not usual in the type 2 human disease but can be consistently demonstrated in individuals with the type 1 disease. In our dog the gangliosides in the spleen were increased approximately fivefold, and 43 percent of the total was identified as GM$_1$. The splenic concentrations of the GM$_1$ fraction was also shown to be more than 70 times greater than those found in the spleen of a normal dog of comparable age. Similar but less marked changes were found in the liver. These results are consistent with a type 1 human gangliosidosis.

Findings in this dog are of special interest because the disorder appears to have features of both types of GM$_1$ gangliosidosis seen in children. As in the human conditions, an autosomal recessive pattern of inheritance is also probable. Evidence for this include (i) pedigree consanguinity, (ii) phenotypically normal parents, and (iii) clinical or morphologic observations that indicate that both male and female siblings are affected. Data on the ratio of normal to diseased dogs are fragmentary, but would be expected to approach unity if enough litters were available for evaluation.

On the basis of the genetic, clinical, morphologic, and biochemical studies presented here, we have identified this disease as neuronal-visceral GM$_1$ gangliosidosis with β-galactosidase deficiency. Since canine examples of GM$_1$ gangliosidosis have not been reported, dogs from this family show promise as models for the study of gangliosidosis in man.

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2a. Symbols proposed by L. Svennerholm [*J. Neurochem.* **10**, 613 (1963)] are used for abbreviation of gangliosides as follows: GM$_1$, galactose-(β1 → 3)-*N*-acetylgalactosamine-(β1 → 4)-*N*-acetylneuraminic acid-(α2 → 3)-galactose-(β1 → 4)-glucose → ceramide; GM$_2$, *N*-acetylgalactosamine-(β1 → 4)-*N*-acetylneuraminic acid-(α2 → 3)-galactose-(β1 → 4)-glucose → ceramide.

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8. Gray matter was homogenized in ten volumes of 0.25*M* sucrose containing 1 m*M* EDTA, *p*H 7; the homogenate was treated with ultrasound for 30 seconds with cooling and then centrifuged at 4000*g* for 10 minutes. The resultant supernatant was used as the enzyme source. Complete incubation mixtures contained, in a final volume of 0.35 ml, the following: acetate buffer, *p*H 4.5, 100 moles; *p*-nitrophenyl-β-D-galactopyranoside, 2.5 moles; and 0.4 to 0.6 mg of enzyme protein. Incubations were at 37°C for 1 hour. Reactions were terminated with trichloroacetic acid and the amount of *p*-nitrophenol liberated was determined [S. Gatt, *Methods Enzymol.* **14**, 156 (1969)].
9. Tritium was introduced into the terminal galactose of GM$_1$ and lactosyl ceramide by oxidation with galactose oxidase and reduction with NaB^{3}H$_4$ [Y. Suzuki and K. Suzuki, *J. Lipid Res.* **13**, 687 (1972)]. Specific activities of the resultant products were 2000 and 420 count/min per nanomole for GM$_1$ and lactosyl ceramide, respectively. Complete incubation mixtures contained 20 nmole of acetate buffer, *p*H 4.5, 100 nmole of glycosphingolipid, 1 mg of sodium taurocholate, 0.5 mg of Triton X-100, and 4 to 6 g of gray matter supernatant protein in a final volume of 0.2 ml. Incubations were carried out at 37°C and reactions were terminated with 0.2 ml of ethanol. Liberated galactose was separated on thin-layer chromatography plates, and radioactivity was determined by standard methods.
10. Purdue University Agricultural Experiment Station Paper No. 6256. Supported in part by NSF grant PCM75-11908. T.W.K. is supported by NIH research career development award GM-70596.

