# LACK OF GENIC VARIATION IN THE ABUNDANT PROTEINS OF HUMAN KIDNEY

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ABSTRACT

Abundant proteins of 25 human kidneys were surveyed for genic variation by means of two-dimensional electrophoresis. Eighty-three (83) proteins were scored, and no genic variation was detected. This reduction in genic heterozygosity corroborates results determined with two-dimensional electrophoresis in mice and flies. These results suggest that previous estimates of electrophoretic variation may have been in error because of biased selection of loci.

THE level of genic variation in human and natural populations of animals has been intensely studied since the introduction of electrophoretic techniques to population genetics. Researchers have continually sought to extend the range of proteins examined. A recent review reports that over 100 loci have been surveyed in humans (HARRIS, HOPKINSON and EDWARDS 1977). An important consideration in electrophoretic estimates of heterozygosity is the degree to which sampled loci and their polymorphic alleles are representative of genic variation in the genome as a whole. With most electrophoretic techniques, biases towards reporting the more variable loci are to be expected.

Estimates of the proportion of heterozygous loci in humans appear to have declined steadily over the past 10 years (HARRIS 1966). This is largely due to the inclusion of more loci that are less polymorphic. Most determinations of heterozygosity are not attempted without prior knowledge that some variation exists for most of the loci under study. With the exception of human sutdies, heterozygosity determinations in most organisms depend on the same 20 to 30 enzymes, of which many are exceptionally polymorphic (*e.g.*, esterase, phosphatases).

The proportion of amino acid substitutions detected in routine electrophoretic surveys varies with loci sampled and the technique employed (COYNE, FELTON and LEWONTIN 1978). At present, most such surveys involve one-dimensional electrophoresis under native conditions, followed by enzyme-specific staining. There is some uncertainty as to the proportion of amino acid changes detectable under these conditions. This reservation, along with the sampling bias discussed above, makes it difficult to extrapolate total genic variability from the results of routine allozyme surveys.

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An alternative approach utilizes the two-dimensional electrophoresis technique (2-D PAGE) introduced by O'FARRELL (1975). Recent work in this laboratory and others has shown this technique to be suitable for surveys of populations for genic variation (LEIGH BROWN and LANGLEY 1979; ELLIOTT 1979; COMINGS 1979; MCCONKEY, TAYLOR and PHAN 1979; WALTON, STYER and GRUENSTEIN 1979; and RACINE and LANGLEY 1980a,b). The 2-D PAGE separates crude protein mixtures (such as tissue homogenates) according to isoelectric point (pI) in a tube acrylamide gel (first dimension) followed by separation according to molecular weight in a slab acrylamide gel (second dimension). The slab gels are then stained nonspecifically for proteins or autoradiographed if the proteins are labeled. In either case, the detectability of a protein is dependent on its abundance.

The technique reliably detects charge changes in the primary sequence of polypeptides over a wide range of molecular weights and isoelectric points. COMINGS (1979) reported a polymorphism detected by 2-D PAGE of human brain homogenates. He surveyed some sixty other proteins that showed no variation. MCCONKEY, TAYLOR and PHAN (1979) and WALTON, STYER and GRUENSTEIN (1979) surveyed several lines of human diploid fibroblasts with 2-D PAGE. Both studies revealed an average heterozygosity of less than 1% for changes involving charged amino acids. In *Drosophila melanogaster* and *Mus musculus*, 2-D PAGE has been used to survey population genetic variation (LEIGH BROWN and LANGLEY 1979; RACINE and LANGLEY 1980a,b). In this report, we document by 2-D PAGE the lack of detectable genetic variation in the protein pI's of 83 abundant human kidney proteins. These findings confirm previous results, suggesting a lower level of variation in the most abundant human proteins as revealed by 2-D PAGE than has been reported for allozymes using conventional native electrophoresis.

