Table 2. COMPARISON OF GENE FREQUENCIES OF CATTLE OF DIFFERENT COUNTRIES

Breed	α-Lactalbumin		β-Lactoglobulin	
	A-gene	*B*-gene	*A*-gene	*B*-gene
British breeds (ref. 2)	0·00	1·00	0·28	0·72
Icelandic (ref. 3)	0·00	1·00	0·34	0·66
Holstein (ref. 6)	0·00	1·00	0·52	0·48
White Fulani (Nigeria) (ref. 3)	0·15	0·85	0·21	0·79
Boran (Kenya) (ref. 4)	0·13	0·87	0·19	0·81
Indian Zebu*	0·24	0·76	0·09	0·91

* From Table 1 *a* and *b*.

of whey proteins of buffalo's milk, and cow's milk with α-*A*, β-*B* seem to be almost identical, α-*A* migrating approximately the same distance as buffalo α-lactalbumin. Thus, α-lactalbumin polymorphism and existence of combinations such as α-*A*, β-*B* in zebu milk rule out the possibility of differentiation of cow's and buffalo's milk in a mixture, as previously suggested[7].

Cow α-lactalbumin-*A*, purified and crystallized by the method[10,11] used for α-*B*, and buffalo α-lactalbumin show, as expected, very similar mobilities (u) and shapes of patterns in free electrophoresis at *p*H 6·0, 7·58 and 8·6. The values of u (descending) for cow α-*A* and buffalo α-lactalbumin are $-2{\cdot}40 \times 10^{-5}$ and $-2{\cdot}43 \times 10^{-5}$ cm²/sec/V (*p*H 6), $-4{\cdot}92 \times 10^{-5}$ and $-4{\cdot}70 \times 10^{-5}$ cm²/sec/V (*p*H 7·58) and $-4{\cdot}40 \times 10^{-5}$ and $-4{\cdot}40 \times 10^{-5}$ cm²/sec/V (*p*H 8·6) respectively. In respect of other physical chemical properties, cow α-*A*, α-*B* and buffalo α-lactalbumin show close parallelism. They appear to have the same crystalline form, similar nitrogen contents, specific extinction coefficients at 280 mμ and tyrosine and tryptophan contents. Their sedimentation coefficients ($s_{20,w}$) fall within the range $1{\cdot}87 \times 10^{-13}$ sec and $1{\cdot}99 \times 10^{-13}$ sec. The molecular weight of buffalo α-lactalbumin, 16,200 g/molo, calculated from sedimentation and diffusion data is also close to that of the values[11,12] reported for cow α-lactalbumin. Some of the protein preparations, even after several recrystallizations, show the presence of a faster moving minor component (4–6 per cent) in electrophoresis and sedimentation, the mobilities and s values of which indicate it to be probably the serum albumin contaminant.

Thus, it is interesting to observe that cow α-*A* and buffalo α-lactalbumin show close similarity in their properties in almost the same manner as shown by cow β-*B* and buffalo β-lactoglobulin, which have approximately the same molecular weights[9] and are practically indistinguishable in crystal form[7], electrophoresis[8], sedimentation and ultra-violet absorption[9].

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Significance of the Michaelis Constant

THE Michaelis–Menten[1] equation relating initial velocity of a simple enzyme-catalysed reaction to initial substrate concentration is :

$$v = \frac{k_3 es}{K + s} \qquad (1)$$

where v is the initial velocity of reaction, e is the initial enzyme concentration, s is the initial substrate concentration, k_3 is the velocity constant of the breakdown of the enzyme-substrate compound into enzyme and product of the catalysed reaction and K is the substrate concentration at which v is half-maximal.

Michaelis and Menten[1] supposed K to be the equilibrium constant of the reversible reaction between enzyme and substrate. Briggs and Haldane[2] derived an expression showing it to be the ratio of the sum of the two monomolecular velocity constants governing breakdown of the enzyme-substrate compound to the bimolecular constant governing association.

Both pairs of workers based their derivations on the equation :

$$(e - p)s = Kp \qquad (2)$$

where p is the initial equilibrium or steady-state concentration of enzyme-substrate compound. The full equation is :

$$(e - p)(s - p) = Kp \qquad (3)$$

and the assumption was made in both cases that p was negligible relative to s. However, there are now known numerous cases in which the half-saturation concentration of the enzyme is very low. For example, the half-saturation concentration of D-amino-acid oxidase with flavin adenine dinucleotide (FAD) was reported by Warburg and Christian[3] to be in the region of 10^{-7} M. Since this means that the practicable range of substrate concentrations that can be used for kinetic studies will extend over about one order either side of this, the validity of equation (2) in all cases as an approximation to equation (3) seems doubtful.

The purpose of this communication is to point out that when the quadratic (3) is solved for p, one of the roots gives a solution which approximates to the form of equation (1), but K is replaced by $(K + e)$.

It would seem wise, therefore, in experiments with enzymes of unknown molar concentration, which have low half-saturation concentrations of substrate, to perform at least two series of experiments at different enzyme concentrations before equating the half-saturation concentration with the Briggs–Haldane[2] K. Where the enzyme concentration is known, an approximate idea of the error involved in ignoring enzyme concentration can be obtained by inspection.

When the original data of Warburg and Christian[3] are re-examined using modern techniques of fitting the Michaelis–Menten[1] relation it is found that the fit is very close and that the original estimates of K and k_3e are fully confirmed. Using more modern data for the half-saturation concentration for alanine, the second substrate used by Warburg and Christian[3], and for the turnover number of the pure enzyme (corresponding to k_3) which are given by Long[4], it can be calculated that the enzyme concentration used was roughly $1{\cdot}4 \times 10^{-7}$ M. Since the half-saturation concentration for FAD was found to be $2{\cdot}5 \times 10^{-7}$ M, it follows that the Briggs–Haldane[2] constant is about half the published value for K_m.

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