tration of epinephrine(3). In addition, in this report, puromycin-effected enhancement of phosphorylase activity could be detected in rat liver slices under conditions that permitted the initial control level to fall, and where, after a latent period, a small but consistent activation was observed in control slices.

Of interest are the findings of Ferguson (7), in which puromycin alone had no effect on phosphorylase in steer adrenal slices, but enhanced the stimulation observed in the presence of adrenocorticotropic hormone. A plausible explanation for the potentiation of the effect of hormonal agents on phosphorylase would be an inhibition of phosphorylase phosphatase or cyclic-3′,5′-adenosine monophosphate phosphodiesterase as has been suggested(1). Further studies will be needed to elucidate the mechanism of the puromycin-stimulation of hepatic phosphorylase activity, and to evaluate the role of this effect in glycogenolysis.

Summary. Liver glycogen phosphorylase activity was studied in mice at early times subsequent to *in vivo* administration of puromycin, and following puromycin addition to incubations of rat liver slices. No effect of puromycin on phosphorylase could be detected following injection of unanesthetized mice. Puromycin treatment of pentobarbital-anesthetized mice, however, resulted in an enhancement of the phosphorylase activity as compared to control values. Phosphorylase activity in rat liver slices incubated with puromycin was greater than those incubated without puromycin. These results indicate that puromycin can elevate liver phosphorylase activity, a finding consistent with the hepatic glycogenolytic action of the antibiotic *in vivo*.

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Erythrocyte Inorganic Pyrophosphatase Activity in the Newborn Infant.* (30823)

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The activity of several enzymes is different in young *vs* older erythrocytes, and in the blood of newborn *vs* adult individuals. Inorganic pyrophosphatase activity has been

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studied in rabbits and the activity in reticulocytes found to be many times increased over that in mature erythrocytes(1,2). Our investigation concerns the measurement of this enzyme in normal newborn infants, infants with the reticulocytosis of acquired hemolytic anemia and normal adults. In addition, we have compared the pyrophosphatase activity of erythrocytes separated by density into older and younger cell fractions.

Materials and methods. Erythrocyte inorganic pyrophosphatase activity was determined in 19 adults and 71 full-term infants during the first 3 days of life. The adult subjects were normal healthy volunteers with no evidence of hematologic disease. The newborn infant subjects included 35 normal infants chosen at random from the nursery and 22 infants with Rh hemolytic disease and 14 with ABO hemolytic disease. ABO hemolytic disease was diagnosed according to criteria previously stated(3).

Venous blood samples from adults and capillary blood samples from heel sticks from infants were studied. No differences in pyrophosphatase activity were noted between venous and capillary blood samples. The blood was collected in sequestrene and the red cells were washed 3 times with 20 volumes of ice-cold 0.15 M NaCl solution by centrifugation at 1500 rpm for 5 minutes. The supernatant liquid and buffy coat were carefully removed after each centrifugation and an approximately 50% cell suspension in saline was prepared after the last washing. This suspension was diluted 1:9 in ice-cold distilled water and the resultant lysate was assayed in duplicate for enzyme activity. Since pyrophosphatase activity is associated exclusively with the cytoplasm(4), removal of red cell stroma was not necessary. No significant activity was detected in assays with white cell preparations.

The reaction system for determination of pyrophosphatase activity contained in 3.5 ml: 1.6×10^{-2} M $Na_4P_2O_7 \cdot 10H_2O$ (neutralized according to Naganna *et al*(5)); 1.6×10^{-2} M $MgCl_2 \cdot 7H_2O$; 5×10^{-2} M tris-HCl buffer, pH 7.4; and 0.5 ml of hemolysate. After 30 minutes incubation at 37°C the reaction was stopped by precipitation with 10% trichloroacetic acid. The amount of orthophosphate formed was determined by the method of Fiske and SubbaRow(6). Appropriate enzyme and substrate controls were run with each assay. Variations between duplicates were less than 5%. Specific activity was expressed as micromoles of orthophosphate liberated at 37°C in 30 minutes per mg hemoglobin, the latter measured at 540 mμ as cyanmethemoglobin. Preliminary tests have shown that with this assay system, hydrolysis of pyrophosphate was linear with time for the initial 120 minutes of the reaction at 37°C. A direct proportionality was also noted between hydrolysis of the substrate and enzyme concentration during a 30-minute incubation period.

Erythrocytes were segregated into fractions of older cells and of younger cells by the microgravimetric technique previously described(7). The supernatant from thrice washed erythrocytes was suctioned off and the packed cells were mixed by repeated inversions of the tube. A 0.2 ml aliquot was adjusted with saline to a hematocrit of approximately 50% and set aside for determination of the "original" enzyme activity. Six microhematocrit capillary tubes were filled with the thoroughly mixed cell suspension and one end of each tube sealed with vinyl plastic putty (Biological Research, St. Louis, Mo.). The capillary tubes were centrifuged for 5 minutes at 12,500 rpm in a microhematocrit centrifuge (Clay Adams, Inc., New York) and then stored vertically. The upper part of the capillary tube containing supernatant was broken off and discarded. Pressure was applied with putty at the lower end of the capillary tube so that the top 10% of the red cell column was expelled into 0.25 ml of saline. The capillary tube was then inverted and after breaking off the putty, pressure was applied from the upper end so that the bottom 10% of the column was transferred into another test tube containing 0.25 ml of saline. Pyrophosphatase activity was measured in the "original" sample and in both fractions. The adequacy of the erythrocyte separation into older and younger cell fractions by this method was estimated by reticulocyte concentrations.