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Halictine Social Evolution: The Australian Enigma

Abstract. *Australian halictines belong to the primitive genus* Lasioglossum *or related subgenera. The underground nests have lined cells in series or clusters and sometimes at the end of laterals. Two full generations per year are produced in the communal nests. Overwintered and newly emerged females form unique "pseudosocieties" rather than matrifilial societies along Holarctic patterns. Several* Chilalictus *species produce a "male caste" of big-headed, flightless males, in addition to normal individuals. Oviposition of unfertilized eggs on large pollen balls causes such allometric bees.*

Halictine bees form a rich assemblage of species that embraces every conceivable degree of presocial and fully social behavior. The studies of many observers have contributed greatly to our understanding of the evolution of bee societies (*1, 2*). North America and Europe were especially active centers of research, since the two major halictine groups, *Lasioglossum* and *Halictus*, reach their greatest development there. *Lasioglossum* is found all over the world, with the related species of *Evylaeus* and *Dialictus* dominating the Holarctic region. *Halictus* is mostly confined to the Northern Hemisphere, where solitary and primitively social members occur. Their nests consist of burrows with sessile cells, except for the combs that are constructed by *H. quadricinctus* in Europe.

Nothing illuminates the diversity of nest architecture and the range of behavior better than the many species of the genus *Evylaeus*. One generation per year is produced by *E. lucidulus*, *E. rufitarsis*, and *E. minutissimus*, whereas two independent generations occur in *E. villosulus* and *E. quadrinotatulus*. Primitive as well as highly evolved societies abound in most regions; sometimes several social levels are represented by a few related species. *Evylaeus pauxillus*, *E. linearis*, *E. laticeps*, and *E. malachurus*, for example, show a gradual increase in caste differences, nest population, and number of worker broods produced, but at the same time they delay the production of males until their appearance coincides with that of the future queens (*3*). Much lower levels of sociality are found in the siblings *E. calceatus*, *E. albipes*, and *E. duplex* where caste differences are not distinct, males are produced throughout the summer, and nest populations remain small (*4*). *Evylaeus marginatus* is the only perennial social halictine whose huge societies last for up to 6 years but where queens and workers are morphologically identical (*5*).

Great variety is also found in the nest architecture of *Evylaeus*. Solitary species usually have a simple burrow from which lateral tunnels lead to a single terminal cell or, as in *E. nitidiusculus*, to a few cells in series. Social species have sessile cells or combs surrounded by a cavity. The most advanced halictine societies of *E. marginatus*, *E. linearis*, *E. malachurus*, and *E. cinctipes* leave the brood cells open for greater interaction between the two generations and better nest sanitation (*6*). No communal nests are known from *Evylaeus*, but several species, including *E. linearis*, *E. glabriusculus*, and *E. calceatus*, form semisocial societies of overwintered females in spring. A hierarchy that results in a division of labor becomes established among the nest mates. A functional queen remains in the nest and lays all the eggs; the rest of the females forage and act very much like workers. This arrangement is replaced by a matrifilial society in summer (7). The more aggressive species—*E. marginatus*, *E. malachurus*, and *E. cinctipes*—never tolerate another queen but are only found in monogynous nests (8).

Australian halictine bees are numerous, with many sets of sibling species occurring side by side. The main component of the fauna is the genus *Lasioglossum*, with a few related groups that are incompletely known. Despite this dearth of biological information, several interesting facts have emerged. The nest architecture, for example, is dominated by cells constructed in series or in clusters; sessile cells have yet to be found in any Australian halictine (Fig. 1). This is in complete contrast to the Holarctic situation, where sessile cells are not only common in many species but are thought to be ancestral to comb construction in others (9). The possible development of such clusters was observed by one of us (G.K.) in the sandy cliffs along the river Dordogne in southwestern France. *Halictus (Seladonia) subauratus* was nesting there in great numbers and building the usual sessile cells along the burrow in the higher reaches of the cliff. However, close to the water's edge where the sand was wet, the females removed the material around the cell concentrations in order to form a cavity.

The biology of the species studied by us in Australia was surprisingly uniform. Two generations were usually produced in communal nests that were used year after year. Overwintered or summer females rarely established their own burrows but shared their natal nest with other females. During summer, more than 20 females from both generations foraged in these nests. There was no difference between the females of the two generations as far as morphology, ovarian development, or sperm content was concerned. The wing and mandible wear of the long-lived, overwintered females was naturally greater than that shown by their daughters. It was clear that no matrifilial society ever became established in these halictines, yet the aggregations were not comparable to andrenid communal nests because the latter lack all the social traits shown in the Australian bees. For example, several *Lasioglossum* species, especially *L. lanarium*, exhibited the most aggressive nest guarding seen anywhere. *Lasioglossum cyclurum* cooperated in cell construction when several foragers shared a single cell comb, and most of the other populous nests had cell concentrations in confined spaces, thus making interaction among nest mates unavoidable (Fig. 1).

