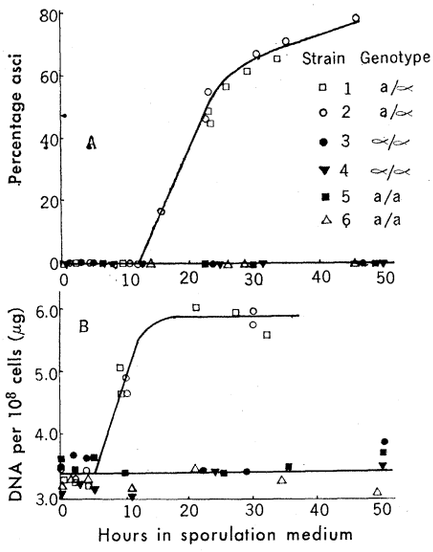

Fig. 1. Sporulation and DNA synthesis in yeast. Vegetable cells were harvested during early logarithmic growth, washed, and resuspended at zero hours in sporulation medium. At intervals samples were withdrawn for the determination of the percentage of asci (A) by phase contrast microscopy, and total DNA (B) with Burton's modification of the diphenylamine reaction.

their genotypes with respect to the mating type alleles are given in Fig. 1; each strain grew with a doubling time of about 3 hours in acetate presporulation medium (*4*). Strains 1 and 2 are heterozygous for the mating type alleles; strains 3 through 6 are homozygous for either the α or a allele. The ability of each of these strains to sporulate was examined by growth of the cells in presporulation medium followed by transfer of the cells to a potassium acetate sporulation medium free of nitrogen (*5*). All media were supplemented with 50 mg of adenine per liter. Strains 1 and 2 sporulated abundantly, while strains 3, 4, 5, and 6 failed to form asci (Fig. 1A). The cells of both the homozygous and the heterozygous strains increased about equally in mass during incubation in sporulation media, an indication that their ability to utilize acetate under these conditions was apparently normal (*6*).

Samples of cells from each of the above strains were harvested throughout the period of incubation in sporulation media, frozen, and subsequently analyzed for total DNA (*7*) (Fig. 1B). The total number of cells, including buds, was determined with a hemacytometer after the clumps were broken up by sonication. Cell numbers did not change appreciably (± 10 percent during the course of the experiment.

Upon inoculation into sporulation media (zero hours), each of the strains had approximately the DNA content expected of a growing diploid (*8*). Both heterozygous strains underwent net DNA synthesis which began 4 to 6 hours after the cells were placed in the sporulation media (*3*). In contrast, four homozygous diploids exhibited little if any net DNA synthesis during incubation in sporulation media (up to at least 50 hours).

Croes (*7*) first showed that DNA synthesis was an early event during sporulation, beginning about 8 hours before the first appearance of mature spores. When the net synthesis of DNA in the heterozygous strains is compared with the absence of synthesis in the homozygous strains, it is clear that the α/a alleles have already exerted a profound effect on development early in the sporulation cycle. This conclusion is further supported by our results which show that commitment to recombination, which also begins early in the development of heterozygous strains, is blocked in the homozygous strains (*9*, *10*).

Spore formation in yeast follows meiosis and recombination (*7*, *10*) and results in the segregation of the haploid nuclei to the individual ascospores. Synthesis of DNA is usually regarded as a prerequisite step in meiosis, which increases the diploid DNA content of the cell to the $4n$ level necessary for the formation of four haploid nuclei (*8*). Synthesis of DNA has also been implicated in aspects of the recombination process in yeast (*11*). Although DNA synthesis may be necessary for sporulation and recombination, the net synthesis of DNA, in itself, may not be sufficient to insure the completion of these processes. In our experiments the homozygous cells were harvested during logarithmic growth and were, therefore, heterogenous with respect to vegetative DNA replication; a small portion of them have surely undergone the DNA replication required for the ensuing division and, consequently, should contain enough DNA for meiosis. Nevertheless, the homozygous cells exhibited no sporulation whatever.

