

With the strength of hypertonic solutions used, it is not possible to dilute blood much more than 10% or some laking results. In the case of BaCl₂, 24% solution even in 10% dilution caused laking.

In the various experiments, the figures were all in the same direction. Thus, in the 11 experiments with hypertonic dextrose (Series 1), every experiment showed decreases of total protein which varied between .079 gm. and 1.913 gm. with an average decrease of .513 gm. In the 15 experiments using hypertonic NaCl (Series 7), the decreases varied between .052 gm. and 1.003 gm. with an average decrease of .326 gm. These phenomena are probably connected either with a reversal of charges on the corpuscles and plasma protein in concentrated solutions or with an alteration of the lipid protein equilibrium between the cells and plasma. These possibilities are being investigated.

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Effect of Saliva on Coagulation of the Blood.

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It was observed that the addition of human saliva to dog blood greatly shortened the coagulation time. Hunter¹ alone observed that the addition of his own saliva to his own blood hastened the coagulation. He did not try to determine the nature of the active substance. There is nothing specific about this material. Saliva hastened the coagulation of the following types of blood: (1) Other humans; (2) recalcified oxalated human blood of another individual; (3) dog blood; (4) recalcified oxalated ox blood; (5) recalcified oxalated ox plasma. Figures for dog blood are given below.

Human saliva was obtained from various individuals after rinsing the mouth with warm physiological saline and discarding the first samples. Salivary flow was increased by chewing paraffin. All samples of saliva were centrifuged for 30 minutes before using. Unless otherwise indicated, the saliva was unheated. When heated, the saliva was placed on a water bath for 20 to 45 minutes and then diluted to its original volume, cooled to room temperature, and filtered before using. Blood was obtained directly from the cannulated femoral artery of dogs into tubes containing the known

¹ Hunter, John B., *Brit. J. Surg.*, 1928, **16**, 203.

amount of saliva. Four to 8 samples were used for each determination; the times given below are averages. The saliva and tubes were at room temperature. The times for coagulation are given in minutes and seconds.

TABLE I.
Ratio of Saliva to Blood, cc. and Clotting Times.

Dog	Normal	0.6:2	0.4:2	0.1:2
1	6:00	1:00	2:35	4:30
2	5:30	1:30	2:00	3:30
3	2:00	:30	1:00	1:30
4	5:00	1:00
5	4:30	1:00	1:00	4:00
6	9:00	3:50	6:45

It will be seen that a reduction of 80% in clotting time can be obtained with proper proportions. Experiments in which saliva was added to blood in proportions of 1:400 showed a decreased coagulation time. When the proportion of saliva to blood is very great, such as 20:1, the coagulation time is still reduced, but not very much. Similar reductions in clotting time were observed in the case of the other types mentioned above. The clots formed after the addition of saliva seemed to retract more than normal blood.

Saliva may be heated to 60° with little reduction in coagulative power. Boiling, however, causes considerable reduction in power but it is not entirely destroyed by 5 minutes boiling over a free flame.

Saliva alone added to oxalate blood has little effect on the coagulation. If added in large amounts (equal or twice the volume of blood) some coagulation may be observed after a number of hours. We are inclined to believe this is due to the calcium in the saliva rather than to a thrombin, because if the saliva be oxalated before its addition to the blood no coagulation occurs. If we believe with Mills and Mathews² in the two types of coagulation, this would mean saliva contained no thrombin but a tissue fibrinogen or kephalin. However, as Loucks and Scott³ showed, oxalates destroy thrombin. The nature of this phenomenon is being studied further.

² Mills, C. A., and Mathews, A. P., *Arch. Int. de Physiol.*, 1924, **24**, 6.

³ Loucks, M., and Scott, F. H., *Am. J. Physiol.*, 1929, **91**, 27.