Table 1. The intracellular sodium $[Na]_i$ and potassium $[K]_i$ concentration (millimoles per kilogram of fiber water) of guinea pig right ventricular strips during anoxic incubation and two cooling-rewarming cycles. Calculations were based on extracellular space (inulin) of 267 ml per kilogram of wet tissue and water content of 766 ml per kilogram of wet tissue. Calculated potassium equilibrium potentials (E_k) are based on mean of $[K]_i$ and the resting potentials (E_m) measured in papillary muscle incubated under similar conditions. All values are mean ± S.E.M. Numbers in parentheses are either number of preparations analyzed for sodium and potassium or number of E_m measurements made.

Time (min)	Medium	T (°C)	$[Na]_i$ (mmole/kg)	$[K]_i$ (mmole/kg)	E_k (mv)	E_m (mv)
0	$G_{50}O_2$	35	36.7 ± 4.4 (14)	103.8 ± 2.4 (14)	−82.6	−81.1 ± 1.0 (12)
15	$G_{50}N_2$	8	64.8 ± 5.2 (14)	78.9 ± 4.8 (14)	−69.3	−67.9 ± 1.5 (12)
30	$G_{50}N_2$	35	60.2 ± 4.9 (12)	76.5 ± 3.8 (12)	−74.0	−81.8 ± 1.1 (12)
480	G_5N_2	35				−76.4 ± 1.7 (12)
485	$G_{50}N_2$	35	117.0 ± 5.0 (13)	36.7 ± 1.4 (13)	−55.3	−83.0 ± 1.5 (12)
500	$G_{50}N_2$	8	136.5 ± 3.8 (13)	30.5 ± 1.6 (13)	−46.3	−46.4 ± 2.5 (12)
515	$G_{50}N_2$	35	118.0 ± 3.0 (11)	29.4 ± 1.8 (11)	−48.8	−85.9 ± 2.2 (12)

intracellular sodium concentration ($[Na]_i$) had increased sharply and $[K]_i$ had decreased. On rewarming to 35°C for 15 minutes, E_m returned to control level some 8 mv greater than E_k. Neither potassium nor sodium changed significantly, although a slight loss of both may have occurred.

The muscles were then incubated for 450 minutes in G_5N_2 medium. This environment has been shown previously to induce large losses of muscle potassium and gains of sodium (*6*). At the end of this period of incubation, E_m had decreased slightly to −76.4 mv, but on elevation of the glucose concentration to 50 m*M* a slight increase (6.6 mv) in E_m was noted. The E_k at this time was −55.3 mv. An increase in the glucose concentration from 5 m*M* to 50 m*M* has been shown to reverse anoxia-induced shortening of the action potential duration in cat (*13*) and guinea pig (*5*) cardiac muscle. This effect was associated with an increased ATP production (*11*).

During rapid cooling to 8°C and incubation for 15 minutes, additional sodium was gained and potassium was lost (*14*), and E_m fell to a value that approximated E_k. On rewarming to 35°C, there was no change in potassium, a sharp loss of sodium, and a hyperpolarization of the membrane.

The observed responses during these cold-warm cycles indicate that the resting potential of anoxic cardiac muscle is dependent on the activity of an electrogenic pump rather than on a cellular compartmentalization of potassium. If the concentration of potassium in a particular compartment were the same in fresh muscle ($G_{50}O_2$) as in anoxic muscle, and if the potassium gradient between this compartment and the extracellular phase were responsible for the resting potential, E_m would be expected to decline to the same value during each period of cooling. Furthermore E_k, a function of total cell potassium, would not approximate E_m at 8°C. In experiments to be described elsewhere, $10^{-5}M$ ouabain decreased the membrane potential of anoxic muscle, and almost completely blocked the hyperpolarization and extrusion of sodium following hypothermia. Raising the external potassium concentration to 15 m*M* increased the hyperpolarization following hypothermia. This evidence, combined with the absence of chloride contribution to the hyperpolarization after hypothermia in cat papillary (*10*) and uterine muscle (*15*), does not support a role for chloride in the present case.

The results support the concept that the resting potential of anoxic guinea pig ventricular muscle can be separated into at least two components. One component is predicted by the potassium distribution and one is dependent on the activity of an electrogenic sodium pump. Energy from glycolysis has been implicated in the electrogenic pump of mammalian nonmyelinated nerve (*16*). Although glycolytic ATP provides sufficient energy to drive the pump described here, it is not sufficient to maintain the action potential duration and contractility, nor prevent large changes in ion content.

