THE MECHANISM OF THE ACTION OF SALIVA IN BLOOD COAGULATION

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It is well known that the addition of saliva to blood will accelerate its coagulation (1, 2, 7). This property of saliva is not species specific. It has been shown that dog, cat and human saliva will indiscriminately accelerate the clotting of blood obtained from the dog, cat, cow or man (1, 7). Bellis, Birnbaum and Scott (1) noted that while the clotting factor of saliva was stable at 60°, it was destroyed by boiling at 100°. Bellis and Scott (2) found that the addition of saliva decreased the coagulation time of hemophilic blood, and that a similar effect was obtained with peritoneal and cerebrospinal fluids.

The present investigation was undertaken to determine the function of saliva in the coagulation mechanism and to investigate the nature of the active material. The results obtained indicate that saliva contains a factor which acts like the thromboplastic material present in blood platelets, brain and lung tissues and that it probably is protein in nature.

EXPERIMENTAL. The reagents employed in this investigation were prepared as follows: *Plasma*—from beef blood obtained by the addition of 0.2 per cent potassium oxalate. *Prothrombin*—prepared according to the method of Mellanby (8). The product was not activated by calcium ions alone when incubated for the maximum length of time of the experiments. *Fibrinogen*—obtained by repeated precipitation with sodium chloride as described by Eagle (3). *Saliva*—human saliva was collected by first rinsing the mouth with saline and the chewing of paraffin to stimulate the flow. Samples were used within a few hours of collection. *Calcium chloride*—0.025M solution. *Thromboplastin*—a fresh saline extract of blood-free calf-brain.

The clotting mechanism may be separated into two well-recognized stages; 1, the formation of thrombin, and 2, the formation of fibrin from fibrinogen. The first step in analyzing the rôle of saliva was to determine which of these two stages was influenced by the presence of saliva. The following observations show that saliva has no important action on fibrinogen but that it is active in the formation of thrombin.

When fresh, filtered saliva is added to purified fibrinogen, no clot

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forms. After several hours' standing, a few strands of a fibrin-like material do appear. However if the saliva is boiled for a few minutes before being added to the fibrinogen, no fibrin is formed. Since the heated saliva is still active in accelerating the coagulation of blood as is shown later, the action of saliva must be on the formation of thrombin.

The slight action on fibrinogen is probably due to the presence of proteolytic enzymes in saliva (4) which are destroyed on heating.

The formation of thrombin is dependent on a reaction involving prothrombin, ionic calcium and a thromboplastic substance such as cephalin or certain tissue extracts. The amount of thrombin which has been formed may be approximately measured by adding the reaction mixture to a solution of purified fibrinogen in physiological saline, and measuring the coagulation time. The coagulation time has been found to be directly proportional to the amount of thrombin present (9).

Using this method it can be demonstrated that saliva cannot take the place of prothrombin or calcium ions. It can replace the thromboplastin of brain. The obvious conclusion to be drawn from these experiments is that saliva contains a thromboplastic material.

Thromboplastic activity may be assayed by the following method similar to the one reported by Ferguson (6) for cephalin.

A mixture of 0.7 ml. of prothrombin solution, 0.2 ml. calcium chloride solution and 0.1 ml. of thromboplastic material is incubated in a water bath at 38°. After definite intervals of time (marked *incubation time* in the figures), 0.1 ml. portions are pipetted off and added to 1.0 ml. of fibrinogen solution or to plasma (diluted 1:4 with saline to remove the effects of anti-thrombic substances which are present) in 10 by 75 mm. serological tubes maintained at 38°. The *coagulation time* is measured by tilting the tubes every 5 seconds until the mixture ceases to flow. The elapsed time is measured from the addition of the thrombic mixture with a stopwatch.

