Heterozygote detection in angiokeratoma corporis diffusum (Anderson–Fabry disease)

Studies on plasma, leucocytes, and hair follicles

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SUMMARY  Heterozygote detection for angiokeratoma corporis diffusum (Anderson-Fabry disease, ACD), an X-linked disorder of glycosphingolipid metabolism, was examined using α-galactosidase activity, an α-galactosidase/β-galactosidase activity ratios (α/β ratio) in leucocytes, plasma, and hair follicles. For leucocytes, 22 obligate heterozygotes, 25 suspected heterozygotes, and 47 control subjects were studied, while for plasma, the groups were 17 obligate heterozygotes and 35 controls. The α/β ratio in plasma and leucocytes was clearly a better discriminator between obligate heterozygotes and controls than α-galactosidase activity alone, but still failed to detect 3 obligates with leucocytes and 2 with plasma. Discrimination was not improved by joint use of plasma and leucocyte α/β ratios, but was improved by measurement of hair-follicle α/β ratios. The interdecile range of log (α-galactosidase/β-galactosidase activity) in 20 hair follicles from each of 4 obligate and 7 suspected heterozygotes was clearly different from 11 control subjects. Accordingly, for rapid screening for carriers of ACD, we recommend use of leucocyte or plasma α/β ratios which should detect > 85% of heterozygotes. When results are equivocal, and ancillary information suggests heterozygous status, the more time-consuming measurement of hair-follicle α/β ratios is a useful additional test.

Angiokeratoma corporis diffusum (Anderson-Fabry disease, ACD), a hereditary systemic disorder of glycosphingolipid metabolism, is characterized by progressive accumulation of trihexosyl ceramide (galactosylgalactosylglucosyl ceramide; CTH) and to a lesser extent dihexosyl ceramide (galactosylgalactosyl ceramide; CDH) in many body tissues (Sweeley et al., 1972). The metabolic abnormality is a deficiency of an α-galactosidase, trihexosylceramide galactosyl hydrolase (CTH-hydrolase). The enzyme deficiency has been shown in plasma and several tissues from hemizygous males, with both natural (CTH and CDH) and artificial (4-methylumbelliferyl-α-D-galactopyranoside, 4-MU-α-gal) substrates (Sweeley et al., 1972; Brady et al., 1967; Mapes et al., 1970; Kint, 1970; Desnick et al., 1973; Mapes and Sweeley, 1973a, b). The enzymes catalysing the hydrolysis of α-linked galactose from natural substrates may differ from those active with artificial substrates (Mapes and Sweeley, 1972, 1973a), but because of their ready availability and simplicity of assay, the latter have been used in most studies of ACD (Kint, 1970; Desnick et al., 1973; Beutler and Kuhl, 1972; Wood and Nadler, 1972; Ho et al., 1972); enzyme activity thus measured is referred to as non-specific α-galactosidase activity. At least two enzyme activities are measured; a major, heat-labile form, α-galactosidase A that is present in normal persons but absent in males with ACD, and a minor, heat-stable form, α-galactosidase B that is present in males with ACD and normal controls (Beutler and Kuhl, 1972; Wood and Nadler, 1972; Ho et al., 1972).

The disease is transmitted in an X-linked manner (Sweeley et al., 1972). Two distinct cell populations have been shown (Romeo and Migeon, 1970; Grzeschik et al., 1972) in fibroblasts cultured from

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ACD heterozygotes—one with normal \( \alpha \)-galactosidase levels, and one with levels similar to those of male hemizygotes—supporting the Lyon hypothesis (Lyon, 1961) of X-chromosome inactivation for the \( \alpha \)-galactosidase locus. The degree of mutant X-chromosome inactivation may vary in different tissues (Gartler et al., 1969, 1971a), because of the size of the embryonic cell population at the time of X-chromosome inactivation, and more particularly the size of the embryonic pool from which a particular tissue develops. The number of cells with the mutant phenotype may also depend on selection favouring one of the two populations produced following inactivation.

