تأثير الخزن على نتائج قياسات بعض المواد الكيموحياتية في مصل الدم

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الخلاصة

المقدمة: يعتبر حامض اليوريك والكولسترول و اليوريا و الكلوكوز و إنزيم الفوسفاتيز القاعدي من المواد ذات الأهمية من الناحية الكيماوية و الحياتية وقد وجدت أنماط متنوعة من التغيير في تركيز هذه المواد بمرور الفترات الزمنية المختلفة. تهدف هذه الدراسة إلى دراسة العلاقة بين أنماط التغيرات المحتملة في مستويات المواد الموضحة أعلاه في المصل مع الزمن.

طريقة العمل: تم أخذ عشرة عينات من ألدم من أناس أصحاء وبعد فصله والحصول على المصل وتم قياس بعض المواد الكيموحياتية (حامض اليوريك والكولسترول و اليوريا و الكلوكوز وإنزيم الفوسفاتيز القاعدي) في مصل الدم. أعيدت هذه القياسات لدراسة لتأثير الخزن على تركيز هذه المواد في مصل الدم عند فترات زمنية مختلفة (1, 2, 3, 4, 24, 48) ساعة.

النتائج: أظهرت النتائج أن جميع المواد المدروسة في البحث تتناقص بتأثير الخزن ويكون هذا ألتناقص ضئيل أو كبير فسرت النتائج اعتمادا على الخواص الكيميائية لكل مادة ويتعاظم النقصان بمرور الزمن.

الاستنتاج: استنادا للتحاليل المختبرية هنالك تأثير للخزن على نتائج حامض اليوريك والكولسترول و اليوريا و الكلوكوز وإنزيم الفوسفاتيز القاعدي في مصل الدم ويوصى بإجراء التحليل المطلوب مباشرة بعد سحب العينة. إذا تأخرت العينة فيجب أن تؤخذ النسب المستخرجة من هذا البحث بنظر الاعتبار لتعديل النتائج إلى قيمها الحقيقية.

مفاتيح الكلمات: حامض اليوريك ,الكولسترول, الكلوكوز , إنزيم الفوسفاتيز القاعدي, اليوريا, الخزن, المصل.

EFFECT OF STORAGE ON THE LEVEL OF SOME SERUM BIOCHEMICAL PARAMETERS

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Abstract:

Background : Uric acid, cholesterol, urea, glucose, and alkaline phosphatase are of biological and chemical importance. A variety of changes in the concentration of these parameters were found with time. The aim of the present study is to determine the possible changes in concentration of biological parameters under storage.

Methods: The serum was separated from ten healthy persons and the biochemical parameters were measured immediately using the routine methods that used in the laboratories. The measurements were repeated at different intervals (1, 2, 3, 24, and 48 hours).

Results: The results showed a decrease in all the measured biochemical substances in the research and the amount of decrease depends on the time. The decrease depends on the chemical type of each substance.

Conclusion: It was found a negative relationship between the storage time and each of the measured biochemical parameters in serum; uric acid, cholesterol, urea, glucose, and alkaline phosphatase. It is recommended that the analysis should be done immediately after blood aspiration. If the sample delayed then the results should corrected according to the ratios that obtained in this work.

Key words: Uric acid, cholesterol, urea, glucose, and alkaline phosphatase, storage.

Introduction:

In the daily routine work in clinical laboratories, the storage of some biological samples (e.g. serum, urine, and body fluids) may be required for different reasons. In most Iraqi hospitals, the blood aspiration started from 8 AM and the measurement carried out at 11AM in order to be measured in one batch. Hence there is a gap of about 2-3 hours between the blood aspiration and the measurements. This delay affects the results and subsequent judgment obtained from these results for diagnosis, treatment, and follow up. In many cases, some samples have been received to the laboratory at a late time in the night, some reagents may be exhausted, and some samples may be transferred into other laboratories for analysis that is not found in the laboratory. In addition to the fact that, some patients are unable to come to the laboratory due to their severity of illness. Hence, the blood is aspirated at their houses and transferred into the laboratory. In Iraq, there is another chronic problem that may be not found in other country; the electricity supplied may be shutdown at any time during the analysis for prolonged time. Hence the storage of biological samples especially serum is required for these reasons. In order to obtain an accurate results, the analyst should know the possible biochemical changes (qualitatively and quantitatively) that occurred if he had to store the serum for different intervals.

