THE EFFECT OF SODIUM FLUORIDE AND SODIUM IODOACETATE ON GLYCOLYSIS IN HUMAN BLOOD*

By ERNEST BUEDING and WALTER GOLDFARB

(From the Medical Service of the Psychiatric Division, Bellevue Hospital, and the Departments of Medicine and Psychiatry, New York University College of Medicine, New York)

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It has been generally accepted that a concentration of 1 per cent sodium fluoride is adequate to prevent glycolysis in non-precipitated blood and that no appreciable glycolysis results if the blood proteins are precipitated within 5 minutes after the blood is drawn (1). Determinations of lactic acid in the blood precipitated within 5 minutes or preserved in 1 per cent sodium fluoride have yielded a range of 8 to 20 mg. per cent for normal subjects under basal conditions.

We have had occasion to analyze pyruvic acid and lactic acid on the same blood samples. The analysis of pyruvic acid requires the use of iodoacetate as a stabilizing agent (2). The lactic acid values we observed in blood from normal fasting subjects at rest were found to be appreciably lower than those hitherto reported. We therefore reinvestigated the inhibitory effect of sodium fluoride and iodoacetate on glycolysis in human blood.

Methods

Drawing of Blood—The blood was drawn from the antecubital vein without stasis. If stasis were required to puncture the vein, the blood was drawn after the pressure on the arm had been released. The sodium iodoacetate used was prepared from iodoacetic acid (Eastman Kodak) (3) as a 30 per cent solution. The iodoacetate solution was measured into the syringe used to draw the blood and mixed with the blood in the syringe before

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delivery into the sampling bottles. The glucose and lactic acid values observed were corrected for the dilution. The sodium fluoride was added to the sampling bottles as a 4 per cent solution and then dried to a powder over a hot-plate. 0.2 per cent potassium oxalate was used for all blood samples in addition to the preservative used.

Analytical Methods—The blood sugar was estimated in an aliquot of 0.2 cc. of blood (2 cc. of blood filtrate) by a modification (4) of the Hagedorn-Jensen method, 0.002 instead of 0.005 N sodium thiosulfate being used for the final titration. The samples were analyzed in duplicate and the duplicate determinations never varied more than 0.7 mg. per cent. Non-fermentable reducing substances were determined by yeast fermentation (5).

For the lactic acid determination 15 cc. of blood were used. A 20 cc. aliquot of the carbohydrate-free filtrate (representing 1.6 cc. of blood) was determined according to Wendel's modification (6) of the method of Friedemann, Cotonio, and Shaffer (7). All determinations were made in duplicate or triplicate.

In eleven analyses known amounts of lithium lactate were added, corresponding to a lactic acid concentration varying from 3.0 to 12.5 mg. per cent. In six instances the blood contained 0.2 per cent oxalate and recoveries varied from 92 to 104 per cent (average, 97 per cent). In the other five analyses the blood contained 0.2 per cent potassium oxalate, 1 per cent sodium fluoride, and 1 per cent sodium monoiodoacetate and 93 to 98 per cent (average, 95 per cent) of the added lactic acid was recovered.

Experimental Method—In the present experiments we have estimated the glycolysis in human blood over a period of 4 hours. The blood was drawn without stasis and added to various concentrations of iodoacetate or sodium fluoride. The blood proteins of an aliquot were precipitated (8) immediately and the filtrate was analyzed for glucose and lactic acid. A second aliquot was precipitated 4 hours later.

Observations were made on the changes in non-glucose reducing substances with and without iodoacetate and sodium fluoride. We have repeatedly observed that there was no change in the non-glucose reducing substances of the blood during the 4 hour period of the experiment. We therefore feel that the values observed in the following experiments represent changes in the true glucose of the blood.

Results

Effect of Sodium Fluoride on Glycolysis—The changes in the non-precipitated blood during 4 hours after withdrawal from the vein are presented in Fig. 1, *A* to *E*. It may be seen that various concentrations of sodium fluoride up to 2 per cent did not prevent completely the decrease in glucose nor the rise in lactic acid of

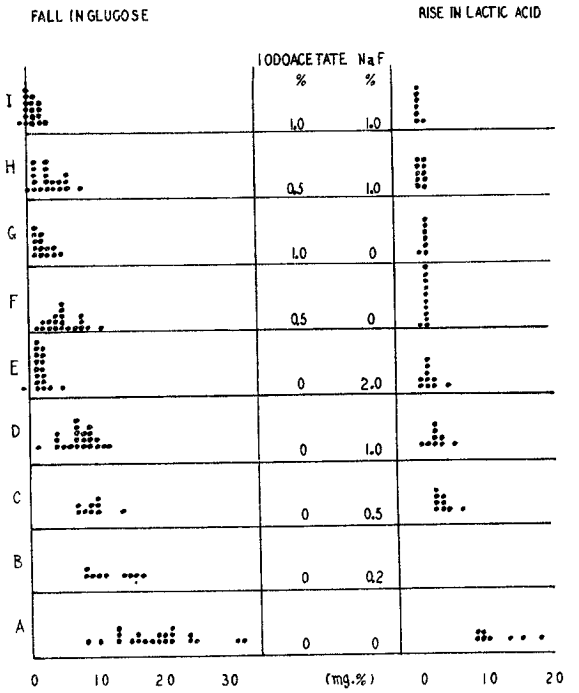

FIG. 1. Effect of various concentrations of sodium fluoride or sodium iodoacetate, and concentrations of both on the changes in sugar and lactic acid of human blood on standing at 20° for 4 hours. (All samples contain 0.2 per cent oxalate.)

the non-precipitated blood samples. In practically all cases the loss of glucose exceeded the rise of lactic acid. The averages of each group were analyzed by Fisher's method (9) and were found to be statistically significant. In order to test the effect of sterility on glycolysis, we have analyzed a series of blood samples preserved with 1 per cent sodium fluoride in which one aliquot was kept in sterile containers, and a second aliquot was left open to

the air. No difference was found between the fall in glucose concentration in the two sets of specimens in 4 hours.

