It is of interest to compare our data on lead absorption with those found in men; blood levels of lead up to 0.2 ppm are considered normal, clinical signs of toxicity appear upward of 0.35 ppm[8]. In the blood of control mice 0.05 ppm lead was detected. The blood levels in the lead-treated mice rose according to the dose of administered lead up to 2 ppm. Symptoms of toxicity could be seen in the mice only at the latter high blood level of lead. The doses given were the same as those reported by Hemphill et al.[6].

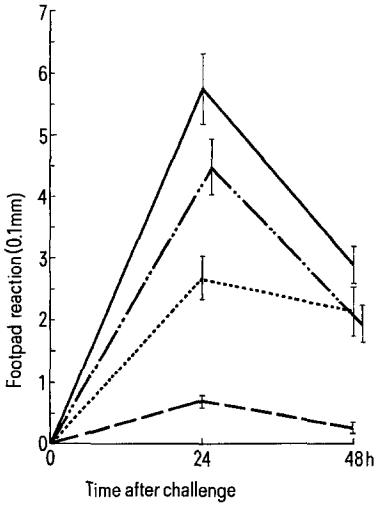

Fig. 2. Inhibition of the secondary DTH-response by lead. 10 mice were injected with 10^5 SRBC i.v. and challenged 4 days later with 10^8 SRBC into the right hind footpad. After 4 days DTH was elicited by inoculation of 10^8 SRBC into the left hind footpad. The inflammatory response was followed for 2 days. ———, saline; — — —, 0.25 mg lead acetate; ..... 0.10 mg lead acetate; –..–..–, 0.025 mg lead acetate. Each point represents the mean value detected in each group of mice. Vertical bars represent the SE of the mean.

The lymph nodes and thymi of the lead-exposed animals showed, as compared to the controls, no significant morphological changes. However, in some cases the marginal zone of the white spleen pulp of lead-treated animals seemed to be smaller and contained a lower number of cells than that of controls. The results of 2 independent experiments summarized in figure 1 clearly demonstrate a dose-dependent suppression of DTH-response by lead acetate.

To assess the effect of lead on the secondary DTH-response, groups of mice receiving various doses of lead for 30 days were sensitized with 10^5 SRBC i.v.; challenged 4 days later with 10^8 SRBC into the right hind footpad and rechallenged with the same antigen dose into the left hind footpad after another 4 days (figure 2). Similar to the primary response lead-treated animals showed also a suppressed secondary DTH-response.

The question arises whether lead exposure may affect the afferent or efferent arc of DTH. In other words, does the exposure of the animals to lead affect the generation of sensitized lymphocytes responsible for DTH, or does lead affect the expression of already established DTH? The result of a preliminary experiment (data not given here) seems to argue against the first possibility. Spleen cells of sensitized normal mice, as well as spleen cells derived from donors exposed previously to lead, both elicited DTH when transferred into normal recipients. If this is true, the most probable targets for lead effects seem to be monocytes known to be involved in the expression of cell-mediated immunity[9] and macrophages participating in induction (or modulation) of humoral immune responses[10].

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Degradation of [^{3}H]thymidine by a pentosyltransferase (EC 2.4.2.4) in the plasma of man and different animals[1]

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Summary. [^{3}H]Thymidine is degraded by an enzyme (thymidine phosphorylase; EC 2.4.2.4) which we have identified in the plasma of man and some animals. The presence of this enzyme in plasma or sera used to supplement culture media may, under certain experimental conditions, limit the validity of measuring the uptake of radiolabeled thymidine as a means of defining DNA synthesis.

Thymidine phosphorylase (EC 2.4.2.4; thymidine-orthophosphate deoxyribosyltransferase) catalyses the reversible reaction of thymidine (TdR) + orthophosphate ⇌ thymine (Th) + 2-deoxy-α-D-ribose-1-phosphate[2,3]. This enzyme is present in many normal and neoplastic cells of man and different animals[2–13] as well as in various pathogenic and nonpathogenic prokaryotic cells[2,3,14]. We report here the identification of TdR phosphorylase in the plasma of man and some animals, and discuss those limitation which this enzyme may have on assays in which [^{3}H]TdR uptake is used as a means of defining DNA synthesis.

