[^{3}H]TdR degraded (mean ± SE) in 1.5, 3.0 and 6.0 h reactions was 11.1 ± 1.5, 16.3 ± 2.6 and 21.4 ± 2.8%, respectively. Corresponding value for background samples was 2.1 ± 0.1%. Studies were performed to determine whether TdR phosphorylase was also present in the plasma of different animals. Figure 2 shows the results of a study of 55 animals representing 11 species (N = 5 animals per group); for comparison, human plasma from 5 donors served as a 'positive-control' group. Reaction mixtures containing plasma from monkeys, baboons, humans, dogs and horses showed significant ($p < 0.005$) [^{3}H]TdR degradation. In contrast, the amount degraded in reaction mixtures with plasma from other animals ranged from low (mice and pigs) to negligible (cows, guinea-pigs, goats, rabbits and rats).

We have also compared the amount of [^{3}H]TdR degraded in reaction mixtures containing plasma from healthy and diseased subjects. Values recorded in 3.0 h reactions of plasma from 59 healthy donors and 146 patients hospitalized with uncontrolled neoplastic diseases were 26.0 ± 1.6 and 48.2 ± 1.6%, respectively. This difference was highly significant ($p < 0.005$); nevertheless, values within each group were distributed over a wide range (healthy donors, 5–55%; patients, 12–95%). Results of a representative experiment comparing TdR phosphorylase activity in the plasma of a normal subject and a patient with uncontrolled chronic myelocytic leukemia is presented in figure 3.

Discussion. In addition to demonstrating the presence of TdR phosphorylase in the plasma of man and some animals, these studies also suggest that levels of activity in the plasma of some patients is markedly higher than that of healthy subjects. Whether this finding is of clinical importance remains to be determined. Particularly noteworthy, however, is that [^{3}H]Th, the product of [^{3}H]TdR catabolism, is not incorporated to any appreciable extent by replicating eukaryotic cells[5,9–11]. It is also known that plasma or serum is required for sustaining maximal cell viability, proliferation and function of cells of primary or established cultures. Thus, the presence of this enzyme in plasma or sera used to supplement culture medium may, under certain experimental conditions, limit the utility of assays measuring [^{3}H]TdR uptake as a means of defining DNA synthesis. Evidence supporting this hypothesis and results of studies characterizing this enzyme will be presented elsewhere.

Red blood cell glycolysis and potassium type in sheep[1]

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Summary. Based on the observations made on human and dog red blood cells, it was recently proposed that the response of red cell glycolysis to variations in the levels of specific cations is an evolutionary adaptation in response to a specific cellular environment. We have now examined the effect of K^+ and Na^+ on the activity of pyruvate kinase (PK) in the red blood cells from 2 genetically different types of sheep high potassium (HK) and low potassium (LK). The results indicate that K^+ stimulate glucose consumption and the activity of PK in both types of sheep. It thus appears that red cell PK from LK sheep does not fit into the concept of cellular environment and PK activity.

The red blood cells of man and several other mammalian species have high potassium and low sodium concentrations, while dogs and cats and possibly other carnivores, have red blood cells which have low potassium and high sodium levels[2]. In sheep and certain other ruminant species a polymorphism exists with respect to red blood cell electrolyte levels[3]. Normal adult sheep have either high (HK) or low (LK) red blood cell potassium levels (and low or high sodium levels respectively). These potassium types are determined genetically by a simple allelic pair in which the LK allele is dominant[4]. Most sheep breeds examined so far have both phenotypes[5].

The possession of high K^+ levels by the human red blood cell has not yet been shown to confer any distinct advantage. However, it has been shown that pyruvate kinase (PK), one of the rate limiting enzymes of glycolysis, requires a high K^+ level for its activity[6]. In contrast dog red blood cell PK activity is not K^+ dependent[7,8]. These observations have led to the theory that the response of red blood cell glycolysis to variations in the levels of specific cations is an evolutionary adaptation in response to a specific cellular environment[7,8]. In order to substantiate the validity of this hypothesis, we have examined the effect of K^+ and Na^+ on red blood cell glycolysis and the activity of various glycolytic enzymes in HK and LK sheep. It is also hoped that these studies will help towards an understanding of the significance of red blood cell potassium polymorphism in animal adaptation, production and reproduction, an area which has received considerable attention in recent years[5,9].

Blood was obtained from 8 HK and 8 LK Merino sheep. After centrifugation at 3000 × g for 20 min the plasma and buffy coat were removed and the packed red cells were washed 3 times with 150 mM sodium chloride before being hemolyzed with an equal volume of water. The stroma was removed by centrifugation at 30,000 × g for 30 min at 4 °C, and the hemolysate passed through a Sephadex G-25 column equilibrated with 50 mM Tris-HCl (pH 7.8) and 1 mM reduced glutathione. This gel-

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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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