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14. There was a greater loss of $[K]_i$ during the 15-minute cold period in fresh muscle than during the 15-minute cold period after 8 hours anoxia. This finding has been confirmed in a further series of experiments. $[K]_i$ loss during the 0- to 15-minute period at 8°C was compared in fresh muscles and in muscles whose $[K]_i$ had been reduced by 33 or 66 percent after 3 or 8 hours of anoxia at 35°C. The $[K]_i$ loss was less in those muscles having a reduced $[K]_i$ than in fresh muscles, and the relationship between the rate of loss and $[K]_i$ was found to be linear.
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5 November 1970; revised 21 January 1971 ■

Lesch-Nyhan Syndrome: Rapid Detection of Heterozygotes by Use of Hair Follicles

Abstract. *A method is described which permits rapid phenotypic diagnosis of the Lesch-Nyhan heterozygote by direct assay of hypoxanthine guanine phosphoribosyltransferase activity in single hair follicles obtained from the scalp.*

The Lesch-Nyhan syndrome is a rare metabolic disorder of purine metabolism due to a deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT) (*1, 2*). The gene for HGPRT is X-linked and fibroblast skin cultures derived from female heterozygotes show two cell populations consistent with the Lyon hypothesis, one expressing the mutant *HGPRT*− allele and the other expressing the normal *HGPRT*+ allele (*3*). However, the cells of the hematopoietic system in heterozygotes appear to exhibit only one phenotype, that of the normal allele (*4, 5*). This has been attributed to a selective overgrowth by the normal (*HGPRT*+) cells in the bone marrow and may be related to some special requirement of the bone marrow for the salvage pathway of purine utilization (*6*). Consequently,

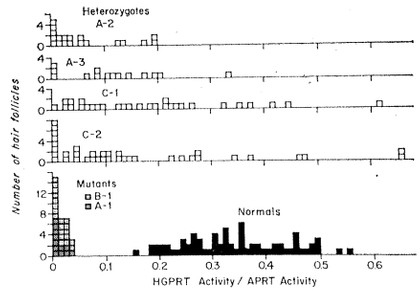

Fig. 1. Ratio of HGPRT activity to APRT activity of single hair follicles from normals (closed squares), Lesch-Nyhan mutants (hatched and dotted squares), and female heterozygotes (open squares). Patients A-1, A-2, and A-3 are members of the same family. Patients C-1 and C-2 are members of a family previously studied with respect to HGPRT activity in blood and fibroblasts and correspond to II-2 and II-3 as previously reported (*5*). Neither the A family nor the B family has been reported before. Each square represents one hair follicle.

the diagnosis of heterozygosity in the Lesch-Nyhan syndrome up to now has only been achieved by cell culture techniques involving either cloning (*3*) or growth in a selective medium (*7*). The cloning approach takes at least 6 weeks, whereas growth in selective media requires half this time.

In this report we demonstrate the diagnosis of the Lesch-Nyhan heterozygote by direct assay of HGPRT activity in single hair follicles obtained from the scalp. This is possible because the hair follicle starts from a small number of cells, and the probability of obtaining a follicle with only one of the X-linked alleles being expressed is great. Furthermore, the frequency of such nonmosaic follicles is greater than would be expected by chance because the cells of the scalp are not distributed at random but exhibit a certain degree of clonal growth (*8*). Thus, a female heterozygous for an X-linked gene, as demonstrated for glucose-6-phosphate dehydrogenase heterozygotes, will show three phenotypic classes of hair follicles—those consisting of cells expressing only the wild type gene, those with cells expressing only the mutant allele, and a third class consisting of both kinds of cells (*8*).

