STUDIES OF BLOOD GLYCOLYSIS

II. Sugar and Phosphorus Relationships during Glycolysis in the Blood of Infants and Children with Various Diseases [1]

By GEORGE MARTIN GUEST

(From the Infants' and Children's Hospital and the Department of Pediatrics, Harvard Medical School, and the Children's Hospital Research Foundation and the Department of Pediatrics, College of Medicine, University of Cincinnati)

(Received for publication January 23, 1932)

The significance of the rate of blood glycolysis as it occurs in health and in disease has been the subject of a great many investigations which are cited in the articles by John (1925), Falcon-Lesses (1927) and Barer (1931). The rate of blood glycolysis is fairly constant in health but varies greatly in certain conditions of disease, the most rapid rates having been observed in leukemia (Falcon-Lesses (1927)). Acceleration of the glycolytic rate has been observed also in diabetes, in heart disease, in nephritis, in septicemia, and in other unrelated conditions. However, it is likely that the acceleration of blood glycolysis observed in these conditions is not a peculiarly characteristic manifestation of any of the diseases named, but is due to some common changes in the state of the blood. In previously reported clinical studies, comparatively little attempt has been made to correlate with the determinations of blood glycolysis other chemical alterations of the blood which might influence glycolysis, and apparently no attention has been paid to the behavior of the blood phosphorus during glycolysis in different pathologic conditions.

In many of the diseases in which alterations of the rate of glycolysis have been reported, marked changes of the blood phosphorus are known to occur and some abnormality of the phosphorus metabolism often may be recognized. The studies to be reported here were undertaken with the idea that in those pathologic conditions in which glycolysis is altered, the related order of changes of the blood phosphorus observed in normal blood during glycolysis (as described in the preceding paper) might also be found altered.

METHODS

The methods employed were the same as those mentioned in the preceding paper.

---

[1] An abstract of this paper was read at a meeting of the American Pediatric Society, Montreal, June 18, 1930.

RESULTS

The glycolysis and inorganic P curves shown in the first eight of the figures may be regarded as nearly normal; they are presented to demonstrate the slight variations in the pattern of these changes which may be observed in the milder disturbances of various diseases. More marked alterations of the normal pattern of the glycolysis and inorganic P curves are shown in Figures 9 to 17, inclusive.

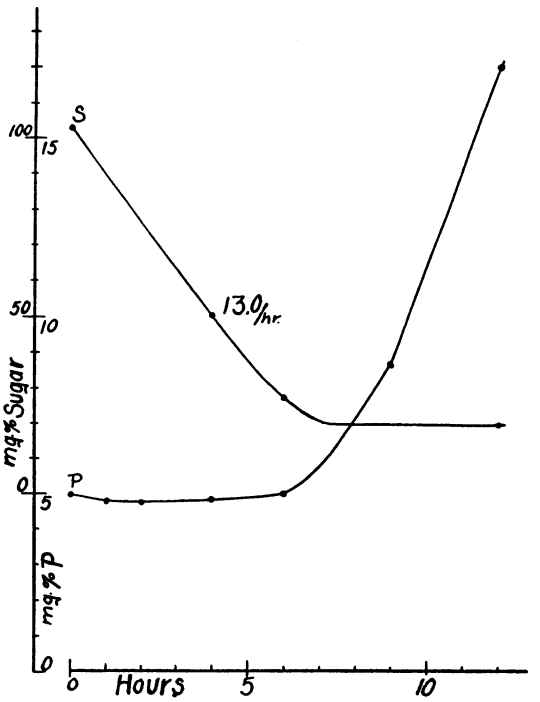

FIG. 1. PURPURA HEMORRHAGICA

E. D., female, 6½ years, not acutely ill.

Case 1, Figure 1. Thrombocytopenic hemorrhagic purpura: E. D., female, age 6½ years, not acutely ill. Physical findings were normal except for extensive purpuric lesions over the body. The bleeding time was markedly prolonged and the clotting time of the blood was normal. The curves representing changes of sugar and inorganic P during glycolysis in this blood may be accepted as characteristic of normal blood. Note that there was little change in the inorganic P until after the sugar was exhausted.

Case 2, Figure 2. Celiac disease: P. T., male, age 4 years, not acutely ill. Typical history of celiac disease, with onset at about one year of age. He had been under treatment several months and was doing well when this blood sample was taken.