Difficulties in detecting with certainty all female carriers of an X-linked trait have been documented for glucose-6-phosphate dehydrogenase (G6PD) deficiency (Stamatoyannopoulos et al., 1967; Kattamis, 1967). In ACD, however, with only few exceptions (Romeo and Migeon, 1970; Avila et al., 1973; Philippart et al., 1974), the reported enzyme activities in heterozygote leucocytes (Kint, 1970; Desnick et al., 1973), plasma (Sweeley et al., 1972; Desnick et al., 1973), urine (Desnick et al., 1973), fibroblasts (Wood and Nadler, 1972; Ho et al., 1972), and tears (Del Monte et al., 1974) indicate that \( \alpha \)-galactosidase levels are clearly abnormal, and that heterozygotes can be clearly identified by these methods.

During a case- and carrier-detection study of a kindred with ACD we have identified some obligate heterozygotes in whom leucocyte and in some cases plasma \( \alpha \)-galactosidase activities were indistinguishable from normal. It is the purpose of this report to document the danger of classifying heterozygotes as normal when only \( \alpha \)-galactosidase activity is measured in leucocytes or plasma, and to recommend the measurement of a second lysosomal enzyme (\( \beta \)-galactosidase), to help in heterozygote detection. When equivocal results are obtained, measurement of \( \alpha \)- and \( \beta \)-galactosidase activity in hair follicles is recommended: these have been used to identify heterozygotes for two X-linked disorders, G6PD deficiency and hypoxanthine-guanine phosphoribosyl transferase deficiency (Gartler et al., 1969, 1971a, b; Francke et al., 1973; Silvers et al., 1972), and this paper reports their use in ACD.

Subjects

Classification of Heterozygotes

All but 3 of the 47 suspected and obligate heterozygotes are members of the same kindred. Females were classified as obligate heterozygotes if they were either (a) mothers of two or more affected sons, (b) mothers of one affected son and sisters or aunts of affected males, or (c) daughters of proven affected fathers. Suspected heterozygotes included persons at risk in the kindred in whom slit-lamp ophthalmoscopic examination showed typical corneal opacities and who were (a) daughters of deceased males suspected of having Fabry's disease or (b) sisters or maternal aunts of affected males. Also included is one female (Case 7), mother of an affected son, who could not be definitely linked to the main kindred and who had normal ophthalmological findings. The diagnosis of ACD in affected males >15 years of age was made on the basis of the presence of clinical findings considered typical of the disease, such as painful crises, and skin and corneal lesions. In all affected males at all ages examined (14 months to 62 years) plasma \( \alpha \)-galactosidase was <16%, and leucocyte \( \alpha \)-galactosidase <6% of mean control levels, clearly separable from the lowest controls.

Control Subjects

Control samples were obtained from females, aged 1 to 76 years, with no known relationship to the kindreds with ACD.

Methods

Plasma and leucocytes were obtained from non-fasting controls and suspected carriers (McKnight and Spence, 1972; Spence et al., 1974) and were stored at \(-20\)°C for periods of <3 weeks before analysis. Hair follicles (at least 30) were plucked in groups of 4 or 5 from at least 6 areas of the scalp, and were placed in a humid Petri dish at 0 to 4°C for <12 h (preliminary studies showed little loss of enzyme activity during storage for up to 48 h; freezing (\( -20\)°), however, reduced activity).

4-Methylumbelliferyl-\( \alpha \)-D-galactopyranoside (4-MU-\( \alpha \)-gal), 4-methylumbelliferyl-\( \beta \)-D-galactopyranoside (4-MU-\( \beta \)-gal), and bovine serum albumin were obtained from Sigma Chemical Co. (St Louis, Mo.). 4-Methylumbelliferone (4-MU) was obtained from Pierce Chemical Co. (Rockford, Ill.). All other reagents were obtained from Fisher Scientific Co. (Dartmouth, Nova Scotia).

Plasma and Leucocytes

All assays were run in duplicate at two enzyme concentrations under reaction conditions which were determined to be optimal for the estimation of the different enzyme activities.