The simultaneous absence of DNA synthesis, recombination, and sporulation in the homozygous strains probably results from the blockade of some step, or steps, early in development which is controlled by the mating type alleles; this step need not be DNA synthesis itself.

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References and Notes

1. H. Roman and S. M. Sands, *Proc. Nat. Acad. Sci. U.S.* **39**, 171 (1953).
2. J. Friis and H. Roman, *Genetics* **59**, 33 (1968).
3. A. F. Croes, *Exp. Cell Res.* **41**, 452 (1966); M. S. Esposito, R. E. Esposito, M. Arnaud, H. O. Halvorson, *J. Bacteriol.* **100**, 180 (1969).
4. These strains were provided by Prof. H. Roman. Details are given in (*2*). All strains required adenine for growth. The original designation of these strains are: 1, 11-35A (190); 2, 11-5B (190); 3, N3 (170); 4, 11-35C (170); 5, 11-5D (160); and 6, 54-8B (170). The numbers in parentheses are the mass doubling time (minutes) at 30°C in presporulation medium.
5. R. Roth and H. O. Halvorson, *J. Bacteriol.* **98**, 831 (1969).
6. R. Roth, *ibid.* **101**, 53 (1970).
7. A. F. Croes, *Planta* **76**, 209 (1967).
8. M. Ogur, S. Minckler, G. Lindegren, C. C. Lindegren, *Arch. Biochem. Biophys.* **40**, 175 (1952); G. K. Darland, thesis, University of Washington (1969).
9. Commitment to intragenic recombination was detected by the appearance of adenine-independent clones as described (*10*) for isoleucine-requiring diploids. This was possible here since each strain was heteroallelic for the ad_{6-b} and ad_{6-e} pair.
10. F. Sherman and H. Roman, *Genetics* **48**, 255 (1963).
11. S. Fogel and R. K. Mortimer, *Proc. Nat. Acad. Sci. U.S.* **62**, 96 (1969).
12. Supported by NIH grant GM 16522 and NSF grant GB 8534. We thank A. Weiland and R. Louden for assistance with manuscript preparation.

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Starch Accumulation Associated with Growth Reduction at Low Temperatures in a Tropical Plant

Abstract. *Growth of* Digitaria decumbens *is severely reduced by night temperatures of 10°C or below. Ultrastructure of leaves and chemical analyses show a high starch content in chloroplasts of plants illuminated and kept at a temperature of 30°C. This starch disappears after a period in the dark at 30°C, but it remains if the temperature during the dark period is 10°C. The inhibition or slowing of starch translocation out of chloroplasts appears to account for reduced photosynthesis and growth at low night temperatures.*

Digitaria decumbens (Pangolagrass), a grass grown in tropical areas throughout the world, exhibits marked reductions in growth when subjected to low night temperatures (*1*). Since photo-

synthetic processes are thought to occur in the mesophyll chloroplasts (*2*), we examined the effects of low night temperatures on starch content of mesophyll chloroplasts of *Digitaria decumbens* in order to study the relation between deposition of photosynthate and growth. We also measured the effects of temperature treatments on photosynthetic potential, as measured by the packing volume of isolated chloroplasts (*3*). Plants were grown outdoors at summer temperatures (mean maximum daylight temperature, 32°C) and were moved into growth chambers for 12-hour dark periods at 10°C or 30°C.

For visualization with the electron microscope, tissue from young, but fully expanded, leaves was fixed in 5 percent glutaraldehyde in 0.2*M* phosphate buffer, *p*H 7.6, for 4 hours at 4°C, then fixed in osmium tetroxide in 0.2*M* phosphate buffer plus 2 percent sucrose, *p*H 7.6, for 1.5 hours at 4°C. Fixed tissue was dehydrated in an ethanol-acetone series and embedded in an Epon-Araldite mixture. Sections were stained with uranyl acetate and lead citrate. Electron micrographs were made with a Hitachi HS-8 electron microscope at 50 kv. We examined tissue from plants which had continuous 30°C nights and from plants which had 30°C nights followed by 1 night at 10°C. Samples were taken just prior to the end of the 12-hour light period and again just after the 12-hour dark period with both 10°C and 30°C temperature treatments.