## MATERIALS AND METHODS

Human kidney samples (25) were selected from random autopsies in London. The kidneys came from 15 females and 10 males whose ages ranged from 8 to 89 years. The samples were kept frozen (—70°) and were packed in dry ice for transport. They were kept at (4°) during preparation. Samples were prepared utilizing the modifications of WILSON *et al.* (1977). Two-dimensional electrophoresis was carried out as described by O'FARRELL (1975) with modifications of RACINE and LANGLEY (1980a). The samples were homogenized in 1% SDS; then urea, ampholytes and nonionic detergent were added. This homogenate was centrifuged, and the supernatant was applied to the isoelectric focusing gel.

Isoelectric focusing was done with the same carrier ampholytes (pH 3.5–10 and 5–8) used by O'FARRELL (1975), but 1% DTT from Eastman replaced 5% $\beta$-mercaptoethanol. Focusing was carried out in 11.5 cm long pyrex tubes with 2 mm internal diameter. After isoelectric focusing, the gel was equilibrated in SDS and placed across the top of a SDS 10% acrylamide slab gel. After electrophoresis, as described by O'FARRELL (1975), the slab gels were fixed overnight in 45% methanol and 10% acetic acid. Staining was done in the same solution containing 0.02% Coomassie Blue R-250. The destaining solution contained 25% methanol and 7% acetic acid. The gels were photographed through a red filter on Polaroid 55 positive/negative film.

The pH gradient was established in the first dimension by chopping two rod gels into 9 sections and homogenizing each section in 1 ml distilled water. Calibration of the second dimension was performed by running four proteins with known molecular weights in parallel. These were RNAase (13,000), chymotrypsin (25,000), aldolase subunit (40,000) and ovalbumin (45,000), all from Pharmacia.

## RESULTS

A typical 2-D PAGE of human kidney proteins is shown in Figure 1. Notice that the spots are primarily found in the central portion of the gel. No spots from the extreme basic or acidic regions of the isoelectric focusing dimension were included in the analysis because the pH gradient becomes too nonlinear at the edges. A variant due to a charge-change amino acid substitution would appear as an extra spot in the isoelectric focusing dimension on the same molecular weight level as the original. Variants of this type were found for proteins from Drosophila (LEIGH BROWN and LANGLEY 1979, 1980) and mice (ELLIOTT 1979; RACINE and LANGLEY 1980a,b). COMINGS (1979) has reported such a 2-D variant in human brain proteins. No simple variants by this definition were found in the 2-D PAGE of the 25 human kidneys. Three regions of the gels did show a more complex variation. These regions are displayed in Figure 2.

The variation illustrated in the various panels of Figure 2 did not show recognizable patterns of homozygotes and co-dominant heterozygotes. The large spot

