Serum Enzymes in Diabetes Mellitus

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Serum enzymes that show changed activities in diabetes mellitus can be divided into four groups: Group I includes some lysosomal enzymes—β-glucuronidase, N-acetyl-β-glucosaminidase, acid phosphatase, and amylase—that show increased activity correlated with blood sugar concentration. Because lysosomal enzymes as well as liver amylase show latency and may be "activated" by several agents, their increased activity in the serum of diabetics might be a manifestation of an activation occurring in tissues. Group II includes alkaline phosphatase and trehalase, which are increased but not correlated with blood sugar concentration. Their enhanced activity may reflect tissue metabolic disorders. Group III includes enzymes that increase in the postketotic period almost regularly—phosphohexose isomerase—or in only the most severe cases—aminotransferases and several dehydrogenases—because of tissue damage caused by metabolic and circulatory alterations. Cholinesterase, on the other hand, is decreased. Group IV includes any of the above-mentioned enzymes, and still others, that may be more active in diabetics with complications such as hepatic and renal involvement and obesity.

In diabetes mellitus, several serum enzymes have been studied. The data reported are apparently unrelated and often conflicting. However, when they are considered together, a general scheme may be envisaged that provides an understanding of the changes seen in the activities of the various enzymes. The scope of this review is to present and discuss available data on serum enzymes in diabetes mellitus set in such a scheme, as well as to consider some special problems concerning particular enzymes.

The serum enzymes investigated in diabetes mellitus can be divided into (1) enzymes whose activity is usually increased, and (2) enzymes whose activity is usually normal, but which may change in special conditions and in some patients. The first group, in turn, may be divided into (a) enzymes whose serum level is correlated with the concentration of blood sugar, and (b) enzymes that do not show such a correlation. The second group may be divided into (a) enzymes that change in the postketotic period, and (b) enzymes whose activity may increase in diabetics with complications. Therefore, altogether, four groups of enzymes are to be considered (Table 1).

Enzymes of Group I

This group includes β-glucuronidase,1 N-acetyl-β-glucosaminidase, acid phosphatase, and amylase, and is characterized by the occurrence of a correlation of enzyme activity with the blood sugar concentration, and therefore, approximately, with the degree of metabolic decompensation.

The observation of an increased serum activity of β-glucuronidase (1-5) and N-acetyl-β-glucosaminidase (4, 6) in diabetes mellitus, with higher values in women than in men (1, 3-5), has been given conflicting interpretations. Some workers (1, 4) have regarded the enzyme change as being linked to the diabetic condition. Others (2, 3), on the grounds of some data (7, 8) showing a correlation between the activity of these enzymes and atherosclerotic disease, have postulated that the increased activity is linked to the increased susceptibility of diabetics to atherosclerosis. Because β-glucuronidase activity increases in hepatic diseases (9-11), its increased activity in diabetes has been also ascribed to underlying liver involvement (12). Further evidence would suggest that the serum activity of these two enzymes is linked to both the diabetic state (5, 6, 13) and the presence of vascular lesions in either the small or large vessels (5, 6). In fact, in severely decompensated diabetics with various degrees of ketoacidosis the activity of both enzymes in serum is markedly ele-