(a) \( \alpha \)-Galactosidase

The reaction mixture consisted of 20 \( \mu \)mol sodium acetate buffer, \( p \)H 5·0 containing 4-MU-\( \alpha \)-gal (1·2 \( \mu \)mol for plasma and 0·5 \( \mu \)mol for leucocytes), and 20 or 40 \( \mu \)l plasma, or 4 to 20 \( \mu \)g leucocyte protein in a final volume of 0·2 ml. Incubation was for 2 h for...
plasma and 1 h for leucocytes at 37°C; the reaction was terminated by immersion in an ice bath, and the addition of 1 ml 0·25 mol/l glycine-carbonate buffer, pH 10·0.

(b) \(\beta\)-Galactosidase
The reaction mixture consisted of 50 \(\mu\)mol NaCl, 100 \(\mu\)mol sodium acetate buffer (pH 4·1 for plasma and 4·5 for leucocytes), containing 4-MU-\(\beta\)-gal (0·5 \(\mu\)mol for plasma and 0·2 \(\mu\)mol for leucocytes) and 20 or 40 \(\mu\)l plasma, or 4 to 20 \(\mu\)g leucocyte protein in a final volume of 1·1 ml. Incubation was for 2 h for plasma and 1 h for leucocytes at 37°C, and the reaction was terminated by immersion in an ice bath and the addition of 2 ml 0·25 mol/l glycine-carbonate buffer, pH 10·0.

HAIR FOLLICLES
Only hairs with visible bulbs and sheaths were used; they were trimmed to just above the sheath and were placed in 6 × 50 mm test-tubes at 0 to 4°C. Each assay tube contained 1 follicle; 20 follicles were studied from each hemizygote, heterozygote, and control.

(a) \(\alpha\)-Galactosidase
The reaction mixture added to each assay tube containing 1 follicle consisted of 0·6 \(\mu\)mol 4-MU-\(\alpha\)-gal, 0·5 \(\mu\)mol NaCl, 11 \(\mu\)mol sodium acetate buffer (pH 5·0) in a final volume of 0·1 ml. Incubation was for 45 min at 37°C. At the conclusion of incubation the tubes were placed in an ice bath (0-4°C); the medium was carefully aspirated to avoid removing the follicle and was transferred to a second series of tubes containing 0·5 ml 0·25 M glycine-carbonate buffer, pH 10·0.

(b) \(\beta\)-Galactosidase
The single hair follicles, still in the original 6 × 50 mm test tubes used for \(\alpha\)-galactosidase incubation, were washed with 0·1 ml sodium acetate buffer, 0·02 mol/l, pH 4·6, containing 100 mmol/l NaCl; the wash solution was discarded. The hair follicle was then immersed in a reaction mixture consisting of 0·1 \(\mu\)mol 4-MU-\(\beta\)-gal, 5·5 \(\mu\)mol NaCl, and 11 \(\mu\)mol sodium acetate buffer, pH 4·6, in a final volume of 0·1 ml. Incubation was for 45 min at 37°C. The reaction was terminated by immersion in an ice bath, and the addition of 0·5 ml 0·25 mol/l glycine-carbonate buffer pH 10·0.

Fluorescence of the released 4-MU was determined with an Aminco Bowman spectrophotofluorometer (American Instrument Co., Silver Springs, Md.), at previously determined optimal wavelengths for excitation (363 nm) and emission (448 nm). The product was quantified by reference to appropriate standards and reaction blanks.

Protein concentration of the leucocyte preparations was determined by the method of Lowry et al. (1951).

Results

PLASMA AND LEUCOCYTES
Only leucocyte enzyme activity was determined in the initial phases of this study; accordingly, the number of patients in whom both plasma and leucocytes were examined is lower. Leucocyte \(\alpha\)-galactosidase values for 8 of 22 obligate and 12 of 25 suspected heterozygotes overlapped the normal group (Fig. 1). Plasma \(\alpha\)-galactosidase levels for 6 of 17 obligate heterozygotes were well within normal range (Fig. 2). When \(\alpha\)-galactosidase activity was expressed in terms of a second lysosomal hydrolase, \(\beta\)-galactosidase, the \(\alpha/\beta\) ratio in leucocytes from 3 of 22 obligate and 2 of 25 suspected heterozygotes, and in plasma from 2 of 17 obligate heterozygotes was within the normal range. (Analysis of the \(\beta\)-galactosidase values of controls and heterozygotes (obligate and/or suspected) indicated that there was no significant difference between the groups (Langley, 1971)).