Accurate determination of uric acid concentrations in different biological samples is essential for the diagnosis and classification of gout according to uric acid metabolism derangement. Various studies indicated a significant decrease in uric acid concentration after preservation in different samples [(1)], regardless of the storage temperature. Uric acid crystals were often observed in urine of these cases which showed a marked decrease in urinary uric acid concentration after storage [(2)]. The results of serum uric acid measured by the convenient methods and newly developed methods[(3)] for estimation of serum uric acid, were affected by serum storage for different times[(3-4)].

Ono *et al* (1981) [(5)] studied the effects on 25 analytes of duration of contact of serum with non-anticoagulated blood and of temperature. Serum was separated after blood was allowed to stand, for 0, 2, 4, 6, 8, 24, or 48 h at 4, 23, or 30°C and different profiles of changes were obtained i.e. some biochemicals changed while others are not changed [(5)].

The effect of different substances partly used as preservatives for the blood storage and at different stages of manufacturing of human blood preparations on the dynamics of nonenzymatic deamidation of commercial protein preparations and on their heat stability was being studied [(6)].

In renal failure (acute or chronic), the management either conservative or dialysis depends on the correct results of the laboratory, and if these results wrong or changed [(7, 8)], it may causes a crisis for the patients state.

Some studies were carried out on the effect of storage on animal blood components[(9, 10)] because the time lost between the aspiration of blood in the field of the study and measurement time in veterinary clinics. In the present work, the human sera are used in order to study the effect of storage on serum components.

Many studies focused on the effect of storage time and conditions on the measurement of different blood parameters and different results were obtained [11]. Hence, serum parameters that selected for the present work were chosen because they are routinely measured. Serum cholesterol is an important parameter for diagnosis of atherosclerosis and other heart disorders [12-13]. Hence, the estimation of serum cholesterol precisely is an important issue for treatment, diagnosis and follow up.
Serum ALP level changes in different disorders, liver cholestasis, Paget's disease [14], bone disorders [15], and some types of cancer [12]. In cholestasis state (intra- or extra-hepatic), serum ALP decide whether the treatment surgical or medical.

The correct estimation of serum sugar is very important for clinical evaluation of the patients (diagnosis, classification, and choosing the suitable treatment) [12]. The goal of the present work is to give a factor can be used to correct the measured values (if present) depending on the time of delay between aspiration of blood and laboratory work.

Materials and Methods:

Blood Samples: Ten milliliters of venous blood samples were collected from ten healthy males aged (22-43 years). The samples were taken after 3-5 hours of food ingestion and before taking any medications. Sera were separated and each serum samples distributed into aliquots of 0.5ml in seven tubes. Serum alkaline phosphate, cholesterol, urea, uric acid, and glucose were measured at different intervals (1, 2, 3, 4, 24, and 48 hours) at room temperature.

Cholesterol Estimation:

Total serum cholesterol was measured by using cholesterol kit (PAP 100 bioMerieux) according to Richmond (1973) [16]. The principle of this method was to lysis the cholesterol ester to the cholesterol and fatty acids, and then oxidized it to get the quinoemine:

$$Cholesterol\ ester \xrightarrow{Cholesterol\ esterase} cholestrol + fatty\ acids$$

$$Cholesterol \xrightarrow{Cholesterol\ oxidase} cholest-4-en-3-one + H_2O_2$$

$$2H_2O_2 + phenol + 4-a\min oantipyrine \xrightarrow{peroxidase} quinoe\min e + 4H_2O$$

The absorbance was measured spectrophotometrically at 500 nm after five minutes at 37˚C.The intensity of the color produced was directly proportional to the total cholesterol concentration in the sample [16].

