with methanol and characterized as *d*-methadone N-oxide (R_F 0.08), 2-ethylidene-1,5-dimethyl-3,3′-diphenyl pyrrolidine (R_F 0.27), *d*-methadone (R_F 0.58) and *p*-hydroxyphenyl derivative of *d*-methadone (R_F 0.96), respectively, by co-chromatography with authentic samples.

The polar fraction (R_F 0.0) proved to be predominantly glucuronide conjugates by enzymic hydrolysis with β-glucuronidase (Fig. 3*c*). Acid-hydrolysis of conjugates and subsequent extraction with chloroform-isopropanol (3:1, v/v) and ITLC with ethyl acetate-methanol-ammonia (17:2:1, v/v) (Fig. 3*d*) and with benzene-ethyl acetate-methanol-ammonia (80:20:1.2:0.1, v/v) (Fig. 3*e*) showed the formation of *p*-hydroxyphenyl *d*-methadone N-oxide (R_F 0.04), *p*-hydroxyphenyl pyrrolidine derivative (R_F 0.27), *d*-methadone (R_F 0.68), and *p*-hydroxyphenyl *d*-methadone (R_F 0.96) with approximate distribution of radioactivity as 25.3, 14.3, 5.7, and 54.7%, respectively (Fig. 3*e*). Acid hydrolysis of the methanol-insoluble residue of the eluate from 'Amberlite XAD-2' column, extraction and ITLC of extract with ethyl acetate-methanol-ammonia (17:2:1, v/v) (Fig. 3*f*) and subsequent resolution with system: benzene-ethyl acetate-methanol-ammonia (80:20:1.2:0.1, v/v) provided a pattern of metabolites identical to that given in Fig. 3*e*.

The major metabolic pathways for *d*-methadone with qualitative similarity to *l*-isomer[15] are: (*a*) *p*-aromatic hydroxylation by hepatic microsomal mixed-function oxidase system which has a low order of substrate specificity, (*b*) glucuronide conjugation, and (*c*) N-oxidation[18]. By contrast with *l*-methadone only minor amounts of pyrrolidine[19,20] and *p*-hydroxyphenyl pyrrolidine metabolites[21] were formed; thus mono-N demethylation and subsequent cyclization are a very minor metabolic route in *d*-methadone. These *in vivo* findings with *d*-methadone are consistent with earlier observations from *in vitro* liver microsomal[22,23] and isomeric substrate studies[24] where the active *l*-isomer was shown to be more readily N-dealkylated than inactive *d*-isomer, and N-dealkylation rather than N-oxidation was shown to be stereoselective.

These observations on comparative metabolism *in vivo* of optical enantiomorphs of methadone-1-^{3}H at identical 10 mg kg^{-1} subcutaneous doses strongly suggest that (1) the stereoselective N-demethylation pathway as previously postulated[14] is a preferential route with active *l*-isomer; (2) significant differences exist in half-lives of isomers in plasma and brain; and (3) the formation of an apparently active metabolite in rat brain occurs with *l*-isomer[14], but not with *d*-isomer.

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Milk Proteins: Reply

WE are grateful to Dr Wheelock[1] for drawing our attention to some mistakes in our paper[2]. The α_s-casein was inadvertently labelled α-casein and in error had been included as a glycoprotein, which it is not. This correction, which reduces the percentage of milk proteins which are glycosylated, further emphasizes the wider argument presented in the paper, based on various analyses of which bovine milk is only one, that extracellular proteins do not, of necessity, require glycosylation for export from the cell.

With regard to the other points raised, K-casein referred to in refs. 3 and 4 is the monomeric reduced form, not the native disulphide bridged polymer. Although only 28% of monomeric K-casein is glycosylated[4], the situation concerning the polymer is uncertain. We did not list α-lactalbumin as a glycoprotein because the glycosylated form (the existence of which was stated) accounts for less than 10% of the total α-lactalbumin in bovine milk.

The role of the carbohydrate moiety of K-casein in the interactions of the caseins has not been resolved yet by the workers in this field. Lysozyme, a glycosidase, has a similar coagulation action on casein micelles to that induced by rennin[5], while Talbot and Waugh[6] related the stabilizing capacity of K-casein to the content of one of the carbohydrates, N-acetyl-neuraminic acid. Even the critic of our paper has expressed the opinion that the rate of action of rennin is influenced by the nature of the saccharide unit[7]. Therefore, on the evidence available the use of K-casein as an example of anti-recognition is not invalid.

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