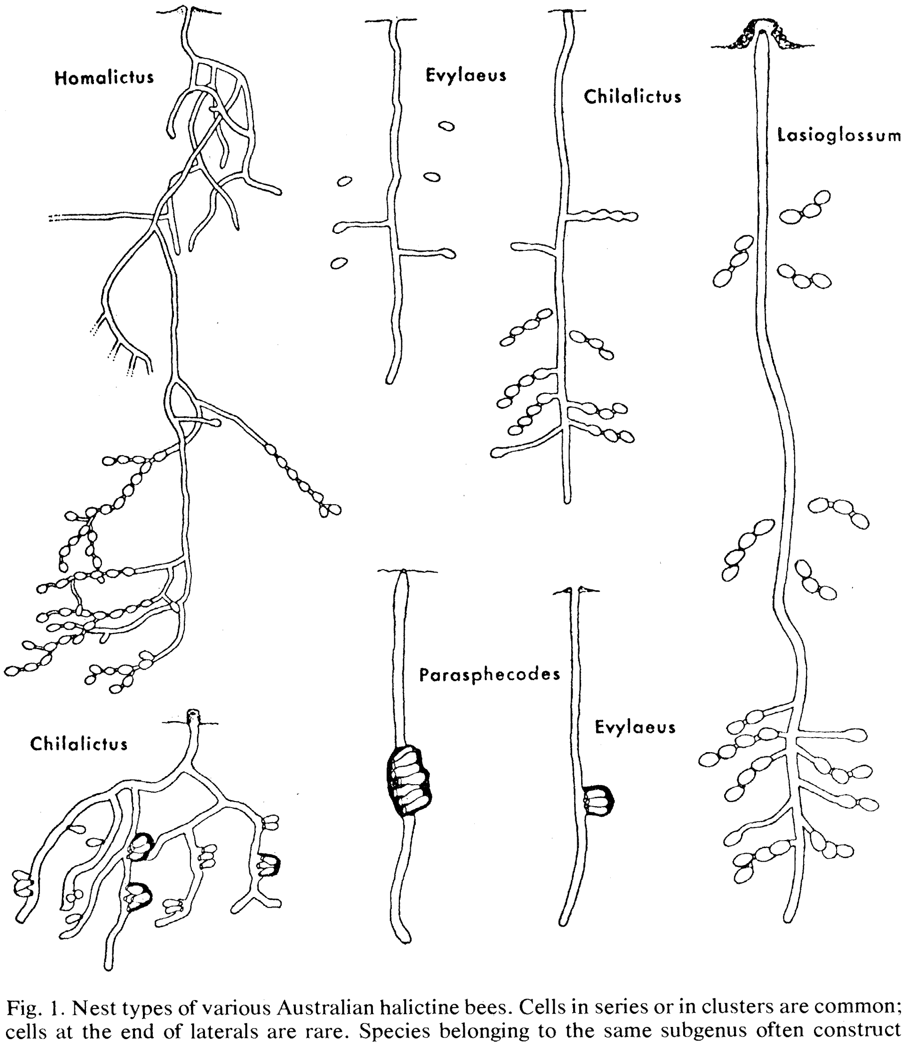

Fig. 1. Nest types of various Australian halictine bees. Cells in series or in clusters are common; cells at the end of laterals are rare. Species belonging to the same subgenus often construct strikingly different nests, for example, *Chilalictus inclinans* (top) and members of the *C. erythrurum* complex (bottom left). Similar differences occur in the two *Evylaeus* species illustrated. *Homalictus demissus*, *Lasioglossum lanarium*, and a *Parasphecodes* species complete the range.

In view of the primitive sociality of Australian halictines, it was of general interest when the existence of a "male caste" was first reported for this group. A small species close to *Lasioglossum (Chilalictus) erythrurum* was found to have two types of males, one normal and another with large heads and rudimentary wings. A defensive function was attributed to the latter because of its great resemblance to ant soldiers (10). This idea was received with reservation, mainly because no instance of other male castes has ever been recorded in the social Hymenoptera (1). We recently excavated many nests of *L. erythrurum* and several related species in a few localities in New South Wales. The burrows usually contained both types of males but the entrances were never found to be guarded or in any way defended by the bigheaded males (Fig. 2). These males have not been found to react to virgin females caged with them. At best they lack any purpose in the society, but more probably they are parasitic, being unable to forage afield.