Random Blood Sugar Estimation:

Random blood sugar levels were estimated using a kit supplied by DiaSys® according to the enzymatic colorimetric test (glucose oxidase method). The principle of the method depends on the oxidation of glucose molecules to produce hydrogen

peroxide which reacts with 4-aminoantipyrine in the presence of phenol to produce a colored substance that absorbed maximally at 500nm.

$$Glucose + O_2 \xrightarrow{GOD} Gluconic\ acid + H_2O_2$$

$$H_2O_2 + 4-Aminoantipyrine + Phenol \longrightarrow Chinonimine + 4H_2O$$

Principle of Urea Estimation:

Serum urea was measured using urease enzyme methods according to the following reaction:

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

Ammonium ion reacts with Salicylate and hypochlorite to give colored complex [17].

Principle of Alkaline Phosphatase Estimation:

Colorimetric determination of ALP activity according to Sigma® kit manual depending on the following reaction :

$$Phenylphosphate \xrightarrow[PH\ 10]{AlKaline\ Phosphatase} phenol + phosphate$$

The phenol liberated is measured in the presence of amino-4-antipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction [18].

Principle of Uric Acid Estimation:

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide, according to the following equation.

$$Uric\ acid + O_2 + H_2O \xrightarrow{Uricase} Allantoine + CO_2 + H_2O_2$$

$$2H_2O_2 + 4Aminophenazone + 2\text{-}4Dichloro\text{-}phenol\text{-}sulfonate \xrightarrow{Peroxydase} Quinoneimine + 4H_2O$$

Results:

The results obtained were shown in Figures (1-5) which indicate the decrease in serum urea at different intervals and a maximum decrease occurred after 48 hours Figure (1). Figure (2) showed a decrease in serum ALP with time. Figure (3) showed a decrease in serum uric acid at different intervals. The results showed a maximum decrease after 48 hours. Random blood sugar showed a decrease at different intervals Figure (4). The decrease in random blood sugar was more than any other serum components. Figure (5) showed the decrease in random blood cholesterol at different intervals. The results showed that cholesterol is the most stable component for storage among the other measured parameters.

Discussion:

The results of the present work showed a decrease in all the measured serum components. These results are not agreed with the results of other researchers. Ono *et al* (1981) [5] results for serum components including ALP, total cholesterol, serum urea nitrogen, and uric acid were not influenced by storage at 4, 24, or 30 ºC for as long as 48 h. Statistically significant changes were seen for total protein and calcium after 48 h at 30 ºC; for glucose at 24, 4, or 2 h of storage at 4, 23, or 30 ºC [5].The reason for decrease serum urea is due to degradation of urea into ammonia with time due to decrease activity of the enzyme as a result for biodegradation or change in the activity of serum. While the decrease in the serum glucose may be due to the fermentation by the living organisms that may affect the serum during the storage at room temperature.

Alkaline phosphatase as enzyme can be stored effectively at different temperatures and maintain high percentages of its activity by addition of albumin and trehalose [19]. In serum, albumin can be considered as a partial protector for ALP activity [19] and hence the decrease in the activity was not high. Addition of Zn^{+2} plus Mg^{+2} markedly stimulated and completely protected the ALP activity [20]. However, other researchers consider these differences were thought to be due to laboratory variability [21].

