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PRELIMINARY STUDIES ON THE EFFECT OF CHOLINE CHLORIDE ON BLOOD COAGULATION

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CHOLINE chloride has been extensively used as a constituent in high fat atherogenic and thrombogenic diets.[1-4] Howard and Gresham[3] also observed that the ability of a fat to produce thrombosis was enhanced by the addition of choline chloride. However, coagulation studies, carried out in rats maintained on such regimens, revealed that the prothrombin time was shortened on one hand and the thromboplastin generation time prolonged[5] on the other, and the blood rendered less coagulable than normal.[6] No satisfactory explanation for these opposing effects was given. Since choline chloride was common in all these studies and its effect on blood coagulation was not fully known, a preliminary study in this direction was undertaken.

The experiment was conducted on 36 normal, male albino rats of C.D.R.I. colony of average weight 120 g. Blood of 6 rats was utilised for *in vitro* study and the remaining animals were divided into 5 groups of 6 each and used for *in vivo* study. Group I was used as normal control. Each animal of Groups II, III, IV and V was forced fed a single dose of 100 mg. choline chloride dissolved in 2 ml. of distilled water and coagulation studies in each group performed at ½, 1, 2 and 4 hours respectively. The animals were put under light ether anæsthesia and blood withdrawn directly from abdominal aorta in a glass syringe, kept in oxalated bottles and plasma separated.

To test the coagulation mechanism, calcium clotting time, prothrombin time and fibrinolytic activity of plasma were performed in all the groups. Calcium time was done as described by Dacie[7] and prothrombin time by Quicks' one stage technique,[7] using 0·01% solution of Russel's Viper venom in place of brain extract. Calcium time and prothrombin time techniques were modified as described earlier (Srivastava, *et al.*[8]) in case of *in vitro* tests in which 10%, 25%, 50% and 100% solution of choline chloride were added to the clotting mixtures. Fibrinolytic activity of plasma was done as reported earlier.[8]

Table I shows the mean values of *in vitro* tests.

It is seen that both calcium time and prothrombin time became markedly prolonged when a 10% solution of choline chloride was added to the clotting mixture. With 25% solution, the clotting times registered further prolongation until in 50% and 100% solutions the clotting was either totally inhibited or greatly retarded upto a period of 2 hours. These observations showed that choline chloride interfered with the normal coagulability of blood *in vitro*.

TABLE I

Group	Calcium time (Sec)	Prothrombin time (Sec.)	Fibrinolytic activity (%)
1. Control ..	44·5 ± 1·5 (6)	13·5 ± 0·8 (5)	50·8±2·4 (3)
2. 10% choline chloride ..	126·5*±10·0 (6)	46·4*± 4·1 (5)	48·5±2·2 (3)
3. 25% ,, ..	498·5*±51·3 (6)	137·0*± 11·3 (5)	49·2±0·7 (3)
4. 50% ,, ..	No clotting (4)	1110·0*±210·0 (5) (clotting in only 2 samples)	52·3± ·1 (3)
5. 100% ,, .. ,,		No clotting (5)	50·0±2·5 (3)

* Highly significant ($P < ·01$). Figures in parenthesis indicate the number of observations.

TABLE II

Group	Calcium time (Sec)	Prothrombin time (Sec.)	Fibrinolytic activity (%)
I Zero hour ..	39·7 ±1·8 (5)	12·1 ±0·5 (6)	48·8±1·2 (6)
II Half hour ..	46·2*±3·7 (5)	15·0*±0·8 (6)	46·8±1·3 (4)
III One hour ..	53·6*±3·1 (5)	15·5*±0·5 (6)	48·3±3·1 (6)
IV Two hours ..	45·9*±2·4 (4)	15·3*±1·0 (6)	51·8±2·6 (6)
V Four hours ..	39·7 ±3·0 (5)	11·4 ±0·5 (6)	44·8±1·6 (6)

* Highly significant ($P < ·01$). Figures in parenthesis indicate the number of observations.

The mean values of coagulation tests at ½, 1, 2 and 4 hours after *in vivo* administration of choline chloride are summarised in Table II.

The study of the table shows that the calcium time and prothrombin time increased from ½ hour interval lasting upto 2 hours and tending to return towards original values at 4 hours interval.

Fibrinolytic activity of plasma in both experiments did not show an appreciable change.

Both these studies indicated that choline chloride had an anticoagulant effect. This effect was more pronounced *in vitro* than *in vivo*. This was perhaps due to the oral route of administration where only a comparatively lower concentration of choline chloride was attained in the plasma. Higher or repeated oral doses might cause a similar effect as *in vitro*.

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