sporozoites in hen blood did become infected when subsequently bitten by infected mosquitoes. It is felt that these findings suggest the existence of a factor in the blood of the hen that prevents the initiation by the sporozoite of the exoerythrocytic cycle in the canary.

Subsequent note: Further studies, completed after the above manuscript was submitted for publication, have revealed that (a) the inhibitory action of hen's blood on the sporozoites is exerted though sodium citrate is used as anticoagulant instead of heparin; and (b) it is not lessened by agitating the blood at room temperature for an hour before adding the sporozoites to it.

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Activity of Hexokinase Preparations from Rat Muscle.*

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Hexokinase activity involves the conversion of an easily hydrolyzable phosphate group to one not easily hydrolyzable (glucose-6-phosphate). Since the P_7† value of the reaction mixture includes both the P_0† and the $\Delta 7P$† but not the P in glucose-6-phosphate, any conversion of $\Delta 7P$ to glucose-6-phosphate will be reflected in a decrease in the P_7 values during incubation. However, the calculation of hexokinase activity in complex systems by means of such changes poses a difficult problem since transphosphorylation from ATP may fail to account for all of the phosphate changes actually occurring.

In rat muscle preparations at least two other types of transfer, in addition to transphosphorylation, are often observed. Large increases in inorganic phosphate may occur, even in the presence of fluoride, due to the dephosphorylation of ATP by the apyrases[1] present in such preparations. In addition part of the inorganic phosphate formed during incubation may be derived from sources other than the $\Delta 7P$. The action of the apyrases is accompanied by a large increase in the P_0 content, a decrease in the $\Delta 7P$ and no change in the P_7 value. The increase in inorganic phosphate from extraneous reactions is manifested by increases in both the P_7 and P_0 values. The increase in the P_7 value indicates that fraction of the total increase in inorganic phosphate that is attributable to the dephosphorylation of esters other than those containing easily hydrolyzable phosphate.

In order to demonstrate hexokinase activity by means of phosphorous transfers, it is essential to compare the changes occurring during incubation in the absence and presence of glucose. If the addition of glucose failed to influence phosphate changes other than those occurring as the result of the transphosphorylation catalyzed by hexokinase, calculation of the activity from a determination of the effect of glucose on the easily hydrolyzable phosphate content would be entirely feasible.[2] There is good evidence, however, that the presence of glucose may influence other reactions. Meyerhof and Geliazkowa[3] demon-

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† The following symbols are used: P_0—inorganic phosphate determined prior to hydrolysis; P_7—inorganic phosphate content determined after 7 minutes' hydrolysis at 100°C in 1 N HCl; $\Delta 7P$—that fraction of esterified phosphate converted to inorganic phosphate by 7 minutes' hydrolysis at 100°C in 1 N HCl (*i. e.*, $P_7 - P_0$).

1 Meyerhof, O., *J. Biol. Chem.*, 1945, **157**, 105.

2 Colowick, S. P., and Price, W. H., *J. Biol. Chem.*, 1945, **157**, 415.

3 Meyerhof, O., and Geliazkowa, N., *Arch. Biochem.*, 1947, **12**, 405.

TABLE I.

Calculation of Hexokinase Activity in Extracts and Homogenates of Rat Muscle.

Each flask contained the following components: Side arm—0.1 ml 0.05 M or 0.1 M Na-ATP (pH 7.5), 0.15 ml 0.9 M NaF and 0.05 M $NaHCO_3$, 0.1 ml 1% glucose or water. Main compartment—0.4 ml 0.15 M $NaHCO_3$, 0.4 ml 0.05 M $MgCl_2$, 0.5 ml extract or homogenate and H_2O to 2.05 ml. Incubation for 30 min. in 95% N_2, 5% CO_2 at 30°C. All values are expressed in terms of μg per ml of reaction mixture. The examples given are typical of a great number of similar results.

Preparations	Glucose	Before incubation			After incubation			Hexokinase activity expressed as decrease in P_7 during incubation (B)-(A)		Hexokinase activity expressed as decrease in P_7-P_0 during incubation (B)-(A)	
		P_0	P_7	P_7-P_0	P_0	P_7	P_7-P_0				
Extract	——— (A)	104	231	127	127	235	108	—4		19	
	Present (B)	104	231	127	104	186	82	45	49	45	26
Homogenate	——— (A)	119	440	321	230	458	228	—18		93	
	Present (B)	119	440	321	217	426	209	14	32	112	19

strated that, in both homogenates and extracts of brain tissue, dephosphorylation of ATP occurs to a much greater degree in the absence than in the presence of glucose. We[4] reported similar findings in the case of homogenates and extracts obtained from rat muscle. In the presence of fluorides the activity of the apyrases although markedly inhibited still remains appreciable. The inclusion of glucose in the reaction mixture enables the hexokinase reaction in extracts to proceed through its successful competition with the apyrases for the participation of ATP. Obviously, since the presence of glucose results in an inhibition of apyrase activity, calculation of the hexokinase effect by a comparison of the changes in the $\Delta 7P$ after incubation in the absence and presence of glucose represents the summation of hexokinase and apyrase activity. On the other hand, although apyrase activity is accompanied by a decrease in the $\Delta 7P$ and an increase in the P_0, it fails to influence the P_7 values. It is therefore perfectly justifiable to calculate hexokinase activity from a comparison of the P_7 changes during incubation in the presence and absence of glucose provided that these two reactions are the only ones occurring which involve phosphate transfers to an appreciable degree.

As a matter of fact, in rat muscle extracts of the type described by Colowick, Cori, and Slein,[5] the sole phosphate changes observed to any degree are those due to these two reactions. In homogenates, on the other hand, extraneous phosphate formation is also observed. Since this latter type of transfer involves changes in both the P_7 and P_0 values, calculation and demonstration of hexokinase activity in homogenates forms an almost insoluble problem.

Since the method of obtaining hexokinase-containing extracts, as described by Colowick *et al.*,[5] yields preparations which fail to present such complex problems, it is obvious that this type of extract should preferably be employed for the demonstration of activity.

[4] Broh-Kahn, R. H., and Mirsky, I. A., *Fed. Proc.*, 1947, **6**, 83.

[5] Colowick, S. P., Cori, G. T., and Slein, M. W., *J. Biol. Chem.*, 1947, **168**, 583.

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 treatment 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 μg 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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