TABLE I. Erythrocyte Inorganic Pyrophosphatase Activity.*

	Enzyme activity			
	No.	Range	Mean ± SD	Mean % reticulocytes
Normal adults	19	.46-1.02	.78 ± .15	(1.0)†
" newborns	35	.35-1.02	.70 ± .19	6.3
ABO disease	14	.82-2.16	1.40 ± .39	14.4
Rh "	22	.50-3.64	1.26 ± .73	16.8

* Specific activity expressed as micromoles orthophosphate formed at 37°C in 30 minutes per mg hemoglobin.

† Adult value taken from Wintrobe, M.M.: Clinical Hematology, 5th Ed., Lea & Febiger, Philadelphia, 1961.

Results. Erythrocyte inorganic pyrophosphatase activity was determined on samples of blood from 90 subjects and the results are presented in Table I. Pyrophosphatase activity was essentially the same in erythrocytes from normal infants and normal adults, despite the increased number of young erythrocytes in the blood of newborn infants. A significant increase in mean enzyme activity was observed in infants affected with ABO or Rh hemolytic disease of the newborn compared to normal newborn infants and normal adults ($p = <.001$). There was no difference between the mean pyrophosphatase activity of children with ABO hemolytic disease compared to Rh hemolytic disease.

When erythrocytes were separated into lighter and heavier cell fractions, pyrophosphatase activity was increased in the lighter fraction with higher reticulocyte content, and reduced in the heavier (older cell) fraction. Results of four typical separations are presented in Table II.

Discussion. Erythrocyte, pyrophosphatase activity is age dependent like that of other erythrocyte enzymes. Separation of human erythrocytes *in vitro* by density differences revealed increased enzyme activity associated with the lighter cell fractions containing the largest proportion of young erythrocytes. This age-dependency had previously been reported following *in vivo* studies of rabbits with induced reticulocytosis(2).

From the higher reticulocyte content one would expect the mean pyrophosphatase activity of erythrocytes from normal infants to be higher than that of adults; however, no difference could be found between the activities of the two groups. This pattern resembles that obtained in studies of acetylcholinesterase and methemoglobin reductase during the neonatal period. The activity of these enzymes is also higher in younger cells, but the mean activity in red cells from normal newborns is in fact lower than that from adults (3,8). In marked contrast to the cells from normal newborns, the erythrocyte pyrophosphatase activity in infants affected with Rh or ABO hemolytic disease was considerably increased. In the latter groups the elevated enzyme activity may be a reflection of the presence of a larger proportion of young erythrocytes.

Inorganic pyrophosphatase appears to be a sulfhydryl enzyme and maintenance of intracellular glutathione in the reduced state is necessary for maximum enzyme activity(9). The decrease of pyrophosphatase activity as erythrocytes mature has been correlated with the increased lability of the SH-groups of the enzyme(9). The increase in pyrophosphatase activity in younger cells reported in the present study is similar to the increase reported for other sulfhydryl enzymes, such as hexokinase(10). These observations are consistent

TABLE II. Pyrophosphatase of Human Erythrocytes Separated into Light and Heavy Fractions.

	Original		Top		Bottom	
	% reticulocytes	Specific activity*	% reticulocytes	Specific activity*	% reticulocytes	Specific activity*
Newborn (normal)	2.8	.92	9.8	1.78	.8	.52
" (Rh disease)	15.0	3.79	49.6	7.74	2.6	2.21
" (ABO disease)	13.2	1.16	80.0	3.55	.6	.97
Adult (normal)	1.7	.92	4.8	1.59	.2	.86

* Expressed as micromoles orthophosphate formed at 37°C in 30 minutes per mg hemoglobin.

with the decreased levels of reduced glutathione observed in older erythrocytes of rabbits with acetylphenylhydrazine-induced anemia(11,12), of fava bean-sensitive subjects (13) and of a group of hematologically normal adults(14). The finding that erythrocyte pyrophosphatase activity of normal newborn infants does not differ significantly from that of adults is compatible with the observations that there is no significant difference in glutathione content between the red cells of newborns and of adults(15).

Summary. Erythrocyte inorganic pyrophosphatase activity was measured on 90 samples of human blood. The enzyme activity was found to be similar in normal newborn infants and normal adults. Erythrocyte pyrophosphatase activity was increased in infants affected with Rh and ABO hemolytic disease. When erythrocytes were separated into light and heavy cell fractions, pyrophosphatase activity was increased in the lighter fraction with higher reticulocyte content.

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Effects of Amebicides on Growth of *Acanthamoeba* sp. (30824)

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The effects of 3 chemically distinct types of amebicides: carbarsone, a pentavalent arsenical; emetine, an alkaloid of *Ipecacuanha*; and glaucarubin, derived from the fruit of *Simarouba glauca*, were tested during the initial 14-day period of growth of *Acanthamoeba* sp. under axenic conditions. This organism is a small free-living, usually non-pathogenic, soil amoeba which can be easily grown in large numbers in a relatively well-defined medium. Certain strains of *Acanthamoeba* sp. which have similar appearance under the light microscope are known pathogens and can cause death in rodents and monkeys(1, 2). This genus has also been isolated from routine throat cultures of humans.

For these reasons, a study of the growth inhibiting effects of amebicides on this amoeba seemed warranted as a first step to future comparisons of the biochemical and ultrastructural differences which may exist between the free-living and pathogenic forms of this genus. There is some morphological evidence that a fundamental relationship does exist between parasitic and free-living forms of amoebae(3).

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