Mesophyll chloroplasts contained prominent starch grains at the end of the light period (Fig. 1A). At the end of the dark period at 30°C, starch grains were absent from the chloroplasts (Fig. 1B), but chloroplasts from plants receiving low night temperature contained starch grains similar to those found at the end of the light period (Fig. 1C). These micrographs indicate that starch is synthesized during the light period and is deposited in the mesophyll chloroplasts. In other species starch accumulates only in bundle sheath chloroplasts (*2, 4*). In *Digitaria decumbens* starch accumulates in chloroplasts of both the bundle sheath and the mesophyll. During the dark period at 30°C starch is rendered soluble and translocated from the chloroplasts. Apparently this dissipation is interrupted if the plant is exposed to cold night temperature, and the interruption may involve temperature-sensitive enzymes in the system that solubilizes starch in mesophyll chloroplasts.

For chloroplast isolation, equal samples of fresh green leaves from each temperature regime were ground in 0.4*M* sucrose and filtered through cheesecloth. The filtrate was centrifuged at 60*g* for 5 minutes; then the supernatant was centrifuged at 500*g* for 30 minutes. The pellet contained intact chloroplasts which were osmotically lysed before starch determination (*5*). The chloroplasts in plants given 1 night at 10°C contained more than six times as much starch as chloroplasts from plants grown with continuous night temperatures of 30°C (Table 1). With two consecutive nights of 10°C the difference was increased to a factor of 10. These results indicate that starch is retained in chloroplasts as a result of low night temperatures, and further, the presence of accumulated starch in chloroplasts after 1 night at 10°C reduces the amount of subsequent starch synthesis. Plants that were returned to 1 night of 30°C following 2 nights of 10°C had a greatly reduced starch content. This indicates that starch accumulation in chloroplasts is reversible.

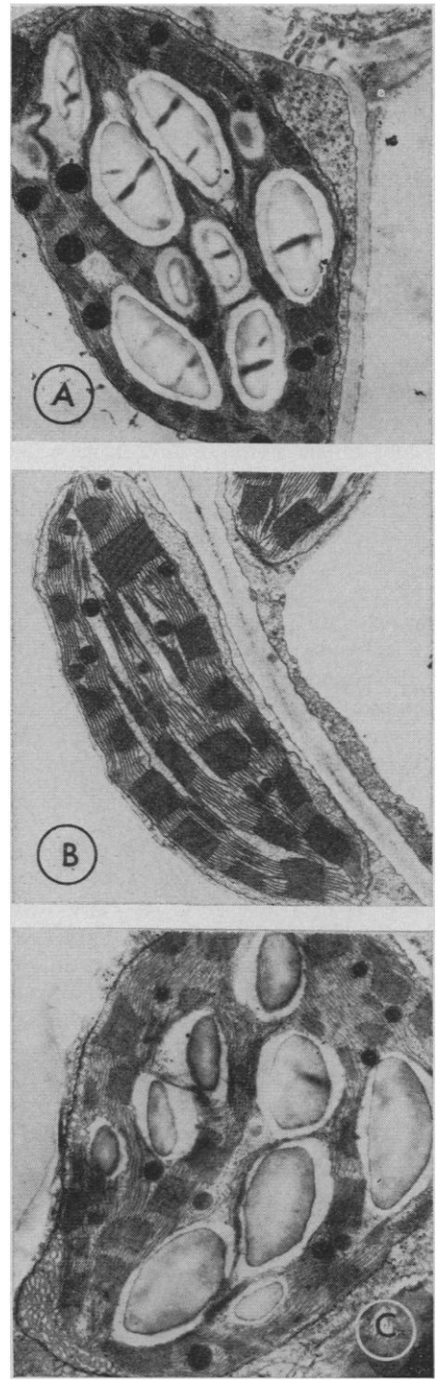

Fig. 1. Representative chloroplasts from leaf mesophyll cells of *Digitaria decumbens*. (A) After 12-hour illumination at 30°C, starch grains have accumulated in the chloroplasts (× 12,375); (B) after 12 hours of darkness at 30°C, starch grains have disappeared from the chloroplasts (× 15,000); but in (C) starch grains remain in the chloroplasts after 12 hours of darkness at 10°C (× 15,000).

