Brief Notes and Comments

An Evaluation of Ketostix Strips

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SUMMARY

The usefulness of Ketostix as a semiquantitative screening procedure for the estimation of acetoacetate concentration in both plasma and urine has been established. Because of the reasonably constant relationship of β-hydroxybutyrate to acetoacetate, Ketostix also gives a good indication of the β-hydroxybutyrate concentration. By reading the Ketostix after thirty seconds instead of the recommended fifteen seconds, the sensitivity of the reaction is increased. The use of Ketostix to examine the plasma from patients with diabetic acidosis will reliably distinguish ketotic from non-ketotic cases and is strongly recommended for this purpose. DIABETES *17*:398-401, June, 1968.

A remarkable variety of qualitative tests for the detection of acetone and acetoacetate exists, and they continue to be devised. The first and one of the best known followed the observation of Gerhardt (1865)[1] that a few drops of ferric chloride added to the urine from diabetic patients often produced a characteristic dark red-brown color indistinguishable from that given by acetoacetic acid. The test was unfortunately nonspecific and gave a positive result with salicylates (as well as phenol and antipyrine) and, although the color developed with salicylates was resistant to heat and that with acetoacetate was not, the usefulness of the test was diminished. Thus, by 1895, Allen[2] in his description of urine examination included more tests involving distillations, iodoform formation, indigo-blue formation, Legal's nitroprusside reaction, and others. Many other color reactions have been described, including 2, 4-dinitrophenylhydrazine, furfural, salicylaldehyde, nitrobenzaldehyde and vanillin.[3] The most recently devised test uses parabenzoquinone[4] but this has no obvious advantages over the nitroprusside reaction, and several considerable disadvantages.

Sodium nitroprusside was originally used for the detection of creatinine in Weyl's reaction (Quoted by Rothera, 1908).[5] The observation by Legal (1884)[6] that a color reaction was also given by acetone provided the basis for the most successful test which has so far been devised. The reaction with creatinine is of interest, and interference by acetone and acetoacetate with the Jaffé reaction for creatinine determination has also been observed.[7] Rothera[5] improved Legal's test by using neutral ammonium salts to increase the intensity of the color and the reaction is now generally known by his name. Its application to the detection of plasma ketones was described by Wishart (1915, quoted by Lee and Duncan, 1950[8]) and this was known as the Rothera-Wishart test. Duncan has for many years advocated this test in the routine management of cases of diabetic ketosis and described a scheme for the calculation of insulin dosage on this basis, but no quantitative studies have been reported.[22]

The nitroprusside reaction detects acetone as well as acetoacetate but is very much more sensitive to acetoacetate.[9,10] For example, Nash et al. (1954)[9] showed that the Acetest tablet can detect as little as 5 mg. per 100 ml. of acetoacetate but only 25 mg. per 100 ml. of acetone. Since the proportion of acetone compared with acetoacetate is always small, the nitroprusside reaction in practice detects only acetoacetate.[9,11,12] Although high concentrations of creatinine could theoretically give false positive results, this is unlikely to occur in practice, and would never occur in plasma.[13] This reaction has, therefore, a high degree of specificity and is also unaffected by a wide range of drugs.[14] The sensitivity of the nitroprusside reaction is greater than that of the ferric chloride test of Gerhardt,[9,14] and this together with its greater specificity, makes it the more useful test.

Development of the nitroprusside reaction for both qualitative and quantitative investigations has resulted in improvements both in speed and accuracy. The use of glycine both to intensify and to stabilize the color has been described[7] and glycine has been incorporated in the Acetest tablet* which presented the nitroprusside test for the first time in a compact form, simple to use, and semiquantitative. The tablet test was not useful for the assessment of plasma acetoacetate, although a crushed tablet or nitroprusside powder is adequate for this

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*Acetest Tablet, Ames Co.: These contain sodium nitroprusside, glycine, dibasic sodium phosphate, and lactose.

purpose.[22] The development of nitroprusside impregnated paper strips, Ketostix,* is a great advance on the older methods. The nitroprusside reaction, including glycine, has recently been employed in two quantitative methods for the determination of acetoacetate,[13,15] but the instability of acetoacetate makes it difficult to assess their accuracy or usefulness.