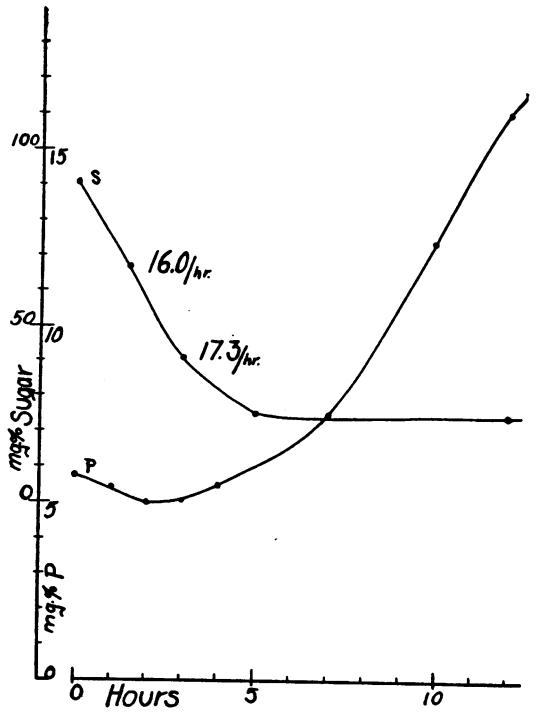

FIG. 2. CELIAC DISEASE

P. T., male, 4 years, not acutely ill.

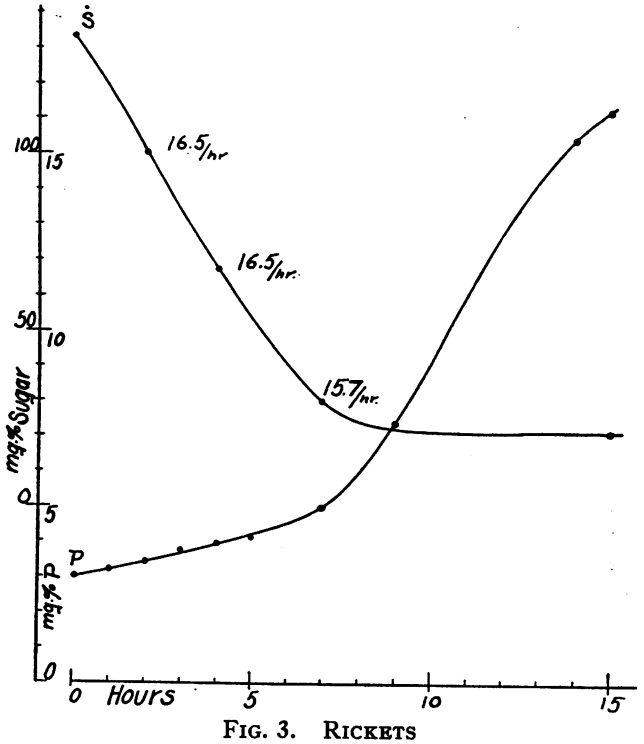

FIG. 3. RICKETS

A. J., female, 3 years, not acutely ill.

Case 3, Figure 3. Rickets: A. J., female, age 3 years, not acutely ill. There were gross signs of severe active rickets, confirmed by x-ray. The glycolytic rate was normal but the inorganic P increased slowly even during the early hours of incubation. In the blood of a few other infants with rickets, less severe, the glycolysis and inorganic P curves were normal.

Case 4, Figure 4. Tuberculous meningitis: J. F. B., male, age 1 year. Onset of symptoms two weeks before admission to the hospital. Convulsions began

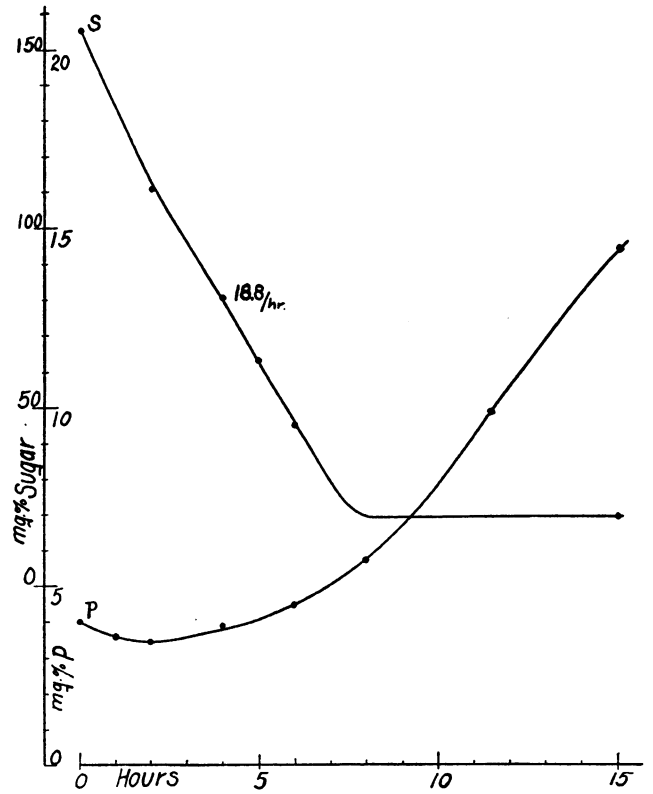

FIG. 4. TUBERCULOUS MENINGITIS

J. F. B., male, 1 year.

5 hours prior to admission, and were practically continuous until death occurred 4 days later. The blood sample was taken 2 days before death, when the temperature was 103° F. Glycolysis and inorganic P curves were essentially normal. Normal curves were likewise obtained from blood samples of 3 other infants with the same diagnosis and similar symptoms.

Case 5, Figure 5. Upper respiratory infection, with recovery: J. McC., male, age 3 years, well developed and nourished, complaining of sore throat. Temperature was 103° F. Physical examination was essentially negative except for redness of pharynx. Lungs were clear. Temperature fell to normal after 3 days. In Figure 5 the curves S_1 and P_1 are from a blood sample taken the

day after admission to the hospital; S_2 and P_2 are from a sample taken 5 days later, after apparent complete recovery.

Case 6, Figure 6. Dysentery (Flexner type): F. M., male, age 6 years, acutely ill. Temperature was 104° F. Preceding admission to the hospital there had been diarrhea, fever, and headache of 2 days' duration. Stool culture yielded Flexner dysentery bacillus, and 3 weeks later the blood serum agglutinated a standard antigen of this organism. The curves in Figure 6 are from a blood sample taken on the day of admission. The inorganic P rose slightly during the first 3 hours, before glycolysis was completed, but the pattern of the curves is nearly normal.