Chloroplasts active in photosynthesis shrink in size, presumably because energy transfers lead to synthesis of adenosine triphosphate and the effusion of ions (*3*). The packing volume of isolated chloroplasts is a measure of photosynthetic potential as evidenced by shrinkage. We determined the packing volume of isolated chloroplasts after continuous 30°C night temperatures and after continuous 30°C night temperatures followed by 1 night at 10°C. Plants were illuminated at 30°C after night temperature treatments; then packing volumes were measured in hematocrit tubes by centrifuging resuspended isolated chloroplasts in 0.4*M* sucrose at 1000*g* for 30 minutes. The packing volume of chloroplasts from the 10°C treated plants was 1.3 times that of plants receiving 30°C. This finding suggests reduced photosynthetic potential due to low nighttime temperatures, or packing volume may be increased due to accumulated starch.

Results from chemical starch determinations support our electron microscope findings; starch remains in mesophyll chloroplasts as the result of cold night temperatures. Presumably this starch is translocated during the dark period at higher temperatures. Repeated low temperatures during dark periods provide for continued starch accumulation, but at a progressively lower rate, because the presence of previously accumulated starch interferes with photosynthesis and further starch accumula-

Table 1. Starch content and packing volume of chloroplasts isolated from *Digitaria decumbens* treated at 10°C and 30°C. Sample was 8.5 g (fresh weight) for each treatment.

Night temperature	Starch content (mg/g)	Packing volume (cm/g)
Continuous 30°C	0.61	0.09
Continuous 30°C + 1 night of 10°C	4.11	0.12
Continuous 30°C + 2 nights of 10°C	6.01	
Continuous 30°C + 2 nights of 10°C + 1 night of 30°C	1.62	

tion. The larger size of chloroplasts after low night temperatures, as indicated by a larger packing volume, gives evidence of lowered photosynthetic potential. Thus, the severe reduction of growth in *Digitaria decumbens* by low night temperatures may be the result of the failure of translocation of products of photosynthesis from the mesophyll chloroplasts and consequent interference with photosynthesis.

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References and Notes

1. W. G. Blue, N. Gammon, Jr., H. W. Lundy, *Soil Crop Sci. Soc. Fla. Proc.* **21**, 56 (1961); D. E. McCloud, O. C. Ruelke, J. M. Creel, Jr., *Fla. Agr. Exp. Sta. Res. Rep.* **2** (1), 8 (1957).
2. C. R. Slack, M. D. Hatch, D. J. Goodchild, *Biochem. J.* **114**, 489 (1969).
3. P. A. Siegenthaler and L. Packer, *Plant Physiol.* **40**, 785 (1965).
4. T. Bisalputra, W. J. S. Downton, E. B. Tregunna, *Can. J. Bot.* **47**, 15 (1969).
5. E. Madsen, *Physiol. Plant.* **21**, 168 (1968).
6. Supported in part by an NDEA fellowship grant. We thank Dr. H. C. Aldrich and staff, Biological Ultrastructure Laboratory, University of Florida, for assistance and facilities. Florida Agricultural Experiment Station Journal Series No. 3496.

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Obesity: Absence of Satiety Aversion to Sucrose

Abstract. *In obese subjects, ingestion of glucose did not cause the transformation of the gustative sucrose sensation from pleasant to unpleasant as in normal subjects. This result is consistent with the theory of a decreased sensitivity to internal signals in the control of food intake of obese people.*

Food intake is controlled by a system receiving multiple inputs, which can be grouped according to their origin as (i) internal signals, such as humoral modifications, gastric contraction or distention, and internal temperature changes and (ii) external stimuli, such as the taste, smell, or sight of food.

