg of dry matter" (*9*).

Varietal differences in mobilization of nitrogen from leaves are known (*9–12*) and some gain by breeding may be realized in increasing the proportion of plant nitrogen in grain at harvest (PNG) or harvest nitrogen index, a term used by Canvin (*13*). The PNG to a large extent is influenced by the harvest index. A negative correlation between grain protein percent and harvest index or grain/straw ratio has been reported in wheat (*14, 15*). The high yield potential of the current semidwarf varieties is due to their higher harvest index, and such cultivars already have a high PNG [> 70 percent in many (*6*)]. Further, the energetic considerations and the finite limits to what can be remobilized from leaves puts a constraint on breeding gains. However, increased mobilization of nitrogenous material from leaves and the development of root systems that make more efficient utilization of available soil nitrogen are the only avenues for obtaining higher grain protein concentrations without additional nitrogenous fertilizers.

In this context, ideas concerning the introduction of a nitrogen fixation capacity into cereals as a symbiotic process (*16*) need to be considered in terms of energetics as well. Reduction of nitrogen from the atmosphere requires energy. Even if the genetic obstacles to introducing this process into cereals are overcome, there will be an energetic cost for symbiotic nitrogen fixation, and this most likely will compete with grain filling for photosynthates just as in the legumes.

Alteration in protein composition. Opaque-2 maize, mutant 1508 barley, and high-lysine sorghum show increased lysine contents in their endosperm pro-

Table 3. Amino acid composition of normal and high-lysine maize and barley. Values are grams of amino acid per 100 g of protein. Sources were: W64A and W64Ao_2, from Nelson (*20*); 'Eva,' from D. Boulter, University of Durham, Durham, England; Hiproly, from Munck (*21*); and 1508, from the Seibersdorf Laboratory of the International Atomic Energy Agency.

Amino acid	Maize		Barley		
	W64A	W64Ao_2	'Eva'	Hiproly	1508
Lysine	1.38	3.53	3.65	4.07	5.37
Tryptophan	0.26	0.67	0.81	0.81*	0.81*
Histidine	2.49	3.06	2.13	2.14	2.74
Arginine	2.92	4.97	4.56	4.48	7.46
Aspartic acid	6.02	10.31	6.39	6.31	8.00
Glutamic acid	22.36	18.91	25.86	24.41	17.00
Threonine	3.01	3.53	3.15	3.56	4.50
Serine	4.82	4.58	3.96	4.68	5.04
Proline	7.39	8.21	12.07	12.21	7.02
Glycine	2.58	4.49	4.26	3.76	5.37
Alanine	8.68	6.88	4.26	4.27	5.26
Valine	4.64	5.06	5.58	5.39	6.47
Cystine	1.55	0.86	2.43	1.73	2.63
Methionine	1.72	1.72	1.42	2.03	1.54
Isoleucine	3.87	3.72	3.75	3.97	4.06
Leucine	16.17	11.08	7.71	7.12	7.35
Tyrosine	4.56	3.72	2.54	2.95	4.61
Phenylalanine	5.59	4.68	5.48	6.10	4.71

*Actual values are not known; standard barley values have been used for calculations.

teins. This, in general, is brought about by a decrease in the prolamin fraction in the endosperm protein with a compensatory increase in other, mainly albumin and globulin, protein fractions. Another characteristic common to high-lysine stocks is their reduced grain yield. The substrate and energy requirements for the synthesis of normal and opaque-2 maize and normal and high-lysine barley proteins were calculated by considering the biosynthetic pathways for different amino acids. The amino acid compositions on which the calculations were based are given in Table 3. Calculations were made by using the computer program described by Penning de Vries *et al.* (*4*). The results (Table 4) show that the energy and substrate requirements for the synthesis of high-lysine proteins are slightly higher than those for normal proteins in both maize and barley. A maximum increase of about 2.5 percent in glucose requirement is seen for the proteins of 1508 barley mutant, in comparison with 'Eva,' a barley cultivar recently released in Sweden. This additional requirement would be for altering the amino acid composition alone. Increasing the protein concentration, if that were a breeding objective, would further increase the assimilate and nitrogen requirements.