Other study showed the effects of storage at room temperature (23-25 °C) and refrigeration (4-5 ºC) on various biochemical constituents of camel serum were investigated. Some parameters including cholesterol and alkaline phosphatase (ALP) did not change when stored at 4-5ºC over 9 days, glucose remained stable for 6 days; total protein for 7 days; and blood urea nitrogen for 8 days. Stability at room temperature for ALP was 8 days[9]. Also, the effect of temperature and duration of storage on clinico-chemical variables was investigated in Na heparinized plasma and plasma or serum of Na heparinized whole blood and whole blood of horses, respectively. The values of AST, Gamma-GT, ALP, bilirubin, cholesterol, urea, total protein, albumin, and electrolytes varied in all the sample substrates investigated at 20-22 ºC and 4 ºC less than 10% during the observation period of up to four days[10].

Many studies found that the addition of different substances to the stored samples reduces the changes in the level of the stored serum constituent. Examples are the addition of exogenous pyridoxal phosphate to serum improved stability of AST storage at ambient temperature (22 ° C) from 1 day to 7 days [22], glycerol added. To the reconstituted serum maintained maximum activity before refrigeration during either storage for 30 days or on repeated freezing and thawing [23], and a porcine collagen peptide fraction recently used as a safe and advantageous stabilizer for addition to biological products with a view to long-term lyophilized storage and short-term liquid storage [24].

In other research suggested a new method for estimation of serum uric acid, serum storage for 72 hours at room temperature resulted in a significant ($p < 0.0005$) increase in measured uric acid [3]. This result interpreted in the means of the interferences of the chemicals formed in the serum with the reagent of the kit [3]. Certain diagnostic kits that measure serum urate by the principle of enzymic liberation of oxygen and its

combination with chromogens can give results for urate in fresh serum that are approximately 20% lower than results from serum stored at ambient temperature for 72 h [4]. In fresh serum, antioxidants compete with chromogen for liberated peroxyl-oxygen. So it was postulated that during storage the interfering antioxidant substances are destroyed. In some diagnostic kits, L-ascorbate oxidase is added to the reaction, eliminating some but not all of this effect. [4]. Same interpretations may be applied to explain the decrease in uric acid concentration in the present work.

Other workers [25] compared the differences between results of serum aliquots assayed immediately for 12 constituents and frozen aliquots accumulated and assayed. Storage at -20ºC for 15 weeks had a mild destructive effect on two enzymes in serum. The control serum data revealed significant linear trends in magnesium (upwards) and ALP (downwards). In the other 10 constituents tested, comparison of variances indicated that long-term (weeks) variation in control serum assays is similar to the difference of variation between aliquots assayed immediately and those frozen and assayed at the same time [25].

To study the effect of storage on the measurement of blood constituent from different sources, the following enzymes were determined in the serum and plasma of man, dog and rat: alanine aminotransferase (ALT), asparate aminotransferase (AST), ALP, lactate dehydrogenase and alpha-hydroxybutyrate dehydrogenase. The enzyme-activities changed by variable amounts during standing of the blood at 25 ºC . This concerned mainly lactate dehydrogenase, alpha-hydroxybutyrate dehydrogenase and AST in the serum of the rat. In human serum and in dog serum, and in the plasma of man, dog and rat this effect was only less pronounced [26].

In other study which measured the concentrations of 29 commonly measured analytes in fresh sera and in sera that had been stored as whole blood at seven different temperatures for 24h. Significant differences were observed for concentrations of creatinine, glucose, inorganic phosphorus, potassium, and both aminotransferases. The extent of these differences was temperature dependent. Values for the remaining 23 analytes examined were essentially unaffected by the storage [27].

Conclusions and Recommendations:

The investigation for serum; uric acid, cholesterol, urea, glucose, and ALP give false results when the serum stored for different intervals. It was found a negative relationship between the storage time and each of the measured biochemical parameters. This may lead to misleading clinical diagnosis, treatment, and follow up of the patients.

It is recommended that the analysis should be done immediately after blood aspiration. If the sample delayed then the results should corrected according to the ratios that obtained in this work.

