A Comparison of the Variability of Muscle, Hair, Lens and Plasma Proteins

Structural proteins form a heterogenous group including collagens, keratins, histones and the proteins found in the lens, muscle, ribosomes and membranes. Most have complex structures, and their functions usually involve interactions with other macromolecules. Estimates of variability indicate that while there is some variation between species[1-3] many structural proteins have evolved in a strikingly conservative fashion[4-6]. In contrast many enzymes are not only very variable between species but commonly exhibit intraspecific polymorphisms[7,8]. This report is concerned with whether there is a genuine difference between the variability of structural and plasma proteins.

Materials and methods. The animals used were inbred lines of *Oryctolagus cuniculus, Cavia porcellus, Mesocricetus auratus* and *Cricetulus barabensis*. Polyacrylamide electrophoresis of the lens and hair proteins is reported elsewhere[9,10]. Muscle extracts were prepared by homogenizing the exsanguinated femoral complex of muscles in 0.05 *M Tris*-HCl buffer pH 7.2 containing 8 *M* urea. The homogenate was left overnight at 0–4 °C, centrifuged at 3000 *g* for 30 min and the resulting clear viscous solution applied to polyacrylamide gels containing 6 *M* urea. The protein patterns obtained were essentially the same as those obtained by using partially purified actin and myosin solutions[11]. The crude muscle protein contained no proteins in common with the pattern obtained when urea treated whole blood was electrophoresed. Contamination of the muscle with significant quantities of blood proteins can therefore be eliminated.

Plasma was obtained by collecting blood into an equal volume of 0.6% sodium citrate followed by centrifugation at 3000 *g* for 10 min. The supernatant was electrophoresed in gels not containing urea.

The mobilities of all protein bands were expressed as mobilities relative to a standard marker (bromophenol blue) included in every gel.

Results. In order to compare the variabilities of muscle and plasma proteins (Figures 1 and 2) and those of lens and hair[9,10], Jaccard's coefficient of similarity[12] was calculated using each set of proteins in turn and every possible pair of species. Jaccard's coefficient is given by: Number of bands in common/Total number of comparisons.

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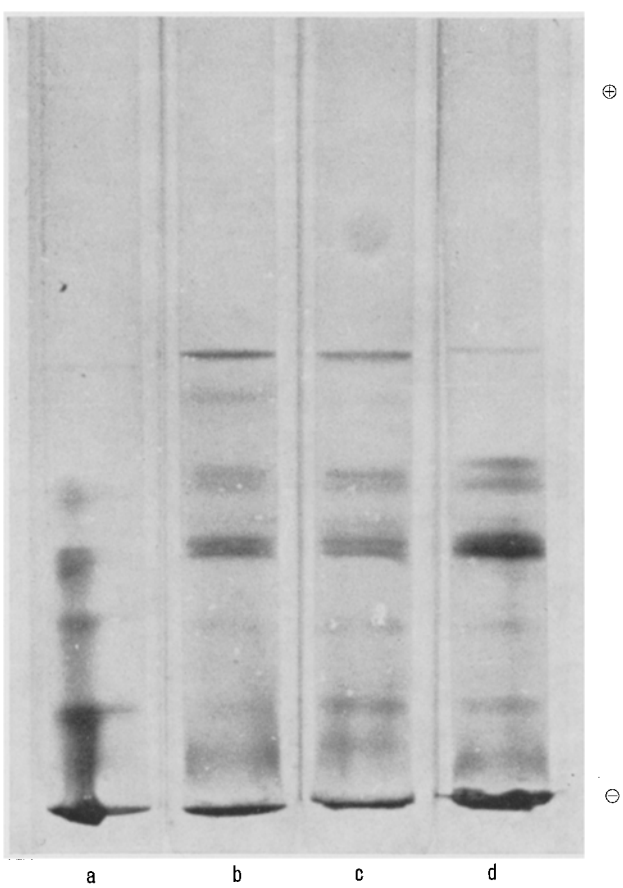

Fig. 1. Muscle protein patterns after electrophoresis in polyacrylamide gels. a) *Cavia porcellus*; b) *Cricetulus barabensis*; c) *Mesocricetus auratus*; d) *Oryctolagus cuniculus*.

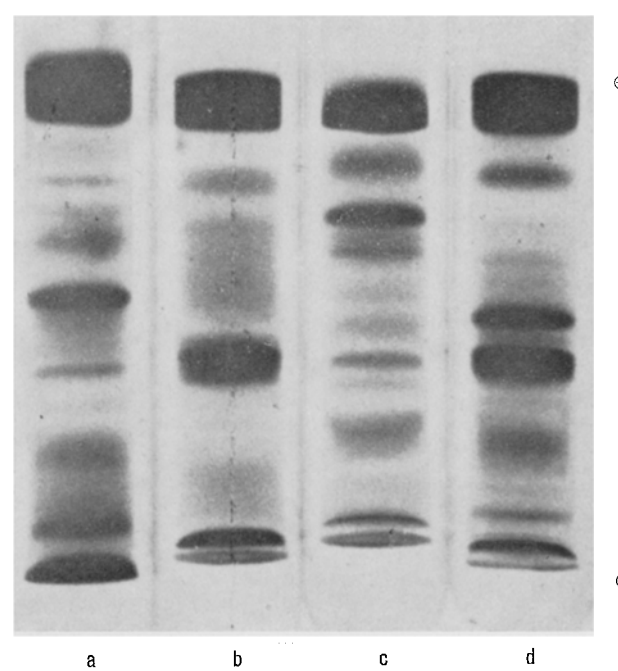

Fig. 2. Plasma protein patterns after electrophoresis in polyacrylamide gels. a) *Oryctolagus cuniculus*; b) *Mesocricetus auratus*; c) *Cavia porcellus*; d) *Cricetulus barabensis*.