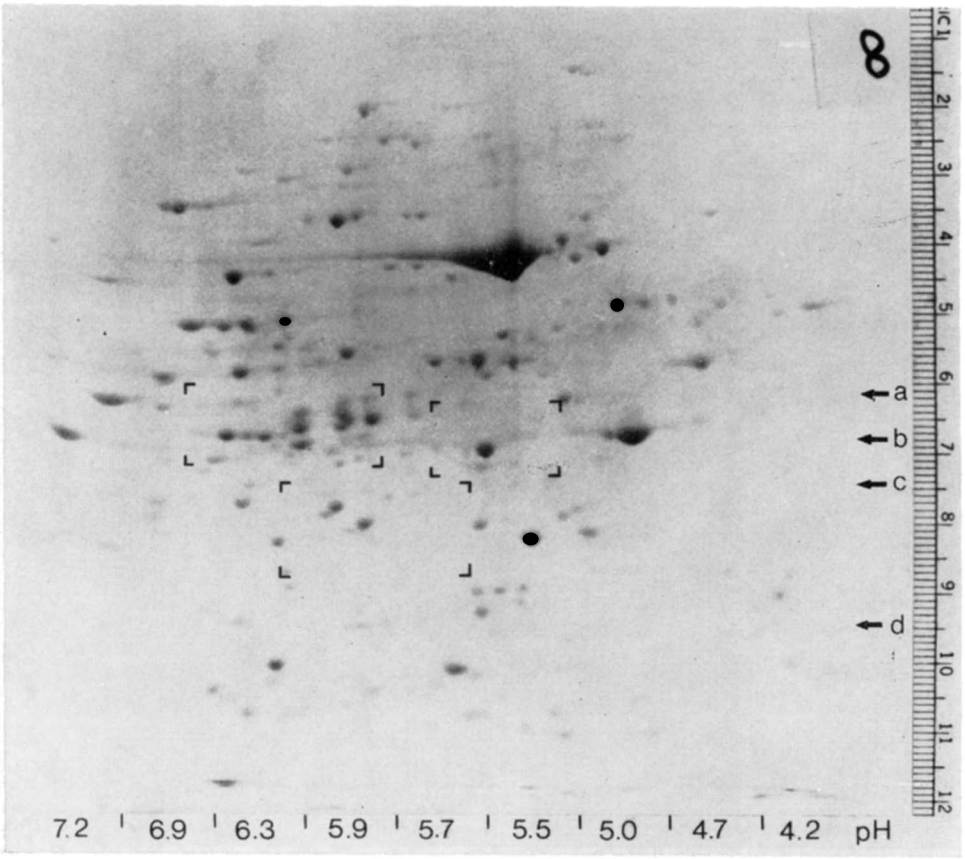


FIGURE 1.—Two-dimensional gel pattern of human kidney proteins. The brackets denote the three variant regions described in the text. Typical mobilities of molecular weight standards (see text): (a) 45000, (b) 40000, (c) 25000 and (d) 13000.

