

mada male cephalic glands is emitted and presumably sprayed onto the female partner (Fig. 3). Gas chromatographic and mass spectrometric data indicate that this is the case. The low volatility of F6/G8, and perhaps also the waxy coating and the structure of the cuticle, make these compounds adhere to and be emitted from the *Nomada* female for a long period of time. The F6/G8 may be the signal that establishes the nonaggressive relationship (18) between females of *Andrena* and *Nomada*. The perfuming of the female by the *Nomada* male might make the signaling more effective than if the compounds were produced by the *Nomada* female herself. If females perfumed with F6/G8 have advantages over unperfumed females or females perfumed with other compounds, the males producing F6/G8, and also this signal system, will be evolutionally favored. Males perfuming females during copulation have been reported in butterflies (19), who transfer a substance that acts as an antiaphrodisiac signal.

The chemical-physiological-ethological link between *Andrena* or *Melitta* (hosts) and *Nomada* (parasite) is supposed to be mediated partially through precise chemical compounds. These are volatile substances influencing behavior through the olfactory sense. In this case, they constitute a liaison among three elements in the biocenosis and facilitate their coevolution. The possible advantage of transfer of volatile compounds from male to female *Nomada* (over the female's producing these substances herself) presumably arises by binding the parasite male closer to the relationship between the host and the parasite females.

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5. We have neither observed any aggression between *Nomada* and *Andrena* females nor seen any such reports in the literature. About other genera of solitary bees, contradictory statements about conflicts between host and cleptoparasite females have been made. The parasite females have often been observed to sit outside the host nest waiting for the host female to leave, which has been interpreted as an indication that the host female would defend her nest. However, the parasite must wait for an appropriate moment in the nest cell construction for her egg laying, which must cause her to check the stage of development of the nest after each occasion the host female has been in the nest. From social bees there are also reports of both aggressive and nonaggressive relationship between host and cleptoparasite. H. Friese [*Zool. Jahrb. Abt. Syst. Oekol. Geogr. Tiere* **3**, 847 (1888)] postulated concerning the non-aggressive relationship of *Andrena* and *Nomada* females, about the odor of *Nomada*. "Vielleicht liegt in diesem Individual-geruch das freundschaftliche Verhältnis begründet." Also, W. M. Wheeler [*Proc. Am. Philos. Soc.* **58**, 1 (1919)] discussed the significance of odor in the nest parasite relation to the host.

6. A. Haas, *Z. Tierpsychol.* **17**, 402 (1960).
7. A. Haas [*Z. Naturforsch.* **1**, 596 (1956)] describes how bumblebee males mark their flight paths with odor spots. In our studies we have observed the *Andrena* males to do practically the same thing.
8. C. G. Butler, *Proc. R. Entomol. Soc. London Ser. A Gen. Entomol.* **40**, 77 (1965).
9. The techniques of chemical analysis rely to a large extent on capillary gas chromatography coupled to mass spectrometry. The inlet system used has been described in S. Stållberg-Stenhagen, *Chem. Scr.* **2**, 97 (1972); S. Stållberg-Stenhagen, E. Stenhagen, G. Bergström, *Zoon Suppl.* **1**, 77 (1973); G. Bergström, *Chem. Scr.* **4**, 135 (1973).
10. The behavior of *Melitta* species is essentially the same as that of the *Andrena* species.
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15. The chemical correspondence between *Andrena* and *Nomada* is constituted through esters of isoprenoid alcohols, geraniol, and farnesol. The compound in the *Melitta-Nomada* relationship is an ester of a fatty alcohol, octadecanol. The isoprenoid esters and octadecyl butyrate each represent separate biosynthetic pathways.
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17. R. R. Askew, *Parasitic Insects* (Elsevier, London, 1971).
18. Most bees defend their nests. The significance of odor in aggressive relationship between host and parasite have been demonstrated by S. W. T. Batra [*J. Kans. Entomol. Soc.* **38**, 367 (1965)] concerning a mutillid wasp and a halictid host bee, *Lasioglossum zephyrum*, and by E. Ordway [*J. Kans. Entomol. Soc.* **37**, 139 (1964)] concerning the bee *Sphecodes pimpinellae* and its two host species of the halictid bee *Au-gochlorella*. The visit of a female *S. pimpinellae* to a host nest causes a change in host bee behavior and has an inhibitory effect on the nesting activity, lasting for a few weeks. Not leaving an unfamiliar smell in the host nest must be advantageous to *Nomada* females with the F6/G8 odor.
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20. *Nomada panzeri* is closely related or synonymous to *N. ruficornis*. The *N. ruficornis* group is not clear taxonomically. Most authors regard *A. helvola* as one of the hosts of *N. ruficornis*.
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Hypoxanthine Phosphoribosyltransferase: Two-Dimensional Gels from Normal and Lesch-Nyhan Hemolyzates

