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دراسات من التحلل الدموى - خارج الأوعية وتأثيره على بعض
مكونات مصل الدم

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اجرى البحث على عدد أربعون عينة من مصل الدم فى الماعز المصرى ولقد عينت نسبة الهيموجلوبين فى هذه العينات وكان متوسطها ١٢٣٣٧٣ مجم / سم^٣ وكذلك عينت نسب بعض المكونات الأخرى مثل البيليروبين وانزيم الاسبرتات أمينوترانسفيريز وكذلك اجمالى بروتين الدم والبوتاسيوم والكلوريد وعينت معدلاتها الطبيعية .

وكذلك اجرى تجربة تحلل الدم خارج الأوعية ومدى تأثيره على المكونات الأخرى التى أجريت الدراسة عليها . هذا وقد لوحظ أن الدرجات البسيطة من التحلل الدموى لها تأثير واضح على الهيموجلوبين والانزيمات بينما هى عديمة التأثير والتغيير فى نسبة الهيموجلوبين وتركيز انزيم اسبرتات أمينوترانسفيريز والبوتاسيوم وكذلك البروتين الاجمالى .

EFFECTS OF IN - VITRO HAEMOLYSIS SOME CONSTITUENTS
OF GOATS SERUM
(With 3 Tables)

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SUMMARY

Serum hemoglobin as well as some other serum parameters, were determined in 40 healthy Egyptian Goats. Mean values of serum hemoglobin was 120.78±5.95 mg/100 ml; also the values of serum - aspartat amino transferase; serum total bilirubin; serum total protein; serum potassium and serum chloride were estimated. In vitro hemolysis was made; the effect of in-vitro hemolysis was studied. A significant adultrations of values of serum hemoglobin; serum spartat amino transferase; serum potassium were marked with silght and severe degree of hemolysis, while other parameters were less or not affected.

INTRODUCTION

In the use and interpretation of laboratory data for the dignosis and mangment of the disease, many factors come in consideration. When errors arise one think immediatly of the analysis itself or the computation of the results just as important in this respect however are errors which arise from improper collection and preservation of specimens for analysis. In order to be assured of over - all accuracay of results - some degree of quality control must necessarily practiced in clinical chemistry. This required adequate personal and equipment accurate standard, clean glassware and so on. As more instrumental analysis is used awarness of limitation and potential sources of errors in such equipment imperative.

Serum is preferable to whole blood or plasma for biochemical analysis because of better preservation of constituents, better correlation with interstitial fluid levels, less chance of alteration due to hemolysis and avoidance of possible errors from added anticoagulent, (MARTINEK, 1966 and CARAWAY, 1962). Normal serum appears visibly hemolytic when the concentration of hemoglobin exceeds 0.02 gm%. Much higher levels could remain undetected in jaundce serum (BEHRENDT, 1957). EL-SEBAIE (1981) reported that the bovine serum showing hemolysis when the serum hemoglobin level exceed 162 mg/100 ml, while hemoglobin value in bovine serum was 116.48 mg/100 ml.

Hemolysed specimens do arrive at laboratory quite regulary and the convenience difficulty or even impossibility of obtaninig more a clear specimens from the patients makes it nessary to decide whether the assay will be refused or performed. In hemolysed serum the cellular contribution to the analysis will be governed by the intercellular concentration of the substances be analysed. The problem of hemolysis is most important if the concentration of measured constituents is much greater in the red cells than in the plasma. Depending upon the ratio of these concentration slight hemolysis can produce significant errors. Concentration of serum potassium, serum aspartat amino transferase, serum lactic dehydrogenase and serum bilirubin were significantly elevated with moderate degree of hemolysis (McGANN and CARTER, 1960; LAESSIG *et al*, 1976; DORNER, *et al*, 1981 and EL-SEBAIE, 1981).

Studies on hemoglobin concentration in goats serum are very scanty in literarature, also the adultration effects of hemolysis on serum constituents were not sufficiently studied. The purpose of this investigation is to determine the values of serum hemoglobin in healthy goats as well as the estimation of some parameters, in addition to the determination of the extent of alteration of values of such parameters under the influence various degree of hemolysis.

MATERIAL and METHODS

1- Blood samples were collected from 40 native Egyptian goats. Samples were collected from the jugular vein, and serum was separated by ordinary methods of hematology. Collection of blood samples and serum-separation were carried out very carefully to avoid hemolysis.

2- Preparation of hemolysate:

Anti-coagulated blood sample (EDTA), was used in this purpose. Blood was centrifugated at 3000 r.p.m. - plasma was separated. Red blood cells were washed three times by the use of normal saline (0.9%). Washing and centrifugation were repeated several times, and the plasma poured-out. Washed red cells were exposed to repeat freezing and thawing - to ensure complete destruction of the red blood cells. Destructed red blood cells were centrifugated, and clear supernatant poured off and used as hemolysate. According to the visual appearance of the serum, serial dilutions from the hemolysate were made by the use of clear non-hemolysed serum of goats.

The samples were classified according to the density of colour as follows:

- 1- Non hemolysed serum (-)
- 2- Slight hemolysed serum (+)
- 3- Severe hemolysed serum (+++)

Biochemical methods:

The following parameters were determined in each samples:

- 1- Serum hemoglobin was determined by the method modified after RICHTERICH, (1978).
- 2- Serum potassium was determined flamephotometry by Corning - flamephotometer.
- 3- Serum chloride was determined by Corning - chloride meter.
- 4- Serum aspartat amino transferase (AST), serum total bilirubin, serum total protein were estimated by the use of Boehringer reagent - kits (W. Germany).