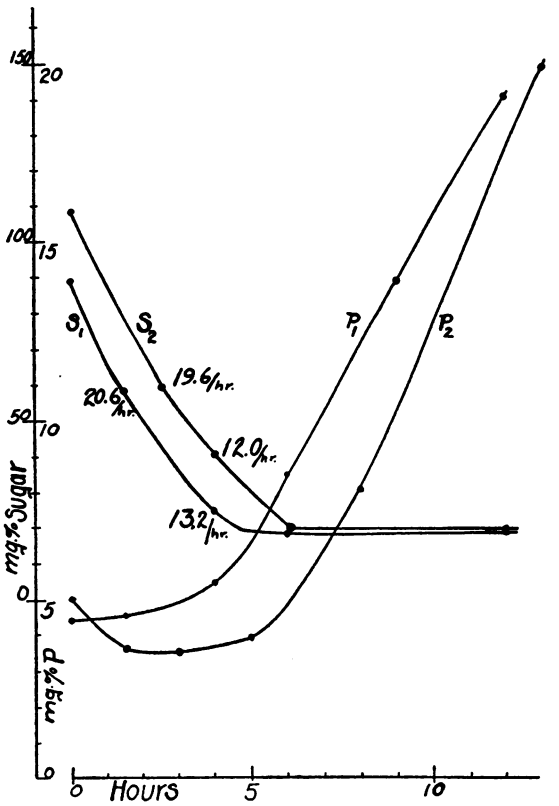

FIG. 5. UPPER RESPIRATORY INFECTION

J. McC., male, 3 years. S_1 and P_1, from blood taken during the acute infection; S_2 and P_2, from blood taken 5 days later, after recovery.

Case 7, Figure 7. Typhoid fever: W. S., male, age 3 years. Acute onset of symptoms was 2 weeks previously. Stool culture yielded *B. typhosus*, and the Widal test became positive. There was a typical course of typhoid fever, moderately severe, with slight improvement at the time the blood sample was taken for the glycolysis studies. Temperature at this time was 101° F., and fell to normal about 10 days later. Glycolysis and inorganic P curves were

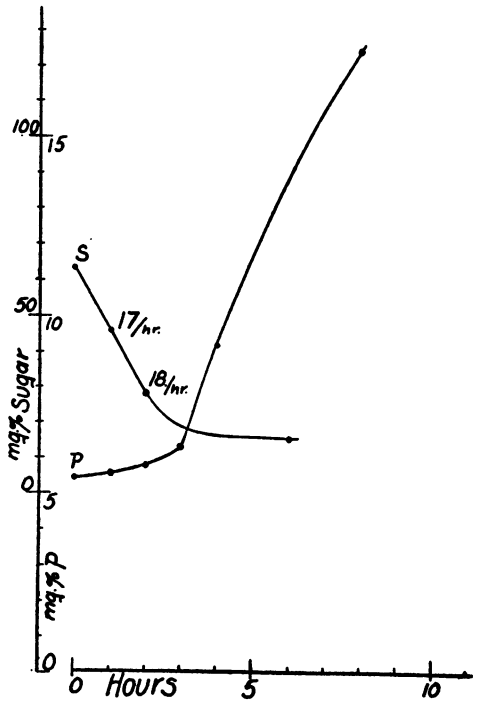

FIG. 6. DYSENTERY. FLEXNER TYPE

F. M., male, 6 years, acutely ill.

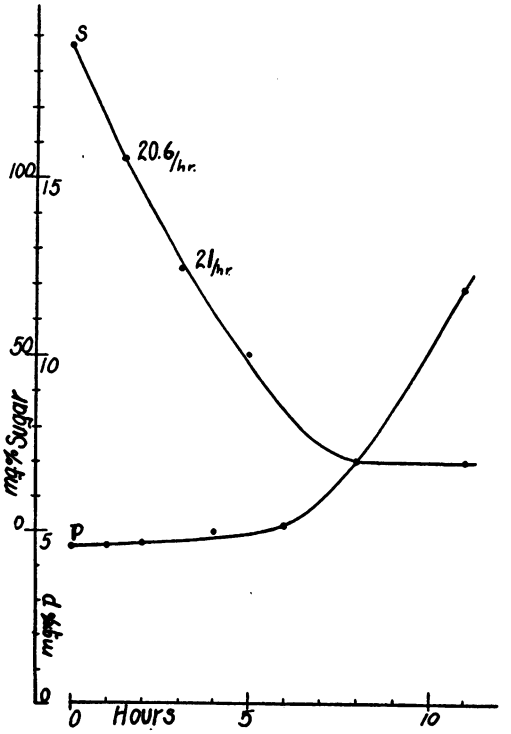

FIG. 7. TYPHOID FEVER

W. S., male, 3 years; blood sample taken at about the middle of the period of the illness.

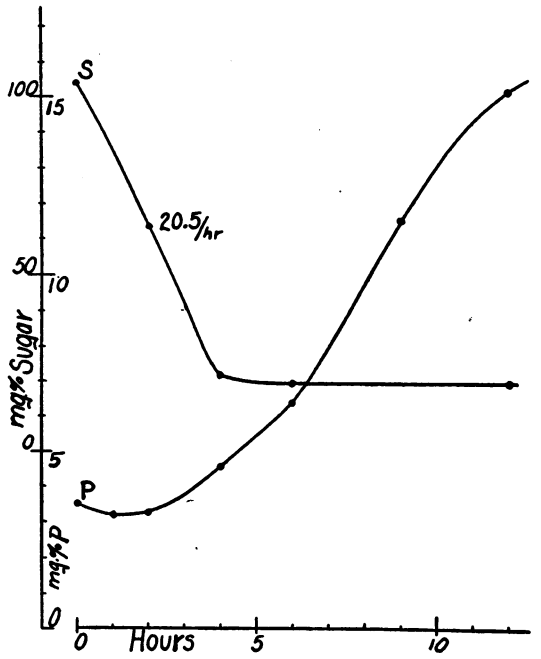

FIG. 8. LEAD POISONING
K. T., male, 18 months; blood sample taken 48 hours antemortem.

