of the above cell types were grown in FCS or MP-containing medium (data not shown).
Table 2 shows the incorporation of ^{3}H-thymidine by different cell types grown in the presence of FCS or MP. The thymidine incorporation was lower in the case of Vero, BHK and RVF grown in MP-containing medium when compared with the incorporation by these cells in the presence of FCS. On the other hand, HeLa had a higher incorporation when grown in MP. BSC-1 cells incorporated approximately the same amounts of thymidine when grown either in FCS or in MP.
Discussion. From these studies it is evident that bovine milk obtained later in the lactation period (60 days after calving), can substitute serum in supporting cell growth. For the initial attachment of cells, it is necessary to add FCS, since the cells do not attach in the presence of milk plasma alone. The addition of attachment factors is not required when colostrum is used in place of milk[8]. It would be interesting to grow cells where attachment is not essential for growth, such as transformed cells, in milk plasma without prior preincubation in medium containing FCS.
Klagsbrun and Neumann[8] have shown the presence of different types of growth factors in milk and serum. Since milk contains EGF[4], it has been observed that epithelial types of cells like HeLa and BSC-1 grow quite well in milk plasma. Vero, BHK and RVF which are fibroblastic cells do not grow to the same extent in milk plasma as in FCS. This may be due to the absence of fibroblast growth factor (FGF) or other factors needed for the growth of fibroblasts.
Milk plasma can replace the requirement of serum for some types of cells as it can meet the requirements of the cell in terms of its need for proteins, carbohydrates, fats, hormones and growth factors. The present studies using milk plasma have shown that the casein part of milk is not essential for the growth of cells.
Colostrum, used by Klagsbrun and Neumann, mainly contains the constituents of blood plasma and therefore is not very different from it. Milk plasma is easily available as compared to colostrum and has the added advantage that it can be filter-sterilized, without difficulty.

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The response to potassium of the Na-K pump ATPase in low-K red blood cells from cattle at birth and in later life

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Summary. It is shown that in low-K red blood cells of cattle the apparent affinity for K($1/K_K^{app}$) at an inhibitory site of the Na-K ATPase increases markedly during the first 3 months of life. This site probably is the Na accepting site at the internal membrane surface and the change in K_K^{app} reflects an increase in K_{Na}/K_K, the ratio of the true dissociation constants. This effect may explain the concomitant fall in cellular K concentration.

As in other ruminants the red cell potassium concentration in cattle is low (20 mmoles/l cells) in a majority and high (up to 70 mmoles/l cells) in a minority of the adult animals from the same breed[1,2]. In both types the sum (Na + K) is approximately 100 mmoles/l cells[1]. In the low-K red cells the number of Na-K pump sites per cell is reduced[3-5], the passive permeability of the membrane is elevated[6] and the kinetics of the pump are different as compared with high-K red cells. The kinetic peculiarity in low-K cells is that K activates at an external site as usual but in addition competes at low cellular concentration with Na at the internal cation binding site of the pump protein[4,7-10].
Interestingly, 'low-K animals' are born with red cells high in K and low in Na[11,12]. It is not clear whether the adult state is reached by replacement of the foetal cells or by an increase in the average age of the cells in the circulating blood[3,9,11,12]. The leak flux of K increases slightly up to 40 days[11] and the steady-state pump flux and the Na-K ATPase activity decrease[11,12]. However, it is unknown whether the kinetic behavior of the pump changes concomitantly. Therefore, we examined the K-dependence of the activity of the Na-K pump ATPase at birth and later on. Blood was taken from the same 7 Simmenthal cattle first at the average age of 2.3 days (when the mean cellular K concentration was 116 ± 8 mmoles/l cells) and again at the age of 78.7 days (when the cell K had fallen to 31.6 ± 1.9 mmoles/l cells).
Red cell membranes were prepared as described before[13]. The assay medium for Na-K ATPase is given in the legend to the figure. Na was kept constant (150 mM) and K was varied by keeping (K + choline) constant at 100 mM. The ATP concentration was saturating (1.25 mM). Na-K ATPase was taken as the difference of phosphate liberation between a sample without and one with 0.17 mM ouabain.
The result is shown in the figure: In membranes taken at the early age K activated the ATPase in the range between 5 and 50 mM (curve A). The curve for the same animals at 79 days of age is the result of superimposed activation and inhibition by K. Inhibition is prominent beyond 5 mM K (curve B). (No measurements were done in the activating range below 5 mM K.
Assuming that activation requires occupation of 2 equivalent binding sites by K at the external membrane surface, that K binding to one of the 3 equivalent internal Na-binding sites blocks the system[10] and that K-binding does not affect the affinity for ATP, the curves of the figure were