Standardization of both the Acetest tablet and Ketostix strip tests has in the past been performed by the addition of solutions of acetoacetate to urine and plasma[9,10] but comparisons between the actual blood and urine ketone body concentrations and the Ketostix reactions performed in clinical practice have not previously been reported. The present investigation correlates acetoacetate and β-hydroxybutyrate concentrations (determined by an enzymatic method) with the Ketostix reactions obtained in samples of urine and plasma. Although Ketostix do not react with β-hydroxybutyrate, the ratio of β-hydroxybutyrate to acetoacetate falls within the range 2·73 ± 0·73 (S.D.),[16] and similar ratios have been reported by others.[17,23]

MATERIALS AND METHODS

Samples of plasma and urine were obtained from diabetic patients. Each sample was tested with Ketostix and the resulting purple color compared with the color chart supplied by the manufacturers. The results were recorded as follows:

Ketostix reaction:	o	No color development after thirty seconds.
Trace	±	Definite purple coloration but less than that described as + on the color chart, developing after fifteen seconds; or slight but definite color development after thirty seconds.
Small	+	Purple coloration developed after fifteen seconds and the result read as indicated on the color chart. If the purple color was intermediate, it was recorded as the higher reading.
Medium	++	
Large	+++	

Ketone body concentrations were determined in each of the samples by a modification[17] of the enzymatic method of Williamson et al. (1962).[16] β-hydroxybutyrate determinations are more easily and reliably performed[17] and for this reason have been undertaken more frequently than acetoacetate determinations. The acid-base measurements were made with the Astrup microelectrode (Radiometer, Copenhagen) and the blood sugar determinations were performed with the ferricyanide AutoAnalyzer method.

*Ketostix, Ames Co.: These are impregnated with sodium nitroprusside, glycine and alkaline phosphate buffers.

RESULTS

The results of the comparisons of Ketostix reactions with acetoacetate and β-hydroxybutyrate concentrations are shown in figure 1. Although there was considerable overlap between the groups, the distinction was sufficiently clear to make the Ketostix reaction useful as a semiquantitative screening test. The ranges of acetoacetate and β-hydroxybutyrate concentrations corresponding to each Ketostix strip reaction are shown in table 1. These results were obtained from figure 1 omitting not more than two of the highest and lowest outlying results in each group. The acetoacetate concentrations compared favorably with those indicated by the manufacturers. The highest β-hydroxybutyrate concentrations in each group varied between two and four times the acetoacetate concentrations in the same group, which corresponds with the known ratio of β-hydroxybutyrate to acetoacetate in blood.[16,17,23]

A more detailed analysis of the relationship of acetoacetate concentrations with Ketostix reactions o and ± (as defined here) shows that by reading Ketostix after thirty seconds instead of the recommended fifteen seconds, acetoacetate concentrations of 0·3 mM can readily be detected and in twenty-one determinations, only one false negative result was obtained.[7] The sensitivity of Ketostix to an acetoacetate concentration of 0·3 mM was confirmed by testing with a standard solution. False positive results are unusual, and in the present study only one weakly positive result was recorded at an acetoacetate concentration of less than 0·2 mM.[7]

Table 2 shows the relationship of the plasma Ketostix reaction performed on admission of twenty unselected patients admitted to hospital as emergencies between November 1965 and October 1967, and clinically suspected to have diabetic ketosis. Two patients on initial assessment (Number 1 and 3) were confirmed as true cases of "nonketotic diabetic coma" and in one (2) the acid-base disturbance was entirely due to hyperlactatemia (blood lactate 19·4 mM). Cases 4 and 5 clearly did not suffer from ketoacidosis and the plasma Ketostix reactions (± and + respectively) assisted in the differential diagnosis. Only one patient (6) with a medium