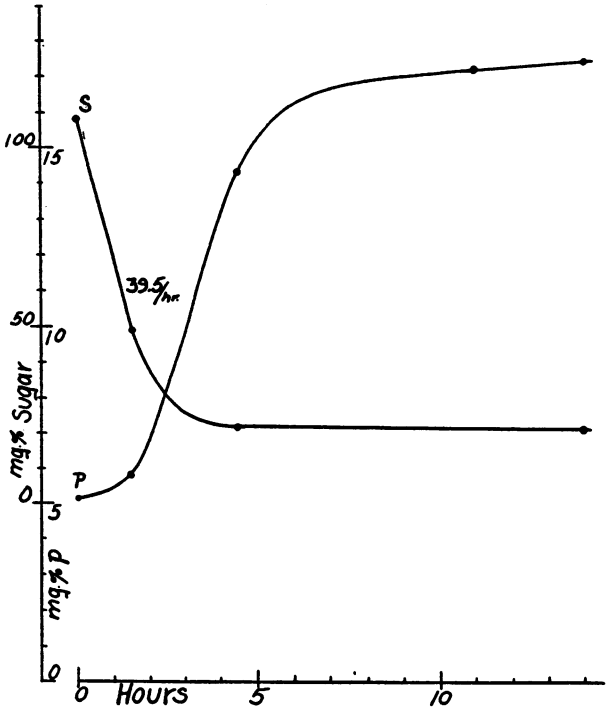

FIG. 9. LEAD POISONING
R. S., male, 18 months; blood sample taken 20 hours antemortem.

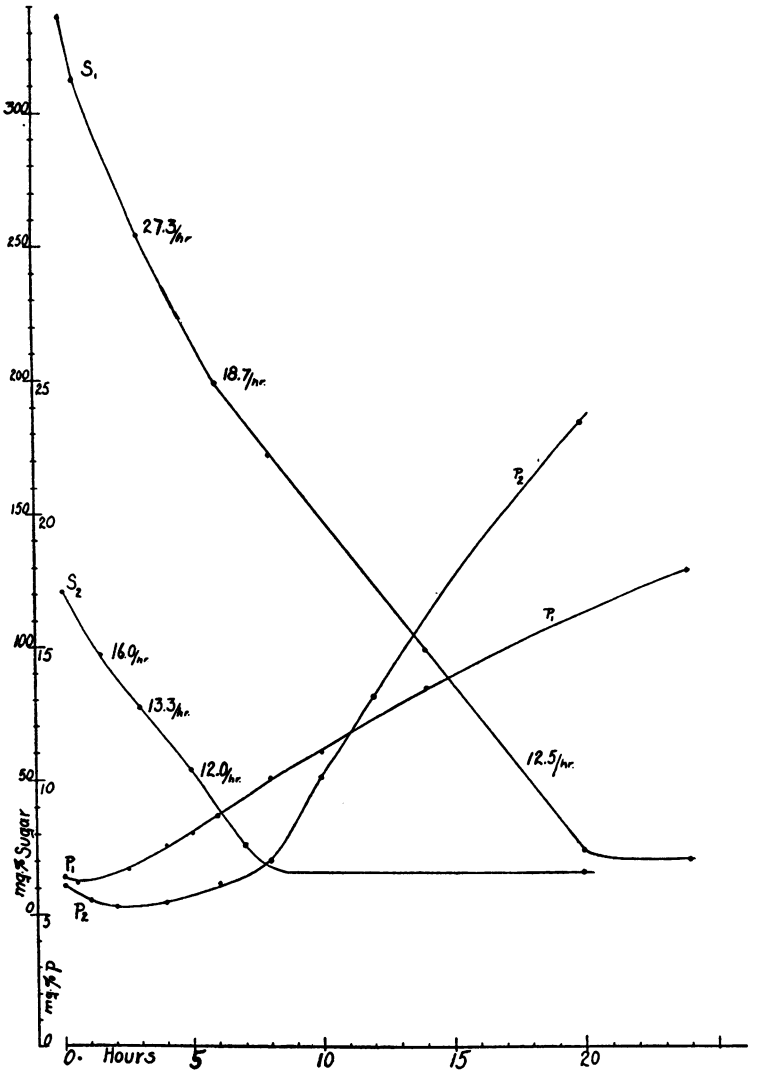

FIG. 10. ENCEPHALITIS WITH HYPERGLYCEMIA

H. H., male, 7 years; S_1 and P_1, from a blood sample taken at time of admission to hospital; S_2 and P_2, from a blood sample taken a week later.

essentially normal, although the rate of glycolysis was slightly accelerated (21 mgm. per cent loss per hour).

Case 8, Figure 8. Lead poisoning: K. T., male, age 18 months. History of eating paint from furniture. Patient had been vomiting once a day for 2 weeks and became stuporous 24 hours before admission to the hospital. There were no convulsions (until after this blood sample was taken). Patient had fever and mild pharyngitis. Stained blood film showed many stippled cells. Patient died 2 days after admission.

