carbohydrate units. It is interesting that the asparagine-linked heteropolysaccharide unit does not increase in amount in the diabetic membrane. This could be due to the fact that asparagine residues are put into the peptide chain in a coded manner during the synthesis of the peptide chain and cannot be increased in number after the protein leaves the ribosome, as is possible for the hydroxylysine residues.

The sequence of events between insulin deficiency and this structural alteration in the basement membrane is not understood. The assembly of the basement membrane involves several steps after the ribosomal ones, including hydroxylation and carbohydrate attachment. These steps are open to regulation by environmental influences. It is conceivable that in diabetes the high concentration of glucose or of some metabolic derivative of this sugar could function in regulating both the hydroxylation and the carbohydration of the basement membrane. In addition, the overall rate of the synthesis of this membrane could be influenced by the availability of sugar nucleotides for attachment to the peptide chain.

The alterations which occur in the diabetic membrane involve lysine, or its derivatives hydroxylysine and glycosylated hydroxylysine. Both lysine and hydroxylysine participate in the formation of cross-links of the peptide chains of collagens and elastin (*13*). It has been postulated that glycosylation may help regulate cross-linking of collagen molecules (*14*). The substitution of carbohydrate on hydroxylysine residues may have the function of making them no longer available for participating in the ε-deamination and the subsequent condensation which are involved in cross-link formation.

If such cross-links also exist in the glomerular basement membrane, the increase in glycosylation of hydroxylysine would reduce the availability of this amino acid to participate in their formation. Such a defect in cross-linking and the effect of the extra and bulky carbohydrate substituents on the packing of the peptide chains could contribute to the increased permeability of the basement membrane seen in diabetes mellitus.

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L-Glutamic Acid Decarboxylase: A New Type in Glial Cells and Human Brain Gliomas

Abstract. *Human glial cells grown in culture and gliomas and white matter contain an* L-*glutamic acid decarboxylase which is stimulated markedly by carbonyl-trapping agents. In contrast,* L-*glutamic acid decarboxylase activity of human cerebral gray matter is strongly inhibited by carbonyl-trapping agents. These results suggest a glial localization of the new type of* L-*glutamic acid decarboxylase.*

The α-decarboxylation of L-glutamic acid is catalyzed in mammalian kidney and other nonneuronal tissues by a second L-glutamic acid decarboxylase (GAD II) (*1, 2*) that differs markedly from the previously described, partially purified and characterized L-glutamic acid decarboxylase (GAD I) (*3*). The GAD I is inhibited by anions and completely inhibited by various carbonyl trapping agents at $10^{-3}M$ (*4*) and has largely a synaptosomal localization (*5*). In contrast, GAD II is activated by high concentrations of anions and carbonyl trapping agents (*1, 2*). The GAD II activity measured in the presence of $10^{-3}M$ aminooxyacetic acid hemihydrochloride (AOAA) is primarily mitochondrial in kidney and in developing chick embryo brain (*2*). The GAD II activity became apparent when purified adult mouse brain mitochondrial fractions were assayed with $10^{-3}M$ AOAA, which suggests that in homogenates GAD II activity may be obscured by the presence of GAD I under the usual assay conditions (*6*).

Table 1. L-Glutamic acid decarboxylase activities in cortical gray and white matters of human cerebrum. The values are averages of closely checking triplicate determinations performed on adult human brain obtained 2 hours after death.

Tissue	Specific activity (μg GABA/g protein/min)	
	Standard assay	+AOAA ($10^{-3}M$)
Cortical gray matter	98.6	29.6
White matter	44.1	80.2

Samples of cortical gray and white matters of human brain (2 hours postmortem, taken at autopsy) were homogenized in ice-cold distilled water and the protein concentrations per assay were adjusted to 0.5 mg/ml. Protein was determined by the method of Lowry *et al.* (*7*). The GAD activity was determined in the absence and presence of $10^{-3}M$ AOAA by the radiometric method of Roberts and Simonsen (*4*). The standard assay was performed in $0.1M$ potassium phosphate buffer (*p*H 6.5) containing $10^{-3}M$ aminoethylisothiouronium bromide and $10^{-4}M$ pyridoxal phosphate. Human glial cells cultured from autopsy material in medium 199 containing 10 percent newborn calf serum and 5 percent fetal calf serum and subcultured in the usual manner were supplied by H. Kihara (Pacific State Hospital, California). Human gliomas (astrocytomas, surgical specimens) were supplied by B. Crue (City of Hope).

Table 2. L-Glutamic acid decarboxylase activities of glial cells grown in culture and gliomas of human brain.

