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THE INFLUENCE OF DIFFERENT ANTICOAGULANTS ON THE RATE OF CHANGE OF BLOOD SUGAR

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ABSTRACT

In this, study, the rate of decrease in blood sugar concentration measured from blood preserved in Dispotassium ethylenediaminetetraacetic acid (K_2EDTA) and fluoride Oxalate containers, respectively, was evaluated. The mean sugar concentration at zero hour and room temperature (29°C) for all the containers was not significantly different (P>0.01). The mean sugar concentration after one hour of keeping the samples at room temperature was 4.4 ±1.5 millimole per litre (mMol/L) and 3.8 + 1.5 millimole per litre (mMol/L) for the fluoride oxalate and K_2EDTA containers, respectively, thus showing a 13% and 25% decrease in sugar concentration respectively. The major difference in level of decrease of the sugar contained in the two anticoagulants after 2hours of blood collection, suggests a greater instability of sugar collected in K_2EDTA containers. This makes its use unreliable for sugar estimation in diabetes or other sugar related ailments for diagnosis and prognosis. From the result of this preliminary studies, it would seem most appropriate that blood samples collected in fluoride oxalate for sugar estimation be analyzed within the 1st hour if kept at room temperature because there is a greater instability of the sugar concentration after one hour when stored at room temperature of 29°C.

Keywords: Anticoagulants, blood sugar, hyperglycemia).

INTRODUCTION:

The level of blood glucose has been reported as the balance between intake of carbohydrate and endogenous glucose synthesis and release by the liver on the hand and glucose storage, utilisatiion and excretion on the other (Baron et al,1994). In adults with hyperglycemia, a random venous plasma glucose of 11.1 mMol/L or more on two occasions or a fasting value of 7.8 mMol/L or more on two occasions is diagnostic of Diabetes mellitus (cheesebrough 1992). Blood glucose or sugar estimation is a very important tool in the diagnosis and follow-up of Diabetes mellitus patients. Methodologies that eliminate variation in sugar values with time are therefore important. Apart from the differences in procedures for blood sugar estimation , there are also various anticoagulants or preservatives used in blood collection. It has been reported that many anticoagulants or preservatives often influence the final results of assays by interfering with the analytical procedure (William et al.1978). The anticoagulant, fluoride oxalate, exerts its effect by inhibiting the glycolytic process, together with its weakly anticoagulant action (Baron et al, 1994).On the contrary, the sodium and potassium salts of ethylenediaminetetraacetic acid are powerful anticoagulants which act by chelating calcium molecules in the Blood (Dacie and Lewis, 1995). Potassium EDTA is reported to have little effect on the glycolytic pathway of erythrocytes (Mayne, 1996)

Since glycolysis is known to reduce glucose(Chan et al, 1989), the maintenance of blood sugar level for reliable diagnosis therefore, requires blood collection with a substance having both anticoagulant and antiglycolytic effect. This work assesses the effect upon the stability of blood sugar level by the collection of blood with substances having either anticoagulant or antiglycolytic effects.

MATERIALS AN D METHOD

SUBJECTS:

The subjects for the study were randomly chosen from medical laboratory science students of the University of Calabar. Their consent was sought and their blood samples were obtained from the cubital area of the arm by venopuncture after thoroughly clearing the site with methylated spirit.

Five milliliters of blood sample was collected and 0.1 milliliters of blood from the syringe was placed straight into a test tube containing 3.7 milliliter isotonic sodium sulphate, copper sulphate solution followed by addition of 0.2 milliliters of 10% sodium tungstate. This served as the control. Half of the remaining blood sample in the syringe was placed in fluoride oxalate container and the other half was placed in dipotassium ethylenediaminetetraacetic acid (EDTA) container. Each of the two containers were swirled gently, to mix the blood with the different anticoagulants in the respective containers. The mixing was done gently to avoid clotting and lysing of the specimens. Since sodium fluoride is poorly soluble, mixing was done thoroughly to allow for effective anti-glycolysis. From each of the two containers,

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0.1milliliter of blood was used for blood sugar determination.

The two values obtained served as the zero time values for the respective anticoagulant. The copper reduction method of Nelson (Nelson, 1944), was used for estimation of blood sugar.

The containers were then left at room temperature (29°C) for 2hours. Blood sugar estimation was carried out hourly on samples from the two anticoagulant containers.

STATISTICAL ANALYSIS:

Results are expressed as mean ± SD. Pair wise comparisms were made using the student t-test. Values ($P< 0.05$) were regarded as significant.