These observations do not agree with the results hitherto reported on the effect of sodium fluoride on glycolysis. Most of the previous investigators have estimated the rate of glycolysis from analyses of blood sugar values (10–15). These authors have used methods for blood sugar estimation inadequate to detect changes of the order of magnitude reported in this paper.

Effect of Monoidoacetate on Glycolysis—It may be seen (Fig. 1, *F* and *G*) that the disappearance of glucose was inhibited by both 0.5 and 1.0 per cent iodoacetate, but neither concentration

TABLE I

Effect of 1 Per Cent Iodoacetate Plus Sodium Fluoride on Glycolysis within 5 Minutes after Blood Was Drawn

Sample No.	1	2	3	4	5	6	7	8	9
1% iodoacetate + 1% NaF	5.6	5.4	7.0	5.9	6.9	8.2	6.4	7.4	6.5
None	6.6	6.6	8.1	7.4	8.7	10.0	7.2	7.4	7.0
Sample No.	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1% iodoacetate + 1% NaF	7.6	4.9	8.4	8.4	18.3*	7.9	5.3	6.8	5.8
None	8.5	6.2	10.4	8.0	19.5*	9.3	6.5	7.2	7.2

* No basal conditions.

of iodoacetate completely stopped the glycolysis. In both there was a significant loss of glucose and rise in lactic acid in 4 hours.

Effect of Iodoacetate Plus Fluoride on Glycolysis—The rate of glycolysis was studied on a series of blood samples preserved with 1 per cent sodium fluoride plus either 0.5 or 1 per cent iodoacetate (Fig. 1, *H* and *I*). There was a perceptible diminution of the rate of glucose disappearance in 1 per cent sodium fluoride plus 0.5 per cent iodoacetate and practically complete preservation of the blood sugar and lactic acid could be accomplished with 1 per cent sodium fluoride plus 1 per cent iodoacetate. The inhibition of lactic acid production was observed with the 0.5 per cent iodoacetate plus 1 per cent sodium fluoride.

Lactic Acid Values in Blood Precipitated within 5 Minutes—Blood was drawn through a single needle into a dry syringe and added to enough oxalate in a sampling bottle to make the concentration 0.2 per cent. An additional sample was drawn through the same needle containing a sufficient amount of iodoacetate to make a 1 per cent solution, and added to a sampling bottle with sufficient sodium fluoride and oxalate to give a final concentration of 1 per cent sodium fluoride and 0.2 per cent potassium oxalate. Both samples were precipitated within 5 minutes and lactic acid analyzed. The data are presented in Table I. It may be seen that the lactic acid values of most of the bloods containing iodoacetate and sodium fluoride were appreciably lower than the sample without the preservatives, indicating that some glycolysis did occur in the first 5 minutes.

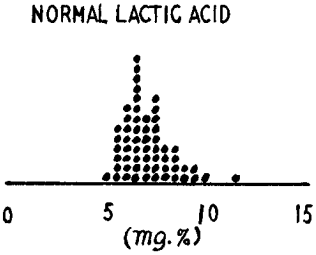

FIG. 2. Lactic acid concentration of normal human blood

Normal Lactic Acid Values in Blood—It has been generally accepted that glycolysis can be prevented if sufficient sodium fluoride is added to make a 1 per cent solution. In the present report our observations do not confirm the previous findings, and we believe that the lactic acid values reported previously have at least two sources of error. The conversion of pyruvic acid to lactic acid occurs so rapidly that the ordinary method of drawing blood in a dry syringe would introduce an error regardless of the type of preservatives used (2, 16). In addition we have observed a perceptible rise of lactic acid in blood containing 1 per cent sodium fluoride before protein precipitation. We have therefore analyzed a large series of blood samples from resting patients for lactic acid, using the following precautions. The observations were made on patients at least 14 hours after the last food intake,

and after a rest of at least 1 hour in bed. Blood was drawn without stasis from the antecubital vein into a syringe filled with sufficient iodoacetate to make a 1 per cent solution. The blood was delivered into a sampling bottle with sufficient oxalate and fluoride to give a final concentration of 0.2 per cent oxalate and 1 per cent fluoride. The proteins were precipitated (8) within 5 minutes and the lactic acid analyzed. The results are presented in Fig. 2. It may be seen that the bulk of the determinations falls between 5 and 10 mg. per cent. The average of the 58 determinations was 7.0 mg. per cent.

SUMMARY

1. The rate of glycolysis in human blood was estimated from the changes in glucose and lactic acid. Either iodoacetate or sodium fluoride alone did not stop glycolysis completely. Complete inhibition of glycolysis was observed with a mixture of 1 per cent sodium fluoride and 1 per cent iodoacetate.

2. When suitable precautions were used, it was found that the normal lactic acid values in human blood ranged from 5 to 10 mg. per cent.

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