Implications for plant breeding and production. The additional energetic demands do not appear to be formidable if the goals for changing protein contents and amino acid compositions are not too ambitious (1 to 2 percent increase in protein or lysine). Moreover, an increased lysine concentration would enhance net protein utilization and ultimately contribute to a higher yield of utilizable nitrogen and greater nutritional efficiency (*17*). Nevertheless, there is still the question of why the goals of higher protein and higher grain yields have not been realized more frequently. The typical yield reduction in improved-protein cultivars exceeds the predicted reduction in most cases. The possibility that some high-protein or high-lysine genes are linked to factors that impair endosperm development cannot be ignored. A high-protein wheat cultivar, CI 17389 (NE 701132), recently released by the University of Nebraska and perhaps some opaque-2 maize hybrids in certain areas are exceptions to this general trend. In two successive years the yield and protein concentration of Centurk, the highest-yielding Nebraska variety, and CI 17389 were compared under Nebraska conditions. The averages were grain yields of 3100 and 3060 kg/ha and protein concentrations of 13.8 and 15.3 percent, respectively (*18*). The disparity in yield (1.6 percent) associated with increased protein concentration (1.5 percent) was very close to what our calculations suggest. However, in the International Winter Wheat Performance Nursery in 1972 and 1973 (*19*), at 25 test sites, CI 17389 was lower in grain yield by about 6 percent in comparison with Centurk (*18*).

Table 4. Glucose requirement for synthesis of maize and barley proteins with normal and increased lysine contents. The nitrogen source is assumed to be NO_3^-. The PV values are for protein with the amino acid composition specified in Table 3 and are defined as in Table 1. Energy requirement factor (ERF) = (gram moles ATP required)/(weight of end product). Hydrogen requirement factor (HRF) = (gram moles NADPH required)/(weight of end product).

Type	PV	ERF	HRF
Maize			
W64A	0.4085	0.0781	0.0292
W64Ao_2	0.4078	0.0836	0.0335
Barley			
'Eva'	0.4112	0.0827	0.0314
Hiproly	0.4090	0.0831	0.0323
Risø mutant 1508	0.4007	0.0885	0.0371

It is apparent that an increase in protein or lysine by breeding in most cereals would demand additional production of photosynthate. Alternatively, such increased photosynthetic production could be channeled into increased grain yield, but with a normal or even reduced protein concentration. Hence improved-protein cultivars are likely to slightly lag the top-yielding cultivars in grain yield. The nitrogen fertilizer requirements of increased protein cultivars would be also higher. For instance, a rice cultivar such as IR-8 may yield about 6000 kg/ha, with 8 percent protein in grain, under optimal growth conditions. If it were replaced by a new cultivar having the same yield but with 10 percent protein in grain, according to the calculations in Table 2 the nitrogen requirement would increase by 24 percent.

Grains with 8 and 10 percent protein would give harvests of 480 and 600 kg/ha for protein or 76.8 and 96.0 kg/ha for nitrogen, respectively, considering protein to be 16 percent nitrogen. If it is assumed that 70 percent of plant nitrogen is harvested in grain, the actual nitrogen requirements for such cultivars would be 109.7 and 137.1 kg/ha respectively. Therefore, the high-protein cultivar should have a plant type suitable for the projected level of nitrogen as well—that is, lodging resistance. Slightly reduced grain yield or greater inputs of nitrogen fertilizers would increase the production costs of such cultivars, and hence they might not be attractive to grow unless they fetched a premium for higher protein.

A further implication is that in breeding programs aimed at improving grain protein concentration, segregating generations and subsequent selections should be evaluated at higher soil nitrogen levels than those considered optimal for the current commercially grown cultivars.

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22. We are grateful to F. W. T. Penning de Vries and H. H. van Laar for the computer analysis of comparative energy requirements for the different maize and barley genotypes. Reprint requests should be sent to R.R.