HAIR FOLLICLES
Enzyme activity (\(\alpha\)- and \(\beta\)-galactosidase) was tightly bound to the follicle; there was no evidence of solubilization or enhancement of activity with (a) freezing and thawing \(\times\) 7 between −70°C (methanol-

Fig. 1 Leucocyte \(\alpha\)-galactosidase activity and \(\alpha\)-galactosidase/\(\beta\)-galactosidase activity ratio in obligate heterozygotes (OBL), suspected heterozygotes (SUSP), and control subjects (CONT).
solid CO₂) and 0 to 4°C, (b) addition of Triton X-100 (0-1%), or (c) preliminary incubation for 45 min at 0 to 4°C in distilled water, 0·02 mol/l sodium acetate buffer (pH 4·6), or 0·01 mol/l sodium phosphate buffer (pH 7·0). Increasing preliminary incubation temperature to 37°C caused >30% loss of enzyme activity in normal and ACD hemizygote follicles at pH 7·0 but not at 4·6. Addition of bovine serum albumin (0-1%) to the medium did not enhance enzyme activity during 1 h incubations.

In view of the tight binding of activity to the follicle we studied reaction conditions with initial measurement of α-galactosidase activity, followed by β-galactosidase activity in individual follicles. β-D-N-acetylhexosaminidase activity, which also was examined (O'Brien et al., 1970), exceeded that of α-galactosidase 5- to 10-fold; however, interfollicular variations in α-galactosidase and hexosaminidase activity did not correlate as well as α-galactosidase and β-galactosidase (levels of these two enzymes changed similarly in control follicles) and, therefore, β-galactosidase was used as the second enzyme. Optimal reaction conditions were determined, using hair follicles and serial incubations with either 4-MU-α-gal or 4-MU-β-gal; unless stated otherwise, conditions were similar for β-galactosidase during the first and second incubation periods, irrespective of whether the α- or β-galactoside was the substrate in the first incubation. The pH optimum was 4·6 to 5·0 for α-galactosidase and 4·4 to 4·6 for β-galactosidase. The amount of substrate cleaved was similar in both first and second incubations for 30, 45, and 60 min, showing probable linearity of reaction rates over 2 h with intervening wash and substrate renewal; a time of 45 min for first and second incubations was chosen. Reaction velocity became independent of substrate concentration above 6 mmol/l for α-galactosidase and above 1 mmol/l for β-galactosidase.

Hair follicle α- and β-galactosidase activity was expressed as an α/β ratio, to counter interfollicular variation: for example, low α-galactosidase activity might indicate merely a follicle type in which all enzyme activities were low. α/β activity ratios of control follicles were chiefly in the range 0-6 to 1-2 (Fig. 3; all 11 controls as a group and three randomly chosen controls G, E, F are shown); a few follicles had very similar changes of measurement (levels 4–200").

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Fig. 2 Plasma α-galactosidase activity and α-galactosidase/β-galactosidase activity ratio in obligate heterozygotes (OBL) and control subjects (CONT).

Fig. 3 Hair-follicle α-galactosidase/β-galactosidase activity ratios in control (letters) and heterozygous (numbers) subjects. Twenty follicles were sampled from each individual, and each point represents the ratio on one follicle. G, E, and F are representative controls, picked at random, and are included in the pooled total control group (11 subjects). Numbers 2, 5, 8, and 10 are obligate heterozygotes (OBL); numbers 1, 3, 4, 6, 7, 9, and 11 are suspected heterozygotes (SUSP).
Fig. 4 Interdecile range of log (α-galactosidase/β-galactosidase activity) in obligate heterozygotes (OBL), suspected heterozygotes (SUSP), and control subjects (CONT).

