Effect of K^+ on glucose consumption and pyruvate kinase (PK) activity in high potassium (HK) and low potassium (LK) sheep red blood cells (mean ± SEM)

K^+ (mM)	HK Glucose consumption μM/g Hb/h	HK PK activity μM/g Hb/min	LK Glucose consumption μM/g Hb/h	LK PK activity μM/g Hb/min
0	1.62 ± 0.466	3.07 ± 0.940	1.74 ± 0.277	4.45 ± 1.18
50	2.65 ± 0.499	6.66 ± 2.49	2.81 ± 0.490	9.93 ± 2.99
100	3.74 ± 0.818	5.68 ± 1.47	3.85 ± 0.846	8.51 ± 2.03
150	4.12 ± 0.439	6.45 ± 2.40	4.54 ± 0.772	9.01 ± 3.20

filtered hemolyate, which was virtually free of K^+ and Na^+, was used for the measurement of glycolytic rate and enzyme activities. For glucose consumption measurements a portion of the hemolysate was incubated for 1 h at 37 °C in a buffer mixture containing several co-enzymes, nucleotides and substrates[7] and varying concentrations of K^+ as KCl. Glucose was measured at 0 and 1 h by the glucose oxidase method (Sigma Technical Bulletin No. 510). The activities of the enzymes hexokinase, glucose phosphate isomerase, phosphofructokinase, aldolase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and glutathione reductase were measured at 37 °C by standard techniques[10].

The results given in the table show that glucose consumption increased from 1.62 ± 0.47 to 4.12 ± 0.44 μM/g Hb/h in hemolysates from HK animals, and from 1.74 ± 0.28 to 4.54 ± 0.77 μM/g Hb/h in hemolysates from LK animals when increasing amounts of K^+ were added to the incubation medium. The 2–3fold rise in glucose consumption is evidence of the stimulatory effect of K^+ on red blood cell glycolysis in both groups of sheep. Of the enzymes studied, only pyruvate kinase activity was stimulated by the presence of K^+. PK activity rose from 3.07 ± 0.94 to 6.45 ± 2.40 μM/g Hb/min in hemolysates prepared from HK cells and from 4.45 ± 1.18 to 9.01 ± 3.20 μM/g Hb/min in those prepared from LK cells (table). Further characterization of PK from HK and LK sheep red blood cells was carried out using enzyme preparations partially purified by DEAE cellulose chromatography and ammonium sulphate precipitation[11,12]. No significant difference in the Michaelis constant (K_m) for phosphoenol-pyruvate was observed between enzyme preparations from the 2 sheep types. Fructose-1, 6-diphosphate, which is known to be an allosteric activator of human red blood cell PK[13], had no stimulatory effect on the enzyme from either HK or LK sheep blood cells. In addition to the effect of K^+, the effect of Na^+ and (K^+ + Na^+) was also studied on the purified enzyme preparations. The activity of PK was again enhanced by the presence of K^+, while Na^+ appeared to have an inhibitory effect, both alone and when added in conjunction with K^+. Preparations from both HK and LK red blood cells showed a similar response to K^+ and Na^+.

The data presented shows that glycolytic rate and PK activity are capable of being stimulated by K^+ in both high and low potassium sheep red blood cells. In this respect, the LK red blood cell of the sheep differs from that of the dog, in which glycolytic rate and PK activity are unaffected by the presence of K^+[7,8]. It appears therefore that the low potassium sheep red blood cell does not fit into the concept of a glycolytic response to Na^+ and K^+ being an evolutionary adaptation in response to a specific cellular environment. Our results provide evidence to the hypothesis that the LK allele in sheep is of fairly recent origin compared to the HK allele, or that LK is the result of duplication or mutation of the original HK gene. In both dogs and LK sheep, reticulocytes are known to have higher potassium concentrations than the mature red blood cells[5,9,14]. In the sheep, foetal and neonatal lambs of both potassium genotypes have high red blood cell potassium concentrations, which, in the case of genetically LK animals, decline gradually to the normal adult LK level during the 50–100 days after birth[5,9]. Whether or not the same phenomenon exists in the foetal and neonatal dog is not known.

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Effect of folate and folinate on ^{3}H-thymidine incorporation by transforming human lymphocytes in vitro

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Summary. The incorporation of ^{3}H-thymidine by Concanavalin A-stimulated human peripheral blood lymphocytes in vitro is inhibited by the presence of folate or folinate in the culture medium in a concentration-dependent manner.

Blast cell transformation produced by exposure of human peripheral lymphocytes to mitogenic stimuli is a widely used experimental tool. Relatively little work has been directed to determining the importance of the various components of the complex culture media employed in this process. The majority of the media used for this purpose were originally developed for long-term propagation of cell lines in tissue culture rather than for the short-term culture conditions of lymphocyte transformation. Folic (pteroylglutamic) acid and folinic (5 formyltetrahydrofolic) acid are incorporated in a number of these media and are known to be taken up by transforming

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lymphocytes[3], presumably for utilisation in biochemical syntheses requiring 1-carbon unit transfers. In this study we have investigated the effects of variation of the folate and folinate concentration of the medium.

