ceptors. However, our results clearly show that hypothalamic glucoreceptors are not responsible for feeding and hyperglycemia elicited by brain glucoprivation. The cerebral receptors that mediate glucoprivic feeding and sympathoadrenal discharge are located in the hindbrain.

ROBERT C. RITTER
PETER G. SLUSSER
STEVEN STONE

Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman 99163, and *WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow 83843*

References and Notes

1. G. P. Smith and A. N. Epstein, *Am. J. Physiol.* **217**, 1083 (1969).
2. T. R. Houpt and H. E. Hance, *J. Comp. Physiol. Psychol.* **76**, 395 (1971); T. R. Houpt, *Am. J. Physiol.* **227**, 161 (1974); D. A. Thompson and R. G. Campbell, *Science* **198**, 1065 (1977).
3. R. R. Miselis and A. N. Epstein, *Am. J. Physiol.* **229**, 1438 (1975).
4. H. R. Berthoud and G. J. Mogenson, *ibid.* **233**, R127 (1977).
5. A. N. Epstein, S. Nicolaides, R. Miselis, in *Neural Integration of Physiological Mechanisms and Behavior*, G. J. Mogenson and F. R. Calaresu, Eds. (Univ. of Toronto Press, Toronto, 1975), p. 148.
6. R. L. Himsworth, *J. Physiol. (London)* **206**, 411 (1970).
7. The current belief that the glucoreceptors that mediate sympathoadrenal discharge are in the hypothalamus stems mainly from Himsworth (*6*). In his experiments, injections (3 μl) of local anesthetic (lidocaine) were made bilaterally into the lateral hypothalamus of pentobarbital-anesthetized rats. Lidocaine-injected rats displayed a smaller increase in blood glucose than did controls in response to intraperitoneal injection of 3-*O*-methyl glucose. Himsworth concluded that he had anesthetized the hypothalamic glucoreceptors. However, it is also possible that he simply anesthetized neurons required in the mobilization of the effector response. Furthermore, there is no assurance that the local anesthetic used did not diffuse, via the ventricles, to exert its effect in other brain regions. Finally, considering that the injection volume was 3 μl on each side, the lidocaine would almost certainly have diffused into the ventromedial area. Anesthetization of ventromedial region is known to release insulin and such an effect could mask the sympathoadrenal activation by enhancing removal of mobilized glucose from the blood.
8. R. J. DiRocco and H. J. Grill, *Science* **204**, 1112 (1979).
9. 5-Thioglucose is an analog of glucose in which sulfur is substituted for the pyranose ring oxygen [M. Chen and R. L. Whistler, *Arch. Biochem.* **169**, 392 (1975)]. Recently we demonstrated that 5TG causes dose-dependent increases in feeding and sympathoadrenal hyperglycemia when administered systemically or by ICV cannulae. We also found that 5TG elicits feeding at 2.5 percent of the molar dose required of 2DG when infused by ICV cannulae [R. C. Ritter and P. G. Slusser, *Am. J. Physiol.* **238**, E141 (1980); P. G. Slusser and R. C. Ritter, *Brain Res.* **202**, 474 (1980)].
10. The concentration of 5TG in the infusate was only 154 m*M*. The 5TG with the 0.9 percent NaCl solution was, therefore, about one and one-half times the osmotic concentration of cerebrospinal fluid. In other experiments, we have infused nonglucoprivic osmotically active substances (0.25*M* fructose or 0.125 to 1.20*M* glucose in 0.9 percent NaCl) into the lateral or fourth ventricles. Such infusions have consistently failed to elicit increased feeding. Furthermore, Miselis and Epstein (*3*) have obtained feeding in response to lateral ventricle infusions of 3.0*M* 2DG. They also found that ICV infusion of equiosmotic glucose, sucrose, or urea failed to elicit feeding. Thus, it seems clear that the feeding elicited by intracranial administration of glucose analogs is a specific effect and is not dependent on osmotic properties of the infusate.
11. G. P. Smith and A. W. Root, *Endocrinology* **85**, 963 (1969).
12. J. B. Simpson and A. Routtenberg, *Science* **181**, 1172 (1973); M. I. Phillips. D. Felix, W. E. Hoffman, D. Ganten, in *Neuroscience Symposium II*, W. M. Cowan and J. A. Ferrendelli, Eds. (Society for Neuroscience, Bethesda, Md., 1977), p. 308.
13. W. E. Hoffman and M. I. Phillips, *Brain Res.* **110**, 313 (1976).
14. M. Russek, *Physiol. Behav.* **5**, 1207 (1970); D. Novin, D. A. VanderWeele, M. Rezek, *Science* **181**, 858 (1973).
15. Supported by PHS grant AM20035 to R.C.R.