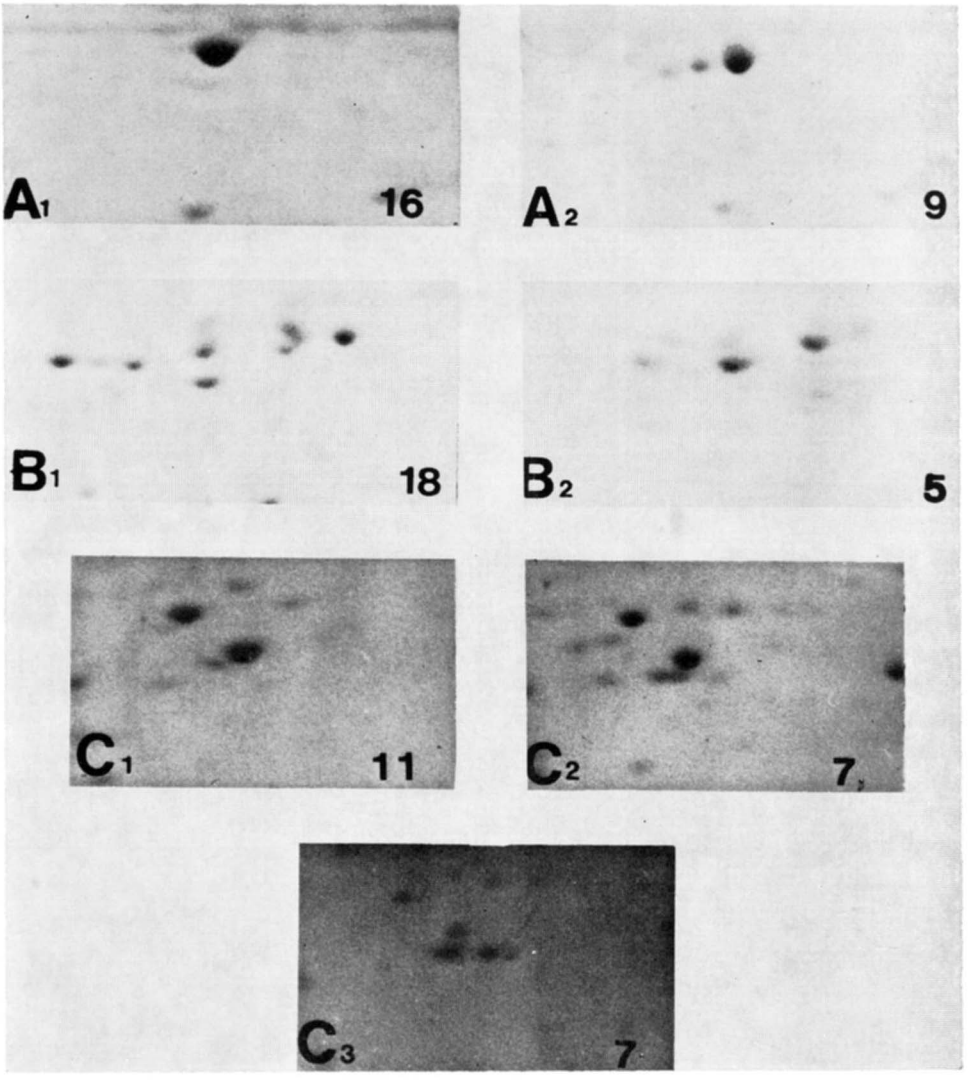


FIGURE 2.—Enlargements of the three variant regions indicated in Figure 1. The numbers in the lower right corner of each box represent the number of gels in which each variant occurred. Two gels were lightly staining for region B. These were omitted from the classifications.

in Figure 2A$_1$ appeared as three spots in nine samples. Surprisingly, the three spots seemed to differ in molecular weight. This can be explained several ways. STEINBERG *et al.* (1977) noted that differentially phosphorylated forms of cAMP-dependent protein kinase ran on 2-D PAGE gels with slightly different mobilities in the molecular weight dimension. Thus, it is conceivable that the three spots represent the same protein in various phosphorylated states. Other protein modifications, such as glucosylation, could result in molecular weight changes, but in this case differential glucosylation must be accompanied by a

charge change to account for the observed result. A variant in a cleavage site could produce changes in molecular weight, as well as in charge. Alternatively the "extra" spots could be the products of an inducible system. A final possibility is that degradative proteolysis occurred in some samples, resulting in multiple spots. This would require specific proteolysis since the other 83 spots were apparently not degraded.

These same possibilities apply to the other two variant groups. Figure 2B shows a group of spots that underwent large changes in intensity and, in several cases, completely disappeared. This group has a shape, pI and molecular weight similar to 2-D PAGE protein spots identified as the IgG chain from human serum (ANDERSON and ANDERSON 1979). The spots shown in Figure 2C have three common configurations. In this case, there is no significant molecular weight change. However, the distribution of the three configurations did not lend itself to interpretation as simple genetic variation.

All of the above variants were reproduced in subsequent electrophoretic analyses of the same kidney samples, indicating that the variants were not artifacts of the preparative procedure. Fifty-nine spots were scored in all 25 samples; 24 additional spots were sufficiently intense to be scored in the gels of 23 of the 25 samples. None of these proteins showed simple changes in pI dimension that were readily interpretable as genetic variation.

## DISCUSSION

The results of this two-dimensional electrophoretic survey suggest a considerably lower estimate of heterozygosity than previously reported for human allozymes. This conclusion, however, depends on several critical assumptions and observations. The fundamental rationale of this survey is that a meaningful estimate of genic variation can be made with 2-D PAGE. The type of variation sought is a simple co-dominant shift in pI. STEINBERG *et al.* (1977) provides chemical evidence that single charge changes in the primary amino acid sequence are detectable on 2-R PAGE. In their study, cAMP-dependent protein kinase was carbamylated to varying extents by heating in the presence of 9.5 M urea. A calibration plot was constructed of charge change *vs.* distance in the isoelectric dimension. They showed that substitutions of single charged amino acids result in discrete and detectable shifts in pI. WILSON *et al.* (1977) observed the migration on 2-D PAGE of numerous unidentified rat proteins in varying states of carbamylation. Again, the results indicated that single charge changes are generally detectable by these techniques. The magnitude of the pI shifts varies from protein to protein depending on the total number of charged amino acids and the polypeptide's position in the pH gradient. The overall impression is that most proteins in the 4.5 to 7.0 pH range show clearly detectable shifts in pI associated with charge changes in amino acids.