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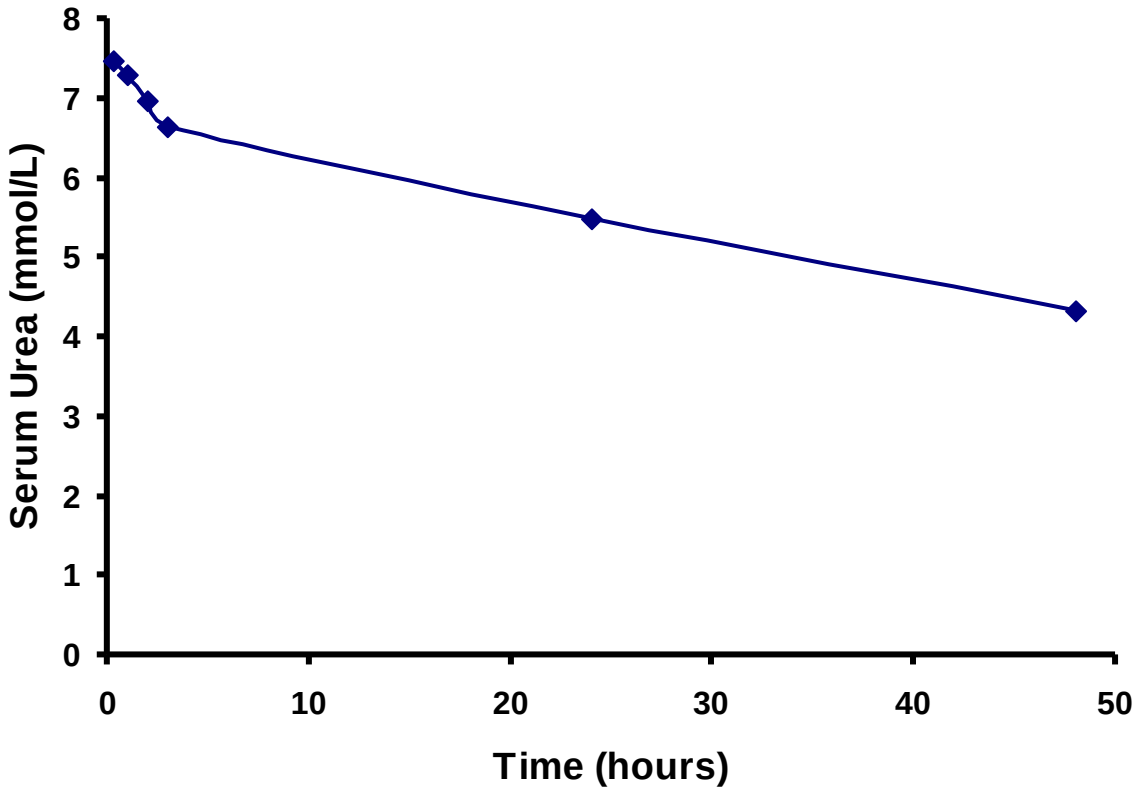


Figure (1): Change in Serum Urea with Time.