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Evidence for Extensive Overlap of Sporophytic and Gametophytic Gene Expression in *Lycopersicon esculentum*

Abstract. *Male gametophyte (pollen) isozyme profiles were compared with those of the sporophyte for nine enzyme systems. Sixty percent of the structural genes coding for these enzymes in the sporophyte were also found to be expressed by the gametophyte. All the genes tested were found to be expressed after meiosis, apparently transcribed and translated in the haploid gametophytes.*

Compared to the sporophyte, the gametophytes of higher plants have been the subject of relatively little genetic and physiological research. This is especially true of genetic research. A basic question about gametophyte-sporophyte relations in higher plants concerns the extent to which the same genes are active in the two phases. Interest in this question has been stimulated by a recent proposal (*1*) that selection among haploid male gametophytes (pollen grains) might have a positive, correlated effect on the sporophytic generation resulting from selection of genes expressed in both stages. With such a scheme, higher plants, particularly angiosperms, would enjoy a unique mode of evolution in which adaptive advances could be made at minimal cost by selection at the haploid gametophytic stage (*1*). Furthermore, the plant breeder could select with great effectiveness in the haploid generation. The hypothesis is supported by experiments that demonstrate correlations in specific fitness parameters between sporophytic and gametophytic generations (*2*).

A fundamental requirement of this selection model is that a portion of the genes expressed by the sporophyte also be expressed by the haploid pollen genome. Previous studies have revealed gametophytic control of such characters as pollen composition and dimension (*3*), self-incompatibility of the gametophytic type (*4*), pollen tube growth rate (*5*), selective fertilization (*6*), genic lethality (*7*), and specific proteins (*8*). The fact that even small chromosomal deletions are not transmitted through the pollen suggests that the number of genes functioning in the gametophyte is large (*9*). Other studies, particularly on isozymes, demonstrate that some of the genes expressed by the haploid pollen genome are also expressed at one or more stages in the sporophyte (*10*); however, there have been no direct estimates of what proportion of the sporophytic genome is expressed in the male gametophyte.

The tomato, *Lycopersicon esculentum*, was selected for our investigations because many of its enzyme systems have been genetically analyzed. This information provides a basis for estimating the overlap of sporophytic and gametophytic genes as well as the portion of gametophytic genes expressed after meiosis. The strategy was to use starch gel electrophoresis, coupled with histochemical staining techniques for specific enzymes, to generate isozyme profiles for sporophytic and gametophytic tissues. These data, combined with previously published genetic data for the individual isozymes, were used to estimate gene overlap.

For dimeric enzymes present in pollen, it is possible to determine whether the corresponding genes are expressed after meiosis. The test involves obtaining plants known to be heterozygous at the locus in question. Extracts from diploid, sporophytic tissue of such plants display on the gel two homodimeric bands and an intermediate heterodimeric band revealing enzymes composed of one fast

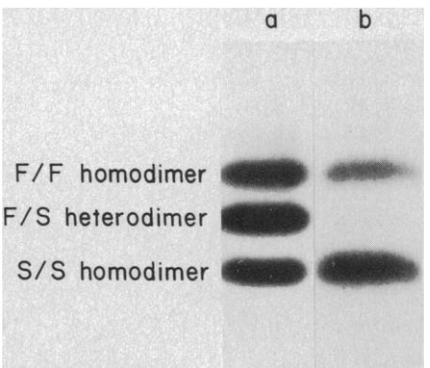

Fig. 1. Zymograms of F_1 heterozygote: (a) leaf tissue (sporophyte) and (b) pollen.