Case 9, Figure 9. Lead poisoning: R. S., male, age 18 months. History of eating paint. Onset of symptoms with occasional vomiting was 2 months previously. Two hours before admission the infant vomited, became unconscious and was having continuous mild convulsions when brought to the hospital. Stippled cells 4.2 per cent. Patient died 24 hours after the blood sample was taken for glycolysis studies. Glycolysis was rapid, 39.5 mgm. per cent loss per hour.

Case 10, Figure 10. Hyperglycemia of encephalitis, with recovery: H. H.,

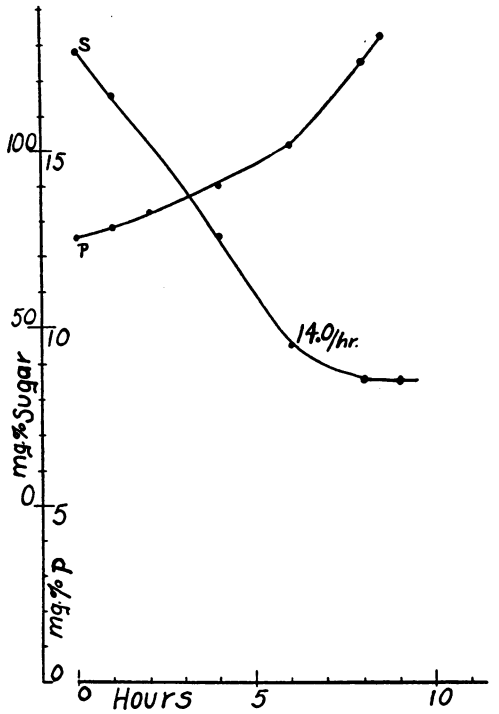

FIG. 11. ACUTE NEPHRITIS

J. K., male, 10 years, on verge of coma; blood sample taken about 12 hours antemortem.

male negro, age 7 years, admitted to the hospital in an unconscious state, with a history of sudden onset of convulsions beginning 2 hours previously. Temperature was 102° F. Spinal fluid contained fresh blood, but was sterile on culture. On the second day he was quieter, but did not regain complete consciousness until the 8th day. Right-sided weakness persisted for about 2 weeks. Patient was discharged from the hospital after 3 weeks, seemingly perfectly well.

In Figure 10, S_1 and P_1 represent respectively sugar and inorganic P changes in the blood sample taken 1 hour after admission. The blood sugar was 336 mgm. per cent, the hyperglycemia being due presumably to some meningeal irritation. The curves S_2 and P_2 represent changes in sugar and inorganic P in a blood sample taken 1 week after admission when the boy was quiet, with normal temperature. This figure may be compared with Figures 3 to 8 in

the preceding paper, which show the effect of a changed sugar concentration upon the inorganic P curve during glycolysis in normal blood. The effect of a high blood sugar content in normal blood is to delay the rise of inorganic P, whereas in the hyperglycemic blood of this patient the inorganic P rose steadily (P_1) after the first hour of incubation.

Case 11, Figure 11. Acute nephritis: J. K., male, age 10 years, acutely ill, with generalized edema and on the verge of coma when admitted to the hospital and the blood sample was taken. There was marked hyperpnea, and probably extreme acidosis although the blood CO_2 was not determined. Blood

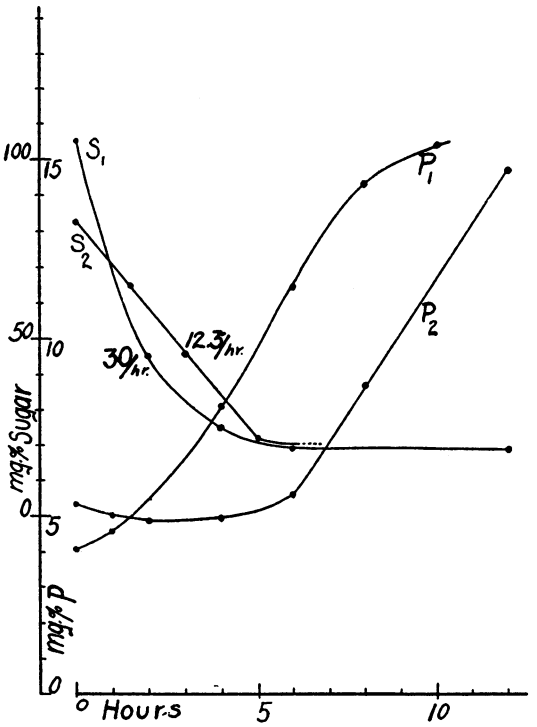

FIG. 12. PYELONEPHRITIS, WITH RECOVERY

I. S., female, 2½ years; pyuria and fever; S_1 and P_1, from blood sample taken at time of admission; S_2 and P_2, from blood taken after recovery.

nonprotein nitrogen was high and coma, convulsions and death ensued a few hours after this sample was taken. The glycolytic rate was normal (14 mgm. per cent per hour), but the inorganic P in the blood was 12.6 mgm. per cent and increased from the start of incubation.

