Differences Between Serum and Plasma Osmolalities and Their Relationship to Lactic Acid Values[1] (36133)

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Measurements of blood osmolality for clinical or research purposes are carried out on serum or plasma. The former is utilized more often than the latter since the use of anticoagulants requires special precautions. It is generally assumed that serum and plasma osmolality values are identical, leaving it up to the investigator to select the material which he prefers (1, 2).

It is shown below that significant differences exist between plasma and serum osmolality, that they are related to the formation of lactic acid in blood samples during the clotting period, and that they can be minimized by immediate cooling and rapid separation of the cellular elements from the blood serum (3).

Materials and Methods. Serum and plasma osmolality was determined from 2 ml samples by measurement of freezing point depression. It is expressed in milliosmoles per kilogram of water. For plasma, a small correction (0.6 mOsm/kg H_2O) was made to account for the sodium heparin added to the collecting tubes. All data were obtained from duplicate determinations. The accuracy of the instrument used, as stated by the company, is ± 0.2 mOsm/kg H_2O.[2] Our own serial tests revealed even smaller deviations.

Lactic acid determinations were performed enzymatically measuring the reduction of NAD in the presence of lactic dehydrogenase (4).

For studies of normal serum and plasma osmolality, 27 male patients were selected who had been admitted to the medical service of a Veterans Administration Hospital with conditions which could not be expected to alter osmolality. Venous blood was aspirated in a syringe and two 10 ml portions were immediately transferred into a heparinized and a nonheparinized test tube. The heparinized sample was cooled in an ice water bath and the plasma was separated from the cellular elements by centrifugation at 2°. The time interval between blood withdrawal and the subsequent measurement of plasma osmolality as well as deproteinization of the plasma for determination of lactic acid did not exceed 20 min. The other sample was allowed to clot at room temperature for periods of 1 to 4 hr. The serum was separated by centrifugation and analyzed for osmolality and lactic acid content in the same manner as the plasma.

For comparison of serum and plasma osmolalities in a grossly abnormal group, 12 male patients were selected who had been admitted to the hospital with mild to severe alcohol intoxication. In this series, all blood samples were cooled immediately after collection; and separation of serum from the cellular elements was not delayed beyond a period of 2 hr.

Experiments with addition of pure l (+) lactic acid to blood serum were carried out with a crystalline preparation.[3]

Blood alcohol determinations were performed with the alcohol dehydrogenase method (5, 6).

Results. Analysis of plasma, as well as serum osmolalities, of 27 patients admitted to the hospital with conditions not expected to influence blood osmolality revealed that the latter were in the average 2.8 mOsm/kg H_2O higher than the former (Table I, Column A). Assuming that this difference was related

[1] Supported in part by the Veterans Administration.

[2] Model 31LAS osmometer, Advanced Instruments, Inc., Newton Highlands, MA.

[3] Sigma Chemical Company, St. Louis, MO.

TABLE I. Correlation Between Serum and Plasma Osmolalities and Lactic Acid Values of 27 Normal Adult Males and 12 Patients with Alcohol Intoxication (mean values with ranges).

A. Normal adult males			B. Patients with alcohol intoxication		
Serum[a]	Plasma[b]	Difference	Serum[c]	Plasma[b]	Difference
		Osmolality (mOsm/kg H_2O)			
290.1 (283.4–298.9)	287.3 (279.6–297.8)	+2.8 (−0.7–+8.1) $p < .025$[d]	355.3 (309.2–393.6)	354.1 (309.3–393.2)	+1.2 (−0.2–+4.4) $p < .01$[d]
		Lactic acid (mg/100 ml)			
35.2 (19.5–55.2)	17.7 (10.9–30.9)	+17.5 (+0.8–+38.2) $p < .005$[d]	39.4 (13.6–52.0)	33.2 (12.4–43.1)	+6.2 (+0.4–+12.2) $p < .005$[d]
		Alcohol (mg/100 ml)			
0.0	—	—	263.9 (79.0–418.0)	—	—

[a] Blood samples stored 1–4 hr at room temperature before separation of serum.

[b] Heparinized blood samples cooled and analyzed (or deproteinized for lactate determinations) within 20 min after collection of samples.

[c] Blood samples stored 1–2 hr in refrigerator before separation of serum.

[d] Paired one-tailed t.

to lactic acid formation in the blood samples, which occurs prior to separation of serum from the cellular elements, each sample was subjected to lactic acid analysis. The results indicated that the heparinized samples, which had been cooled immediately after collection of the blood, and in which the time period for separation of the cellular elements was shorter than in the serum samples, had significantly lower lactic acid values.

In order to verify the association between lactic acid formation and serum osmolality changes, blood from a patient was rapidly divided into seven portions. Three of these were kept at room temperature and three in an ice water bath. The seventh sample was collected in a heparinized tube, cooled immediately, centrifuged to obtain plasma and analyzed for osmolality and lactic acid within 20 min after blood withdrawal. One each of the other samples were analyzed 1, 3.5, and 5 hr after collection of the blood. The data plotted in Fig. 1 show a close linear relationship between osmolality and lactic acid values. The observation that both parameters increase more rapidly at room temperature than at 0° supports the assumption that the changes are caused by the persistent glycolytic activity of erythrocytes and leukocytes. Experiments with blood from five other patients yielded similar results with some variations in total increases related to differences in glycolytic rate and initial blood glucose concentration. Sodium and potassium levels in the different serum fractions were analyzed by flame photometry to exclude other mechanisms which could be responsible for the progressive increase in osmolality. They revealed no significant changes during the period of clotting or incubation.