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and one slow subunit (Fig. 1a). The pollen from the same heterozygous individual is expected to display both homodimeric bands, but if the heterodimer is present, the gene presumably is transcribed and translated at least in part from diploid cells of premeiotic stages. If the heterodimer is absent, expression must be postmeiotic (Fig. 1b). These results are expected, since enzyme subunits produced by translation combine to form dimers in vivo. Thus a cell with only a single copy of a gene can make only one type of dimer (homodimers), and it follows that heterodimers can be produced only by diploid cells possessing copies of both genes. Since pollen from a heterozygous individual is a composite of haploid grains possessing one or the other allele, only homodimers are produced.

The question naturally arises whether the subunits from homodimers recombine in vitro during extraction procedures. To test this, we coextracted allozymes from individuals homozygous for different alleles. Invariably, we found no heterodimers after extraction. Even extraction in a 1*M* NaCl solution, which weakens some of the interactions holding subunits together, failed to produce dissociation of dimeric enzymes. Thus the test for postmeiotic gene expression seems valid.

Nine enzyme systems were assayed: alcohol dehydrogenase (ADH) (E.C. 1.1.1.1), phosphoglucomutase (PGM) (E.C. 2.7.5.1), phosphoglucoisomerase (PGI) (E.C. 5.3.1.9), acid phosphatase (APS) (E.C. 3.1.3.2), glutamic-oxaloacetic transaminase (GOT) (E.C. 2.6.1.1), esterase (EST) (E.C. 3.1.1.1), peroxidase (PRX) (E.C. 1.11.1.7), triosephosphate isomerase (TPI) (E.C. 5.3.1.1), and shikimate dehydrogenase (SKDH) (E.C. 1.1.1.25). Previous studies have revealed 28 genes coding for these enzymes in the sporophytic stage, and most of the structural genes have been mapped (*11*). Roots, leaves, and developing and mature seeds were sampled from the sporophyte. Leaves and roots were repeatedly assayed from plants of ages ranging from 2 weeks to 2 months. Developing seeds, which at early stages include embryo and endosperm, were sampled 20, 40, and 60 days after pollination, and mature seeds were sampled from ripe fruit. Two stages in the gametophyte were examined: mature pollen and pollen that had been germinated on artificial media for 8 hours at 15°C. Mean germination by our technique (*12*) was 70 percent, and the germinated grains had a mean tube length of approximately 100 μm. All isozymes considered to be present were detected at least twice in repeated samplings. [Methods of enzyme extraction and electrophoresis used to detect the isozymes can be found in (*10*).]

Table 1 summarizes the data for all tissues and stages. Of the 31 isozymes encountered, 27 have been described and characterized genetically (*11*). A new anodal peroxidase band, PRX-A, appeared during embryogenesis, banding immediately cathodal to PRX-4. A weakly banding acid phosphatase that migrated to a position between APS-1 and APS-2 was also detected in leaves, pollen, and germinated pollen. Two previously undescribed esterases appeared, one in pollen, EST-A (double-banded), the other in leaves, EST-B (single-banded). These two esterases migrate to almost identical positions (between EST-4 and EST-1), suggesting that they might have a common genetic origin; however, a search through our germ plasm stocks has failed to turn up a variant of either isozyme, prohibiting a straightforward genetic analysis.