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1 Trivial and nonstandard names used: β-glucuronidase is β-D-glucuronidase glucuronohydrolase, EC 3.2.1.31; N-acetyl-β-glucosaminidase is chitobiase acetylaminoxydolglycohodalase, EC 3.2.1.29; acid phosphatase is orthophosphoric monoester phosphohydrolase, EC 3.1.3.2; amylase is α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1; alkaline phosphatase is orthophosphoric monoester phosphohydrolase, EC 3.1.1.31; trehalase is trehalose 1-glucosydrolase, EC 3.2.1.28; glucose-6-phosphatase is N-glucose-6-phosphate phosphohydrolase, EC 3.1.3.3; fructose-1,6-diphosphatase is D-fructose-1,9-diphosphate 1-phosphohydrolase, EC 3.1.3.11; phosphohexose isomerase is D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9; aspartate aminotransferase is L-aspartate-2-oxoglutarate aminotransferase, EC 2.6.1.1; alanine aminotransferase is L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2; sorbitol dehydrogenase is L-iditol:NAD oxidoreductase, EC 1.1.1.14; glutamate dehydrogenase is L-glutamate:NAD(P) oxidoreductase, deaminating, EC 1.4.1.3; isocitrate dehydrogenase is three-D-isocitrate:NADP oxidoreductase (decarboxylating) EC 1.1.1.42; malate dehydrogenase is L-malate:NAD oxidoreductase, EC 1.1.1.37; lactate dehydrogenase is L-lactate:NAD oxidoreductase, EC 1.1.1.27; cholinesterase is acetylcholine acyl-hydrolase, EC 3.1.1.8; aldolase is fructose-1,6-diphosphate 3-glycerolphosphate lyase, EC 4.1.2.13; arginase is L-arginine ureohydrolase, EC 3.5.3.1; lipase is glycero-ester hydrolase, EC 3.1.1.3; creatine kinase is adenosine triphosphate:creatinine phosphotransferase, EC 2.7.3.2.

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From the 1st Clinica Medica Generale e Terapia Medica della Università di Catania, Ospedale Garibaldi, 95123 Catania, Italy. Received Jan. 22, 1973; accepted Feb. 27, 1973.
vated, with an increase of up to 283% for $\beta$-glucuronidase and 140% for N-acetyl-$\beta$-glucosaminidase, and is clearly correlated with blood sugar concentration, so that it returns promptly toward normal as metabolic compensation is achieved (13). This indicates that the increased activity is linked to the condition of diabetic decompensation, and not, as postulated by some workers, to a possible presence of liver involvement (12) or to susceptibility to atherosclerosis (2, 3). This view is supported by the normal activity of other enzymes, including aspartate and alanine aminotransferases, observed in most instances (13). The correlation with the degree of glucose metabolic disorder is also confirmed by the statistically significant correlation between enzyme activity and sugar concentration found in large series of diabetics for both $\beta$-glucuronidase (5) and N-acetyl-$\beta$-glucosaminidase (6). On the other hand, the relationship between the activity of these enzymes and the presence of vascular lesions, that previously had been postulated on tenuous grounds (2), appears to be substantiated by the correlation of the higher enzyme activity observed in diabetics with disease of either the small or large blood vessels when compared with the activity in uncomplicated diabetics with similar degrees of hyperglycemia (5, 6). This relationship is also indicated by the finding that the correlation of $\beta$-glucuronidase and N-acetyl-$\beta$-glucosaminidase with blood sugar concentration, present in uncomplicated diabetics, does not occur in diabetics with diseased blood vessels (5, 6). This shows that the presence of vascular lesions is an interfering factor that may modify serum enzyme activity. The failure of some workers (2) to demonstrate the correlation of serum $\beta$-glucuronidase with both the blood sugar and vascular lesions may perhaps be attributed to the fact that the effect of the latter was not studied in diabetics with similar glucose concentrations, and the effect of hyperglycemia was not studied in diabetics without vascular lesions.

Concerning the correlation with sugar concentration, it must be pointed out that experiments in which $\beta$-glucuronidase and N-acetyl-$\beta$-glucosaminidase were assayed before and after addition of glucose to serum showed that glucose per se does not affect the activity of these two enzymes (13).