	Specific activity (μg GABA/g protein/min)	
	Standard assay	+ AOAA ($10^{-3}M$)
	Glial cells	
Sample 1	42.4	160.4
Sample 2	36.1	141.0
Sample 3	45.0	144.5
	Gliomas	
Case 1	19.1	81.0
Case 2	24.1	79.1
Case 3	21.0	85.0

The GAD activity of cortical gray and white matters of human cerebrum was differentially affected by the addition of $10^{-3}M$ AOAA to the regular assay medium. The GAD of cortical gray matter was inhibited 70 percent, and that of white matter was stimulated 80 percent by $10^{-3}M$ AOAA (Table 1). Glial cells grown in culture and gliomas of human brain removed at surgery had lower GAD activities than cortical gray matter. These activities, however, were stimulated approximately fourfold by $10^{-3}M$ AOAA (Table 2).

To date, all the evidence favors the existence of at least two forms of GAD in mammalian tissues. It has been shown that glial tumors contain both γ-aminobutyric acid (GABA) and GAD activity (*8*) and that the GABA system is present in normal glial cells as well (*9*). Now we have shown the presence of GAD activity stimulated by AOAA in human glial cells grown in culture, in gliomas, and in white matter. The magnitude of stimulation by AOAA was consistent with the suggestion of a glial localization of GAD II, which is present in several nonneuronal tissues and in developing chick embryo brain (*2*). Further studies are being made of the functional significance of the presence of GAD II in glial cells. The rapid isotopic GAD assay may be a useful adjunct in helping distinguish at surgery glial tumors metastasizing from other sites if GAD II should be found to be higher in the glial tumors.

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Clutch Size in Birds: Outcome of Opposing Predator and Prey Adaptations

Abstract. *A model is proposed to explain clutch size in birds as the outcome of the interaction between predatory adaptations of birds to increase their feeding efficiency and adaptations of their food resources to avoid predation. Variations in clutch size are consistent with the model. A modification that incorporates the seasonality of food resources is also discussed.*

Although Lack focused attention on the problem over 20 years ago (*1*), variation in clutch size among birds remains inadequately explained (*2*). Songbirds (Passeriformes) exhibit several patterns. (i) Hole-nesting birds generally lay one to two more eggs per clutch than those which build nests in less-protected locations (*3*). (ii) Clutch size generally decreases as one approaches the equator; from five to seven eggs for open-nesting birds in arctic areas and four to five in temperate localities, to two to three eggs in tropical latitudes. (iii) Within the tropics there are local gradients with respect to rainfall (*4*); however, clutch size does not change as one moves up mountains into cooler climates (*5*). Within temperate areas, birds inhabiting stable "coastal" climates tend to have smaller clutches than those exposed to more variable "continental" climates (*6, 7*).

Currently favored to explain variations in clutch size is Lack's "food-limited" hypothesis (*7*) which treats food availability, or the rate at which adults can gather food, as the factor which limits clutch size. Cody (*8*) modified the theory to incorporate the allocation of time and energy between foraging behavior, which affects clutch size, and other behavior involving predator avoidance, self-maintenance, and competitive ability. Murphy (*9*) and Williams (*10*) have pointed out that the intensity of reproduction, which is reflected in clutch size, may alter the survival of the adults, hence the probability of future reproduction. Such compromise must be incorporated into any complete theory of clutch size. Lack's theory, even as currently modified, postulates that clutch size is adjusted to the optimum rate at which the parents can gather food. Thus, if this rate is maximized through natural selection, clutch size is limited by the abundance, or availability of the food resource.

If Lack's hypothesis is true, we must ask what determines the absolute availability and variations of food resources which produce the diversity of clutch sizes among birds. Differences in local primary productivity cannot be solely responsible because, for example, species of arid and humid regions in the temperate zone have similar clutch sizes although productivity of desert vegetation is but a small fraction of that of eastern forests (*11*). Moreover, the ability of adult birds to gather food resources cannot be treated as a simple function of prey availability but must be considered as the outcome of evolutionary responses of the predator and prey to each other. Birds evolve to maximize the efficiency with which they can exploit food resources. Equally important, and generally neglected in considerations of clutch size, prey evolve to minimize the efficiency of their predators.

These considerations lead to a simplistic model of evolutionary interactions whose outcome is reflected in clutch size. The efficiency with which environmental food resources, or prey, are converted into offspring of the predator depends on the balance between predator and prey adaptations. Evolutionary changes in the foraging strategy of the predator will alter the environment of the prey and select for an evolutionary response, which tends to restore the original rate of exploitation of prey. The equilibrium which determines the mortality of the prey and the rate at which food is gathered by the predator will depend, in the final analysis, upon potential rates of evolutionary change and also upon constraints which limit the adaptability of the predator and prey.

We measure the outcome of the adaptive system from the standpoint of the predator by its clutch size (we assume all species to be equally efficient at converting gathered food into offspring). Perhaps most striking is not that clutch size varies among species, but that it is so constant. Among small passerines, for example, most of those which breed in the United States raise broods of four young. Yet these include flycatchers, foliage gleaners, ground