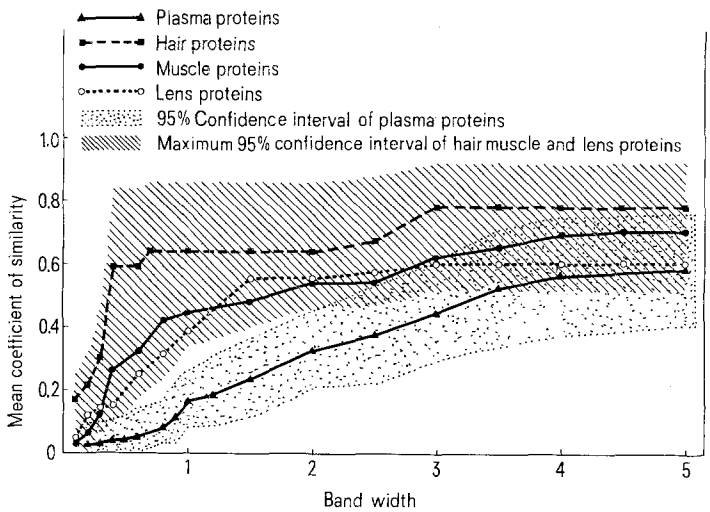

Fig. 3. The relationship between the mean coefficients of species similarity and the theoretical width allocated to each protein band shown in Figures 1 and 2.

Using 4 species there are 6 coefficients of similarity. The mean, variance and 95% confidence interval of these 6 coefficients (using angular transformations of coefficients) were calculated for each of the 4 sets of proteins.

If the mean coefficients are high then the species appear very similar and the set of proteins used must be relatively invariable, whereas if the coefficients are low, the characters are more variable. The coefficients were calculated using a range of band widths from 0.2–5%.

The results (Figure 3) show that at any particular band width the plasma proteins yield consistently lower coefficients of similarity than the other proteins. This difference between plasma and structural proteins is insignificant only if there is far more technical error associated with the plasma protein bands. Two points are relevant. A single sample of lens proteins was repeatedly electrophoresed and the band width that included 95% of all repeats was $\pm$ 0.4% in the presence of urea, and $\pm$ 0.5% in its absence[13]. We should therefore compare the coefficients when the band width is about 0.4–0.5%. Secondly, this is a minimum estimate of technical error since there may be error introduced during sample preparation. However, the extraction procedure for the structural proteins is far more lengthy and involved than that for plasma proteins. We may argue then that if there is a difference in technical error, it could only increase the significance of the result.

There is a further feature of Figure 3 that is worthy of comment. If a set of proteins were completely identical in all 4 species the mean coefficient of similarity would increase sharply with increasing band width and then plateau when all the bands had been recruited. The sharp increase would be in the region where technical error was important. At the other extreme would be a set of proteins that was utterly different in all species. In this case the mean coefficient would increase slowly as more and more bands were gradually recruited. As can be seen from Figure 3 the plasma proteins approximate more closely to the variable extreme and the hair proteins to the constant extreme. The muscle and lens proteins are intermediate (though not significantly different from the hair proteins). This provides further reason for believing the plasma proteins to be more variable than the structural proteins.

There are several points which relate to this conclusion. Firstly it is essentially unaffected by there being different numbers of proteins in each extract. Secondly, the presence of urea in some but not all of the gels may be considered a complicating factor. In fact lens extracts have been analyzed in both types of gels and the two sets of coefficients are indistinguishable. Thirdly the existence of intraspecific polymorphisms cannot be excluded. The coefficients of similarity do not distinguish between inter- and intra-specific variation and so the two must remain confounded. Nevertheless, it should be noted that no variation was detected between replicate animals within inbred lines.

Discussion. The structural proteins may have evolved at a slower rate as a result of greater stabilizing selection. It is certainly not difficult to imagine that the proteins of hair, muscle or collagen should be vitally important to an animal, and that any impairment of function would be disadvantageous if not lethal. Possibly fewer alterations in these proteins are tolerated because there is a more limited array of molecules that will permit interactions with other macromolecules. Certainly the conservatism seen in other structural proteins such as histone IV[4], collagens[6] and muscle parvalbumins[5] is truly impressive.

Résumé. Trois groupes de protéines structurelles (celles de la lentille, celle des muscles, et celles du poil) furent comparés aux protéines de plasma, en utilisant l'électrophorèse en gel polyacrylamide. Les variations apparaissant chez 4 espèces de rongeurs furent examinées et on trouva que les protéines de plasma varient plus que les autres. L'importance de ces résultats dans la recherche d'une évolution possible de ces protéines est considérée.

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