moregulatory mechanisms commonly also impair feeding mechanisms.

The following polynomial equation was derived in an attempt to predict the decrease in $T_r$ for individual cats exposed to 5° from known values of the nutritional index: $\Delta T_r(°) = -50.77 + 343$ Index $-585$ Index$^2$. However, the confidence intervals showed the variability to be too great to provide anything but crude estimations of $\Delta T_r$. These preliminary results merit further investigation of additional ambient temperature effects on a larger sample population.

*Summary.* A nutrition index ($W^{\frac{1}{3}}/L$) was applied to six healthy cats subjected to a restricted diet for a period of 11–23 weeks. During this period, they were exposed each week for 3 hr to an ambient temperature of 5° with $T_r$ measured at the end of each hour. Thermoregulation was considered critically impaired when the $T_r$ fell to 35° or below. In the final test, in each of the six cats, the $T_r$ fell to 35° or below, and in each the nutritional index fell below 0.27 while the mean body weight loss of the six animals was 49.8%. It is suggested that in experiments in which integrity of the thermoregulatory mechanism is vital, use of the nutritional index would eliminate one variable. It is also suggested that the index provides an estimate for the degree of obesity.

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## Glutathione Reduction: Studies Using Deoxyribonucleosides as Substrates* (33416)

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The coenzyme requirement of erythrocyte glutathione reduction remains unclear. In intact cells this reduction is linked primarily to reduced triphosphopyridine nucleotide (TPNH) (1). Disruption of cellular integrity links the reduction to reduced diphosphopyridine nucleotide (DPNH) (2). The purified enzyme catalyzing this reaction, glutathione reductase, can use both TPNH and DPNH as cofactors (3,4).

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Several investigators have attempted to solve this problem using lactate-generated DPNH and oxidized glutathione (GSSG) produced under a variety of conditions (2, 3). Some have suggested that the failure to obtain significant glutathione reduction was due to a failure to generate DPNH through the glyceraldehyde-3-phosphate dehydrogenase reaction (3).

The failure to observe significant glutathione reduction with glucose-6-phosphate dehydrogenase (Glc-6-*P*D) deficient erythrocytes has been used as evidence that glyceraldehyde-3-phosphate dehydrogenase-gener-

ated DPNH does not serve as a cofactor for glutathione reduction (2). Since there is an active efflux of GSSG out of these cells (5), and they have some capacity to oxidize glucose through the hexose monophosphate pathway, this can not be used as conclusive proof (6).

Deoxyribonucleosides can serve as excellent substrates for differentitation of TPNH- and DPNH-linked reactions (7). The deoxyribose portion can not be metabolized through the pentose phosphate pathway, yet lactate production from these compounds compares favorably with glucose, Since this system bypasses the hexokinase step (one of the limiting steps in the erythrocyte metabolism), its rate of DPNH formation as measured by methemoglobin reduction is greater than with glucose (8). Coenzyme reduction occurs through the glyceraldehyde-3-phosphate dehydrogenase reaction (7). The purpose of the present study was to determine if deoxyribonucleosides could serve as substrates for glutathione reduction.

*Materials and Methods.* Deoxyadenosine and deoxyinosine were obtained from California Corporation for Biochemical Research, Los Angeles and glycylglycine, 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), and sodium lactate were purchased from Sigma Chemical Corporation, St. Louis. Methylphenylazoformate was generously supplied by Dr. E. M. Kosower, Stony Brook, New York.

All blood samples were collected from healthy mature human subjects into heparin, and experiments were begun within 0.5 hr.

The glutathione regeneration test was performed as described by Kosower *et al.* (9) except that the initial incubation mixture was 2.0 or 3.0 ml, and 0.2-ml aliquots were taken for GSH determination by the DTNB method (10). In this test the incubation of washed erythrocytes for 10 min at 0° with methylphenylazoformate (azoester) causes a marked decrease in the intracellular reduced glutathione (GSH) and presumably an increased in oxidized glutathione.

*Results.* Addition of glucose to azoester treated erythrocytes causes a rapid linear GSH regeneration for 20–25 min (Fig. 1). If glucose is omitted from the incubation mixture, there is very little, if any, conversion of GSSG to GSH. The substitution of deoxyadenosine or deoxyinosine (not shown) for glucose also does not result in significant glutathione regeneration.