Since $\beta$-glucuronidase, together with N-acetyl-$\beta$-glucosaminidase and other enzymes, is able to degrade mucopolysaccharides and glycoproteins, its increased activity in the serum of diabetics has been regarded as a defense mechanism against accumulation of these compounds in the walls of blood vessels (2), as occurs in diabetics with vascular disorders. However, it has been pointed out (5) that this hypothesis can apply only for the enzyme change occurring in diabetics with lesions of large vessels, but not for those occurring in diabetics with disease of small vessels. In fact, unlike the walls of large blood vessels, the basement membrane of small blood vessels, which is the site of diabetic microangiopathic lesion, does not contain glucuronic acid, but instead glycoproteins, constituted of characteristic carbohydrate units, made up of glucose-galactose disaccharides (14). Based on the consideration that both $\beta$-glucuronidase and N-acetyl-$\beta$-glucosaminidase are lysosomal enzymes (15), and that lysosomes have a rich complement of hydrolytic enzymes capable of degrading a large variety of compounds, the hypothesis has been put forward (5, 6, 13) that the increase of $\beta$-glucuronidase and N-acetyl-$\beta$-glucosaminidase in serum could be the expression of lysosome involvement in tissues, leading to activation of lysosomal hydrolases. This phenomenon probably occurs in response to the metabolic need to degrade any of these compounds that have accumulated in tissues, such as mucopolysaccharides and glycoproteins in diabetics with vascular disease (5, 6), or various constituents of cells themselves, in a context of increased tissue catabolism, as occurs in diabetics at a rate proportional to the degree of met-

| Table 1. Classification of Changes in Serum Enzyme Activity in Diabetes Mellitus |
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| Enzymes whose activity is correlated with glycemia (Group I) | Enzymes whose activity is moderately increased and not correlated with glycemia (Group II) | Enzymes that may change in the post-ketotic period (Group III)<sup>a</sup> |
| Beta-glucuronidase | Alkaline phosphatase | Phosphohexose isomerase |
| N-Acetyl-beta-glucosaminidase | Trehalase | Aspartate aminotransferase |
| Acid phosphatase | | Alanine aminotransferase |
| Amylase<sup>b</sup> | | Sorbitol dehydrogenase |
| | | Glutamate dehydrogenase |
| | | Isocitrate dehydrogenase |
| | | Malate dehydrogenase |
| | | Lactate dehydrogenase |
| | | Cholinesterase<sup>c</sup> |

<sup>a</sup> Enzymes of group I, being correlated with glycemia, are increased during ketogenic episodes, but rapidly return to normal with the normalization of glycemic level, while enzymes of group III remain elevated for some days in the postketotic period.

<sup>b</sup> Amylase elevation becomes appreciable only in severely decompensated diabetics.

<sup>c</sup> Cholinesterase is decreased in the postketotic period, in contrast to the other enzymes of this group.
abnormal decompensation (13). Unpublished data from this laboratory further supports this hypothesis, for it showed that also a third lysosomal enzyme, acid phosphatase, was increased in 40 adult uncomplicated diabetics, with a mean increase of 148%, statistically significant \( P < 0.01 \).

The observation that in experimental activation of lysosomes, lysosomal enzymes are released from cells (16) and increase in serum (17, 18) has been regarded (5, 6, 13) as supporting the hypothesis described above.

As far as serum amylase is concerned, some workers (19-22) have found decreased activity in diabetics, others (23-29) a normal activity, others (30) variable values, and still others (31, 32) increased activity in untreated diabetics and normal values in diabetics receiving insulin. Furthermore, a definitely lowered activity has been reported in cases of diabetic coma (21, 22), and this diminished amylase activity has been regarded as an index of liver involvement (21, 22).

A series of reports have dealt with the relationship between blood amylase and carbohydrate metabolism (32-35), or hormones that affect carbohydrate metabolism (35-37), and have given rise to the suggestion that blood amylase is affected by the anterior pituitary (36) and by ACTH or cortisol (35, 37), and that “plasma amylase falls during states of increased carbohydrate utilization, and rises following the administration of hormones that diminish utilization of carbohydrate or in physiological states of diminished carbohydrate utilization . . . as when insulin is withheld in patients with diabetes” (38).

Based on these statements, an increased serum amylase activity should be expected in decompensated diabetics.