Single hair follicles were plucked from different areas of the scalp; those hairs with visible sheaths and bulbs were trimmed to just above the sheath, placed in 0.03 ml of buffer (0.25*M* tris-chloride, *p*H 7.4; 0.025*M* $MgCl_2$; 0.003 mg of bovine serum albumin per milliliter, and 1.6 mg each of penicillin and streptomycin per milliliter), and frozen and thawed three times. Each follicle was then assayed for both HGPRT and adenine phosphoribosyltransferase (APRT) activity by addition to a final volume of 0.055 ml, of 0.015 ml of phosphoribosyl pyrophosphate (4 mg/ml, Sigma Chemical Company), 0.5 μc [^{14}C]hypoxanthine, 40 mc/mmole (Schwarz BioResearch Corp.) and 5 μc [^{3}H]adenine 1.6 c/mmole (Schwarz BioResearch Corp.) and incubated at 37°C. Portions of 5 μl were obtained from each incubation mixture at zero time and at 1 or 2 hours and were applied to poly(ethylene)imine cellulose thin-layer chromatograms (Baker Chemical Co.), which were then developed with water as solvent. After ascending chromatography for 2 to 3 hours at room temperature, each chromatogram origin containing the radioactive phosphorylated compounds inosinic acid (IMP) and adenylic acid (AMP) (*9*) was cut out and radioactivity was counted in Omnifluor counting fluid (New England Nuclear Corp.) in a liquid scintillation counter. Rate of formation of IMP (nanomoles formed per hour at 37°C) is a measure of HGPRT activity (*2*) and rate of formation of AMP (nanomoles formed per hour at 37°C) is a measure of APRT activity (*9*). The ratio of HGPRT activity to APRT activity (hereafter referred to as HGPRT/APRT) was used as a comparative measure of HGPRT activity for detection of heterozygotes. Heterozygotes could also be identified from the HGPRT assay alone. However, with this latter procedure, it is necessary to select follicles with sheaths of similar size and configuration since HGPRT activity is primarily restricted to the sheath. Therefore, a normal follicle with little or no sheath could be misclassified as a mutant follicle in the absence of a sheath reference such as APRT activity.

The HGPRT activity in single hair follicles of four Lesch-Nyhan heterozygotes (each proved by family analysis, or by demonstration of a double cell population in skin fibroblast cultures, or both), two affected male hemizygotes (each proved by clinical manifestations and HGPRT assays of skin fibroblasts and red cells), and 12 normals (consisting of 6 males and 6 females ranging in age from 8 to 50) was compared with the HGPRT/APRT assay (Fig. 1). Although the normals exhibit a wide range of HGPRT/APRT, there is no overlap with the values for the two affected hemizygotes. Each of the heterozygotes tested showed three classes of follicles: those with only deficient activity, those with intermediate activity, and those with only normal activity. The single heterozygote studied with the HGPRT assay shows the three classes of follicles (Fig. 2). Normals for this study consisted of eight females ranging in age from 20 to 60. Thus, Lesch-Nyhan heterozygotes can be rapidly diagnosed and differentiated from normals and affected hemizygotes by the presence of three classes of hair follicles with respect to HGPRT activity.

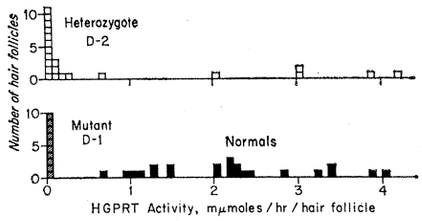

Fig. 2. The HGPRT activity of single hair follicles from normals (closed squares), a Lesch-Nyhan mutant (hatched squares), and a female heterozygote (open squares). Members of the D family have not been previously reported with respect to HGPRT activity. The HGPRT activity is expressed as the nanomoles of IMP formed per hour at 37°C per single hair follicle after subtraction of a zero-time value. Each square represents one hair follicle.

This method may become of value when one is faced with the necessity of making the diagnosis of maternal Lesch-Nyhan heterozygosity during early pregnancy, since it would determine quickly whether or not to offer the patient amniocentesis. In addition to these clinical implications, it seems likely that the hair follicle may become considerably more important in genetic investigations. It could be of special value in studies of X-chromosome inactivation as the hair follicle often consists of cells of only one type present in a mosaic heterozygote and therefore may be used as though they were clones.

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8 February 1971

Deletions in Immunoglobulin Polypeptide Chains as Evidence for Breakage and Repair in DNA

Abstract. *The partial sequence of the light chain of the myeloma-like immunoglobulin Sac shows a large deletion in its variable region. The sequence provides evidence that the corresponding gene was formed by the repair of DNA broken at nonhomologous positions. Data from other immunoglobulin (heavy) chains containing large deletions are compatible with their genes also being the result of DNA breakage and nonhomologous repair. Single homologous reciprocal exchanges in DNA networks at immunoglobulin loci could be the cause of the nonhomologous breaks. The relevance of these events to the generation of normal antibody variability remains to be determined.*