RESULTS

The obtained results in this experiments were presented in tables (1,2,3). The mean values of serum hemoglobin in healthy goats was 120.73 ± 5.95 mg/100 ml. The mean values of other measured serum were, serum aspartat amino transferase (23.6 ± 3.16 U/L); serum total bilirubin (11.35 ± 2.80 μ mol/L) serum total protein (99.08 ± 1.11 g/L), serum chloride (109.6 ± 2.45 mmol/L) and serum potassium (5.26 ± 0.93 mmol/L).

The effect of in-vitro hemolysis on the values of the measured parameters were shown in tables (2,3). The mean values of serum hemoglobin, aspartat amino transferase, serum total bilirubin, serum total protein, serum chloride and serum potassium under the influence of severe hemolysis were 462.06 mg/100 ml, 47.80 U/L; 15.83 mol/L, 99.12 g/L, 101.21 mmol/L and 10.30 mmol/L respectively. The result of slight degree of hemolysis were presented in table (2).

DISCUSSION

The data given here seem to indicate that the normal value of serum hemoglobin in healthy goats was 120.73 ± 5.45 mg/100 ml. Previous data on serum hemoglobin concentration in goat were not available in literature, but the mean value of serum hemoglobin in the present investigation was lied in the range given previously by different authors in bovine serum (SCHULTZLER and SIEGERT, 1963 and EL-SEBAIE, 1981).

A limited number of parameters will be reviewed in this section in order to examine their concentration in serum of healthy goats and the effect of different degree of hemolysis on the picture of such parameters. The obtained data of the examined constituents were generally in agreement with the published data by (GURTNER and

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RICHTER, 1959; SOLBACH *et al.*, 1962; BOYD *et al.*, 1964; YOUNG *et al.*, 1965). There were only relatively small differences between the mean values of the published data, this may be explained due to variations in breed, age, and methods used. On respect of the effect of hemolysis in various degree on the values of studied constituents, it can be concluded that the goats serum appears hemolysed, when the serum hemoglobin concentration exceeds a value of 132.06 ± 0.427 mg/100. The values fall within the range published previously by GARAWAY, (1962); LAESSIG *et al.*, (1976); DORNER *et al.*, (1981) and EL-SEBAIE (1981). Slight degree of hemolysis exert its effect and causes adulteration of values of serum constituents. The marked influence was shown in the concentration of serum hemoglobin which reach a value of 132.06 mg/100 ml. A less effects were showed on the values of serum aspartat aminotransferase which elevated from 23.6 U/L in non-hemolysed serum to reach 25.30 U/L. Serum potassium first of all was markedly elevated when the serum appears slightly hemolysed. It could be explained such rises in serum hemoglobin, aspartat amino transferase and serum potassium due to high concentration of such substances in red blood cells than in plasma.

Other constituents such as serum total bilirubin, serum total protein and serum chloride showed no significant elevation in their values. The above mentioned results are in agreement with the data given by CARAWAY, (1962); LAESSIG, *et al.*, (1976); HENRY, *et al.*, (1977); DORNER, *et al.*, (1981) and EL-SEBAIE, (1981).

A prominent effect of hemolysis was observed when the serum was severely hemolysed, adulteration not only include values of serum hemoglobin, aspartat amino transferase and potassium but also include some other parameters such as serum bilirubin. A sharp elevation in hemoglobin value was observed, the value reached 462.06 mg/100 ml, this value commonly obtained when the serum appears bloody. Similar elevation also observed on the determination of serum potassium and aspartat amino transferase (AST). Serum bilirubin value was less elevated when compared with other parameters. This results were agreed with (HENRY, *et al.*, 1977); LAESSIG, *et al.*, (1976) and EL-SEBAIE, (1981).

Finally it should be concluded that, a slight degree of serum hemolysis had a minimal effect in adulteration of values of some serum constituents such as aspartat amino transferase, and serum potassium, while the same degree of hemolysis had no effect on the other measured serum constituents.

Severely hemolysed serum causes a significant elevation of values of aspartat amino transferase, serum potassium and serum total bilirubin, with exception of serum chloride. From this point of view the hemolysed serum samples from goats used in clinical chemistry assay, should be rejected.

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Table (1): Normal values of some serum parameters in healthy goats

Serum Parameters	Hb mg/100 ml	Aspartat amino transferase U/L	Total Bilirubin mol/L	Total Protein g/L	Chloride mmol/L	Potassium mmol/L
Mean value	120.73	23.6	11.35	99.08	109.6	5.26
S.E.	± 5.95	± 3.16	± 2.80	± 1.11	± 2.45	± 0.93
n = 40						

Table (2): Effect of slight degree of hemolysis on some serum parameters

Serum Parameters	Hb mg/100	Aspartat amino Transferase U/L	Total Bilirubin mol/L	Total Protein g/L	Chloride mmol/L	Potassium mmol/L
Mean value	132.06	25.30	11.80	97.16	102.1	7.84
S.E.	± 0.427	± 1.07	± 1.60	± 1.23	± 0.73	± 0.30
n = 10						

Table (3): Effect of severe degree of hemolysis on some parameters

Serum Parameters	Hb mg/100	Aspartat amino transferase U/L	Total Bilirubin mol/L	Total Protein g/L	Chloride mmol/L	Potassium mmol/L
Mean value	462.06	47.80	15.83	99.12	101.21	10.30
S.E.	± 0.10	± 3.08	± 2.82	± 0.67	± 6.87	± 1.42
n = 10						