Of the isozymes found in pollen, all except perhaps EST-A are also present at one or more of the sporophytic stages. No new isozymes appeared in germinated pollen—in fact, fewer isozymes were observed. PRX-2, PRX-5, and PRX-6, which were found in dry pollen in low amounts, were not detected in germinated pollen, possibly because of dilution, since the water content of germinated pollen is considerably higher than that of dry pollen. With this possibility in mind, we concentrated the germinated pollen extract approximately fourfold by dialysis, but still failed to detect the three isozymes.

Seven of the dimeric isozymes found in pollen were tested for postmeiotic expression (Table 1). Pollen from the F_1 heterozygotes corresponding to each locus displayed a zymogram with only homodimers, indicating that all of these genes are expressed after meiosis from the haploid genome.

In interpreting the results, one must consider whether these isozymic genes are representative of the entire set of sporophytic and gametophytic genomes. All of the genes sampled code for water-

Table 1. Isozymes in various sporophytic and gametophytic tissues and stages (+, present; N.D., not detected; −, data inconclusive).

Isozyme	Sporophyte			Gametophyte		Postmeiotic expression	
	Roots	Leaves	Developing and mature seeds	Pollen	Germinated pollen	Tested	Positive
APS-1	+	+	+	+	+	+	+
APS-2	+	+	+*	+	+		
APS-A	N.D.	+*	N.D.	+*	+*		
GOT-1	+	+	+*	+*	+*		
GOT-2	+	+	+	+	+	+	+
GOT-3	+	+	+	+	+	+	+
GOT-4	+	+	+	+	+	+	+
PRX-1	+	+	N.D.	N.D.	N.D.		
PRX-2	+	+	+	+*	N.D.		
PRX-3	+	+	+	N.D.	N.D.		
PRX-4	+	N.D.	N.D.	N.D.	N.D.		
PRX-5	+	N.D.	N.D.	+*	N.D.		
PRX-6	+	N.D.	+*	+*	N.D.		
PRX-7	+	N.D.	+*	N.D.	N.D.		
PRX-A	N.D.	N.D.	+*	N.D.	N.D.		
EST-1	+	N.D.	N.D.	N.D.	N.D.		
EST-3	+*	+	+	+	+		
EST-4	+	N.D.	N.D.	N.D.	N.D.		
EST-5	+	N.D.	N.D.	N.D.	N.D.		
EST-6	+	N.D.	N.D.	N.D.	N.D.		
EST-7	+	+*	N.D.	N.D.	N.D.		
EST-A	N.D.	−	N.D.	+†	+†		
EST-B	N.D.	+	N.D.	−	−		
PGI-1	+	+	+	+	+	+	+
PGM-1‡	N.D.	+	+*	+*	+*		
PGM-2	+	+	+	+	+		
ADH-1	N.D.	N.D.	+	+	+	+	+
ADH-2	+	+	+	N.D.	N.D.		
SKDH-1	+	+†	+	+	+		
TPI-1‡	N.D.	+	N.D.	+*	+*		
TPI-2	+	+	+	+	+	+	+

*Weakly expressed. †Double-banded. ‡Enzyme localized in chloroplast.

soluble enzymes; none of the hydrophobic or membrane-bound enzymes are assayable by our techniques. However, the systems surveyed include enzymes of intermediary metabolism (phosphoglucomutase, phosphoglucoisomerase, and triosephosphate isomerase) as well as nonspecific enzymes (esterase and acid phosphatase). It is interesting that the only purported isozyme unique to pollen, EST-A, is classified as nonspecific.

Of the 30 isozymes present in sporophytic stages, 18 were also found in pollen. If we count EST-A as unique to pollen, then 18 of the 19 pollen isozymes are also found in one or more of the sporophytic stages. All the pollen genes tested were expressed postmeiotically, apparently transcribed from genes in the haploid genome. The data suggest little divergence in the structural gene repertoire of sporophyte and gametophyte. By the same token, they support the concept that selection for genes expressed in the gametophytic stage could have a marked effect on the sporophytic generation, since many of these genes are expressed in both generations.