The detection of enzyme assay of male hemizygotes with Fabry's disease presents few difficulties; in our experience, with patients aged 14 months to 62 years, the α-galactosidase levels in plasma and/or leucocytes are well below the normal range. We have encountered more difficulty with the identification of female heterozygotes, as has been reported in other X-linked diseases (Stamatoyannopoulos et al., 1967; Kattamis, 1967; Francke et al., 1973). Philippart et al. (1969, 1974) have reported normal urinary glycolipid excretion in 4 potential carriers of ACD and failure to detect obligate heterozygotes by plasma α-galactosidase measurement and search for corneal opacities. Rietra et al. (1972) reported that 3 out of 6 heterozygotes had normal ratios of urinary N-acetyl-β-glucosaminidase/α-galactosidase; Romeo and Migeon (1970) reported normal leucocyte α-galactosidase activity in a proband's mother, in whom subsequent clonal culture of skin fibroblasts established her carrier status. Avila et al. (1973) showed normal α-galactosidase activity in leucocytes and normal urinary glycolipid patterns in two obligate heterozygotes. Such difficulties might be expected. Random X-chromosome inactivation early in development would be expected statistically to produce a final cell population with approximately 50% normal X in most instances. In a few cases, however, the proportion of cells bearing normal X-chromosomes could be considerably higher, obscuring heterozygous status.

**USE OF LEUCOCYTE AND PLASMA α-GALACTOSIDASE AND α-GAL/β-GAL RATIOS IN CARRIER DETECTION**

Our mean control plasma α-galactosidase value (Fig. 2) was twofold and β-galactosidase was tenfold higher than those reported by Desnick et al. (1973). The reason for this difference in the two studies is not clear, particularly since we found only slight differences (<10%) when we measured both activities in parallel by their method and with our own assay system, using similar substrate concentrations.

α-Galactosidase levels in leucocytes and plasma tended to be less in the obligate heterozygotes than in normal subjects, but there was a considerable overlap of the distributions (Fig. 1 and 2). The ratio of α-galactosidase to the β-galactosidase activity was a better discriminator than α-galactosidase alone (Fig. 1 and 2). For example, using leucocyte α-galactosidase activity alone 36% of the 22 obligates and 53% of the 47 normals had overlapping values; the use of the α/β ratio reduced these percentages to 14% and 17%, respectively (P approximately 0-1 for this reduction). Overlap percentages for plasma were also reduced when the α/β ratio replaced the α-gal: from 35% to 12% of the 17 obligates, and from 63% to 14% of the 35 normals (P < 0-001).

The improved discrimination between obligate heterozygotes and controls with the α/β ratio is probably the result of the reduction of such variables as generalized increases in circulating enzyme levels as a result of illness other than ACD or some other cause, and inadvertent enzyme loss during sampling, processing, or storage.

**COMPARISON OF PLASMA AND LEUCOCYTE α/β RATIOS AS DISCRIMINATORS BETWEEN OBLIGATE HETEROZYGOTES AND CONTROLS**

Both plasma and leucocyte enzyme activities were measured for 29 normal subjects and 17 obligate
heterozygotes. A comparison of the discriminatory ability of plasma or leucocyte \(a/\beta\) ratios by the separation of the means relative to the standard deviations (Table 1) showed the standardized differences to be almost identical (and certainly not statistically significant). The magnitude of the classification errors with plasma and leucocyte \(a/\beta\) ratios was also compared (Table 2) by examining the false positive and false negative error rates based on the critical value of the \(a/\beta\) ratio that minimized the total classification error in this study. It appears that the leucocyte \(a/\beta\) ratio is slightly better; however, these differences are not significant for any of the prior probabilities (P is close to 1 in all cases). Thus, there appears to be little to choose between leucocyte or plasma \(a/\beta\) ratios; they are equally good discriminators between normals and obligate heterozygotes.

**USE OF PLASMA AND LEUCOCYTE \(a/\beta\) RATIOS IN CONJUNCTION**

If the plasma and leucocyte compartments were affected independently by the genetic defect in ACD, then the use of data obtained from both should be a better discriminator than either alone. The Spearman rank correlation coefficient (Langley, 1971) between the leucocyte \(a/\beta\) ratios and plasma \(a/\beta\) ratios is 0.4 in the normals (P < 0.05) and 0.85 in the obligates (P < 0.001). The much lower rank correlation coefficient in the normal subjects as compared with the obligates indicates that there is less dependence between the plasma and leucocyte \(a/\beta\) ratios in the normal subjects. The reason for this is not known.