Macrocephaly of both sexes is well known in halictine bees from several continents. A continuous series is usually present, extending from smallish, normal individuals all the way to the larger, bigheaded forms, as illustrated in *E. ohei* from Japan (4). Conversely, Australian halictines have no intermediate-sized males but only show two extremes, that is, the "male castes" and normal individuals. Since the ultimate size of the bees depends on the provisions they receive as larvae, it is obvious that the weight of the pollen balls from which the males developed must have been bimodal too. We had noticed this feature before in other Australian halictines that lacked big-headed males. The pollen balls found in the cells of these species came in two sizes: normal and very small. The small pollen balls appeared to be incomplete except for an egg or a young larva on top. The same species showed a very pronounced sexual dimorphism, with females often reaching twice the size of males. This meant that ovipositing females laid haploid eggs on small pollen balls for the males and diploid eggs on large pollen balls for the females. The species with big-headed males use the same strategy of dimorphic provisions. It is reasonable to assume that an occasional unfertilized egg deposited on a large pollen ball would result in a big but allometric male. Pupal weights of several species confirmed this hypothesis when no weight difference could be found between big-headed males and females (Fig. 3).

Halictine bees have attracted more than their share of speculation about their social evolution (*1*, *2*, *4*). Two hypotheses dominate the literature. (i) The classical idea of solitary females producing a generation whose females stay in the nest as workers is still very popular. (ii) A "parasocial" route proposed the formation of societies by aggregations of related or unrelated females. Whatever the origins, the end result is a monogynous, matrifilial society.

It is difficult to say whether the comparative study of living forms can ever recapture the evolutionary pathways of insect societies beyond any doubt. A curious fact about Australian halictines is their lack of true sociality, in spite of a considerable overlap in the two generations, the sharing of nests, and the addition of several other social characters. Perhaps Australian species are not at an intermediate stage to full social behavior, but have evolved a viable alternative through the perfection of the communal "pseudo-society." A parallel development with the matrifilial system of Holarctic bees would be consistent with a group that has radiated in every possible evolutionary direction.

The probable origin of matrifilial societies from solitary females seems well supported now by discoveries in the genus *Lasioglossum* in the Mediterranean region (*11*). The majority of species, including *L. leucozonium* and *L. zonulum*, are solitary and univoltine, constructing single brood cells at the end of laterals. *Lasioglossum bimaculatum* also produces only one generation a year; however, the cells are in series in deep communal nests that are shared by up to seven females. Finally, *L. aegyptiellum* has a matrifilial society of the most primitive type, with one queen and a few slightly smaller daughters. These do all the foraging, usually remain unmated, but have mature oocytes in their well-developed ovaries. The single brood cells are on very short laterals.

The range of behavioral types found in this primitive genus indicates to us that the communal forms with their serial cells must once have been as abundant in the Northern Hemisphere as they are now on the southern continents. Only a few relicts, such as *L. bimaculatum*, are left in Europe. Instead, they have been replaced by the numerous solitary and