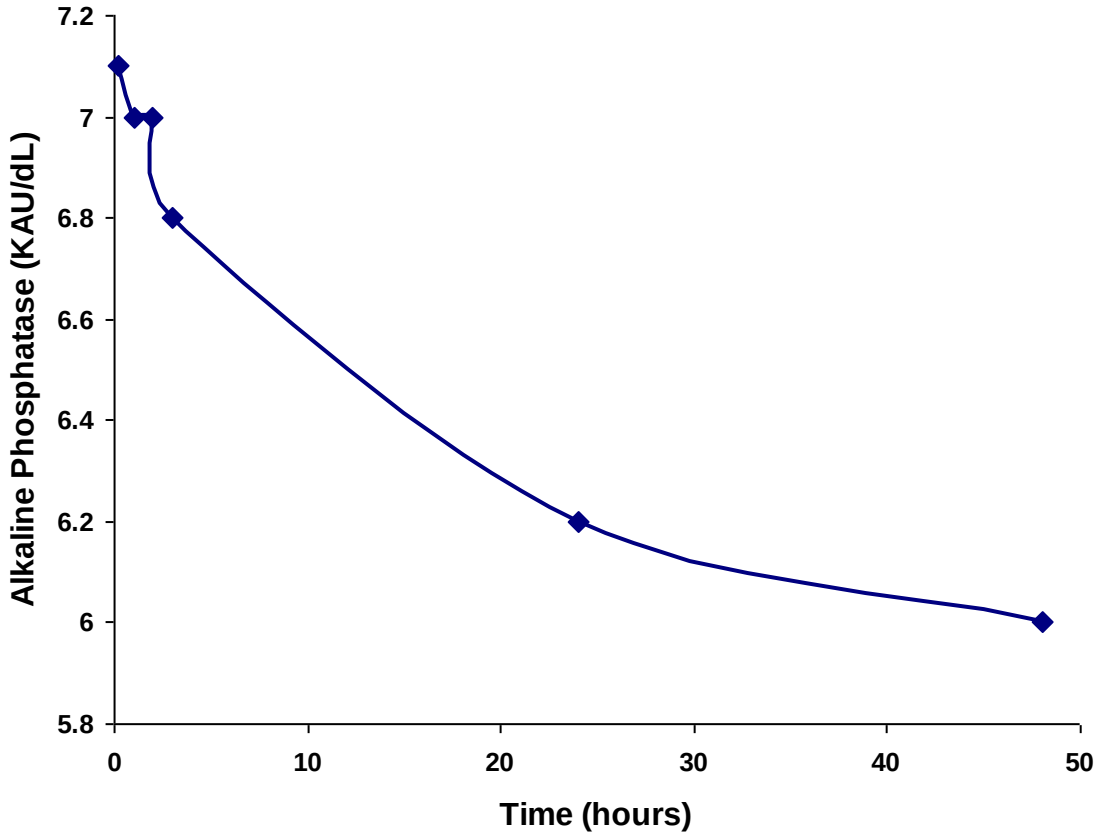


Figure (2): Change in alkaline phosphatase with Time

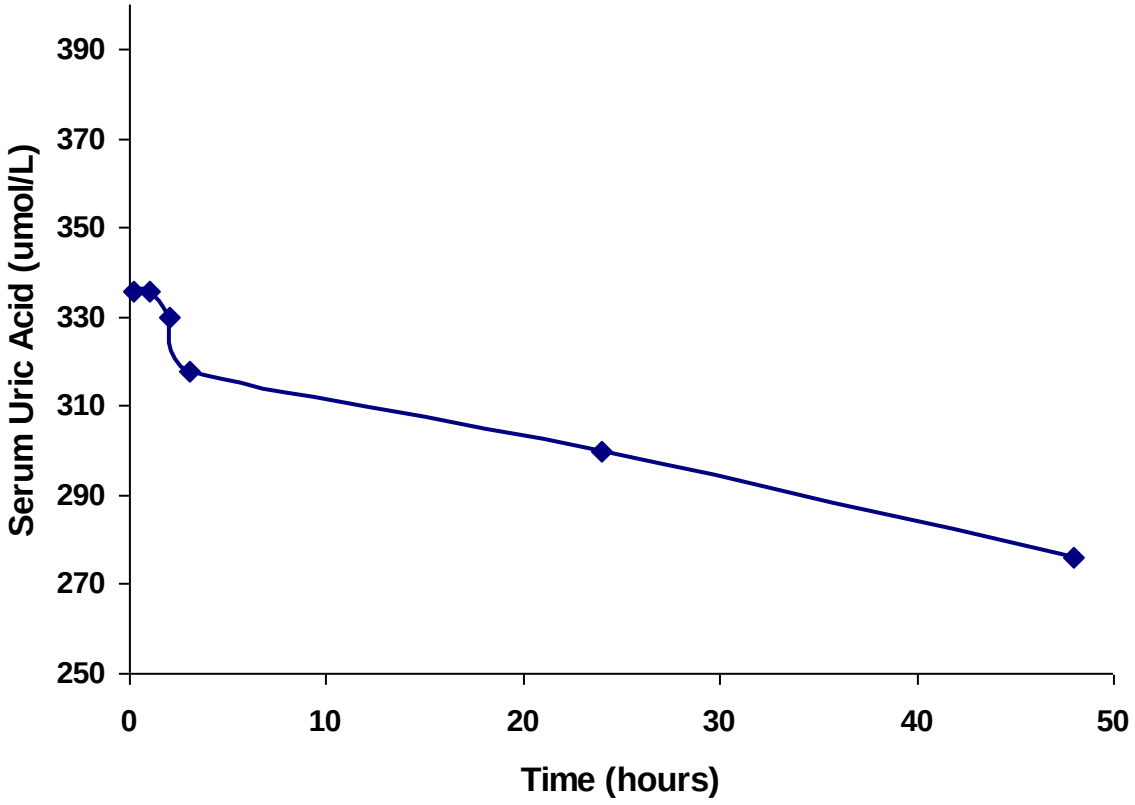
