R factor transfer *in vivo* in sheep with *E. coli* K12

IT has been inferred that the bacterium *Escherichia coli* K12 is a naturally "feeble" strain which can be used in some genetic engineering experiments with little concern that it might "escape" containing a reconstituted plasmid. Also, there seems some doubt as to whether *in vivo* plasmid transfer, especially from the commonly used *E. coli* K12 F^-, can occur to a significant extent within the gastrointestinal tracts of normal, adult humans or other animals[1,2]. This implies that even if a few organisms should be ingested by laboratory personnel, transfer of a plasmid to other bacteria would be negligible and the "feeble" *E. coli* K12 should be quickly eliminated from the human gastrointestinal tract.

In an attempt to ascertain how antibiotic-resistant organisms have become so widespread in farm livestock, I have studied the conditons under which R factors could be transferred from donor to recipient bacteria *in vivo* in sheep[3]. These experiments were repeated using donor and recipient strains of *E. coli* K12 with similar results. By imposing a short period without food (24–48 h duration) on these animals, the environment within the gastrointestinal tract was altered and the administered *E. coli* K12 strains were able to multiply *in vivo*, reaching a population density sufficient to donate or receive an R factor. An experiment was also conducted in which an R factor was transferred *in vivo* from a small number of administered *E. coli* K12 donor organisms (4.0×10^3 cells) to the natural coliform flora of an adult sheep where it survived for 7 d (in up to 10^5 wild-type cells per g faeces). In similar experiments transferring the same R factor from 'animal' donor strains to the natural microflora of sheep, coliform organisms containing the R factor were sometimes excreted for only a few days. At other times, however, these organisms could be excreted for weeks or even months afterwards. It is not known whether the particular donor organism has an effect on the duration of carriage in the microflora of an animal, but this seems unlikely.

These experiments were carried out in the absence of any form of antibiotic treatment, and possession of the R factor should have conferred no special survival value on the host bacterial cells. Using similar nutritional conditions, therefore, it may be possible to transfer other plasmids, perhaps even reconstituted ones, *in vivo* in sheep.

If *in vivo* transfer can be accomplished in one animal species using *E. coli* K12 organisms, it is likely to take place in other animal species, including humans. The difficulty will be to define the conditions in which it occurs.

Short starvation periods or other changes causing temporary alteration of the environment and microflora of the gastrointestinal tract may well potentiate plasmid transfer in monogastric as well as ruminant animals. It is in the interests of all concerned in genetic engineering that this possibility is recognised.

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Human ζ haemoglobin chain

THE embryonic ζ chain occurs in combination with γ chains in haemoglobin (Hb) Portland[1]. Lehmann[2] and Huehns[3] have suggested that the ζ chain is an α-type chain and attempted to confirm the idea by comparison of the amino acid compositions of the tryptic peptides from the ζ chain with those of the human α chain[4,5] and known mammalian α chains[6]. From similar data other workers have proposed the same α chain relationship for the corresponding x-chain found in other mammals[7]. The presence of a Tyr–Arg dipeptide[4,5], the absence of a D helix[4,5], the identification of residues which could participate in α–β contacts[5] and cooperative oxygen binding by Hb Portland[4–6] have been used to support the contention that the ζ chain is a primitive α chain.

I submit that the relationship between the ζ and α chains has not been clearly established by the published evidence. Tyr–His, present in non-α chains, could become Tyr–Arg as a result of a single base change. The missing D helix, like the detection of appropriate α–β contacts, could easily result from the deliberate attempt to align the ζ-tryptic peptide compositions with the α-chain sequence. Certainly the β_4 tetramer does not show cooperative oxygen binding but this does not preclude the possibility that some asymmetric tetramers with no α-chain content could have cooperative function.

Examination of the amino acid compositions of the various human Hb chains[1] by any of the proposed methods[8–10] suggests that the ζ chain is most closely related to the γ chain. The available tryptic peptide compositions of the ζ chain can be aligned with the γ-chain sequence to give 46 differences in 131 positions of comparison (the alignment includes a D helix). In the most recent comparison of ζ-chain peptide compositions and the α-chain sequence there were 47 differences in 135 positions.

I do not claim that the ζ chain is closely related to the γ chain. I do suggest that the lack of restraints in aligning tryptic peptide compositions within a family of related sequences can lead to erroneous and misleading conclusions, and urge that at least the tryptic peptide sequences be obtained before the principle of least dissimilarity is invoked and evolutionary or functional relationships proposed.

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Matters arising

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LEHMANN REPLIES—The α and γ chains of man have more than 60% of their sequences in common. It is therefore not surprising that it is possible to align parts of the ζ chain with either. The fact remains that haemoglobin (Hb) Bart's (γ_4) shows no cooperativity, whereas Hbs F ($\alpha_2\gamma_2$) and Portland ($\zeta_2\gamma_2$) do. The cooperativity of Hb Portland is such[1] that it is difficult to visualise it without the conventional $\alpha_1\beta_2$ contacts typical of cooperative haemoglobins. The fact that it is possible to align by homology residues of the tryptic peptides of the ζ chain in a manner which places $\alpha_1\beta_2$ contacts in the expected positions fully justifies that exercise.

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