Venous blood was drawn into heparin at approximately the same time each morning from a panel of 3 donors to obviate diurnal rhythmicity in lymphocyte responsiveness[4] and to reduce intersubject variation. The blood was sedimented by gravity in sterile tubes at 37 °C. The leucocyte-rich plasma was periodically aspirated and the collected cells washed twice in Eagle's minimal essential medium prepared without folinate. The lymphocyte count was determined in a haemocytometer using 0.1% crystal violet in 1% acetic acid and the final volume of the suspension adjusted to give 10^6 lymphocytes/ml medium and enriched with 10% v/v foetal calf serum (BBL Co., Cockeysville, Md., USA). 1 ml cultures were set up in 12×75 mm polyethylene culture tubes (Falcon No. 2052, Falcon Plastics, Oxnard, Ca., USA) and 40 µg concanavalin A in Hank's balanced salt solution (Miles-Yeda Inc., Kankakee, Ill., USA) added as mitogen to each tube. Additions of varying amounts of folic acid (Sigma Chemical Co., St. Louis, Mo., USA) and folinic acid (Gibco, Grand Island, N.Y., USA) in Hank's salt solution were added to triplicate culture sets at the initiation of incubation. After 69 h incubation in water-saturated 5% CO_2 in air at 37 °C, lymphocyte blastogenesis was measured by adding 1 µCi ^{3}H-thymidine (6.7 Ci/mM, New England Nuclear Co., Boston, Mass., USA). After 3 h further incubation the lymphocytes were harvested by addition of 3 ml ice cold Hank's solution and centrifugation. The cells were washed a second time in this medium then successively in 2, 3 ml aliquots of ice-cold 5% trichloracetic acid and, after standing overnight in acid, finally in 3 ml methanol. The insoluble residue was dissolved in 0.5 ml 1M Hyamine-10-X in methanol at 70 °C for 15 min and transferred to counting vials with 3 ml ethanol. After addition of a scintillation mixture the vials were counted in a liquid scintillation spectrometer with an efficiency of approximately 20%.

Incorporation in the absence of folate or folinate was 60,713 ± 3648 cpm (mean ± SEM; N = 39 replicate cultures). In the absence of concanavalin A, incorporation was 453 ± 140 cpm (N = 9). Figures 1 and 2 show the effects of the addition of various amounts of folic and folinic acid on ^{3}H-thymidine uptake expressed as a percentage of uptake by control cultures incubated concurrently. Both folate and folinate exert an inhibitory effect on ^{3}H-thymidine incorporation which is linearly related to their concentration in the medium.

It has been noted previously[5] that supplementing medium 199 with folic acid reduces ^{3}H-thymidine uptake by human lymphocytes stimulated with phytohaemagglutinin. The present work demonstrates a direct relationship between the folate or folinate content of the culture medium and ^{3}H-thymidine uptake. The metabolic activity during blastogenesis presumably requires provision of folates for synthetic reactions. For example the rate of serine synthesis from labelled formate which is a folate-dependent reaction has been found to increase when human lymphocytes are stimulated with phytohaemagglutinin unless the lymphocytes originated from folate-deficient patients[6]. There seems to be little difference in the inhibitory effects of folate and folinate. This is consonant with the finding that 5-methyltetrahydrofolate and folate are equally effective in maintaining DNA synthesis in mouse leukaemia cells[7]. It is uncertain why the presence of folates throughout culture should inhibit thymidine incorporation. One possibility is that in the absence of folate the intracellular thymidine concentration is depleted due to inadequate synthesis. Therefore avid incorporation of exogenous ^{3}H-thymidine occurs when ^{3}H-thymidine is added at the end of the incubation period. The presence of folates in the medium allows adequate synthesis of thymidine to occur during incubation thereby apparently reducing incorporation of the added labelled thymidine. Further work is required to explore these possibilities.

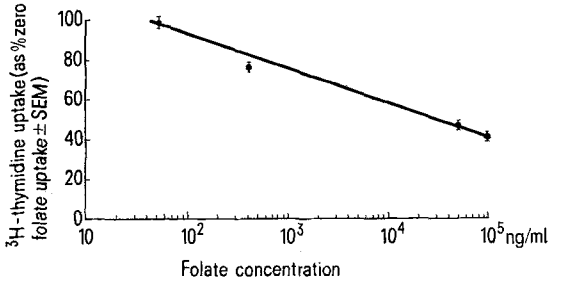

Fig. 1. Relationship of folic acid content of culture medium and ^{3}H-thymidine uptake by human lymphocytes after 72 h incubation expressed as mean ± SEM (N = 9–12) of percentage of control uptake.

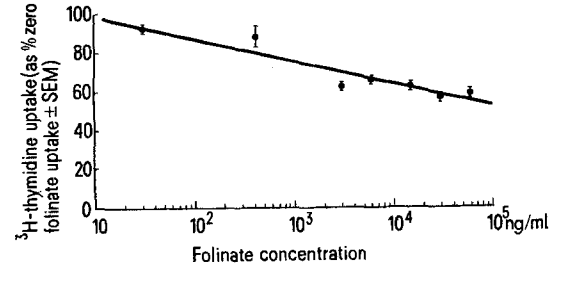

Fig. 2. Relationship of folinic acid content of culture medium and ^{3}H-thymidine uptake by human lymphocytes after 72 h incubation expressed as mean ± SEM (N = 9–12) of percentage of control uptake.

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