Indeed, several studies reporting decreased serum amylase activity in decompensated diabetes (21, 22) are perhaps invalidated by the demonstration that determination of amylase activity by unmodified Somogyi saccharogenic methods gives falsely low values because of the interference of glucose, so that the higher the glucose concentration, the lower will be the amylase value obtained (29). Actually, when amylase activity is determined by the amyloclastic method, which avoids this interference (29), the values found in diabetics are normal (29). Studies from this laboratory (39) are in agreement with this finding, having shown a value of 127 (±51) Somogyi units/100 ml of serum in 75 diabetics, and of 124 ± 36 units in 60 normal subjects. In severely decompensated diabetics with ketoacidosis, on the other hand, serum amylase activity is markedly elevated (39-41), and is accompanied by increased urinary excretion of the enzyme (39, 41). This latter finding allows us to exclude the possibility that the increased activity in serum is the result of impairment in renal excretory function, which is often present in diabetic ketoacidosis. Furthermore, the enzyme activity is correlated with blood glucose, so that the values return to normal with the normalization of the blood sugar (39). No definite explanation has been given for either the source of serum amylase or the mechanism of its elevation in diabetic coma. A correlation has been observed between the increased serum amylase activity and dehydration (40) or plasma hyperosmolarity (41), but the nature of these relationships remains obscure. The pancreatic origin of the serum enzyme has been excluded because of the absence of pancreatic lesions at the postmortem examination (40) and the normality of serum lipase (39, 41). A release of amylase from liver cells has been postulated (40). Since it has been observed that the behavior of amylase in the serum of diabetics with ketoacidosis is closely correlated with that of some lysosomal enzymes such as \( \beta \)-glucuronidase, N-acetyl-\( \beta \)-glucosaminidase, and acid phosphatase, it has been suggested that a similar mechanism could be responsible for the change in both amylase and lysosomal enzymes (39).

Liver amylase, although located primarily in microsomes, is present in a “latency” state and is activated by some agents (42) as are lysosomal enzymes. Therefore, it has been hypothesized (39) that, as for lysosomal enzymes, the increased serum amylase activity might reflect an activation occurring in liver tissue in diabetic ketoacidosis. Possibly liver microsomes are less affected than lysosomes by the diabetic state. This could explain why serum amylase changes appreciably only in severely decompensated diabetes with ketoacidosis, in contrast to lysosomal enzymes, whose level is significantly increased also in nonketotic diabetics, as described above.

**Enzymes of Group II**

This group includes alkaline phosphatase and trehalase. The activity of these two enzymes is moderately increased in diabetes, and does not show correlation with the blood glucose.

Alkaline phosphatase activity has been found elevated by 40% \( (P < 0.001) \), with a frequency of increased values of 44%, in a series of 166 uncomplicated diabetics (43). Enzyme activity was correlated with the daily insulin requirement \( (r = 0.263; P < 0.05) \), but not with the glucose concentration or the known duration of disease (43). The enhanced activity of this enzyme has been tentatively interpreted as a manifestation in serum of the increased phoshatase activity that may occur in tissues in the diabetic state. In this condition, increased activity has been reported for glucose-6-phosphatase (44, 45) and fructose-1,6-diphosphatase (46) in the liver. These phosphatases are enzymes distinct from alkaline phosphatase (47). However, owing to some overlapping substrate specificity shown by the phosphatases under consideration (47), and the possibility that an enhanced alkaline phosphatase might be present in tissues of diabetics, it cannot be excluded that phosphatases released from tissues, mainly liver, might contribute to the elevated serum alkaline phosphatase activity (43). Further studies are needed for a better characterization of serum alkaline phospho-
Enzymes of Group III

This group includes several serum enzyme activities (Table 1) that may change in the postketotic period. Phosphohexose isomerase activity is increased in almost all instances. In fact, in our series of 16 cases of diabetes with ketoacidosis (unpublished observations), activity was augmented in the serum of all the subjects but one. The increased activity of this enzyme might be regarded as a result of diffuse tissue damage caused by the complex metabolic disorder occurring in diabetic ketoacidosis. Actually, owing to the high activity of this enzyme in most tissues, it is probable that damage even of a mild degree, but spread over most organs, may give rise to an appreciable serum enzyme elevation. This assumption is in keeping with the observation that an increased serum activity of phosphohexose isomerase is present (50) in cases of cardiac failure and of bronchial asthma with severe anoxia, probably because of generalized metabolic abnormalities. Other enzymes of this group—such as aspartate and alanine aminotransferases, sorbitol-, glutamate-, isocitrate-, malate- and lactate dehydrogenases—were increased only in some instances in our series, mainly in the most severely ill patients, especially when circulatory failure was severe and the liver was markedly enlarged. This suggests that liver damage, primarily caused by congestion and metabolic disorder, might be the cause of these enzymatic changes. The inconstant occurrence of increased lactate dehydrogenase activity in diabetic ketoacidosis may explain why, in small series of patients, either increased activity (51) or normal values (52) have been observed. As concerns serum aminotransferase activity, it must be mentioned that only findings obtained by using kinetic methods with appropriate controls are reliable in diabetic ketosis, because in this condition, when measurement is made by multi-channel chemical analysis, there is interference from acetocetate (53), which may be responsible for the apparent elevation of aminotransferase activity reported by some workers (54).