Despite this relatively high correlation, it was thought that some degree of independence might exist, and so we examined the use of both leucocyte and plasma data jointly to see if this would yield better discrimination. As one method of joint use of the data, the linear discriminant function was determined (Snedecor and Cochran, 1967); no improvement in error rates at prior probabilities of 0-5, 0-25, and 0-05 was obtained as compared with the use of plasma and leucocyte ratios alone.

A second method examined was a 2-dimensional plot of the leucocyte versus plasma \(a/\beta\) ratios (Fig. 5). Various L-shaped boundaries enclosing the obligate group were examined, based on the premise that if some controls appeared marginally abnormal in only one compartment, they would be classified as normal by this method. There was only a slight improvement in error rates and the difference was not statistically significant. Thus, it appears that either leucocyte or plasma \(a/\beta\) ratios by themselves are as good discriminators of the heterozygote from the control as are the two in combination.

**APPLICATION OF CRITICAL VALUES DEVELOPED FROM DATA OBTAINED WITH OBLIGATE HETEROZYGOTES TO SUSPECTED HETEROZYGOTES**

Among the female members of this deme, there were 25 who by clinical and historical evidence were strongly suspected to be carriers (see Subjects), and for whom leucocyte \(\alpha\)-galactosidase values and \(a/\beta\) ratios were available (Fig. 1). Use of the critical value for the leucocyte \(a/\beta\) ratios (the one that minimizes the total classification error) developed from the

### Table 1  Separation of means relative to standard deviations of controls from obligate heterozygotes: comparison of plasma and leucocytes

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean</th>
<th>SD</th>
<th>n</th>
<th>Obligates Mean</th>
<th>SD</th>
<th>n</th>
<th>Standardized Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocyte (a/\beta)</td>
<td>0.267</td>
<td>0.012</td>
<td>47</td>
<td>0.223</td>
<td>0.024</td>
<td>22</td>
<td>2.73</td>
</tr>
<tr>
<td>Plasma (a/\beta)</td>
<td>0.254</td>
<td>0.015</td>
<td>35</td>
<td>0.214</td>
<td>0.014</td>
<td>17</td>
<td>2.79</td>
</tr>
</tbody>
</table>

*Standardized difference = \(\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}}\)

### Table 2  Comparison of error rates for leucocyte and plasma \(a/\beta\) ratios

<table>
<thead>
<tr>
<th>Prior probability of A Heterozygote*</th>
<th>Leucocyte (a/\beta)</th>
<th>Plasma (a/\beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Critical Level†</td>
<td>False + ve (n = 47)</td>
</tr>
<tr>
<td>0-50</td>
<td>0.3</td>
<td>0.00</td>
</tr>
<tr>
<td>0-25</td>
<td>0.3</td>
<td>0.00</td>
</tr>
<tr>
<td>0-05</td>
<td>0.2</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Three prior probabilities were selected for comparison of error rates. Prior probabilities of 0-5 and 0-25 are encountered in heterozygote detection in a family with male members affected with ACD; 0-05 illustrates the situation where there seems little likelihood of the disease.
†The critical level of the \(a/\beta\) ratio used was one which minimized the total classification error.
Heterozygote detection in ACD

![Graph showing the relationship between plasma and leucocyte aldolase activity ratios](image)

Fig. 5 α-galactosidase/β-galactosidase activity ratios of plasma and leucocytes plotted together for control subjects (○) and obligate heterozygotes (●). The L-shaped boundary enclosing the obligate heterozygote group minimized the total misclassification error at prior probabilities of 0.5, 0.25, and 0.05. Only boundaries of this shape were considered.

obligate heterozygote data to this group classified 22 subjects as normal and 23 as heterozygotes. Assuming all 25 are heterozygotes, the false negative rate of 2/25 is comparable to the rate of 3/22 obtained with the obligate group. It seems, therefore, that the critical value that is best for the obligate group works similarly for this new group of probable heterozygotes.