RESULTS

The values for the blood sugar concentration at various periods are as presented in

Table 1, 11and 111. At the initial period, the mean sugar concentration from the syringe was 5.10 ± 1.75 mMol/L. This value was found not to be significantly different ($P>0.01$) from the zero value sugar concentration for the samples from both the fluoride oxalate and K_2EDTA containers. Despite the fall in blood sugar value in the two different anticoagulant containers after 1hour of storage, the difference in value between the blood sugar measured at zero period (from the syringe) and that from the fluoride oxalate container was not significant ($P <0.01$), while there was a highly significant difference ($P < 0.01$) between this value and the 1hour value of the sample from the K_2EDTA container. After 2hours the mean sugar concentration in fluoride oxalate and K_2EDTA containers fell in value significantly ($P < 0.01$) from the zero time value.

There was a 13% and 25% decrease in concentration of blood sugar for the samples from fluoride oxalate and K_2EDTA containers respectively after 1hour. Also for the 2nd hour there was a 20% and 33% decrease in blood sugar concentration for the samples from fluoride oxalate and K_2EDTA respectively.

DISCUSSION:

The mean glucose concentration obtained immediately after blood collection was found not to be significantly different in samples from both containers with different anticoagulants. Despite the fall in values of the mean glucose concentration in fluoride oxalate and K_2EDTA containers after 1hour, the rate of decease was greater for the latter. This can be explained by the fact that EDTA's anticoagulant action depends on the precipitation or chelation of calcium ions which usually initiates the clotting process. K_2EDTA , unlike fluoride oxalate has little or no effect on the glycolytic process of erythrocytes(Mayne,1996).This therefore influences the comparatively high rate of blood sugar instability in the K_2EDTA container. Fluoride oxalate on the other hand is known to exert its preservative action by inhibiting the enzymes involved in glycolysis (Tietz et al 1994). It has also been observed that without an antiglycolytic agent, the blood glucose concentration

TABLE 1: STABILITY RATE OF SUGAR IN BLOOD COLLECTED WITH FLUORIDE OXALATE

Time of Glucose Assay	Mean glucose concentration (mMol/L)		% Glucose Decrease
	From Syringe	K_2EDTA	
Initial	5.10± 1.75		
1 hr		4.40 ± 1.51	13%
2 hr		4.00 ± 1.59	20%

TABLE 11:STABILITY RATE OF SUGAR IN BLOOD COLLECTED WITH DISPOTASSIUM EDTA

Time of Glucose Assay	Mean glucose concentration (mMol/L)		% Glucose Decrease
	From Syringe	K_2EDTA	
Initial	5.10± 1.75	-	-
1 hr	-	3.80 ± 1.53	25%
2 hr	-	3.30 ± 1.49	33%

TABLE 111: SUMMARY OF STABILITY OF SUGAR IN BLOOD COLLECTED WITH DIFFERENT ANTCOAGULANTS

Time of Glucose Assay		Mean glucose concentration (mMol/L)		% Glucose Decrease	
Time of Glucose	From Syringe	Fluoride Oxalate tubes	K_2EDTA tubes	Fluoride Oxalate tubes	K_2EDTA tubes
Initial	5.10 ± 1.75	-	-	-	-
1 hr	-	4.40 ± 1.50	3.80 ± 1.53	13%	25%
2 hr	-	4.0 ± 1.59	3.30 ± 1.49	20%	33%

52 samples each were analysed.
Results are expressed as mean ± SD

decreases 0.56 mMol/L per hour at 25°c (Tietz et al, 1994). In the present study it was observed that at 29°c blood sugar decreases ~ 1.3 mMol/L in the 1st hour without an antiglycolytic agent.

The trend of glucose instability with time and the high rate of decrease in value for the K_2EDTA preserved samples especially after 2 hours of preservation makes this anticoagulant unsuitable for collection of samples for blood glucose determination

This instability of glucose in blood preserved in fluoride has been observed previously (Chan et al, 1989). This instability. may be due to the time it takes the inhibitor to enter the cells and bring about effective inhibition.

There are conflicting reports on the level of glucose stability in the preserved of fluoride (Nakashima et al 1987, Boyd & Roe, 1977, Sander and Deadman, 1995, Frances et al 1985). These discrepancies have been attributed to poor analytical methods (Wilkerson and Kanto 1987) . The trend towards stability in the mean glucose values in fluoride oxalate presence samples after 2 hours of storage has variously been observed(Chan et al, 1989 and Nelson,1944). In the present study, the major advantage of the fluoride oxalate anticoagulant is attributed to the fact that despite an initial slight drop in sugar values, it can maintain blood sugar concentrations near the initial value for one hour, when the containers are left at room temperature (29°c) whereas for K_2EDTA anticoagulant, the blood sugar concentration decreased continuously upon storage. Since accurate and stable blood sugar values are needed for reliable diagnosis of such aliments as Diabetes Mellitus, it is important to preserve blood samples with anticoagulants like fluoride oxalate that will promote glucose stability. Moreover from the result of this preliminary study, it is advisable for analyses of blood sugar samples to be done within one hour of collection, if samples are normally left on the bench
at the tropical laboratory temperature of 29°c, as obtains in University of Calabar Teaching Hospital, Calabar Nigeria.

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