SHORT COMMUNICATION :

A SIMPLE GRAVIMETRIC METHOD FOR ESTIMATION OF PLASMA FIBRINOGEN

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Summary: The study was undertaken to evaluate a new method of estimating plasma fibrinogen levels, gravimetrically using calcium chloride in albino rats. The results were compared with the standard method in which thrombin was used as clotting agent and were found to be statistically not signficant. Advantages of using calcium chloride instead of thrombin have been discussed.

Key words: plasma fibrinogen calcium chloride thrombin

INTRODUCTION

A variety of acute and stressful conditions like myocardial infarction and surgical trauma etc. have been demonstrated to produce changes in the plasma fibrinogen levels (1,2). Quick and accurate estimation of plasma fibrinogen obviously facilitates assessment of the severity of tissue damage in such conditions. Various biochemical techniques for estimation of plasma fibrinogen are cumbersone, time consuming and require well equipped laboratory (3-5) and are thus rarely used. The gravimetric estimation of plasma fibrinogen, on the other hand, is simple and quick. Fearnley *et al.* (6) have used thrombin as clotting agent for gravimetric estimation of plasma fibrinogen and results obtained by this method are fairly accurate as confirmed by biochemical methods. The present study was designed to devise and assess the accuracy of a new method by using calcium chloride in place of thrombin as clotting agent for estimation of plasma fibrinogen in albino rats.

MATERIALS AND METHODS

Two groups (A and B) of ten albino rats each and of either sex, weighing between 240 to 260 *g* were housed under controlled conditions of temperature and humidity for a week and were provided commercial rat diet. The animals were anaesthetized by pentobarbitone sodium (35 *mg/kg*, ip) and 4 *ml* blood was collected from abdominal aorta in a glass syringe containing 1.0 *ml* of sodium citrate (3.8%) solution. The blood was thoroughly mixed with

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sodium citrate solution to prevent clotting and was centrifuged at 3,000 rpm to separate plasma.

Method employed to estimate plasma fibrinogen was similar to that of Fearnley *et al.* (6) excepting that the thrombin was replaced by calcium chloride. Plasma fibrinogen was estimated in duplicate for each sample. 1.0 *ml* of citrated plasma was added to 10.0 *ml* of physiological saline. The plasma of animals of group A was clotted with 10.0 units of thrombin while the plasma obtained from animals of group B was clotted by addition of 1.0 *ml* of calcium chloride (2.5%); this concentration of calcium chloride has been recommended for estimation of plasma fibrinogen by microkjeldahl (4) and biuret methods (5). The samples were incubated at 37°C for half an hour to complete the clotting. The clot was tipped into the palm of the hand and the fluid was extruded by gentle pressure until the clot was small enough to be squeezed between the fingers into a compact ball. It was kept for 30 minutes in acetone, dried at a constant temperature (50°C) in an oven and weighed. Student's 't' test was applied to compare the results obtained by the two methods.

RESULTS AND DISCUSSION

It is evident from the results obtained (Table I) that plasma fibrinogen levels of the animals of both the groups were nearly equal and no statistically significant difference was observed ($P > 0.05$).

TABLE I : Plasma fibrinogen levels in albino rats estimated by using thrombin or calcium chloride as coagulants.

	Group A (thrombin)			*Group B (calcium chloride)*		
	Weight (g)	*Sex*	*Mean plasma fibrinogen level (mg%)*	*Weight (g)*	*Sex*	*Mean plasma fibrinogen level (mg%)*
1	260	F	184	248	F	186
2	246	F	178	260	F	182
3	260	M	188	250	F	192
4	255	F	176	260	M	190
5	240	F	172	240	M	180
6	244	M	170	242	M	172
7	256	M	176	258	F	180
8	252	F	180	243	M	176
9	258	M	188	246	F	170
10	250	M	176	252	M	172
Mean ± S.E.M.			178.8±2.0			180.0±2.5

Addition of citrate to blood chleates the calcium ions and thus prevents the normal coagulation mechanism. In the present method, addition of calcium chloride to the citrated plasma replenishes, the calcium content and hence initiates the normal coagulation process in the plasma, while, on the other hand addition of thrombin to citrated plasma as used by Fearnley *et al.* (6) directly converts fibrinogen into fibrin which is then estimated gravimetrically. As expected from the foregoing the clot formation with thrombin is quick (within 10 sec.) while calcium chloride takes a little longer time (30 to 60 sec.). However, the advantages of using calcium chloride in place of thrombin as, free availability, low cost and fair stability out weigh the disadvantage of slightly slower coagulation process. In coagulation disorders, the use of calcium chloride for estimation of plasma fibrinogen level may have the disadvantage of delayed coagulation process as well as is expected to give inaccurate results. Such conditions, however, are very rare and predominance of sings and symptoms of coagulation defect may clearly suggest that in such conditions calcium chloride should not be used for estimating plasma fibrinogen. However, in myocardial infarction and other clinical conditions where plasma fibrinogen levels are of diagnostic and prognostic significance (7,8,9,10,11) calcium chloride may safely be employed for its estimation. With further studies in experimental and clinical situations the new method may acquire a place as a routine laboratory test for estimation of plasma fibrinogen levels.

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