In order to investigate whether, in the practical use of osmolality, possible differences between plasma and serum could be eliminated by proper cooling of samples and rapid separation of serum by centrifugation, 12 patients were selected who had been admitted to the hospital with mild to severe alcohol intoxication. The results, as demonstrated in Table I Column B, indicate that even under these conditions there still exists a measurable difference between plasma and serum osmolality values; however, it is less than that observed under the previously described procedure where blood samples were allowed to clot at room temperature. The

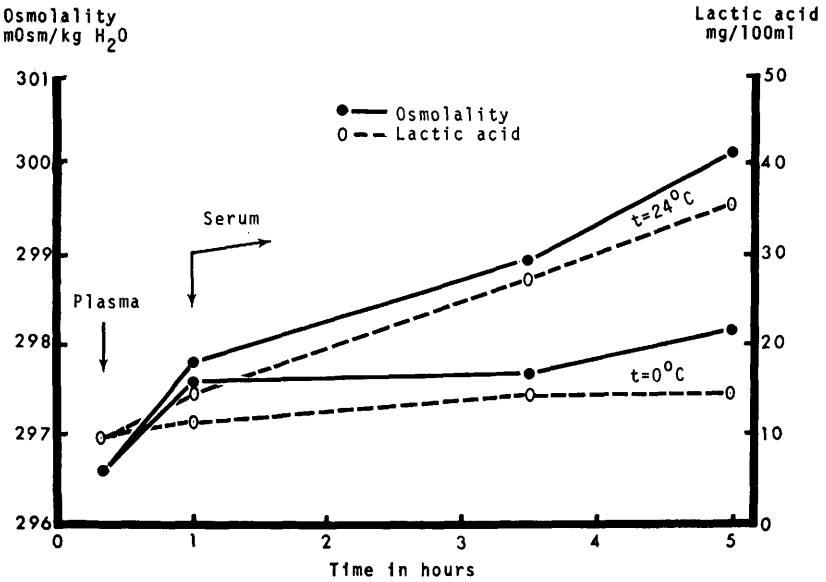

FIG. 1. Influence of storage time and temperature on blood osmolality: Changes in osmolality and lactic acid concentration of a blood sample, portions of which are stored for periods of 1, 3.5, and 5 hr either at room temperature or at 0° before separation of serum for analysis. Plasma from a separate sample taken at the same time serves as control.

high osmolalities of these patients are a reflection of the blood alcohol content which varied from 79 to 418 mg/100 ml of serum. Lactic acid values of the patients with alcohol intoxication exceed those of control patients when plasma levels are compared. Immediate cooling significantly reduces, but does not fully prevent, the unspecific increase in serum lactic acid during storage of blood.

Assuming that lactic acid has an osmotic coefficient of 1.0 and that it is practically fully dissociated (pK 3.13) at the pH of blood, one can expect that each milligram of lactic acid per 100 ml of serum or plasma contributes 0.24 mOsm/kg H_2O to the total osmolality. In order to test these assumptions, known amounts of pure (crystalline) lactic acid were added to four different blood sera. We found that in quantities which did not decrease the pH below 6.80 (up to 90 mg/100 ml of serum), an increase of 1 mg/100 ml corresponded to an osmolality increase of 0.23 mOsm/kg H_2O (range, 0.22–0.24).

Discussion. The material presented here shows that plasma and serum osmolalities are not identical. The findings that differences as high as 8.1 mOsm/kg H_2O can occur when samples are allowed to clot at room temperature and that they can be reduced by proper cooling demonstrate the need for the establishment of special procedures for the handling of blood samples used for osmolality measurements. Using a highly abnormal group of patients (cases with alcohol intoxication), it can be shown that even with immediate cooling it is not possible to eliminate them totally.

The data support the assumption that the unspecific increase in osmolality is related to lactic acid formation. There are no data which pertain to the quantitative contribution of lactic acid to serum osmolality. Our measurements indicate that an increase in lactic acid concentration of 1 mg/100 ml of serum corresponds to an average increase in osmolality of 0.16 mOsm/kg H_2O (Table I, Column A) or 0.19 mOsm/kg H_2O (Column B). These values are lower than those which were obtained by adding pure lactic acid to blood serum (0.23 mOsm/kg H_2O/mg of lactic acid /100 ml). However, it must be realized that 1 mM glucose is utilized for each 2 mM of lactic acid formed; or, considering osmotic equivalents, the metabolism of 1 mOsm of glucose should lead to

a net increase of 3 mOsm. Accounting for the loss of glucose, one can expect that an increase of 1 mg of lactic acid/100 ml of serum would increase the osmolality by 0.17 mOsm/kg H_2O, which closely approximates the obtained values. These correlations cannot be applied to *in vivo* conditions where the carbonate–bicarbonate buffer system modifies the contribution of changing lactic acid concentration to the total osmolality.

The data provide possible explanations for differences in normal osmolality values between various authors and also help to explain some of the discrepancies which are observed between measured osmolality and "predicted" osmolality based on chemical analysis of electrolytes and other osmotically active serum constituents.

Summary. Significant unspecific increases in osmolality can be observed when blood samples are allowed to remain from 1 to 4 hr at room temperature before serum is separated from the corpuscular blood elements. The increase is related to production of lactic acid caused by the persistent glycolytic activity of erythrocytes and leukocytes. This error can be reduced by immediate cooling of the samples or by using plasma which has been separated from the blood cells within 20 min after collection of the sample.

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