The Preservation of Blood Sugar for Diabetes Detection

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A satisfactory preservative for blood sugar is needed for blood specimens that are not analyzed before significant glycolysis occurs. Since the concentration of blood sugar is of primary importance in the diagnosis of diabetes, its chemical determination must be accurate and not subject to undue influences. In a diabetes detection program as well as in clinical medicine it may be desirable to collect blood specimens and transport them to a central laboratory for analysis. Since the amount of glycolysis is related to time and since a varying period of time would elapse between the collection of the blood and its analysis, the need for a substance that would inhibit glycolysis for an appreciable length of time is evident.

A review of literature revealed considerable disagreement concerning the merits of various preservatives.[1–13] However, many investigators have agreed on the usefulness of fluoride. Most of them used sodium fluoride under varying conditions [2, 3, 5, 6, 7, 10–13] with a few using the more soluble potassium fluoride. [8, 9] Some of the discrepancies are probably due to the small number of specimens used by the investigators and to the inaccuracies in the methods of analysis. In this paper, an investigation is reported comparing the efficacy of four preservatives, viz., sodium fluoride and potassium fluoride, with and without thymol, to inhibit glycolysis in whole blood.

PROCEDURE

Approximately thirty milliliters of blood were drawn from patients of the Diabetes Clinic of the Boston City Hospital selected at random. Seven milliliters of blood were added to each container which had previously been prepared as follows: 70 mg. of NaF were weighed on an analytical balance and put into one batch of individual test tubes; 112 mg. of $KF.2H_2O$ were similarly added to another batch of individual tubes. To half of these tubes was added 0.01 ml. of an alcoholic solution of thymol (70 per cent w/v). The concentration of the preservatives was thus:

10 mg. KF per ml. of blood
10 mg. KF and 1 mg. thymol per ml. of blood
10 mg. NaF per ml. of blood
10 mg. NaF and 1 mg. thymol per ml. of blood.

The initial blood sugar determinations were started within an hour after the collection of bloods using the Somogyi-Nelson procedure.[14] The tubes were then mailed from the laboratory, so that they went to the post office and were returned the following morning. Two series of bloods were taken. In the first phase, fifty-one specimens were collected on ten Mondays and blood sugar determinations were repeated 24, 48, 72 and 96 hours afterwards. In the second phase, fifty-six specimens were collected on eight Fridays and blood sugar determinations were repeated after 72, 96, 120 and 144 hours had elapsed. The blood specimens were kept at room temperature between determinations.

RESULTS

The mean of the original four blood sugar determinations was used as an estimate of the "true" blood sugar level, as a base line to determine the efficacy of the four preservatives. An additional experiment showing the immediate effects of sodium fluoride and potassium fluoride as compared to an immediate determination with no preservative showed a mean difference of —.20 per cent for sodium fluoride and —1.65 per cent for potassium fluoride. This small effect indicated that to use the mean of the four original determinations would introduce an error of only 0.7 per cent.

To determine the efficiency of the preservative in preventing glycolysis, the distributions at the various time intervals for the four preservatives were compared to the distribution of the one-hour readings by use of the Chi Square (x^2) test. Thus we were able to test whether the difference between the various distributions could have been due to laboratory procedures. The results of

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these tests showed that all the distributions were significantly different from the one-hour distribution at the .01 probability level. This means that it is highly probable that the preservatives do not completely prevent glycolysis, for the means of all the distributions were lower than the means of the one-hour distribution.

Although the preservatives probably did not prevent glycolysis, they did inhibit this action and therefore can be quite useful. The per cent of blood sugar values that did not differ from the "true" blood sugar value by a given per cent (4, 6, 8 and 10 per cent) after specific time intervals (24 through 144 hours) are shown on table 1 for the bloods drawn on Mondays and table 2 for the bloods drawn on Fridays. From the last column in both tables it will be seen that almost all the blood sugar levels will be maintained within the 10 per cent limit up to 144 hours. Within the 8 per cent limit, 86 per cent or more of all the specimens were maintained under the conditions of the experiment.

It can be noted from the tables that the preservative action on Fridays' bloods after comparable times was better than that on Mondays' specimens. This raises the question as to what effect the multiple withdrawal of blood from a test tube had on the glucose level in this particular experiment.

Bacterial contamination may have been introduced, since nonsterile pipettes were used to measure the blood. If this is so then the single pipetting of blood after a period of four to six days, as would probably be done in field conditions, should yield results somewhat better than those presented here.

DISCUSSION

Although none of the four substances completely prevents the degradation of the blood sugar, any one of them may be used to inhibit glycolysis in blood for several days so that the blood sugar level would not be much different from the original value.

The temperature has considerable bearing on the preservative action of any of these substances. Low temperature retards the rate of glycolysis and thus enhances the preservative action. Since the study, which involved mailing the specimens, was not under temperature control, some of the inconsistent results may be due to exposure to varying temperatures encountered in the transportation. The degree of contamination by microorganisms directly affects glycolysis. Roe, et al., reported that preservation is favorably influenced by sterile conditions.[10] In some of our previous work we have found this to be true.

However, the use of sterile technic in holding blood samples is often impractical from the technical aspect.

The concentration of 10 mg. of the fluoride salt per ml. of blood is a convenient one because of its satisfactory anticoagulant effect. Preliminary work in our laboratory with concentrations below 10 mg. per ml. of blood yielded unsatsifactory results.

The effect of sodium fluoride was similar to that of potassium fluoride. It was noted, however, that the initial blood sugar concentrations of the bloods containing the potassium salt were somewhat lower than those of the bloods with the sodium salt. This slight discrepancy is due, we believe, to the fact that the two salts were weighed on an analytical balance. Since potassium fluoride is used as a hydrate ($KF.2H_2O$) and is also hygroscopic, a small experimental error due to dilution is introduced, but this error is insignificant. There is also a very slight error of dilution introduced by the addition of 0.01 ml. of alcoholic thymol. A check was made to determine the effect of the two fluoride salts upon the Somogyi-Nelson method but no such effect was found.