Material and methods. Plasma assayed was from fresh blood which had been collected aseptically into siliconized tubes containing EDTA anticoagulant (1.5 mg/ml). After depositing the cells by centrifugation (500×g, 12 min), the plasma was collected carefully and, in some instances, passed through a micropore (0.22 μm) filter; particular care was used in handling the blood so as to avoid contamination or cell damage. TdR phosphorylase was evaluated using our microassay[10]; this is a modification of techniques which have been described previously[2,5–7,11] and was constructed to simulate conditions prevalent in microcultures. In this assay, 20 μl of plasma was added

to a 200 µl U-shaped culture-well of a microtiter plate (Cooke Laboratory Products, Alexandria, Va.). To each culture-well was then added 20 µl of culture medium RPMI 1640 (pH, 7.2; Grand Island Biological Co., Grand Island, N.Y.) containing 2.0 µCi of [6-^{3}H]TdR (spec. act., 2.0 Ci/mmole; final conc., 12 mM; Amersham/Searle Corp., Arlington Heights, Ill.). Immediately after mixing (time 0, background samples) and after incubating at 37 °C for various times (usually 3 hr, test samples), 5 µl of the plasma-isotope mixture was spotted onto a Whatman No. 1 chromatography paper at a location which had been spotted previously with 5 µl of a saturated mixture of unlabeled TdR and Th reference markers. Chromatography was performed using the descending method and a biphasic solvent from a mixture of ethyl acetate: water: formic acid (60:35:5, v/v)[5, 10, 12, 13]. Thereafter, TdR and Th reference markers were located with an UV lamp (R_f values, mean ± SE, for TdR and Th were 22.2 ± 0.5 and 37.4 ± 0.4, respectively); no degradation product other than [^{3}H]Th was detected. Radioactivity in grid sections containing TdR and Th reference markers of each chromatography channel was then determined by liquid scintillation counting (total per chromatogram channel, $\cong 2 \times 10^5$ dpm; counting efficiency, 45 ± 0.1%). After subtracting background [^{3}H]Th values (< 3%), the amount of [^{3}H]TdR and [^{3}H]Th present in each test or control sample was defined as a percentage of the total radioactivity (amount recovered, > 95%). Reproducibility of replicate mixtures, usually prepared in triplicate, was good (mean SE, < 5%). Enzyme activity was expressed as the % [^{3}H]TdR degraded; this departure from conventional definitions of enzyme activity was justified in that it more accurately illustrated the magnitude of error which may arise in various in vitro assays of DNA synthesis.

Results. Presented in figure 1 are the results of an experiment measuring [^{3}H]TdR degraded by TdR phosphorylase in the plasma of 13 healthy individuals. Amount of

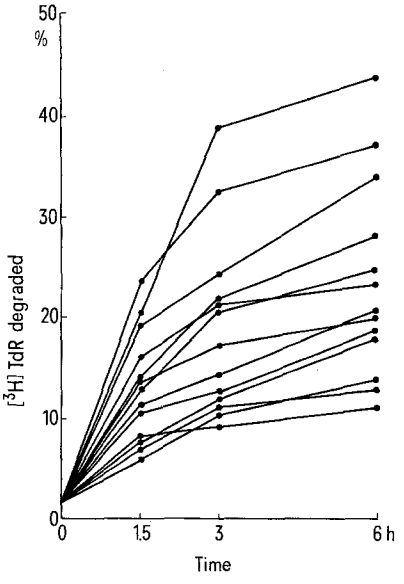

Fig. 1. Analysis of TdR phosphorylase activity in the plasma of 13 healthy men and women.

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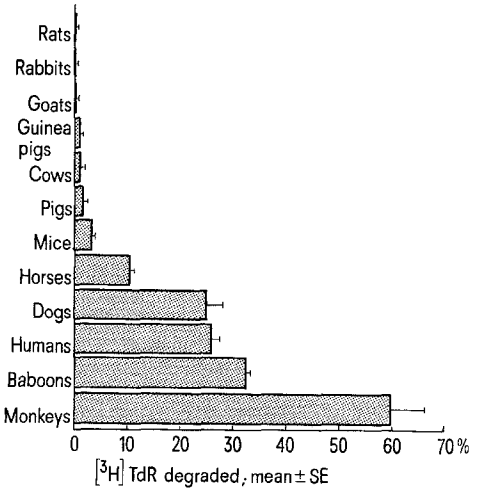

Fig. 2. Results of comparative experiments defining the amount of [^{3}H] TdR degraded in 3.0 h assays of plasma from different animals. Each of the 12 groups contained 5 subjects.

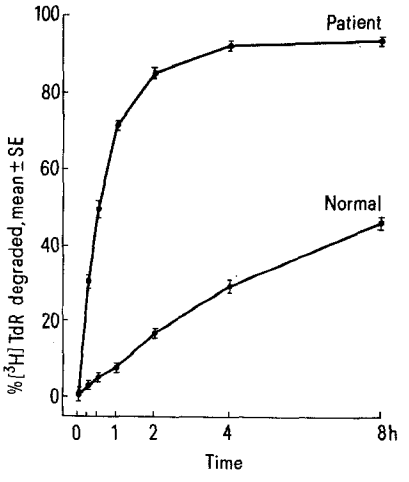

Fig. 3. Assessment of TdR phosphorylase in the plasma of a patient with uncontrolled chronic myelocytic leukemia showing unusually high levels of enzyme activity and that of a normal subject (triplicate assays, mean ± SE).

[^{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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