Franklin *et al.* (*1*) in 1964 described the presence of immunoglobulin heavy chain in the urine of a patient with a generalized lymphadenopathy; the excreted heavy chain did not appear to be complete (*2*). A total of 14 cases of "heavy chain disease" have now been reported or discussed (*3*), and the amino acid sequences of parts of the proteins from three of them have been determined: Zuc (*4*), Hi (*5*), and Cra (*6*). All three have deletions. In 1968 Lewis *et al.* (*7*) published an account of an unusual serum γ_1 immunoglobulin Sac which appeared to have deletions in the variable regions of both its light and heavy chains; the protein was found in the serum of a patient with the tentative diagnosis of a plasma cell neoplasm (*8*). The sequence of the first 20 residues of the Sac heavy chain and of the first 16 residues of the light chain have now been determined by conventional methods (*9*), and the data for the light chain have been extended to include 48 of the first 50 residues (*10*), by means of an Edman-Begg sequenator (*11*). The sequence data fully confirm the observations of Lewis *et al.* (*7*) in that both the light and heavy chains of Sac do indeed contain large deletions in their variable regions. We here discuss some implications of the Sac light chain sequence and of the other large deletions in immunoglobulins.

Figure 1 shows the sequence (*10*) of the light chain of Sac in relation to that of the κ_I Bence Jones protein Roy (*13*). The data show that Sac light chain has the same sequence as Roy up to position 17. From position 31 Sac light chain has a sequence without demonstrable differences from that of Roy from the Roy position 99 to at least ten residues into the constant region, except for a valine/phenylalanine difference at position 106. (Position 99 is near the end of the light chain variable region.) The sequence of the intervening 13 amino acids (Sac 18 through 30) is unusual in comparison with other κ light chain sequences; it differs at 11 positions from the Roy sequence 18 to 30, and at 9 positions from the Roy sequence 86 to 98.

Three simple ancestries for the Sac light chain gene are readily imagined. The gene may be derived from the 1 to 30 region of a κ_I gene joined to the 99 to 214 region of the same or of a second gene. It may be from a κ_I 1 to 17 region joined to an 86 to 214 region. Or it may be derived from a 1 to 30 region and an 86 to 214 region, with the two partners of the overlapping 13 residue region each making some contribution to the final gene. The first possibility can be excluded, since the Sac 18 to 30 sequence differs from the most common κ_I sequence for the same region at 11 positions; none of the other κ_I proteins yet reported differs at more than four positions from this common sequence. The second ancestry, in which the Sac 18 to 30 region is derived solely from a κ 86 to 98 region, is more difficult to exclude since the κ chain 86 to 98 region is highly variable. Thirteen sequences have been reported (*14*) for this region. Twelve of these do not differ by more than four residues (five base pairs) from the sequences most similar to them. The thirteenth, light chain Eu (*15*), differs from the sequence most similar to it by six residues (seven base pairs). The Sac 18 to 30 sequence differs from the most similar of the 86 to 98 sequences by eight residues (eight base pairs), and the differences include two of the four positions in the region not yet observed to vary. It therefore appears unlikely that the Sac 18 to 30 sequence is derived simply from an 86 to 98 region, but we cannot exclude this possibility. The third ancestry for Sac, from a composite of DNA related to both sequences, would reduce the minimum number of unique residues in the two Sac ancestral genes to four as a consequence of being able to use bases from either ancestor. This number is within the normal range of variation, although two of the four required unique residues would be at positions where variations are infrequent; the four invariant residues in the presumed 86 to 98 ancestor would remain invariant. Thus a simple interpretation of the Sac light chain sequence is that its gene was derived from the 1 to 30 region of one gene and the 86 to 214 region of the same or of a second gene with each partner of the overlapping 13 residue region of hybrid DNA making some contribution to the final gene. In addition, mutations may have been introduced by the hybrid region.

There are at least two situations in which hybrid DNA is likely to be formed in vivo: during recombination and during the repair of DNA broken by mechanical or enzymatic action. Recombination normally takes place between DNA regions with some homology. We looked at the regions near 18 to 30 and 86 to 98, but could find no evidence for significant homology between them in the correct register (*16*). We conclude that the Sac light chain sequence is not a direct consequence of an unequal crossover between partially homologous regions.

Consideration of the second possibility, that the Sac sequence is the product of the repair of broken DNA molecules, is handicapped by a lack of knowledge about the structures generated in vivo during such repairs. Khorana and his associates (*17*) have used T4-polynucleotide ligase in vitro to join DNA molecules that had been synthesized with single-stranded ends able to form an overlapping hybrid DNA double helix. DNA double