STEVEN D. TANKSLEY
DANIEL ZAMIR
CHARLES M. RICK
Department of Vegetable Crops, University of California, Davis 95616

References and Notes

1. D. L. Mulcahy, *Science* **206**, 20 (1979).
2. _____ and G. B. Mulcahy, *Theor. Appl. Genet.* **46**, 277 (1975); D. L. Mulcahy, *Science* **171**, 1155 (1971); *Nature (London)* **249**, 491 (1974); E. Ottaviano, M. Sari-Gorla, D. L. Mulcahy, *Science* **210**, 437 (1980).
3. R. A. Brink and J. H. MacGillivray, *Am. J. Bot.* **11**, 465 (1924); M. Demerec, *ibid.*, p. 461; F. R. Parnell, *J. Genet.* **11**, 209 (1921); P. C. Mangelsdorf, *J. Hered.* **23**, 289 (1932); O. Renner, *Z. Bot.* **11**, 305 (1919); S. Satina and A. F. Blakeslee, *Am. J. Bot.* **24**, 518 (1937); K. Sax, *Genetics* **22**, 523 (1937); Y. Sinoto, *Cytologia* **1**, 109 (1929); C. M. Rick, *Proc. Natl. Acad. Sci. U.S.A.* **28**, 518 (1942).
4. E. M. East and P. C. Mangelsdorf, *Proc. Natl. Acad. Sci. U.S.A.* **11**, 166 (1925); D. de Nettancourt, *Incompatibility in Angiosperms* (Springer, Berlin, 1977).
5. P. C. Mangelsdorf and D. F. Jones, *Genetics* **11**, 423 (1926); P. L. Pfahler, in *Gamete Competition in Plants and Animals*, D. L. Mulcahy, Ed. (North-Holland, Amsterdam, 1975), p. 115; E. Ottaviano, M. Sari-Gorla, D. L. Mulcahy, in *ibid.*, p. 125.
6. C. Arnold, *Ergeb. Biol.* **20**, 67 (1958); C. Harte, in *Gamete Competition in Plants and Animals*, D. L. Mulcahy, Ed. (North-Holland, Amsterdam, 1975), p. 31.
7. C. M. Rick, *Genetics* **53**, 85 (1966).
8. D. L. Mulcahy, G. B. Mulcahy, R. W. Robinson, *J. Hered.* **70**, 365 (1979).
9. G. S. Khush and C. M. Rick, *Genetica (The Hague)* **38**, 74 (1967).
10. D. Schwartz, *Genetics* **67**, 411 (1971); N. F. Weeden and L. D. Gottlieb, *Biochem. Genet.* **17**, 287 (1979); S. D. Tanksley, *ibid.*, p. 1159; *Can. J. Genet. Cytol.* **22**, 271 (1980).
11. S. D. Tanksley and C. M. Rick, *Theor. Appl. Genet.* **57**, 161 (1980); S. D. Tanksley and R. A. Jones, *Biochem. Genet.* **19**, 397 (1981).
12. Pollen grains were germinated in petri dishes on a medium containing 18 percent sucrose, 1 percent agar, and 0.015 percent boric acid.
13. We thank S. K. Jain for reviewing the manuscript and D. G. Hunt for editing. Supported in part by NSF grant DEB80-05542.

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Nalidixic Acid, Oxolinic Acid, and Novobiocin Inhibit Yeast Glycyl- and Leucyl-Transfer RNA Synthetases

Abstract. *Nalidixic acid and novobiocin inhibit the aminoacylation and pyrophosphate exchange activities of glycyl- and leucyl-transfer RNA synthetases from bakers' yeast. Similar types of inhibition are observed for both enzymes, suggesting similar mechanisms. The potency of these inhibitors is comparable to that observed for their inhibition of in vivo DNA synthesis in eukaryotic cells.*

Nalidixic acid (NA), oxolinic acid (OA), and novobiocin (NB) are potent inhibitors of DNA replication in *Escherichia coli* through their action on DNA gyrase (*1*). Both NA and OA appear to inhibit the nicking-closing activity of DNA gyrase (*2, 3*), and at low concentrations inhibit DNA replication (*4*). However, NA inhibition of transcription has been observed at higher concentration, and there is evidence that in *E. coli* the target of NA in this inhibition of transcription is also DNA gyrase (*5*).