Already, a number of such simple variants have been observed with 2-D PAGE. As mentioned before, COMINGS (1979) reported a polymorphism in human brain tissue proteins. In *Drosophila melanogaster*, six naturally occurring pI poly-

morphisms were reported by LEIGH BROWN and LANGLEY (1979). Various strain differences in Drosophila have also been observed (*e.g.*, folicle proteins. SPRADLING and MAHOWALD 1979). In *Mus musculus*, seven strain differences have been distinguished with 2-D PAGE (ELLIOTT 1979; RACINE and LANGLEY 1980a), as well as natural population variation (RACINE and LANGLEY 1980b). Protein variants in pI have also been discerned in 2-D PAGE gels for various viruses, bacteria and tissue culture cells (O'FARRELL 1975; STEINBERG *et al.* 1977; MCCONKEY, TAYLOR and PHAN 1979; WALTON, STYER and GRUENSTEIN 1979).

In many of these variants, the genetic demonstration of simple co-dominant inheritance has been carried out. Furthermore, in four instances, the 2-D PAGE surveys independently detected polymorphisms previously observed with the usual native electrophoresis procedures (LEIGH BROWN and LANGLEY 1979; RACINE and LANGLEY 1980a). When all this evidence is considered together, 2-D PAGE qualifies as an appropriate technique for estimating the frequency of allelic variation due to charge-change amino acid substitutions.

A further concern is the quantitative sensitivity of 2-D PAGE relative to the usual native electrophoresis. This is particularly important if direct comparisons are to be made. The proportion of amino acid substitutions detectable with native electrophoresis has always been a subject of controversy. Studies by JOHNSON (1976); COYNE, FELTON and LEWONTIN (1978); and SINGH (1979) indicate that stringent sets of electrophoretic conditions allow detection of more variation than is normally observed under survey conditions. By varying the pH and/or gel concentrations, these investigators discovered previously undetected variation in some enzymes. It is clear that native gel electrophoresis can detect changes that do not involve net changes in the charge of the primary sequence. The problem is to determine the relative sensitivity. Several laboratories are attempting to answer these questions. Preliminary results of SINGH (1979) indicate that significant increases in the overall estimate of genic heterozygosity should not be expected.

The observed differences in variation with 2-D PAGE compared to allozyme surveys seem consistent over all of the species examined to date. Although 2-D PAGE variation is generally reduced, it paralleles the allozymic data in that more variable populations or species are more variable with 2-D PAGE (LEIGH BROWN and LANGLEY 1979; RACINE and LANGLEY 1980b).

It might be argued that this reduction is completely due to the increased sensitivity of native electrophoresis to amino acid substitutions other than those that change the net charge of the primary sequence. LEIGH BROWN and LANGLEY (1979) examined the ability of 2-D PAGE to detect various electromorphs of $\alpha$-glycerophosphate dehydrogenase in Drosophila. COYNE, EANES and RAMSHAW (1979) have identified slight intraspecific mobility differences over and above the well-established three allozymic types. Since $\alpha$-glycerophosphate dehydrogenase is abundant enough on 2-D PAGE to score, sensitivity was easily tested. Their conclusion was that the minor changes were not detectable by 2-D PAGE. There are two additional comments to make about this comparison. First, the differences in question were intraspecific (some relatively distantly related). Second, al-

though they could be detected on routine starch gels, the variants had only slight mobility differences that might be easily overlooked in mass surveys. The mobility differences that contribute to most of the heterozygosity estimates in animals and man are substantially larger and probably due to whole charge changes.

The major difference in levels of genetic variation as reported here by 2-D PAGE compared to those previously reported by traditional allozymic techniques may stem from at least two causes, not mutually exclusive: (1) Differential sensitivities of the two techniques; and (2) inherent differences in levels of genetic variation between the two sets of proteins surveyed, the explanation we favor.

The later explanation is consistent with reported studies of null allele phenotypes and frequencies of allozyme loci. These studies indicate that amorphic mutants at allozyme loci have less severe fitness effects than similar alleles at most loci and that their frequencies are higher than those of recessive lethals and steriles (O'BRIEN and MACINTYRE 1978; VOELKER *et al.* 1980; and unpublished results).

In conclusion, 2-D PAGE appears, on the basis of numerous reports, to be a legitimate technique for estimating genic heterozygosity as reflected in variations in charged amino acids. This study utilized 2-D PAGE to document a lack of charge varation in 83 denatured human kidney proteins.

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