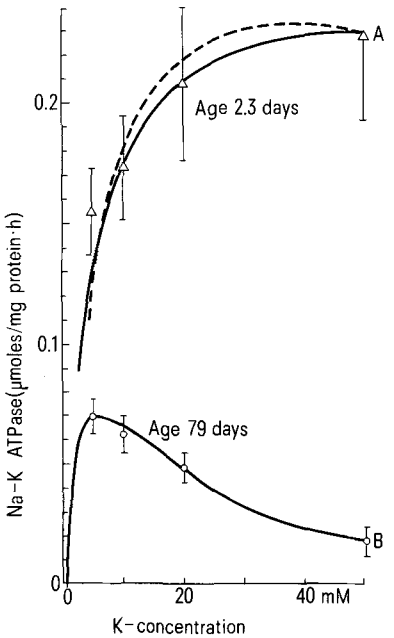
obtained, with the constants given in the legend, using the equation[4,10]

$$\text{observed } v = \frac{v_{max}}{\left(1+\frac{K^a_K}{[K]}\right)^2\left[1+\frac{K^a_{Na}}{[Na]}\left(1+\frac{[K]}{K^i_K}\right)\right]^3} \quad (1)$$

('observed v' is the rate at saturating ATP concentration, K^a_K is the dissociation constant for K at the activating (external) site at 150 mM Na and K^i_K that at the inhibitory (internal) site, K^a_{Na} the dissociation constant for Na at the activating (internal) site and v_{max} the maximal rate).

Using equation (1) to fit the points in curve B seems justified because there is good evidence that the stoichiometry in ruminant red cells is 3 Na:2 K[4,9,10]. For the sake of consistent treatment it was also applied to the data at birth (curve A) although these could be fitted equally well or even better by a rectangular hyperbola. The stoichiometry has not been assessed in young ruminants. K^a_K for curve B was taken = 1 mM in the presence of 150 mM Na, as measured by Ellory and Carleton[9] in adult cows and K^a_{Na} = 20 mM, similar to what was found in goat red cells[4,10]. There is no unique way of transforming curve A into curve B by changing the constants in equation (1). This is exemplified by the 2 approximations shown, one obtained by increasing K^i_K and the other by decreasing K^a_{Na}. However, two points seem clear: a) The ration K^a_{Na}/K^i_K must be > 1 in B[7] and < 1 in A. b) More than one of the 3 dissociation constants must be altered and v_{max} must be decreased when the behavior seen in curve A yields to the behavior seen in curve B.

The qualitative result thus is that during the first weeks in the life of cattle the affinity for K relative to that of Na at the internal metal binding site of the red cell Na-K pump increases, v_{max} decreases moderately (be it by way of a decrease in the number of pumps per cell or by a reduction in the turnover rate) and the affinity for K at the external metal binding site may increase slightly. The rise in K^a_{Na}/K^i_K seems to be more important than the fall in v_{max} for the drop in cellular K concentration observed during maturation of the animal.



Ouabain sensitive ATPase (Na-K pump ATPase) activity of disrupted membranes from red cells of the same 7 cattle at 2.3 days (curve A) and 78.7 days of age (curve B) at variable K concentration. Points: mean of 7 animals ± 1 SE. Medium (mM): NaCl 150, tris-Cl 10 (pH 7.7 at 37 °C), $MgCl_2$ 1.25, EGTA 0.5, ATP 1.25, (K + choline-Cl) 100, with or without ouabain 0.17[8]. Membrane density corresponding to 0.25 haematocrit of original cells. Sample volume 1.5 ml. Incubation 1 h at 37 °C. Start by adding Na_2ATP (Boehringer), stop by adding 0.5 ml trichloroacetic acid 20%. Phosphate liberated was determined according to Martin et al.[14], protein according to Lowry et al.[15]. Curves calculated according to equation (1). A, Solid line: v_{max} = 0.37 μmole/1 cells · h, K^a_{Na} = 20 mM, K^a_K = 2 mM, K^i_K = 1000 mM; broken line: v_{max} = 0.37 μmole/1 cells · h, K^a_{Na} = 2 mM, K^a_K = 4 mM, K^i_K = 6.5 mM. B: v_{max} = 0.19 μmole/1 cells · h, K^a_{Na} = 20 mM, K^a_K = 1 mM, K^i_K = 6.5 mM.

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mtDNA heterogeneity in *Panulirus argus*[1]

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Summary. Restriction endonuclease analysis of mtDNA polymorphisms in *Panulirus argus* has revealed significant heterogeneity and possible species subdivision.

The techniques of both Mendelian genetics and molecular biology have been utilized to quantify intraspecific and interspecific genetic heterogeneity in a variety of organisms[3-6]. Although nucleotide sequences yield maximal information, determination of sequence heterogeneity among large numbers of individuals is not yet feasible. On the other hand, restriction endonuclease analysis permits the indexing of sequence differences, and these techniques may