Serum cholinesterase is unique because its level is decreased 2–3 days after episodes of ketoacidosis (55). Since activity of this enzyme decreases in serum in cases with liver involvement (56), damage to this organ again seems the most probable cause of this enzyme change. As already described, in ketoacidotic patients there is also an elevation of the enzymes of group I. However, as shown in Figure 1, the peak activity of enzymes of group I is simultaneous to the peak of hyperglycemia, and the activity rapidly returns to normal with the normalization of glucose concentration, while the peak of enzyme activities of group III is delayed compared to that of the glucose, and persists for some days during the postketotic period, when metabolic compensation is already achieved. This would indicate that while the enzyme activities of group I are correlated with the degree of metabolic decompensation, the changes in enzymes of group III are rather the expression of tissue damage consequent to the establishment of diabetic ketoacidosis.

Enzymes of Group IV

This group includes a number of enzymes (Table 1) that are usually normal in diabetic patients, but that may increase in some instances as manifestation of complications of the diabetic disease. The most common causes of increased activity of these enzymes in diabetic patients are liver or kidney involvement and obesity. In relatively large series of patients, comprised of 200 (57) and of 130 (58) subjects, increased serum enzyme activity, regarded as an index of liver damage, was reported for aspartate and alanine aminotransferases (57, 58), sorbitol-, glutamate-, and isocitrate dehydrogenases, and arginase (58). The frequency of elevation is different for the various enzymes and reports. In some reports (59) normal activities of both aminotransferases have been found in all instances and enhanced activity of lactate dehydrogenase in some patients. The incidence of enzyme change probably reflects merely the frequency and severity of liver involvement in the series of patients studied. Other enzymes—such as phosphohexose isomerase, malate dehydrogenase and lipase—were found to be almost always normal (unpublished observation), and the same was true for serum creatine kinase (60). In an earlier work (61), a marked elevation of phosphohexose isomerase had been reported in eight of nine diabetics. However, since in that paper the clinical condition of the diabetics studied was not described, it cannot be excluded that they were decompensated diabetics with some degree of ketoacidosis, a condition that almost regularly gives rise to elevation of phosphohexose isomerase activity, as described under Enzymes of Group III. Concerning renal involvement, some workers (62)
have described increased serum activity of phosphohexose isomerase, aspartate and alanine aminotransferases, and lactate dehydrogenase in diabetics with this complication. The same enzymatic changes were observed in diabetics with obesity (62). This last condition has been held responsible also for the increased serum cholinesterase activity (59, 63). The activity of this enzyme, in fact, is increased only in those diabetics who are overweight, suggesting that the elevation is associated with the accompanying obesity rather than with the diabetes itself (63).

Summary

From the data reviewed above we deduce that several serum enzymes may change in diabetes mellitus. The increase of some of these enzymes (groups I and II) seems closely related to the diabetic metabolic alterations, while the change of other enzymes (groups III and IV) are only indirectly related to diabetes, being expression of either acute tissue damage caused by episodes of severe decompensation (changes of enzymes of group III), or complications that may develop during the chronic course of diabetic disease (increase of enzymes of group IV).

We are greatly indebted to Saverio Signorelli, M.D., Professor and Chairman, Department of Medicine, University of Catania Medical School, and Head of the Postgraduate School of Hematology and Metabolic Diseases, for having supported the studies from this laboratory that have been cited in this review.

References