Abstract. Immunoprecipitated hypoxanthine phosphoribosyltransferase (HPRT) from hemolyzates displays two major spots after two-dimensional polyacrylamide gel electrophoresis. HeLa cells or human lymphoblasts display only a single HPRT spot located at the same position as the most basic of the hemolyzate HPRT spots. This suggests that the most basic spot is the form initially synthesized, and the more acidic hemolyzate HPRT spot (a pseudoisozyme) is probably derived from the first by an age-related modification (for example, deamidation). The HPRT pattern of the hemolyzate from a Lesch-Nyhan patient was shifted to a more basic isoelectric pH, implying the mutation of a structural gene.

The purine salvage enzyme hypoxanthine phosphoribosyltransferase (HPRT, E.C. 2.4.2.8) catalyzes the synthesis of inosine or guanosine monophosphate from 5-phosphoribosyl-1-pyrophosphate and hypoxanthine or guanine. Clinically, HPRT-deficiency is associated with the X-linked Lesch-Nyhan syndrome (1). This disorder is characterized by mental retardation, self-mutilation, and high concentrations of uric acid in the blood and urine. Patients with Lesch-Nyhan syndrome usually display virtual absence of HPRT activity in their erythrocytes. Immunological studies indicate that these erythrocytes sometimes contain non-functional HPRT protein which cross-reacts with antisera prepared against erythrocyte HPRT from normal individuals (2, 3).

We have described the analysis of ra-

dioisotope-labeled normal and mutant forms of HPRT from tissue culture cells by immunoprecipitation and two-dimensional polyacrylamide gel electrophoresis (4). In this report, we describe the results of a similar analysis of unlabeled HPRT in red blood cell hemolyzates.

The immunoprecipitation of HPRT protein from hemolyzates was conducted according to a modification of the double antibody procedure previously described (2). All steps were performed at 0° to 4°C. Hemolyzates were prepared as described by Arnold and Kelley from outdated normal blood obtained from a blood bank (5). The Lesch-Nyhan hemolyzate was from patient E.S. (6). A 30- μ l sample of hemolyzate was mixed with 90 μ l of enzyme buffer (20 mM KCl, 6 mM MgCl₂, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 20 mM tris-hydrochloride, pH 7.8)

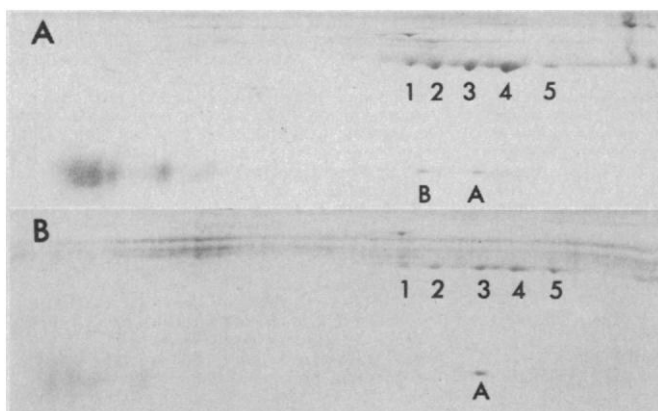


Fig. 1. Two-dimensional polyacrylamide gel of immunoprecipitated HPRT. (A) Hemolyzate prepared from normal blood. (B) Extract prepared from HeLa cells. The direction of sodium dodecyl sulfate electrophoresis is vertical, and of isoelectric focusing is horizontal, with pH 4 on the left and pH 7 on the right.

and centrifuged for 20 minutes at 24,000g. The supernatant was removed and added to 120 μ l of enzyme buffer containing 0.5 percent Triton X-100, 0.5 percent sodium deoxycholate, and 0.5 μ l of goat antiserum to HPRT, and was then incubated for 5 to 8 hours. An equivalent amount of rabbit antiserum to goat γ -globulin (5 μ l) was added and the mixture incubated overnight. The precipitate was sedimented by centrifugation for 10 minutes at 24,000g, and the supernatant was removed with a drawn-out Pasteur pipette. The precipitate was washed once with enzyme buffer containing 0.25 percent Triton X-100 and 0.25 percent sodium deoxycholate, and was either immediately applied to an isoelectric focusing gel or stored in a liquid nitrogen freezer.