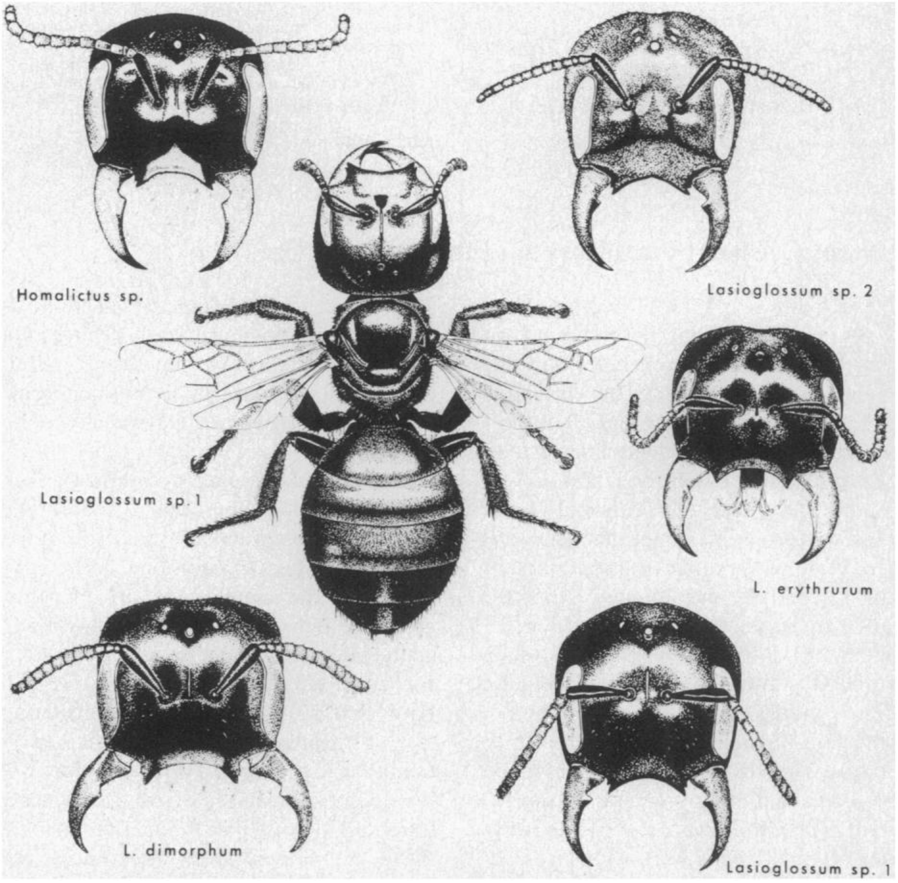

Fig. 2. Head and body shapes of "male castes." *Lasioglossum (Chilalictus)* species 2 is a male of the Eucla nest described by Houston (*10*); *Lasioglossum (Chilalictus)* species 1 is closely related to both species 2 and *L. (Chilalictus) erythrurum*, designated here as a complex.

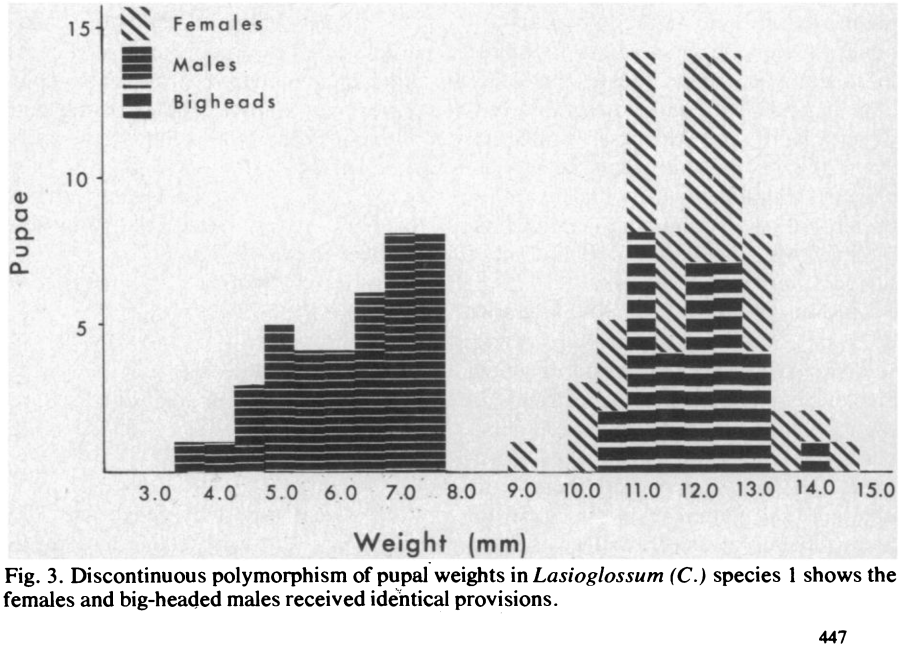

Fig. 3. Discontinuous polymorphism of pupal weights in *Lasioglossum (C.)* species 1 shows the females and big-headed males received identical provisions.

univoltine species. These in turn are at the threshold of social behavior which, in at least one species, has already been crossed.