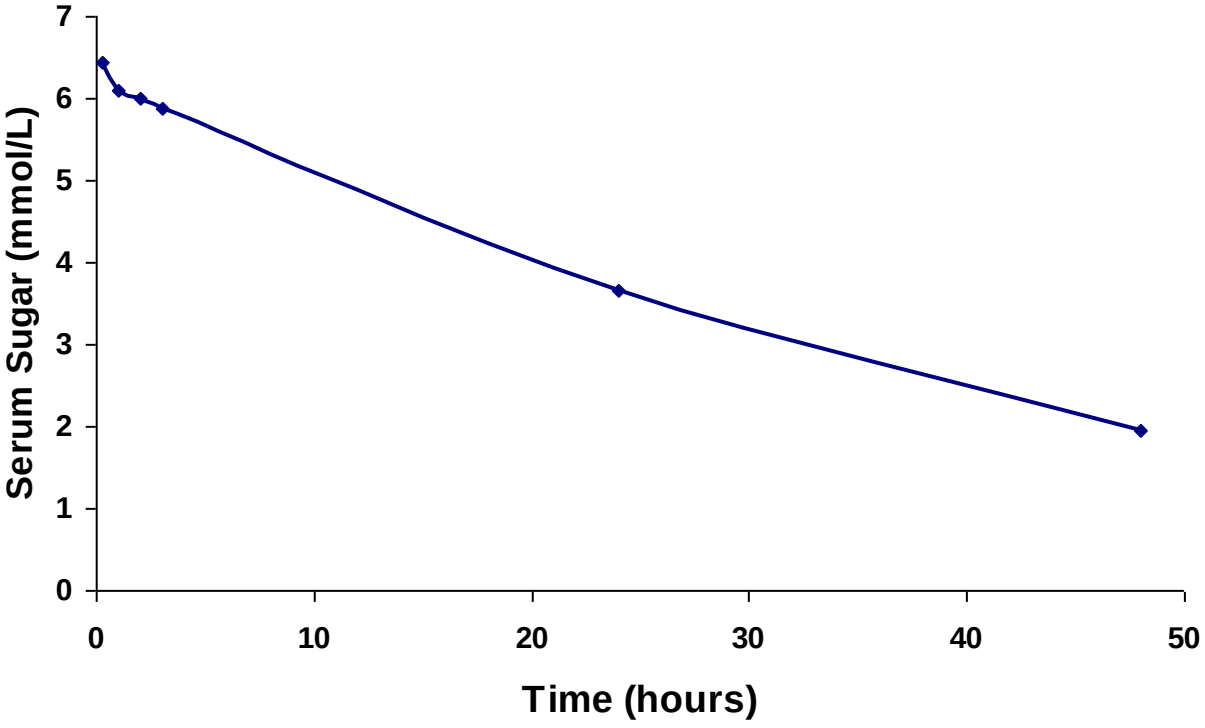


Figure (3): Change of the serum uric acid with time.



