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Interference by Acetaminophen in the Glucose Oxidase–Peroxidase Method for Blood Glucose Determination

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Acetaminophen, *p*-aminophenol, and oxyphenbutazone interfere with the glucose oxidase/peroxidase method for glucose. Structurally related compounds that lack a free phenolic hydroxyl group (acetanilide, aniline, and phenylbutazone) do not interfere. During the analytical procedure acetaminophen is consumed. One mole of acetaminophen leads to an apparent loss of four moles of glucose. The hexokinase/glucose-6-phosphate dehydrogenase method (Boehringer Hexokinase method) is not affected by these substances.

Additional Keyphrases: *2′,2-diazo-di(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)* • *drug interference with clinical chemical tests* • *analytical error*

Acetaminophen, administered in high doses, is said to decrease blood glucose concentrations. A toxic effect on liver cell function was thought to be responsible (*1*). The possibility of a direct interaction of acetaminophen with the blood glucose determination method, however, was not taken into consideration. Such direct interference of drugs with glucose oxidase/peroxidase (EC 1.1.3.4/1.11.1.7) methods have been described for tolazamide (*2*), ascorbic acid (*3*), isoniazid, hydralazine, and iproniazid (*4*). Therefore, it was of particular interest to investigate whether low blood glucose values measured after ingestion of acetaminophen can also be ascribed to a direct interference with the method.

We show here that acetaminophen interferes with this method, and that this interference is not specific for acetaminophen but also occurs with other substrates that contain a free phenolic hydroxyl group.

Materials and Methods

Reagents

Acetaminophen, *p*-aminophenol, acetanilide, and aniline were purchased from E. Merck, Darmstadt, Germany. Phenylbutazone and oxyphenbutazone were gifts of the J. R. Geigy A. G., Basel, Switzerland. Blood sugar test combinations were purchased from Boehringer Mannheim, Germany. Studies in volunteers were performed with commercially available acetaminophen tablets, which were crushed and suspended in 200 ml of water.

Procedure

In the in vivo experiments, acetaminophen was administered orally to three healthy volunteers after an overnight fast. The fast was continued during the first 3 h of the observation period. We obtained 5-ml blood samples for determination of glucose and plasma acetaminophen concentration immediately before the administration of acetaminophen and during the succeeding 4 h. Blood glucose was determined by the glucose oxidase/peroxidase (GOD-Perid) and the hexokinase/glucose-6-phosphate dehydrogenase (EC 2.7.1.1/1.1.1.49) (Hexokinase) methods, according to manufacturer's instruction. Acetaminophen concentration was measured by the method of Büch et al. (*5*), except that extracting solvent was butanol instead of diethyl ether. The analytical recovery of acetaminophen was 75%.

For the in vitro studies we used the same methods for glucose and acetaminophen. We determined the concentration of the oxidized chromogen 2′,2-diazo-di(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by the absorbance change of the radical cation (ABTS-ox) at 420 nm (*6*), using a molar absorptivity of 21.6 liter $mmol^{-1}$ cm^{-1} (7). The absorbance change was followed in a Beckman spectrophotometer, Model DB-GT.

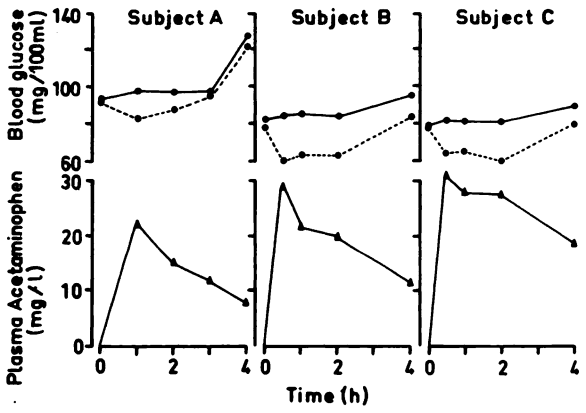

Fig. 1. Simultaneous determination of blood glucose (determined by GOD-Perid and hexokinase methods) and acetaminophen plasma concentration

Glucose determined in serum of three healthy volunteers by the GOD-Perid (● - - ●) and the HK-method (●—●) before (time 0) and during a 4-h interval after a single oral dose of acetaminophen: 2.0 g/80 kg (subj. A, male, 39 y), 2.0 g/70 kg (subj. B, male, 30 y), and 1.5 g/48 kg (subj. C, female, 22 y), resp. Acetaminophen in plasma was concurrently determined (▲ — ▲)