The immunoprecipitate was subjected to two-dimensional polyacrylamide gel electrophoresis as described by O'Farrell (7) with modifications as described (4). Two major spots (labeled A and B in Fig. 1A) appeared in the pattern from hemolyzates at the correct molecular weight location (26,000) and isoelectric pH (5.7 to 6.0) for human HPRT (8). It is not possible to determine if both spots represent active species as isoelectric focusing is performed in the presence of 9M urea. No spots appeared in this region when serum obtained prior to immunization (2) was substituted for HPRT antibody (Fig. 2D), although other spots in the pattern still were present and probably correspond to antibody proteins. A series of spots (labeled 1 to 5 in Figs. 1 and 2) make useful markers in comparing gels, and may correspond to antibody heavy chains. A diffuse pattern of proteins focusing at low pH and having a molecular weight of approximately 25,000 may correspond to antibody light chains.

Only a single HPRT spot occurred in patterns from "wild-type" HeLa cells grown in culture (Fig. 1B) (4), or in the patterns from cultured human lympho-

blasts (data not shown). The most basic of the red blood cell HPRT spots, labeled species A, occurred at the same location as the HeLa or lymphoblasts HPRT, suggesting that it is the form initially synthesized. Species B of red blood cell HPRT is probably a pseudoisozyme derived from species A by an age-related modification (for example, deamidation) of an amino acid residue and is not the product of a separate gene. Similar changes in purine nucleoside phosphorylase have been correlated with red blood cell age in vivo, and with time of storage of fibroblast extracts in vitro (9). We have sometimes observed additional apparent HeLa HPRT isozymes of lower isoelectric pH, but we believe they are artifacts that depend on storage conditions and the handling of extracts.

The significant regions of two-dimensional gel patterns of HPRT immunoprecipitated from normal blood and from the Lesch-Nyhan patient E.S. are presented in Fig. 2. In Fig. 2A, species A and B of normal HPRT lie under markers 3 and 1, respectively. In Fig. 2B, the Lesch-Nyhan HPRT species a and b lie

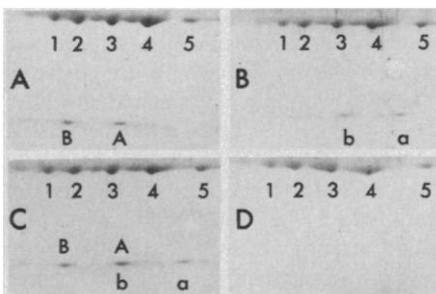


Fig. 2. Significant region of two-dimensional polyacrylamide gel of immunoprecipitated HPRT. (A) Hemolyzate prepared from normal blood. (B) Hemolyzate from a Lesch-Nyhan patient (E.S.). (C) A mixture of hemolyzates from normal blood and from blood from a Lesch-Nyhan patient (E.S.). (D) A control study of hemolyzate prepared from normal blood with serum obtained prior to immunization.

under markers 5 and 3, respectively. Our interpretation is that analogous to normal HPRT, the Lesch-Nyhan species a is the form initially synthesized and species b is a modified form derived from species a. Thus, the two-spot Lesch-Nyhan pattern has undergone a shift to a more basic isoelectric pH. The pattern from a mixture of normal and mutant HPRT in Fig. 2C provides confirmation that the Lesch-Nyhan HPRT pattern is displaced from the normal pattern. The shift suggests there has been the substitution in the mutant protein of a basic for a neutral amino acid or a neutral for an acidic amino acid.

Immunoprecipitation of HPRT and analysis by two-dimensional polyacrylamide gel electrophoresis may be an applicable clinical technique. These procedures can distinguish an immunoreactive abnormal HPRT with an altered isoelectric pH from the normal protein. These types of alterations provide strong evidence for a genetic mutation in the enzyme's structural gene. The clinical characteristics and perhaps methods of treatment of patients with structural gene mutations in HPRT may differ from those of patients who lack HPRT activity as a result of regulatory defects, even though both types of patients lack enzyme activity. An advantage of the two-dimensional gel analysis is that it is not dependent on enzymatic activity. In prenatal diagnosis of potential Lesch-Nyhan infants, the method might be applied to microquantities of fetal erythrocytes (for example, 30 μ l of hemolyzate) or to cells cultured from amniotic fluid to detect abnormal HPRT even in the presence of the normal enzyme.

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10. We thank W. N. Kelley for providing the hemolyzate from the Lesch-Nyhan patient. This research was supported by PHS grant CA-21650 from the National Cancer Institute. Address reprint requests to G.M.

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