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Calcium and Coagulation of Blood.

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Vines[1] called attention to the fact that it requires about 3 times the amount of oxalate to prevent the coagulation of blood than would be expected from the total amount of calcium in the blood. This was confirmed by Loucks and Scott.[2] Vines also called attention to the fact that on recalcifying coagulation occurred before all the oxalate was neutralized. Vines used small amounts of blood and used a biological method for calcium determination and his determinations of the amount of fibrin formed are only relative. It was felt that it was desirable to repeat some of the recalcifying work using larger amounts of plasma. The calcium determinations were made by the method of Kramer and Tisdall[3] while the fibrin formed, was washed, dried and weighed. Dogs that had been anesthetized with nembutal were the source of the blood used.

In a number of cases the amount of oxalate necessary to prevent coagulation was determined and then by recalcification of samples of plasma which had not clotted to see if they would clot at the same deficiency of calcium. Samples of plasma from 15 to 150 cc. were used in these experiments but for comparison all figures are given as if 100 cc. had been used. In all cases a sample of blood was permitted to clot and the calcium estimated in the serum. Loucks and Scott[2] showed the difference in calcium between plasma and serum was within limits of experimental error. To prevent evaporation while centrifuging the centrifuge tubes were always capped with a rubber membrane (Collins and Scott[4]). All the recalcifying experiments were done at room temperature.

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[1] Vines, H. W. C., *J. Physiol.*, 1921, **55**, 86.

[2] Loucks, Milo M., and Scott, F. H., *Am. J. Physiol.*, 1929, **91**, 27.

[3] Kramer, B., and Tisdall, F. J., *J. Biol. Chem.*, 1921, **47**, 415.

[4] Collins, Dean A., and Scott, F. H., *J. Biol. Chem.*, 1932, **97**, 189.

The experiments were carried out as follows: Varying amounts of a 17.4% potassium oxalate solution were put into 15 cc. centrifuge tubes and 10 cc. of the blood run into them, thoroughly mixed and then larger amounts of blood run into bottles containing enough oxalate to prevent coagulation. The larger samples were centrifuged immediately and the plasma removed. The smaller 15 cc. tubes were permitted to stand over night and then centrifuged and the calcium or oxalate estimated in the clear fluid. Typical results like the following were found:

Serum calcium = 10.1 mg. per 100 cc. All tubes of shed blood went solid until there was oxalate sufficient to make a deficiency of calcium of 32.8 mg. per 100 cc. Tubes with a calcium deficiency of 29.2 and 31.1 mg. per 100 cc. went solid. The larger amounts of plasma from the bottles was found to have a calcium deficiency of 38.2 mg. When less than 2 mg. of calcium were added (enough to neutralize only 1/20 of the oxalate present) there was a slight formation of fibrin. In order to get a solid clot one must add approximately enough calcium to neutralize about ⅓ to ¾ the calcium. In the above case the tubes did not go solid until the Ca deficiency had been reduced to 11.4 mg. In other cases it has gone solid with only ⅓ of the oxalate neutralized. The figures for decalcification and recalcification therefore do not indicate any fixed relationship.

As the above figures show one can obtain some fibrin on recalcification when there is still a great excess of oxalate present. One of the surprising things about the partial neutralization of the oxalate is that although there is oxalate present the influence of the calcium is felt for a long time as shown by experiments like this.

Plasma calcium deficiency = 30.1 mg. per 100 cc.
7.5 mg. Ca added after 2 hours slight clot.
" 4 " fair "
" 27 " enough clot so beaker could be inverted. The control without calcium remained fluid. Likewise if one adds a small amount of calcium and lets stand for a time and then removes the fibrin one will get another crop of fibrin. For example a plasma with a calcium deficiency of 28.5 mg. had 14.4 mg. Ca added. After 6 hours it was solid and 0.428 gm. of fibrin were removed. On standing 14 hours longer 0.076 gm. more had formed. The calcium deficiency actually found at the end was 14.4 mg. per 100 cc.

As one adds more and more calcium more fibrin is formed. One obtains figures like the following:

Ca deficiency 38.2 mg. per 100 cc. Ca added	Fibrin formed	Ca deficiency 33.9 mg. per 100 cc. Calcium added	Fibrin formed
1.9	0.024	8.3	.1584
3.8	.020	16.6	.3844
7.6	.053	24.9	.3456
11.4	.052	33.3	.3128
15.2	.049	49.9	.5664
19.1	.046	66.7	.6408
22.8	.064		
26.6	.092		
30.4	.358		
34.2	.580		
38.2	.603		
41.8	.716		

It is seen from the above that the relationships of calcium are by no means simple. That the conversion of fibrinogen into fibrin can take place in the absence of inorganic calcium is certain. When inorganic calcium is added to decalcified plasma the decalcified thrombin can compete at first against potassium oxalate for the possession of the calcium and active thrombin is formed. The active thrombin exerts its action for some time even in the presence of oxalate until it is again inactivated by the loss of calcium. Loucks and Scott[2] showed it takes some time for oxalate to destroy the activity of thrombin. (It is extremely difficult or impossible to destroy it by dialysis.) The regeneration of thrombin, when inorganic calcium is added, is however immediate.

Attention is called to the similarity of the calcium and kephalin in regard to thrombin. Mills[5] showed that thrombin passes into metathrombin because the kephalin goes over to the other blood proteins. Metathrombin may be converted to thrombin by the addition of kephalin and it will pass into metathrombin again as it loses it kephalin to the other proteins again.

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Glucose Tolerance.

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Joslin[1] states that diabetes is hereditary in 25% of the cases. In view of this, the authors were interested in determining whether or not a tendency toward, or an early indication of, diabetes mellitus

[5] Mills, C. A., *Am. J. Physiol.*, 1926, **76**, 651.

[1] Joslin, E. P., *Diabetic Manual*, 1929, 4th edition, **145**.