3 cell lines namely P388, clone 707 Friend, and L5178Y would be 1:1:2. While the L5178Y cells have clearly the highest activity, P388 cells have considerably less activity than would been expected in the event of a direct relationship existing between enzyme activity and gene dosage. The ratios of enzyme activities between P388 and L5178Y cells are similar to those observed earlier by Fox and Anderson[16] It would therefore, appear that gene regulation plays a greater part in determining thymidine kinase activities than does gene dosage.

In conclusion, Anderson and Fox[5] in reporting on a high mutation requency in P388 cells to thymidine kinase deficiency, postulated that they may be heterozygous or hemizygous at the thymidine kinase locus. The latter possibility is clearly the case as the results of the cytogenetic part of the present investigation show that only one copy of chromosome 11, the determinant of thymidine kinase is present per cell.

1 The authors acknowledge a grant from the Department of Health and Social Services for Northern Ireland and the technical assistance of Mr D.J. Mooney and Mr H. Termonia. Author for reprint requests: P.G. McK.

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0014-4754/83/050532-03$1.50+0.20/0

The serum-free growth of different cell types in buffalo milk plasma

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Summary. Buffalo milk plasma can replace the fetal calf serum required for the growth of various types of cells. However, the addition of serum is essential for the initial attachment of the cells.

Serum has been an essential component of the medium for growing almost all types of cells. In addition to nutrients, it also provides growth factors and hormones. Recently attempts have been made to formulate serum-free media using various combinations of hormones and growth factors[1-3]. Human milk has been shown to contain epidermal growth factor (EGF)[4], and it also stimulated the DNA synthesis and cell division in Balb/c 3T3 cells[5]. Bovine colustrum supported the serum-free proliferation of epithelial cells, but not of fibroblasts in long-term culture[6]. We, therefore, tried to grow different established cell lines of epithelial and fibroblastic type in the presence of buffalo milk plasma.

Materials and methods. Fresh buffalo milk was centrifuged at 8000 × g for 15 min followed by another centrifugation at 120,000 × g for 60 min. The clear supernatant, free of micellar casein, was passed through a 0.45-μm millipore filter. HeLa, BSC-1, Vero, BHK cell lines (from National Institute of Virology, Pune) and rat vaginal fibroblasts (RVF) were grown in a medium prepared by mixing equal volumes of Dulbecco's minimal essential medium (DME) and Ham's F-12 (F-12) medium containing fetal calf serum (FCS) or milk plasma (MP). For growing the cells in MP, the cells were initially plated in 4 ml of the medium containing 5% FCS in 6-cm Petri dishes (Sterilin, UK) and incubated in a humidified incubator at 37 °C with 5% CO_2. After the cells were attached, the medium was removed, the cells were washed twice with F-12 medium and incubated further in the medium containing MP. The cells were counted using a hemocytometer. The incorporation of ^{3}H-thymidine was monitored according to a previously described method[7].

Results. The effect of FCS or MP on the total cell count is shown in table 1. Vero, BHK and RVF cells did not grow as well in MP as in FCS-containing medium. In milk plasma, Vero, BHK and RVF cell numbers were 76%, 69% and 58%, respectively, when compared with those in fetal calf serum. On the other hand, the cell number of HeLa and BSC-1 was higher than that in FCS. There was no significant difference in the total protein content per cell when either

Table 1. Total cell-count after the growth of different cell types in fetal calf serum or milk plasma*

Cell type	Initial cell count ($\times 10^4$ cells/ml)	Total cell number ($\times 10^4$ cells/ml ± SEM) FCS	MP
HeLa	9	94 ± 2.3	115 ± 1.1
BSC-1	8	73 ± 0.9	81 ± 1.2
Vero	7	55 ± 1.0	42 ± 0.8
BHK	6	36 ± 2.6	25 ± 2.4
RVF	7	41 ± 1.7	24 ± 0.7

*Counting of the cells was done on the 4th day of culture and the results are average values from 4 Petri dishes in each group.

Table 2. Incorporation of ^{3}H-thymidine by different cell types grown in the presence of fetal calf serum or milk plasma*

Cell type	^{3}H-thymidine incorporation (cpm ± SEM) FCS	MP	Incorporation (%)**
HeLa	78475 ± 6865	125940 ± 6027	160
BSC-1	67533 ± 2621	69087 ± 1873	102
Vero	29125 ± 785	21255 ± 486	73
BHK	25491 ± 643	12831 ± 291	50
RVF	56392 ± 2783	30187 ± 5620	53

*On the 3rd day, 20 μCi of ^{3}H-thymidine was added and the cells were incubated further for 24 h. **Expressed as percentage of incorporation in FCS.

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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0014-4754/83/050534-02$1.50 + 0.20/0

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