Case 12, Figure 12. Pyelonephritis, with recovery: I. S., female, age 2½ years. History of illness of several weeks, with marked pyuria. At the time of admission to the hospital there was marked diminution of urinary secretion. The girl was drowsy and had slight hyperpnea. The blood nonprotein nitrogen was 94 mgm. per cent. In Figure 12, the curves S_1 and P_1 represent changes of the sugar and inorganic P in a blood sample taken at the time of admission. In this blood, glycolysis was rapid and the inorganic P increased rapidly from

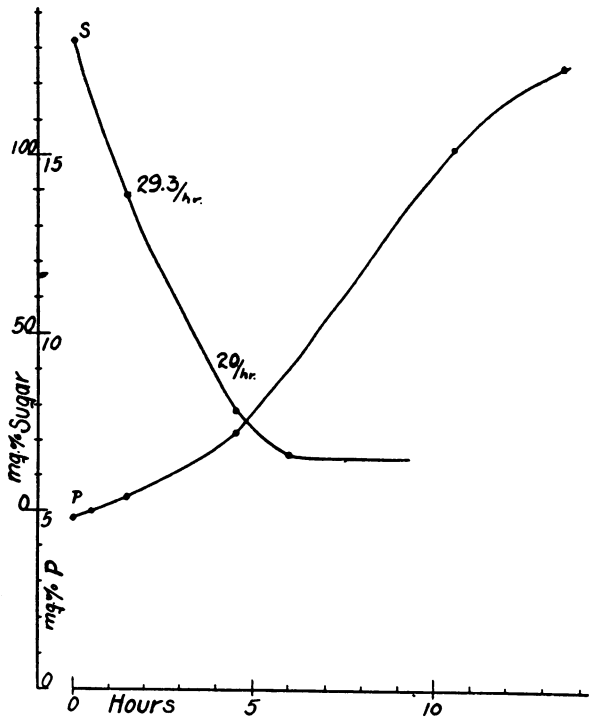

FIG. 13. GASTRO-INTESTINAL INTOXICATION
E. S., female, 2 months, acutely ill.

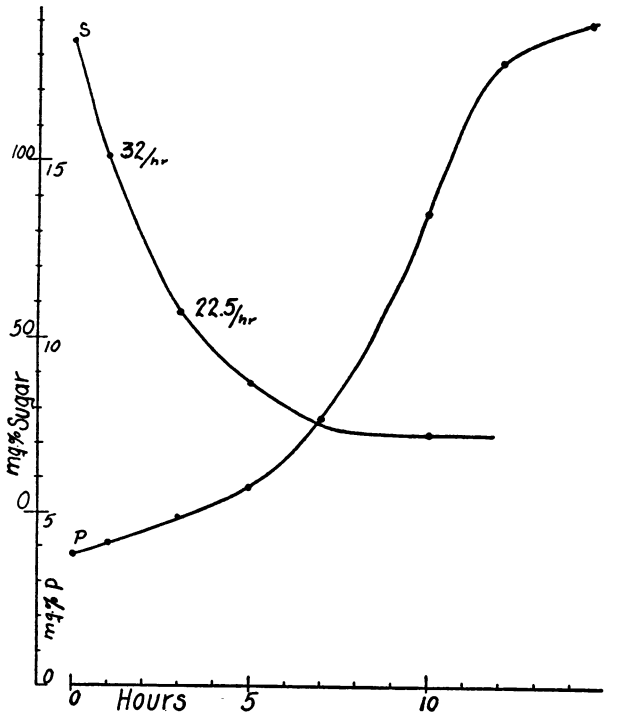

FIG. 14. GASTRO-INTESTINAL INTOXICATION
R. H., male, 7 months, acutely ill.

the start. Eighteen days later all acute symptoms had disappeared, the child appeared well and the blood nonprotein nitrogen was 40 mgm. per cent. The glycolysis and inorganic phosphorus curves S_2 and P_2 from a blood sample taken at this time follow a normal pattern.

Case 13, Figure 13. Gastro-intestinal intoxication: E. S., female, age 2 months, admitted to the hospital with history of mild diarrhea and vomiting for 2 weeks. Patient was a well developed and fairly well nourished infant, weight 8¼ pounds, breathing quietly, skin dry, temperature 101° F., lungs clear, mild furunculosis of scalp. Patient improved after a week of intensive treatment (transfusion, parenteral fluids, etc.) and was discharged after 1 month.

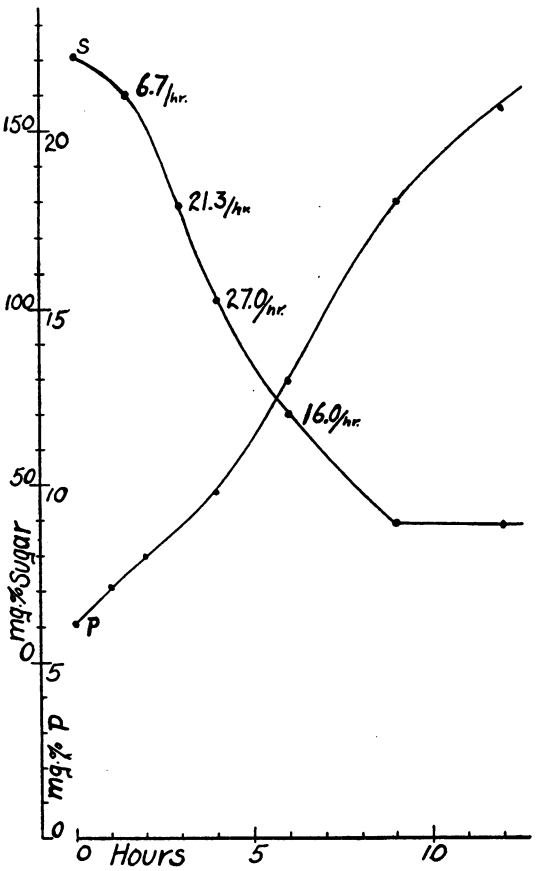

FIG. 15. GASTRO-INTESTINAL INTOXICATION
A. E., male, 4 months, acutely ill.

Glycolysis in the blood sample taken at the time of admission was accelerated, 29.3 mgm. per cent loss per hour, and the inorganic P rose slowly from the start of incubation of the blood.