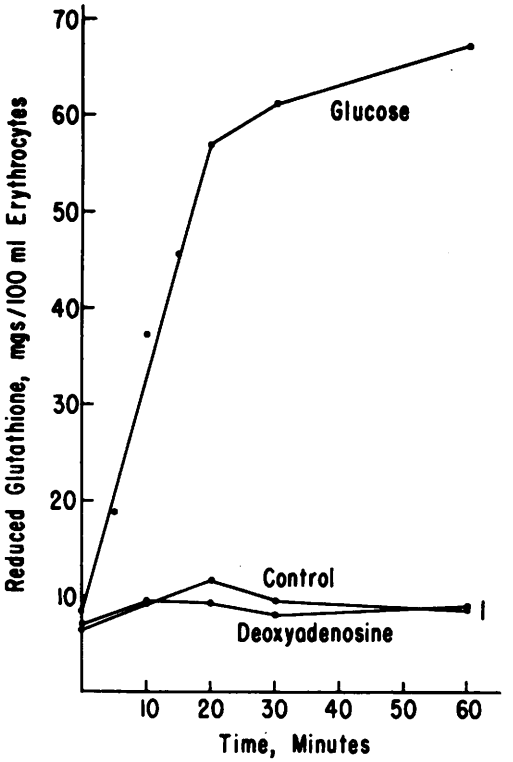


FIG. 1. Glutathione regeneration in azoester treated erythrocytes. The reaction mixtures (2.0 ml) contained glucose, 11 μmoles/ml or deoxyadenosine, 10 μmoles/ml; azoester, 2.17 μmoles/ml; DMSO, 2.67%; and erythrocytes, 42.0%; $T = 37°$.

The failure to observe glutathione reduction with deoxyribonucleosides suggests that glyceraldehyde-3-phosphate dehydrogenase-generated DPNH cannot be utilized for glutathione reduction. These results could be explained in at least two additional ways: glutathione reductase activity could be inhibited by one of the deoxyribonucleoside metabolites, or GSSG might be rapidly transported out of the cell and thus not available for enzymatic reduction. These possibilities were eliminated in the following manner: azoester-treated erythrocytes were incubated with either deoxyadenosine or no substrate for 5 and 30 min. Sufficient glucose was then added to

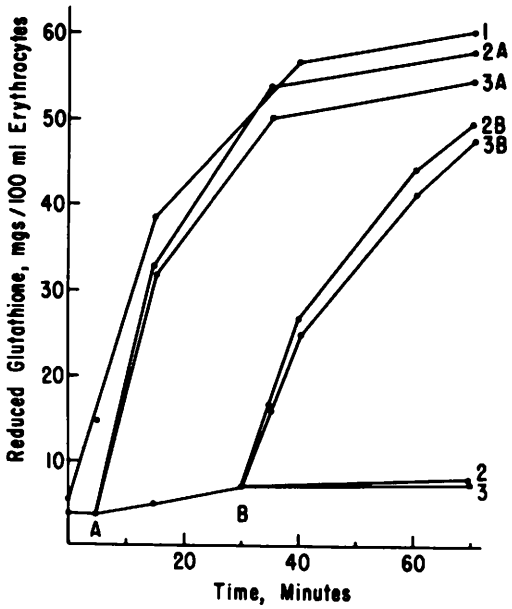


FIG. 2. The effect of deoxyadenosine metabolism on glutathione reduction by glucose. Reaction mixtures (3 ml) were prepared with glucose (1), no substrate (2), and deoxyadenosine (3). At 5 (A) and 30 (B) min 0.9-ml aliquots of samples 2 and 3 were added to a tube containing 1 $\mu$mole of glucose; $T = 37°$.

an aliquot of each sample to give a glucose concentration identical to the control (Fig. 2). The rate of glutathione reduction after glucose addition was not significantly differthione regenerated at the end of 80 min was ent from the control, but the maximum glutadifferent.