Plotting the coagulation time against the incubation time gives a series of curves which represent the relative amounts of thrombin formed at the different intervals. The prothrombin and fibrinogen content may differ considerably from batch to batch, but for any given experiment the properties of these materials are constant. The main controlling variable is therefore the concentration of thromboplastin, which markedly affects the rate of thrombin formation and the amount of thrombin formed. If a standard thromboplastin preparation is used, such as Ferguson's cephalin (5) kept under absolute alcohol, the above method may be used for the quantitative assay of thromboplastic activity. Curves illustrating the experimental procedures carried out on the blood coagulation accelerating properties of saliva in which coagulation time is plotted against incubation time, are given in figures 1 to 4. Because of the variation in

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prothrombin and fibrinogen content, a curve representing the effect of whole or supernatant saliva, on each batch of prothrombin and fibrinogen (to serve as a control), is given in each of the figures representing the results of an experimental series.

It has been found that the thromboplastic material of saliva is about as effective as the thromboplastin of brain in promoting the formation of thrombin, if it is sufficiently concentrated. This is brought out by curves *2* and *3* of figure 1, which gives a comparison of a suspension of sediment from saliva with that of a fresh saline extract of calf brain.

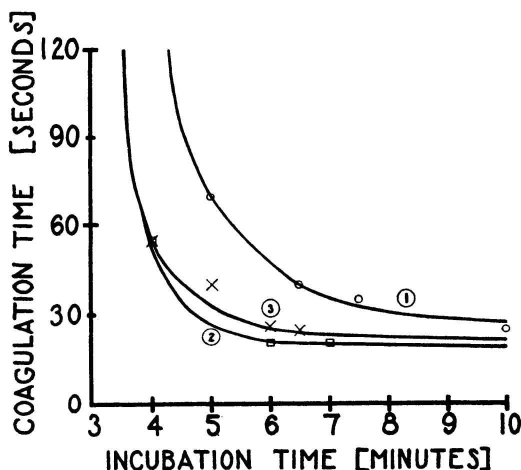

Fig. 1

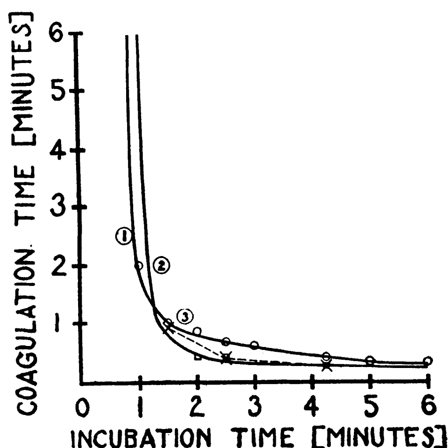

Fig. 2

Fig. 1. Comparison of the thromboplastic action of saliva with that of an extract of calf brain.

Curve *1*. Supernatant saliva after centrifugation. Curve *2*. Saline suspension of sediment from saliva used in curve *1* suspended in ⅓ the original volume. Curve *3*. Fresh saline extract of calf brain.

Fig. 2. Comparison of the thromboplastic action of saliva with that of a suspension of leucocytes and blood platelets.

Curve *1*. Filtered saliva which was boiled one minute over an open flame. Curve *2*. Saline suspension of sediment obtained by centrifuging saliva and washing once. Curve *3* (dotted line). Saline suspension of washed leucocytes and platelets from beef blood.

Curve 1 shows the effect of the supernatant fluid from the same saliva. This is also true if a comparison is made between saliva and a suspension of washed leucocytes and platelets. Such a comparison is shown in figure 2, curves *2* and *3*.

The active material of saliva is probably a tissue extract. This is indicated by the fact that the sediment from centrifuged saliva is highly active in thromboplastic properties (see fig. 1, curve *2*, and fig. 3, curve *1*).

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From figure 3 (curves *1* and *2*) it may be seen that the centrifuged sediment is about thrice as active as is whole saliva. The sediment consists of cellular material and this is probably the source of the thromboplastin in the fluid saliva.

Saliva may be dried in a vacuum oven with little loss of activity. The results of such an experiment are plotted in curve *4* of figure 3. It is stable in the desiccated state, whereas its activity is lost in a few days when it is liquid. The inactivation may be due to bacterial action since the addition of toluene serves to keep it longer.