The individual weighing is laborious and time-consuming. It is more convenient to add aliquots of almost saturated solutions to the blood bottle and evaporate the solution. Sodium fluoride can be prepared as a 4 per cent solution whereas potassium fluoride can be prepared as an 80 per cent solution (80 per cent $KF.2H_2O$ corresponds to 50 per cent KF.) Thus 1.25 ml. of the sodium fluoride solution is required for 5 ml. of blood whereas only 0.1 ml. of the potassium fluoride solution is necessary. Since the potassium fluoride solution is quite alkaline, it should be evaporated at low temperature (50° C.) overnight, otherwise it will react with the glass. It is advisable to store the concentrated solution in a polyethylene container for the same reason. If facilities for evaporation are not available, it may be found convenient to add 0.1 ml. or two drops of a saturated potassium fluoride solution to the blood bottle and make a correction of 2 per cent.

Ordinarily potassium fluoride is the preservative of choice when complete evaporation is feasible. Since sodium fluoride does not absorb moisture from the air, it is preferable where dry powder is to be used in preparing the tubes. Because sodium fluoride is somewhat insoluble, it is important to shake the sample thoroughly in order to prevent clotting.*

*Thymol has been reported to interfere, under certain conditions, with the Wilkerson-Heftmann screening method. For this reason either sodium or potassium fluoride alone is recommended when this test is used. Fortunately, thymol does not enhance the preservative effect of either fluoride compound.

TABLE 1

Distribution of percentage of blood sugar readings falling within specified limits by preservative and time after bloodletting—for fifty-one blood specimens drawn on Mondays

Preservative	Hours after bloodletting	Percentage of reading within indicated limits of original blood sugar level ± 4 per cent	± 6 per cent	± 8 per cent	± 10 per cent
Potassium fluoride	24	58	78	91	97
	48	57	79	92	98
	72	62	86	97	99
	96	60	80	92	98
Potassium fluoride and thymol	24	79	95	99	100
	48	72	90	97	99
	72	66	85	94	99
	96	56	77	89	96
Sodium fluoride	24	71	89	97	99
	48	61	81	92	97
	72	77	93	99	100
	96	64	83	93	98
Sodium fluoride and thymol	24	70	88	96	99
	48	54	73	86	93
	72	61	81	92	97
	96	54	74	86	94

TABLE 2

Distribution of percentage of blood sugar readings falling within specified limits by preservative and time after bloodletting—for fifty-six blood specimens drawn on Fridays

Preservative	Hours after bloodletting	Percentage of reading within indicated limits of original blood sugar level ± 4 per cent	± 6 per cent	± 8 per cent	± 10 per cent
Potassium fluoride	72	68	87	96	99
	96	75	92	98	100
	120	63	83	93	98
	144	57	79	92	98
Potassium fluoride with thymol	72	73	90	97	99
	96	75	92	98	100
	120	76	92	98	100
	144	75	93	99	100
Sodium fluoride	72	71	89	97	99
	96	72	90	97	99
	120	68	87	95	99
	144	68	87	96	99
Sodium fluoride with thymol	72	77	93	98	100
	96	74	91	98	100
	120	65	84	94	98
	144	65	84	94	98

SUMMARY

1. A comparison was made of the efficacy of four preservatives, potassium fluoride, potassium fluoride with thymol, sodium fluoride and sodium fluoride with thymol in inhibiting glycolysis in transported blood specimens.

2. All four substances had approximately the same efficacy.

3. Blood sugar levels were maintained within 10 per cent of the original value in almost all the blood specimens for periods up to 144 hours. Eighty-six per cent or better of the blood sugar values were maintained within 8 per cent.

4. Although these substances do not completely prevent glycolysis, they are sufficiently inhibitory to make them clinically useful in blood sugar analysis and diabetes detection.

SUMMARIO IN INTERLINGUA

Preservation de Sucro Sanguinee pro le Detection de Diabete

1. Esseva comparate le efficacia de quatro agentes in le inhibition de glycolyse in specimens de sanguine. Le agentes esseva fluoruro de kalium, fluoruro de kalium con thymol, fluoruro de natrium, e fluoruro de natrium con thymol.

2. Le quatro substantias habeva approximativemente le mesme grado de efficacia.

3. Le nivellos de sucro sanguinee in quasi omne le specimens esseva mantenite durante usque a 144 horas intra un margine de 10 pro cento. Octanta-sex pro cento del valores (o plus) esseva mantenite intra un margine de 8 pro cento.

4. Ben que iste substantias non preveni glycolyse completemente, illos es sufficientemente inhibitori pro esser de valor clinic in le analyse de sucro sanguinee e le detection de diabete.

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The Bony Skeleton and Body Weight

A final contributor to confusion about the meaning of the total body weight is the bony skeleton. The mineral mass in the skeleton averages something like 6 per cent of the normal body weight of the adult but it may be as low as 4 per cent or as high as 9 per cent.[1] There is no evidence that these variations are in any way related to relative obesity except insofar as they may be erroneously included in the inference of obesity from gross body weight.

Perhaps a more important contribution of the skeleton to the body weight is through its form. Relative body weight and overweight and underweight are commonly computed on the basis of weight for height. But a broad and short skeleton automatically means a large body weight per unit of height and no system has yet been devised to allow for this in a practical manner.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955, Chapter "Body Weight, Body Composition and Calorie Status" by Ancel Keys, Ph.D., p. 15.

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