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9 June 1976; revised 10 August 1976

Calcium and Secretion: Distinction Between Two Pools of Glucose-Sensitive Calcium in Pancreatic Islets

Abstract. *D-Glucose, but not L-glucose or 3-O-methyl-D-glucose, stimulates $^{45}Ca^{2+}$ uptake by both lanthanum-displaceable and lanthanum-nondisplaceable pools in pancreatic islets. The nondisplaceable pool probably represents secretory granules, while the displaceable pool may be located in the β-cell membrane. Kinetic studies with isotopically labeled islets suggest that only the displaceable pool participates in the short-term coupling of the glucose stimulus with secretion.*

Glucose causes a net movement of Ca^{2+} from the extracellular to the intracellular space of pancreatic islets containing more than 90 percent of β-cells (*1*). On fractionation of ^{45}Ca-labeled islets, the isotope taken up in response to glucose was recovered with the insulin secretory granules (*2*). In intact islets the glucose-sensitive calcium pool displayed a marked inertia to provocations aimed at initiating its mobilization (*1*, *2*). Therefore, it is doubtful whether the short-term insulin-releasing action of glucose is mediated by mechanisms for intracellular Ca^{2+} uptake. The rapidity with which changes of the extracellular calcium influence insulin release (*3*) suggests that secretion may depend on a labile pool that is perhaps located in the periphery of the β-cells.

To measure specifically the intracellular calcium, in previous experiments we used lanthanum ions to wash the islets free of extracellular calcium and to prevent losses of intracellular ion (*1*). Lanthanum displaces calcium from cell surfaces. Therefore, if the β-cells contain a membrane-located calcium pool that is important for secretion, it is possible that the lanthanum wash technique prevented the detection of such a pool. In the present study we labeled the islets with ^{45}Ca and studied the rate of disappearance of isotope from both lanthanum-displaceable and lanthanum-nondisplaceable pools. The results indicated that the β-cells contain a glucose-sensitive calcium pool that is displaceable with lanthanum and shows the mobility required of a plausible mechanism for coupling the glucose stimulus with secretion.

Fresh islets containing more than 90 percent β-cells were microdissected from the pancreatic glands of noninbred *ob/ob* mice; the islets were not exposed to collagenase during the isolation procedure. They were incubated at 37°C in a salt-balanced tris or bicarbonate buffer (*1*) supplemented with sugars (see Fig. 1 and Tables 1 and 2). The cells were incubated for 10 or 120 minutes in buffer containing trace amounts of $^{45}CaCl_2$; the calcium concentration was 2.56 m*M* in all media. The ^{45}Ca-labeled islets were incubated for various periods of time in nonradioactive medium, and the radioactivity retained by the islets was measured by liquid-scintillation counting after they were freeze-dried (−40°C, 0.1 pascal) overnight, weighed on a quartz-fiber balance, and dissolved in hyamine. Before being freeze-dried, some islets were washed for 60 minutes with 2 m*M* $LaCl_3$ (*1*). The radioactivity of islets not washed with lanthanum will be referred to as "total" calcium, that of lanthanum-washed islets as "lanthanum-nondisplaceable" calcium, and the difference between the two groups as "lanthanum-displaceable" calcium. Samples of the $^{45}CaCl_2$-containing medium used for labeling the islets were used as external standards in the counting procedure. Islet radioactivities are expressed in terms of millimoles of calcium with same specific radioactivity as the medium used for labeling the islets.

When $^{45}Ca^{2+}$-labeled islets are placed in a nonradioactive buffer, the spontaneous disappearance of isotope is much slower from lanthanum-nondisplaceable than from lanthanum-displaceable pools; virtually all of the $^{45}Ca^{2+}$ retained by islets after 90 minutes of efflux is nondisplaceable with lanthanum (*1*). Therefore, in the present experiments we subjected the ^{45}Ca-labeled islets to very brief periods of incubation (0 to 5 minutes) in nonradiactive buffer. Figure 1 shows the retention of $^{45}Ca^{2+}$ by islets incubated with $^{45}CaCl_2$ for 120 minutes in the presence of 3 m*M* D-glucose, 20 m*M* D-glucose, or 3 m*M* D-glucose plus 17 m*M* L-glucose. D-glucose, but not L-glucose, stimulated the $^{45}Ca^{2+}$ uptake to the lanthanum-nondisplaceable pool, and there was only a negligible loss of isotope from this pool during efflux in nonradioactive buffer. After 5 minutes of efflux, the effect of 20 m*M* D-glucose on the lanthanum-nondisplaceable pool fully accounts for the increase of total islet $^{45}Ca^{2+}$ at this time point. However, after 1 or 2 minutes of efflux, the labeling of the total islet calcium appeared to be greater than that of the lanthanum-nondisplaceable pool. This difference sug-