Case 14, Figure 14. Gastro-intestinal intoxication: R. H., male, age 7 months, admitted with history of mild diarrhea for 3 weeks, and vomiting during the last 5 days. Weight 16½ pounds. Temperature was 101° F. The

infant was drowsy, and the breathing slow and quiet. There were signs of recent loss of weight. The skin was dry and inelastic, of grayish color. Patient died 5 days after admission. In the blood sample taken at the time of admission, the blood glycolysis was accelerated, 32 mgm. per cent loss per hour, and the inorganic P rose from the start of incubation.

Case 15, Figure 15. Gastro-intestinal intoxication: A. E., male, age 4 months, with history and symptoms similar to the last two infants described but apparently less severe. The sugar and inorganic P curves are from a blood sample taken at the time of admission; another blood sample taken 6 days later, after the signs of intoxication had disappeared, gave normal curves.

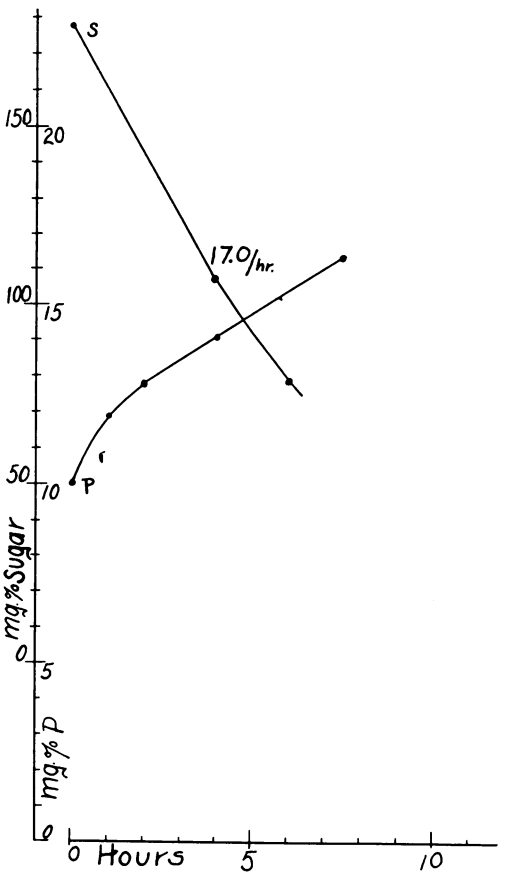

FIG. 16. GASTRO-INTESTINAL INTOXICATION

E. B., male, 3 months, acutely ill, almost moribund, with marked dehydration and acidosis.

Case 16, Figure 16. Gastro-intestinal intoxication: E. B., male, age 3 months, admitted to the hospital almost moribund, with a history of marked diarrhea and vomiting of 6 days' duration. The infant was markedly dehydrated, the eyes sunken, the skin dry and inelastic and of dusky gray color. Respirations were deep and labored. Blood nonprotein nitrogen 92 mgm. per cent; serum

CO_2 content 14 volumes per cent. There was some improvement under treatment for a week, then the infant became worse and died after 3 weeks. In the blood sample taken at the time of admission the glycolytic rate was approximately normal, but the inorganic P was 10 mgm. per cent and rose sharply from the start of incubation.

DISCUSSION

The significance and function of blood glycolysis in the total carbohydrate metabolism of the body is not yet clearly defined. The studies of Warburg and many others have shown that glycolysis has a fundamental importance in the physiology of the tissue cells, constituting part of the respiratory cycle of the cells (Dickens and Šimer (1930)), and Warburg (1925) showed that glycolysis serves as a valuable index of the metabolic activity of various tissues. Blood cannot be regarded as an inert carrier, and in view of its bulk it would be surprising if its own metabolic activities were not of considerable importance in the total metabolism of the body. Aside from the possible quantitative importance, however, the mechanism of glycolysis may well have a considerable significance in reflecting something of the nature of the chemical reactions which occur within the tissues.

Variations in the rate of glycolysis may be due to changes in concentration of the enzyme or enzymes responsible for various steps in the process, changes in concentration of the substrate (the blood sugar), changes in concentration or nature of other substances (such as phosphates) which may be essential to these reactions, or changes in chemical factors (such as the acid-base equilibrium, shifts of pH, etc.) which affect and govern the enzyme activities. As stated in the preceding paper, it appears that most of the glycolysis is due to the erythrocytes. Leucocytes also have high glycolytic power, and this might be regarded as very significant since an accelerated glycolytic rate may be observed in most bloods with a high leucocytosis, but Schmitz and Glover (1927) in studying 7 cases of leukemia found that, although the rate was rapid in the blood of these patients, there was no real correlation between the number of white cells and glycolysis. Probably the most important factors responsible for alterations of glycolysis and alterations of the behavior of the blood phosphorus during glycolysis, as described here, may be found in changes in the chemical state of the blood which affect the enzyme reactions. Martland (1925) suggested that the high concentration of inorganic phosphorus in the blood when observed in states of acidosis in many diseases might be due to the fact that a shift in the reaction of the blood towards acidity diminishes phosphoric ester synthesis and leaves the inorganic phosphorus increasing in the blood because of ester-hydrolysis. Phosphoric ester hydrolysis occurs over a range of pH 6.0 to 9.0 (Rona and Iwasaki (1927)), while ester synthesis is extremely sensitive to such changes, being inhibited by any shift of pH below 7.3 (Martland

(1925)) and stopped below pH 6.8. (This has been discussed briefly in the preceding paper.)