**HETEROZYGOATE DETECTION USING HAIR FOLLICLES**

Studies in which hair follicles have been used to identify heterozygotes for other X-linked disorders (Gartler et al., 1969, 1971a, b; Silvers et al., 1972) suggest that each follicle originates from a small number of cells, so that some of these by chance may be composed of cells of a single type. The continuous spectrum (Fig. 3) of α/β activity ratios in our studies of hair follicles from heterozygotes with ACD suggests that, under our experimental conditions, the follicle's cell population is usually a mixture containing mutant and normal active X-chromosomes. This mixture is responsible for the tailing distribution of the activity ratio, below that observed in normal females, that characterizes the heterozygotes.

In many of the female heterozygotes, the mean hair follicle α/β ratio is indistinguishable from normal, because of a predominance of follicles with normal values. Reliance on a single low follicle value is not advisable, since we have observed one low value (out of 20) in two normal subjects. The occasional high values (Fig. 3) are also suspect, and are generally the result of poor β-galactosidase activity (reason unknown). Accordingly both mean and range do not reliably discriminate between heterozygotes and controls. The range of groups of data (e.g., interquartile, interdecile) has been used as a measure of dispersion (Langley, 1971; Snedecor and Cochrain, 1967), and we have selected the interdecile range of the log (α-gal/β-gal) to indicate an increased dispersion of the heterozygote group (Fig. 4; the interdecile range of the raw data gave less discrimination between obligates and controls). This range gives less weight to the occasional low and high hair-follicle α/β ratios seen in normal subjects and also makes use of all the available data, since the relative rank of the α/β ratios for each follicle is determined by the entire group. We selected the interdecile range on the basis of data of the first seven heterozygotes (obligates 2 and 5; suspects 1, 3, 4, 6, and 7) and controls (A-G) tested; the discrimination of this range was maintained in data of a further 4 heterozygotes (obligates 8, 10; suspects 9, 11) and controls (H-K). Three heterozygotes (obligate 2, 10; suspect 3) had eluded detection by leucocyte assay; 2 (obligate 2 and suspect 3) were also negative by plasma.

Further experience with this method of heterozygote detection is necessary to establish clearly its reliability. Though it may be more discriminating than assays of other tissues or fluids, excepting clonal culture of fibroblasts, complete reliance on this method may not be advisable: Silvers et al. (1972) have reported one heterozygote (by fibroblast analysis) for hypoxanthine-guanine phosphoribosyl transferase deficiency in whom hair-follicle analysis was normal.

Clonal culture of skin fibroblasts has been used to identify heterozygotes for X-linked diseases (Romeo and Migeon, 1970; Davidson et al., 1963; Migeon et al., 1968; Danes and Bearn, 1967), but the technical expertise and labour involved make this method less attractive for dealing with large groups at risk. Philippart et al. (1974) suggested using electron microscopy of skin biopsies, detecting heterozygotes by the observation of cells with lipid-laden organelles. However, this method is very dependent on the electron microscopist's skill and experience.

We have found the leucocyte or plasma α/β ratio equally effective in discriminating heterozygotes from controls, and clearly superior to α-galactosidase measurements alone. No advantage is realized by measurement of the ratio in both plasma and leucocytes. If the results of such examinations are unequivocal, no further testing is necessary. If, however, they are borderline, and particularly if there is ancillary clinical information suggesting heterozygote status, further examination is necessary. We have found hair-follicle assay a useful test that gives a
broad sample, thereby lessening the likelihood of missing mutant tissue; ease of analysis and its apparent discriminatory power make it useful as an ancillary procedure in doubtful cases. Though the hair follicle assay has consistently delineated obligate and suspected carriers in our studies, it must be regarded with the same caution during interpretation as all other detection tests in X-linked conditions.

This work was presented in part at the Canadian Society for Clinical Investigation, Winnipeg, Manitoba, January 1975 (Clinical Research, 22, 741A, 1974). The assistance of Mrs Karen E. MacKinnon during part of this study, and the helpful co-operation of the numerous family physicians, the members of the kindred, and the control group are gratefully acknowledged. The studies were supported by grants from Health and Welfare Canada (602-7-165), the Medical Research Council of Canada (MT-2188), and the Maritime provinces. Dr Spence is a Medical Research Council of Canada Associate.

References


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