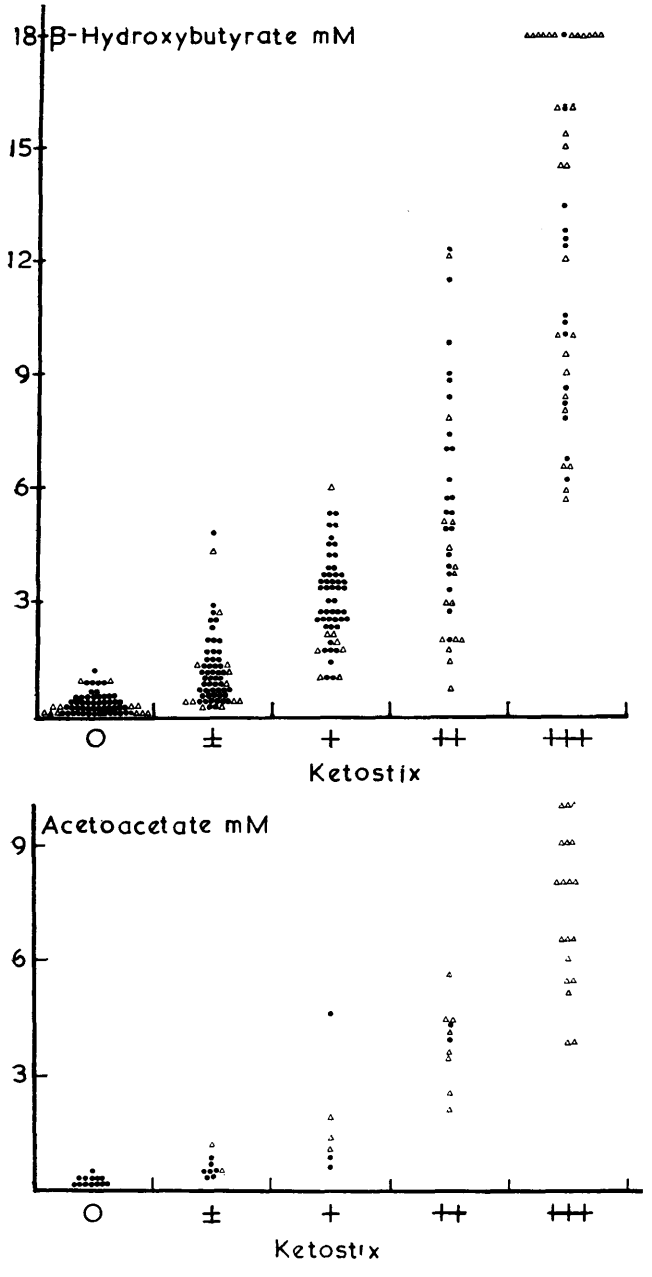

FIG. 1. The correlation of the Ketostix strip reaction with acetoacetate and β-hydroxybutyrate concentrations in plasma (●) and urine (△). + = small. ++ = medium. +++ = large.

(++) plasma Ketostix reaction was not ketoacidotic. Cases 7 to 20 all had medium (++) or large (+++) plasma Ketostix reactions and all these patients were suffering from typical diabetic ketoacidosis, and required large quantities of intravenous fluids and insulin.

Table 3 shows the results of Ketostix tests and β-hydroxybutyrate determinations performed on plasma and urine samples from a healthy nondiabetic subject during a three-day fast. It is clear that relatively low blood levels of ketone bodies may be associated with high urine concentrations and a large (+++) Ketostix reaction.

