

In view of the considerations noted above, attempts to estimate hexokinase activity in homogenates may be of little avail in spite of the fact that such preparations may contain large amounts of the desired system. The data in Table I illustrate these points.

Summary. The activity of apyrase systems and extraneous phosphate formation

interfere with the determination of hexokinase activity in rat muscle homogenates. Hexokinase activity in rat muscle extracts is best calculated from changes in the P_7 values during incubation, a procedure which avoids the complication of the inhibition of apyrases by hexokinase activity.

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Anemia in Rats Infested with *Bartonella muris* and Injected with Pteroylglutamic Acid.*

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An active infestation of *Bartonella muris* in rats leads to an acute anemia, hemoglobinuria, emaciation, and usually death.¹⁻⁴ In our experience the number that die may be reduced somewhat by injecting the infested blood into the rat several days or weeks before the spleen is removed rather than giving it immediately after splenectomy. Death rate has varied considerably in our rats, being around 50% a few years ago^{4,5} and about 25% in the present study. Several efforts have been made to discover a satisfactory treatment for this disease. Some substances such as arsenicals,⁶ extracts of spleen, copper, and iron,⁷ have been found to be beneficial. Negative results were obtained in the treat-

ment of the infestation with sulfanilamide.⁴

Since pteroylglutamic acid (PGA, folic acid) was found to be helpful in anemias of different types^{8,9} it was decided to try it in rats made anemic by *Bartonella muris* infestation.

The rats used in these experiments were nearly full grown albino males fed on Purina laboratory chow (containing 2.4 μ g of PGA per g).[†] They ate approximately 15 g per day at the start and much less during the severe anemia. They were injected intraperitoneally, with saline containing blood taken from rats suffering from acute infestation of *Bartonella muris*. Several days to weeks later they were prepared for operation, etherized and the spleen removed. Hemoglobin estimations and red cell counts were done every 3 to 6 days for several weeks. The hemoglobin was determined by the Sahli method. These readings were checked with the photoelectric acid hematin method for hemoglobin and found to be consistently only slightly higher. The Sahli method was there-

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[†] We are indebted to Mrs. Edith S. Sims for these determinations and for the PGA solutions used.

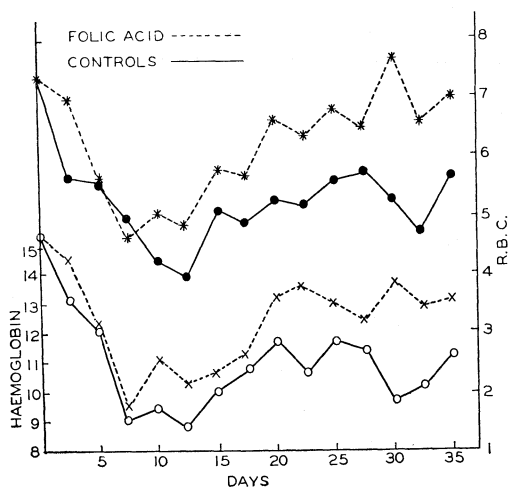


FIG. 1.

Two lower curves hemoglobin in grams of splenectomized rats infested with *Bartonella muris*. Mean values for twenty animals included in each curve. Two upper curves red blood cells in millions.

fore considered to be very satisfactory for these experiments.

Some of the rats in each group were given daily intramuscular injections of 40 μ g of pteroylglutamic acid. The means (for 20 animals in each group) at intervals of 2½ days, arranged as curves of hemoglobin in grams and red cell counts in millions, are shown in Fig. 1. It will be noted that the spread between the treated and control groups is narrow for about 18 days and widens considerably thereafter. This is taken to indicate that pteroylglutamic acid aids new cell formation and in this way keeps the blood count up above the untreated rats during the progressive anemia of the *Bartonella* infestation; after the anemia has reached its lowest level, and blood regeneration exceeds red cell breakdown, then the injected animals have considerable more capacity for new red cell formation than the controls as the curves clearly show. This gives rise to analysis of the curve in two parts. The mean for all the hemoglobin determinations during the first 18 days

in the injected rats has been compared with the mean hemoglobin content of the untreated rats and found to give a mean difference which is very significant, being more than 5 times the standard error of the difference. A similar comparison from the nineteenth to thirty-fifth day showed the mean difference in hemoglobin at this end of the curve to be over 10 times the standard error of the difference. Likewise the red cell count is also significantly higher in the rats treated with pteroylglutamic acid and the results of the analysis for this data are similar to those described for hemoglobin.

From these data it is obvious that pteroylglutamic acid is beneficial in holding the hemoglobin and numbers of red cells in the treated rats above those of the controls. From Fig. 1 it may be seen that the curves for the injected rats are above the controls as early as 2½ days after splenectomy and after injections of pteroylglutamic acid were started. The data are not numerous enough to establish a significant difference between the curves at this point. If the curves are different as early as 2½ days it would be interesting information on the function of pteroylglutamic acid and may suggest a role aside from red cell formation. On the other hand the formation of new cells in numbers sufficient to be detected in the general circulation might occur in a few days in these anemic rats. Since there was some PGA in the food these results would probably be all the more striking in rats on a PGA-free diet.

Summary. Hemoglobin and red cell estimations have been done on the blood of rats made anemic by splenectomy and *Bartonella muris* infestation. Animals injected with pteroylglutamic acid show hemoglobin and red cells at higher levels than those of the untreated controls. Curves made from this data show the treated animals much less anemic than the controls for a period of 35 days or longer.