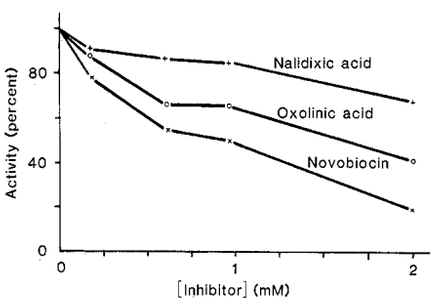

Fig. 1. Dependence of glycine synthetase activity on inhibitor concentration. Purified or partially purified glycyl-tRNA synthetase was mixed with different concentrations of drug. After equilibration at 30°C, a single mix of adenosine triphosphate (ATP), unfractionated tRNA, KCl, $MgCl_2$, and glycine in Hepes buffer at *p*H 7.2, 30°C, was added to the enzyme solution to give final concentrations of 1 m*M*, 2.4 μ*M*, 10 m*M*, 5 m*M*, 33.6 μ*M*, and 40 m*M*, respectively. Reaction was stopped with ice-cold 5 percent trichloroacetic acid after 3 minutes and samples were filtered through 0.45-μm Millipore filters, dried, and counted.

Low concentrations of NB block DNA replication, presumably by inhibiting the adenosinetriphosphatase activity of DNA gyrase in *E. coli* (*6*). Novobiocin also inhibits DNA replication in certain eukaryotic cells at much higher concentrations than those required for in vitro inhibition of prokaryotic DNA gyrase (*7, 8*). DNA polymerase α is also inhibited by NB in extracts of CV-1 monkey cells (*8*), but the target of NB which results in inhibition of eukaryotic DNA synthesis in vivo is not known.

We describe here our finding that NA, OA, and NB inhibit representatives of yet another class of enzymes, the aminoacyl-tRNA (transfer RNA) synthetases. Both yeast glycyl- (GST) and leucyl (LST)-tRNA synthetases are inhibited by these drugs, but at concentrations several orders of magnitude higher than those that inhibit *E. coli* DNA gyrase. Figure 1 shows a dose response curve for the three drugs in the aminoacylation catalyzed by GST. The yeast leucyl- and glycyl-tRNA synthetases used in our study were purified to near homogeneity (*9*).

We have determined the steady-state kinetic parameters for inhibition of the aminoacylation and pyrophosphate exchange reactions by these antibiotics with respect to the adenosine triphosphate (ATP) and tRNA substrates. Figure 2 shows the inhibition patterns for GST with respect to the ATP and tRNA substrates in the aminoacylation reaction. Similar data were obtained for the

Table 1. Summary of inhibition data for glycyl- and leucyl-tRNA synthetases.

Inhibitor	Substrate	Glycyl-tRNA synthetase		Leucyl-tRNA synthetase	
		K_m or K_I (m*M*)	Type*	K_m or K_I (m*M*)	Type*
	ATP	0.1		0.3	
	tRNA	0.6×10^{-3}		0.3×10^{-3}	
		Aminoacylation			
NA	ATP	0.8	C	2.7	C
NA	tRNA	1.3	?NC or C	2.0	NC
NB	ATP	0.9	NC	5.4	NC
NB	tRNA	0.2	UC	3.6	UC
		Pyrophosphate exchange			
	ATP	1.2		1.0	
NA	ATP	6.0	?NC or UC	3.9	NC
NB	ATP	3.5	NC	2.9	NC

*C, competitive; NC, noncompetitive; UC, uncompetitive.

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