TABLE 1

Correlation of Ketostix reaction with acetoacetate and β-hydroxybutyrate concentration in blood and urine

Ketostix reaction	0	(±)	Small (+)	Medium (++)	Large (+++)
β-hydroxybutyrate mM	0-0.9	0.3-2.9	1.0-5.3	1.7-11.5	>6.2
Acetoacetate mM	0-0.3	0.3-0.7	0.6-2.0	2.1- 5.6	>5.1
Acetoacetate mM indicated by manufacturer	0-1.0	—	1.0-2.0	3.0- 5.0	>8.0

TABLE 2

The plasma Ketostix reactions on admission of twenty patients with clinically suspected "diabetic ketosis"

Patient	Plasma Ketostix reaction	β-hydroxybutyrate mM	Blood sugar mg. per 100 ml.	Blood *p*H	Base Excess mM
1	0	0.09	600	7.34	— 2.0
2	0	0.9	650	7.39	—16.6
3	±	1.04	1,010	7.33	— 2.5
4	±	1.0	108	7.60	+ 2.6
5	Small (+)	4.9	248	7.33	— 5.0
6	Medium	3.9	303	7.48	+ 2.6
7	(++)	5.4	1,250	7.27	—12.4
8		7.0	825	7.26	—11.5
9		8.0	280	7.22	—18.2
10		8.4	635	7.28	—16.8
11		9.0	415	7.05	—25.0
12		9.8	278	7.10	—19.5
13		13.2	1,165	6.85	—32.5
14	Large (+++)	6.2	250	7.30	—13.4
15		7.8	740	7.25	—11.6
16		8.3	600	7.16	—15.0
17		8.3	450	7.08	—24.5
18		12.4	335	7.07	—25.0
19		12.5	1,100	6.88	—
20		18.0	1,100	6.97	—28.5

DISCUSSION

The development of nitroprusside impregnated paper strips Ketostix is of great value. The present work establishes their reliability and this together with the great simplicity of plasma testing supports the manufacturers' claim that they have superseded the older methods for this purpose.

Urine testing for the presence of the ketone bodies is of limited value in the assessment of ill diabetic patients and the results are confusing. Thus, a strongly positive Ketostix strip test (+++) may be obtained in urine samples when the blood concentration is rela-

TABLE 3

Urine and blood Ketostix reactions and β-hydroxybutyrate (β-HB) concentrations in a nondiabetic subject during a three-day fast

Day	Urine Ketostix	Urine β-HB mM	Blood β-HB mM	Plasma Ketostix
1	0	0.04	0.1	0
2	Medium (++)	2.1	1.2	±
3	Large (+++)	11.8	1.8	±

tively low and at levels which do not contribute to the acid-base disturbance for which a concentration of at least 4 to 5 mM of organic acids is required.[18] This is shown in table 3 in which the Ketostix tests and β-hydroxybutyrate concentrations of plasma and urine are compared during starvation in a healthy nondiabetic subject. In contrast, the absence of ketonuria in the presence of hyperketonemia may occur if renal function is impaired[19] and this is not uncommon in "diabetic coma."[20] Bradley (1965) stated that blood total ketone body concentrations as high as 50 to 70 mg. per 100 ml. (8.6 to 12.0 mM) can occur in the absence of ketonuria. This can result in serious confusion in diagnosis and it is concluded that the use of Ketostix strips for the semiquantitative assessment of the plasma ketone bodies is important in the investigation of these patients.

The present study establishes the usefulness of plasma Ketostix testing in differentiating true cases of diabetic ketoacidosis from those with nonketotic, nonacidotic "diabetic coma" or those in whom another mechanism is responsible for a metabolic acidosis (table 2). Patients in whom an erroneous clinical diagnosis has been made may also be readily distinguished (cases 4 and 5, table 2). It is concluded that a strongly positive plasma Ketostix reaction (++++) always indicates, and a medium reaction (++) usually indicates, a state of typical ketoacidosis. Weaker plasma reactions reliably indicate that the hyperketonemia is not severe.

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