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gure (4): Change of serum sugar with time.

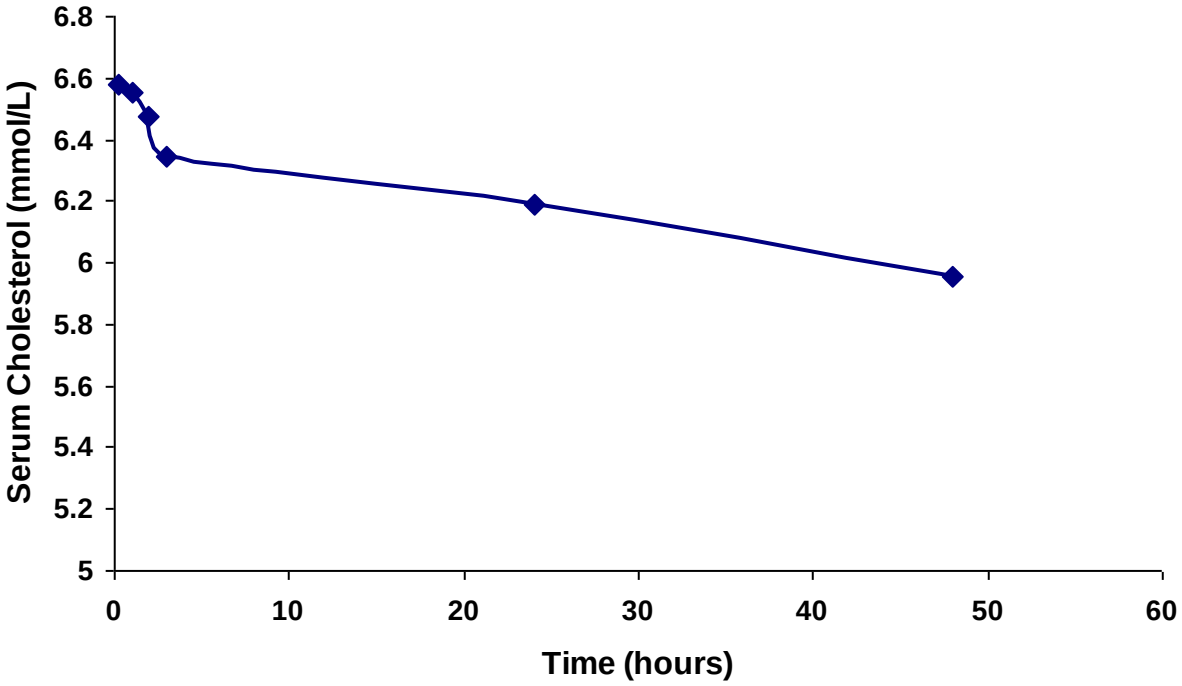

Figure (5): Change of Serum Cholesterol with Time.

Table (1): Factors to be multiplied by results obtained after elapsing of different time intervals.

Time/Hours	Cholesterol	Sugar	Uric acid	Alk. Phosph.	Urea
1	1.005	1.037	1	1.014	1.023
2	1.017	1.049	1.018	1.014	1.071
3	1.038	1.06	1.057	1.044	1.125
24	1.064	1.417	1.12	1.145	1.364
48	1.106	2.074	1.217	1.183	1.731

Table (2): Percentage of deficiencies in some serum analytes after elapsing of different intervals.

Time/Hours	Cholesterol%	Sugar%	Uric acid%	Alk. Phosph. %	Urea%
1	0.394	3.529	0	1.408	2.222
2	1.575	4.706	1.786	1.489	6.667
3	3.543	5.882	5.357	4.225	11.111
24	5.906	29.412	10.714	12.676	26.667
48	9.449	51.765	17.857	15.493	42.222