*Discussion.* Several hypotheses have been proposed to explain the confusing coenzyme requirements of erythrocyte glutathione reduction. Beutler and Yeh (3) suggested that the redox potential difference between the lactate dehydrogenase system ($E'_0 = -0.19$ V) and the glucose-6-phosphate dehydrogenase system ($E'_0 = -0.32$ V) might make the latter more thermodynamically suitable for glutathione reduction. Since the redox potential of the glyceraldehyde-3-phosphate dehydrogenase ($E'_0 = -0.29$ V) is much closer to that of glucose-6-phosphate dehydrogenase, they suggested that the red cell might use this system more effectively than the lactate system. Reiber *et al.* (2) found that lactate is capable of promoting glutathione reduction in both normal and Glc-6-*P*D deficient hemolyzates. Beutler and Yeh (3) also suggested that enzyme compartmentalization within the erythrocyte might make the lactate-generated DPNH unavailable for glutathione reduction.

Both of these hypotheses suggest that the failure to obtain glutathione reduction with DPNH in erythrocytes is a result of not using the glyceraldehyde-3-phosphate dehydrogenase in the coenzyme reducing system. The failure to obtain glutathione reduction with deoxyribonucleosides should dismiss this possibility and give additional support to the hypothesis that erythrocyte glutathione reduction is primarily linked to TPNH formation.

Reiber *et al.* (2) have suggested that the change in DPNH-dependent reduction of GSSG may be due to variations in subcellular concentrations of enzymes and cofactors or enzyme conformation changes with activation of previously inactive sites.

Disruption of intracellular organization by erythrocyte lysis must cause marked changes in enzyme kinetics, substrates and their interrelationships. Hemolyzates (but not erythrocytes) from the Negro type of glucose-6-phosphate dehydrogenase deficiency will regenerate glutathione (11). There is also a discrepancy between the Glc-6-*P*D activity of sheep hemolyzates and the apparent ability of sheep to utilize the hexose monophosphate pathway (12). The differences between the linkage of DPNH to glutathione reduction in hemolyzates and in erythrocytes is probably another example of this alteration.

*Summary.* The deoxyribonucleosides are excellent substrates for studying DPNH-linked reactions under physiological conditions without the interference of TPNH formation. Glutathione was not significantly reduced using deoxyadenosine in intact methyl phenylazoformate-treated erythrocytes. Incubation of samples with deoxyadenosine did not affect GSH regeneration with dextrose.

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# Antimalarial Activity of 1-Methyl-3-Nitro-1-Nitrosoguanidine*† (33417)

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1-Methyl-3-nitro-1-nitrosoguanidine (NMNG) has been shown to have anticancer activity in mice (1-3) and mutagenic activity in bacteria (4). This report presents evidence that NMNG is also an effective antimalarial agent *in vitro*.

*Materials and Methods. Plasmodium berghei* was cultured *in vivo* as previously described (5). The parasitemia in the infected mice is usually greater than 90% at day 7 after inoculation and death occurred within 10 days after inoculation. The mice were anesthetized with diethylether and the blood was removed from the infected mice aseptically by heart puncture. Usually 1.0 ml of blood was removed per mouse. The whole blood was suspended into an equal volume of modified acid-citrate-dextrose (ACD) solution, pH 5.0. Preincubation was performed as follows: 0.50 ml of the cell suspension ($4 \times 10^8$ parasites/ml) was added to 0.95 ml of ACD solution containing the NMNG or the test substance. The preincubation control consisted only of the parasitized blood in the ACD solution. The mixture was allowed to stand at room temperature for 25 min. After the preincubation period, 0.1 ml aliquots were inoculated intraperitoneally into healthy white mice,[1] Walter Reed strain, weighing 25 g each (12 mice per group). To determine the extent of parasitemia, blood smears were prepared at intervals and stained with Giemsa staining solution. Parasitemia is the number of infected blood cells per 500 uninfected blood cells per mouse. The average parasitemia is expressed as the average of the sum of the parasitemias per group (12 mice per group) and was calculated just before the death of the first mouse in a group.

*Results and Discussion.* As observed in Table I, the infectivity of *Plasmodium berghei* is affected by NMNG. The ability of the plasmodia to infect healthy mice was destroyed when preincubated with 200, 400, or 500 μg of NMNG. Preincubation with 50 or 100 μg of NMNG showed a gradual increase in parasitemia in the mice after day 6 and 8 after inoculation respectively. Parasitemia was observed in the control group (no additions) as early as day 5 after inoculation.

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† The opinions and conclusions expressed herein do not necessarily reflect the views of the Department of the Army or of any other agency of the Federal Government and should not imply Department of the Army indorsement of or preference for the commercial products described.

[1] The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.