The response of obese humans has been compared with the behavior of hypothalamic hyperphagic rats, and the differences between internal and external signals were noted (*1*). These observations led to the theory that obese people are highly sensitive to external stimuli but are relatively insensitive to internal signals.

Two components can be distinguished in sensation. (i) The discriminatory component analyzes the nature and the intensity of the stimulus and (ii) the affective component analyzes whether the sensation is pleasant or unpleasant. For example, the taste of a sweet solution is pleasant to fasting subjects but turns unpleasant after a load of glucose in the stomach (*2*). Because the pleasantness or unpleasantness of sweet sensation is controlled in part by a gastric internal signal, it is relevant, according to the above theory, to look at the affective response of obese people to gustative stimulation. The obese subjects were patients entering the hospital for a cure of obesity or detection of potential diabetes, ten women averaging 151.6 cm in height, and 83.5 kg in body weight and five men averaging 173.2 cm and 93.2 kg were used. None of the subjects used in this experiment was diabetic. The subjects, not yet under a controlled caloric food intake, wished to decrease their body weights. Control subjects were six females, averaging 160 cm in height and 50.5 kg in body weight, and four males, averaging 176 cm and 69.9 kg, respectively.

After the subjects were fasted for 12 hours, they were given a taste test with a series of sucrose solutions (2.5, 5, 10, 20, and 40 percent). Taste samples (25 ml each) were presented in random order, with suitable precautions. Each sample was taken in the mouth but not swallowed. After 15 seconds, the subject expectorated the sample and then graded the solution just tasted on an affective scale as follows: + 2, very pleasant; + 1, pleasant; 0, neutral; − 1, unpleasant; − 2, very unpleasant.

The subject was allowed to give any rational number between − 2 and + 2. Immediately after this series of tests, he ingested 50 g of glucose in 200 ml of aqueous solution. This amount is routinely used for blood insulin and glucose responses and gives comparable results regardless of body weight and size in nondiabetic subjects.

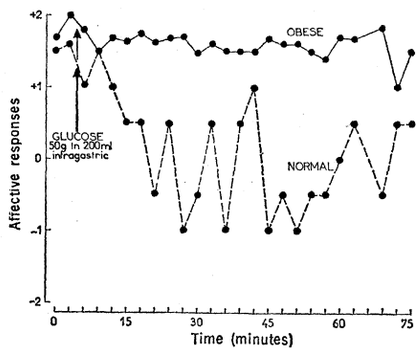

Fig. 1. Development of the affective responses given by one obese and one control subject to gustative stimuli of 25 ml of 20 percent sucose solution. Duration of each stimulus, 15 seconds.

To test the development of the sensation, each subject was given 5 g of sucrose in 25 ml of aqueous solution every 3 minutes after glucose ingestion. One hour after the glucose gastric load, the subject was offered the same range of sucrose solutions as previously and in the same order of presentation.

Blood glucose and insulin were measured 10 minutes before, and 30, 60, and 120 minutes after glucose ingestion. Only two obese subjects had hyperglycemic responses; insulin responses were quite variable. No correlation was found between gustative response and these parameters.

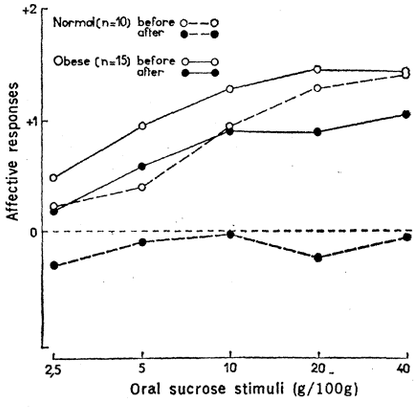

Fig. 2. Affective responses given by ten control and 15 obese subjects after tasting, for 15 seconds, 25 ml of sucrose aqueous solutions at increasing concentrations before and 45 to 70 minutes after ingestion of 200 ml of 25 percent glucose solution. Presentation of solutions was in random sequence and was different for each subject, but the same sequence was used for each subject before and after stomach load. Note that the coordinates are semilogarithmic.