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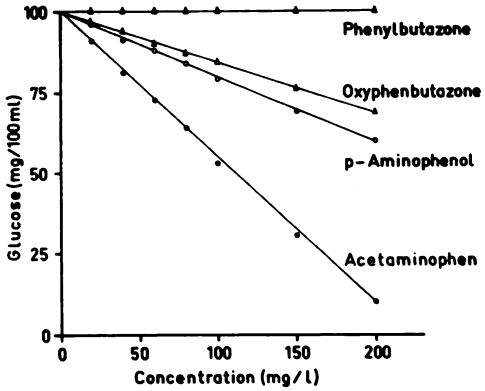

Fig. 2. Interference of acetaminophen and other compounds with glucose determination by the GOD-Perid method

Glucose concentration was determined by the GOD-Perid method in standard solutions containing 1.0 g of glucose per liter of water and various amounts of acetaminophen, *p*-aminophenol, oxyphenbutazone, or phenylbutazone

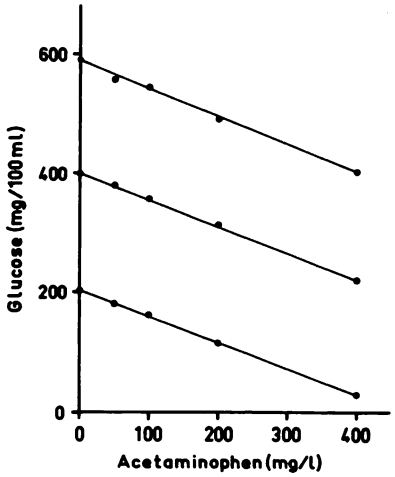

Fig. 3. Influence of acetaminophen on glucose determination by the GOD-Perid method at various initial glucose concentrations

Apparent glucose concentration in solutions containing 2.0, 4.0, and 6.0 g of glucose per liter of water, at various acetaminophen concentrations

Results

In Vivo Experiments

We determined the effect of a single oral dose of acetaminophen on blood glucose values of three healthy volunteers, by the two methods (Figure 1). Immediately before the ingestion of acetaminophen (at time 0), blood glucose concentrations monitored by the GOD-Perid method were either identical or only slightly lower than the corresponding values found with the Hexokinase method. After administration of acetaminophen the blood glucose values obtained with the GOD-Perid method were consistently lower than the values obtained with the Hexokinase method. The difference between both methods was most pronounced at high acetaminophen plasma concentrations and became smaller with decreasing concentrations of acetaminophen in plasma.

In Vitro Experiments

1. The effect of various concentrations of acetaminophen, *p*-aminophenol, oxyphenbutazone, and phenylbutazone on the glucose determination by the GOD-Perid method in an artificial system (1.0 g of glucose per liter of water) is shown in Figure 2. Evidently acetaminophen as well as *p*-aminophenol and oxyphenbutazone interfere with the GOD-Perid method. There was an essentially linear relationship between the concentrations of the interfering drugs and the decrease in glucose readings. None of the three substances interfered with the Hexokinase method. Neither phenylbutazone, acetanilide, nor aniline (not shown in the figure) interfered with the GOD-Perid method.

Figure 3 shows that the effect of acetaminophen is independent of the initial glucose concentration. Over a wide range of initial glucose concentrations, acetaminophen diminished the measured glucose values by the same extent, as can be seen from the parallel shift in the regression lines of the plot for glucose concentration vs. acetaminophen concentration.

2. To study the mechanism of the interference of acetamionphen with the GOD-Perid method in more detail, we measured three components of the system (glucose, ABTS-ox, and acetaminophen) at the end of the usual determination procedure. Table 1 shows the effect of two different acet-

Table 1. Influence of Acetaminophen on Chromogen (ABTS-ox) Formation during Glucose Determination by the GOD-Perid Method

Initial concn[a]		Concn at end of reaction		Concn ratio	
Glucose	Acetaminophen	Glucose	ABTS-ox	Glucose (lost)/ acetaminophen (initial)	ABTS-ox (lost)/ acetaminophen (initial)
		μmol/liter			
20	0	20.5	37.5	—	—
30	0	29.5	53.2	—	—
40	0	39.5	71.8	—	—
20	2	12.5	22.7	4.0	7.4
30	2	21.7	39.4	3.9	6.9
40	2	30.9	56.0	4.3	7.9
20	4	3.3	6.0	4.3	7.9
30	4	12.5	22.7	4.3	7.6
40	4	21.9	39.8	4.4	8.0

[a] Initial concentrations of glucose and acetaminophen were chosen to yield complete consumption of acetaminophen during the incubation period (*cf.* Table 2) in the presence of excess glucose.

aminophen concentrations (2 and 4 μmol/liter) on glucose determination or ABTS-ox formation, respectively. This experiment was performed at initial glucose concentrations of 20, 30, and 40 μmol/liter. Independent of the initial glucose concentration, 2 μmol of acetaminophen per liter diminished the measured glucose values by about 8 μmol/liter as compared with the value obtained in the absence of acetaminophen. At the twofold acetaminophen concentration of 4 μmol/liter, the apparent glucose-diminishing effect was doubled (about 17 μmol/liter). Corresponding results were obtained when ABTS-ox was calculated by using the molar absorptivity (15 and 31 μmol/liter, respectively). From these results we calculated a stoichiometry between glucose and acetaminophen of 4/1. For ABTS-ox and acetaminophen the ratio is about 8/1.