When dealing with substances of the blood which are known to be extremely labile, it should be recognized that the values obtained from their measurements are not static: for example, a given concentration of blood sugar in a diabetic has a different significance when that sugar is increasing than when it is decreasing. The element of change, and rate of change, *in vivo* in such circumstances can be brought out only by repeated blood sugar determinations made at short intervals. The changes in blood constituents which are observed *in vitro*, as in glycolysis, may not be identical with changes which occur *in vivo* but by study of such changes at least one set of chemical reactions characteristic of a given sample of blood may be analyzed. The blood of the infant in Figure 16 had an inorganic phosphorus content of 6.1 mgm. per cent, only slightly above the usual value for a normal infant of this age, but this inorganic phosphorus was in a state of rapid increase, due to the splitting of organic phosphorus compounds within the cells. Whatever may be the cause of the increased hydrolysis of the phosphoric esters (or partial failure of their resynthesis) this increased rate of liberation of inorganic phosphate under such circumstances must have a considerable importance in the total phosphorus metabolism and also in the acid-base equilibrium of the whole body. Normally the inorganic phosphate in the blood is kept at a fairly constant level, and constitutes only 2.5 m Eq. of the total acids (160 m Eq.) of the serum. Except in extreme conditions (in uremia, for example) the inorganic phosphorus rarely increases to more than double its normal value, a concentration that has been considered rather negligible compared to the increases of other acids observed in severe acidosis due to different causes (Peters, Wakeman, Eisenman and Lee (1929)). The maintenance of the inorganic phosphorus at such a constant level is to a considerable degree a function of the kidneys, and the importance of the urinary excretion of phosphates to the acid-base equilibrium of the body is well known. Part of the inorganic phosphorus freed in the blood, from both blood and tissue cells, is resynthesized to organic compounds. An increased speed of liberation of inorganic phosphorus must place a considerable burden upon the adjusting mechanisms which are responsible for keeping constant the level of inorganic phosphorus in the blood, and herein may lie the chief significance of the changed pattern of reactions displayed in the figures.

The formation of lactic acid during glycolysis in these bloods was not determined, but it has been repeatedly demonstrated by others that in blood glycolysis each molecule of sugar is split to two molecules of lactic acid. Relatively little oxidation of the lactic acid occurs as it is formed during glycolysis *in vitro*, but in the body the lactic acid is caused to disappear from the blood by processes of oxidation and also by its resyn-

thesis into carbohydrate. If the oxidative functions of the blood and tissues are inadequate, as they probably were (because of anhydremia, etc.) in the two infants described under Figures 16 and 17, the more rapid formation of lactic acid from the accelerated glycolysis may contribute considerably to the amounts of acids accumulating in the blood. In severe gastro-intestinal intoxication like that observed in these infants a high lactic acid content of the blood is a common finding (Clausen (1925), Hartman (1928)). Here again it may be pointed out that the *rate* of formation of lactic acid may be of greater importance to the acid-base economy of such patients than the actual concentration of that acid determined at any one time.

SUMMARY

The order of the changes of inorganic phosphorus in relation to the blood sugar, usually observed during glycolysis in normal blood, was found markedly altered in certain pathologic conditions, especially in gastro-intestinal intoxication of infants and in nephritis with acidosis. In defibrinated blood samples of these patients the inorganic phosphorus increased progressively from the start of incubation of the blood instead of remaining low until after the blood sugar was exhausted.

The reasons for the altered pattern of these changes are not clear, but it has been pointed out that the *rate of change* of certain blood constituents (changes consequent upon glycolysis, lactic acid formation, hydrolysis of phosphoric esters and liberation of inorganic phosphorus) may be of greater importance than the actual concentration of those constituents determined in the blood at any one time.

BIBLIOGRAPHY

Barer, A. P., J. Clin. Invest., 1931, x, 507. A Study of Glycolysis.

Clausen, S. W., Am. J. Dis. Child., 1925, xxix, 761. Anhydremic Acidosis Due to Lactic Acid.

Dickens, F., and Šimer, F., Biochem. J., 1930, xxiv, 1301. The Metabolism of Normal and Tumour Tissue. II. The Respiratory Quotient, and the Relationship of Respiration to Glycolysis.

Falcon-Lesses, M., Arch. Int. Med., 1927, xxxix, 412. Glycolysis in Normal and Leukemic Blood.

Hartman, A. F., Am. J. Dis. Child., 1928, xxxv, 557. Chemical Changes Occurring in the Body as the Result of Certain Diseases. I. The Effects of Diarrhea, Vomiting, Dehydration and Oliguria on the Acid-Base Balance of the Plasma of Infants with Mastoiditis.

John, H. J., Ann. Clin. Med., 1925, iii, 667. Glycolysis.

Martland, M., Biochem. J., 1925, xix, 117. The Phosphoric-esterase of Blood at Various Hydrogen Ion Concentrations.

Peters, J. P., Wakeman, A. M., Eisenman, A. J., and Lee, C., J. Clin. Invest., 1929, vi, 517. Total Acid-Base Equilibrium of Plasma in Health and Disease. X. The Acidosis of Nephritis.

Rona, P., and Iwasaki, K., Biochem. Ztschr., 1927, clxxxiv, 318. Beiträge zur Frage der Glykolyse, VII. Mitteilung. Über die Beziehung der Verteilung des Phosphors im Blute zur Glykolyse.

Schmitz, H. L., and Glover, E. C., J. Biol. Chem., 1927, lxxiv, 761. Glycolysis in Leukemic Blood.

Warburg, O., J. Cancer Research, 1925, ix, 148. The Metabolism of Carcinoma Cells.