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Wheat Gluten–Schizophrenia Findings

Singh and Kay (*1*) reported that 30 out of 39 measures of psychopathology and social participation reflected non-therapeutic changes during the period of gluten challenge. In fact, only 5 of the 14 reported measures yielded changes sufficiently large to reach the commonly accepted .05 level of statistical significance. Moreover, symptoms characteristic of schizophrenia, such as poor impulse control and thought disorder, failed to attain this criterion. Indeed, 9 out of 13 measures yielded two-tailed probability values of .2 or less, indicating trends in the data, yet not direct confirmation of their hypotheses. Even the one measure on which the authors reported significant improvement, passive or apathetic withdrawal, represents only a weak trend at the .2 level.

The authors stated that group changes during gluten challenge "occurred against the expected course of improvement with neuroleptic treatment." However, deterioration during gluten treatment occurred only in the five most seriously ill patients with a less favorable therapeutic outcome, while the other nine patients with more favorable outcomes were not adversely affected. These figures indicate that the sample was not homogeneous with regard to premorbid history, number of prior hospitalizations, or severity of illness, all of which covary with prognosis.

Perusal of Singh and Kay's graphs does not convincingly indicate pathologic increases during the period of gluten challenge; indeed, decreasing trends in the pathology ratings occurred concurrently with gluten challenge. Moreover, the fact that overall therapeutic outcome and gluten response were not independent of each other suggests that a different type of analysis would have been more suitable in handling the data. The basic methodological question is whether pathologic increase during gluten challenge retains significance after the overall decrease in symptomatology covering 12 weeks is statistically controlled.

Analysis of the data would further require separating subgroups with differing premorbid histories and paranoid-nonparanoid status. In addition, the heterogeneity of the sample indicates that this group of schizophrenics includes both acute and chronic subjects. Moreover, the mean IQ of this sample was 78.57; borderline intelligence is not representative of hospitalized schizophrenics, particularly at the age of this sample, and this points to another source of confounding in the data. Such factors as these may significantly affect drug responsivity and prognosis independently of gluten treatment.

We doubt that the reported findings would persist if the analyses recommended above were carried out. If gluten impairs the psychological status of schizophrenics, it is important to provide sound data to demonstrate that effect. Appropriate statistical tests of the data and replication of the results of the study are called for.

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The recent report by Singh and Kay (*1*) appears to support the hypothesis that wheat gluten is a pathogenic factor in schizophrenia. However, it suffers from several serious methodological flaws.

The chi-square analysis they present utilizes the averages of 33 dimensions of psychopathology obtained with 14 patients as if they were independent observations, a basic requirement in any application of the chi-square test (*2*). However, since these dimensions of psychopathology are correlated, the chi-square analysis is not valid.

The correlated *t*-test comparing group changes across all 33 dimensions of the psychopathology rating schedule is also inappropriate since the sampling distribution for the correlated *t*-test is predicated on the fact that the rows are independent, a condition which again does not exist. The usual approach to answering the question the investigators appear to be asking in the analysis would be to compute a correlated *t*-test (or nonparametric Wilcoxon matched-pairs signed-ranks test) between the gluten and nongluten mean psychopathology scores with the data of the 14 subjects entered as rows.

Since no control groups were studied which received the placebo drink for all 12 weeks, it is impossible to determine how much of the attenuation of clinical effect after 6 weeks of neuroleptic treatment is due to the gluten intervention and how much is due to the normal process of recovery from a schizophrenic episode (that is, rapid neuroleptic effect followed by a more gradual and fluctuating recovery).

While the authors note that "each patient was interviewed for 90 minutes and independently rated by a specially trained psychiatrist and a psychologist," it is unclear whether the nonblind principal investigator (M.M.S.) conducted or participated in these clinical interviews. Since the interviewer is an important factor in any clinical interview situation, participation of the nonblind principal investigator in these interviews would make the ratings based on the information obtained during these interviews less independent of the experimental conditions than one would be led to believe.

Considering these methodological problems, I am reluctant to conclude that the hypothesis that wheat gluten is pathogenic in schizophrenia was confirmed.

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