Table 2 shows the concentration of the interfering substance itself, measured at the end of the determination procedure. The relationship between different glucose concentrations and the amount of acetaminophen detectable at the end of the incubation was studied at initial acetaminophen concentrations of 12 and 24 μmol/liter. Table 2 shows that in the presence of glucose there is a considerable loss of acetaminophen during the reactions, which depends on the glucose concentration. Based on the complete loss of glucose during the determination (*cf.* Table 1) a stoichiometry of 4/1 (glucose/acetaminophen) can be calculated.

Discussion

Acetaminophen, oxyphenbutazone, and *p*-aminophenol interact with the glucose determination by the GOD-Perid method. All interfering substances have a free phenolic hydroxyl group. The corresponding compounds without the phenolic hydroxyl group (acetanilide, phenylbutazone, and aniline) do not interfere with the method. In addition, the conjugation products of acetaminophen (acetaminophen glucuronide and acetaminophen sulfate) also did not interfere (unpublished observation). Evidently the hydroxyl group is involved in the interfering reaction. The loss of acetaminophen during the incubation procedure indicates that this compound undergoes a chemical reaction that has not been clearly defined. It is likely, however, that acetaminophen is involved in an oxidation process, because a loss of acetaminophen was also observed during its incubation with hydrogen peroxide and peroxidase (unpublished observation). Therefore, in the presence of acetaminophen a competition of oxidative equivalents might occur so that the chromogen ABTS is oxidized to a smaller extent. The high ratio of 8/1 (ABTS-ox/acetaminophen) suggests that other reactions than oxidation may also be involved.

Our results show that acetaminophen interferes with the GOD-Perid method even at therapeutic concentrations, but will produce completely misleading results in cases of acetaminophen intoxication.

Table 2. Influence of Glucose on Acetaminophen Concentration during Glucose Determination by the GOD-Perid Method

Initial concn[a]		Acetaminophen concn at end of reaction	Concn ratio: glucose (initial)/acetaminophen (lost)
Acetaminophen	Glucose		
μmol/liter			
12	0	12.0	—
12	24	5.5	3.7
12	48	0.0	4.0
24	0	24.0	—
24	48	10.8	3.6

[a] Initial concentrations of glucose and acetaminophen were chosen to correspond to complete glucose loss as evidenced from the lack of ABTS-ox formation (*cf.* Table 1).

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References

1. Davidson, D. G. D., and Eastham, W. N., Acute liver necrosis following overdose of paracetamol. *Br. Med. J.* **ii,** 497 (1966).

2. Sharp, P., Riley, C., Cook, J. G. H., and Pink, P. J. F., Effect of two sulphonylureas on glucose determinations by enzymic methods. *Clin. Chim. Acta* **36,** 93 (1972).

3. Peterson, J. I., and Young, D. S., Evaluation of the hexokinase/glucose-6-phosphate dehydrogenase method of determination of glucose in urine. *Anal. Biochem.* **23,** 301 (1968).

4. Sharp, P., Interferences in glucose oxidase-peroxidase blood glucose methods. *Clin. Chim. Acta* **40,** 115 (1972).

5. Büch, H., Pfleger, K., and Rüdiger, W., Nachweis und Bestimmung von Phenacetin, *N*-Acetyl-*p*-aminophenol sowie ihren Hauptumwandlungsprodukten in Harn und Serum. *Z. Klin. Chem. Klin. Biochem.* **3,** 110 (1967).

6. Werner, W., Rey, H.-G., and Wielinger, H., Über die Eigenschaften eines neuen Chromogens für die Blutzuckerbestimmung nach der GOD-POD-Methode. *Z. Anal. Chem.* **252,** 224 (1970).

7. Kahle, K., Weiss, L., Klarwein, M., and Wieland, O., Klinisch-chemische Erfahrungen mit einem neuen Chromogen für die Blutzuckerbestimmung nach der GOD-POD-Methode unter Verwendung eines automatischen Analysiergerätes. *Z. Anal. Chem.* **252,** 228 (1970).