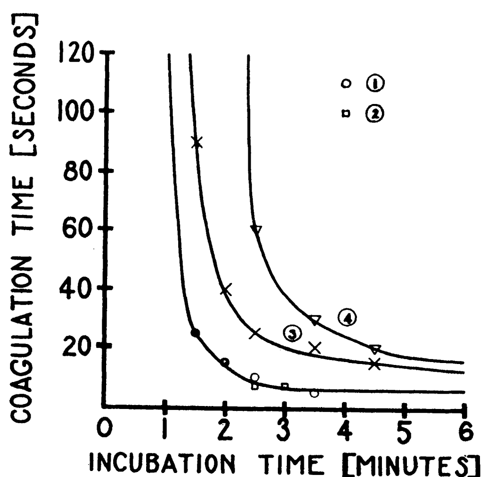

Fig. 3

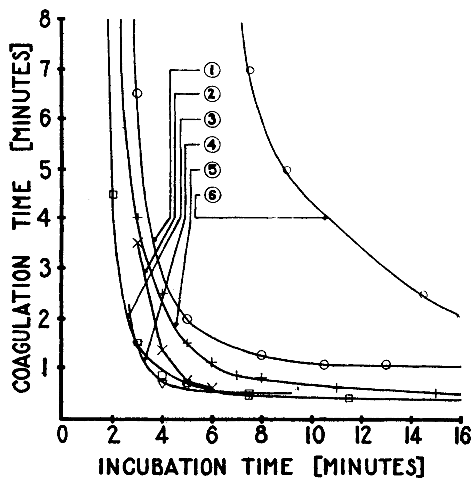

Fig. 4

Fig. 3. Experiments on the activity and stability of the thromboplastin of saliva. Curve *1*. Saline suspension of sediment obtained by centrifuging saliva. Curve *2*. Saliva concentrated 3-fold by ultrafiltration. Curve *3*. Whole, untreated saliva. Curve *4*. Saliva dried in vacuo at room temperature and then redissolved in distilled water.

Fig. 4. The influence of heating on the thromboplastic activity and stability of saliva.

Curve *1*. Supernatant saliva after centrifugation. Curve *2*. Same—heated 30 seconds at 100° and cooled in running water. Curve *3*. Same—heated 60 seconds. Curve *4*. Same—heated 3 minutes. Curve *5*. Same—heated 6 minutes. Curve *6*. Same—heated 10 minutes.

The influence of heating on the properties of saliva is shown by the curves given in figure 4. When centrifuged saliva is heated at 100° its activity is greatly enhanced during the first few minutes and then it is gradually lost. Very little activity is left after 10 minutes' heating (fig. 4, curve *6*), and none after 20 minutes. The maximum activity obtained by heating (fig. 4, curves *3* and *4*) is almost equal to that of the whole saliva (where the cellular elements are still present). The initial

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increase of activity on heating may be attributed to the hot liquid extracting more active material from fragments of cells that remain suspended even after centrifuging. Undoubtedly the active material is steadily undergoing heat destruction from the start of the experiment.

The thermolabile nature of the active material together with its probable origin in the tissues leads one to suspect it may be a protein. This is supported by the observation that it is not dialysable. Saliva can be dialyzed for 24 hours in cellophane bags against running distilled water without loss of activity, also its activity can be increased by ultrafiltration (fig. 3, curve *2*).

Another experimental evidence, which favors the view that the active substance in the saliva is of protein nature, is that all of the activity is removed from solution by full saturation with ammonium sulfate, and that the active material may be recovered from the precipitate. The thromboplastin of saliva does not appear to be lipoidal. Ether or benzene extracts of saliva showed no thromboplastic activity. This perhaps may be due to the minute amounts extracted. However the residue from the extracts are not as active as the original material. Therefore the possibility exists that the active material contains a lipoid combined with a protein and that small amounts of the lipoid are being removed by extraction with the lipoid solvents.

SUMMARY

1. Saliva owes its blood coagulation-accelerating properties to the presence of a substance acting as a thromboplastin.

2. The active material in saliva is probably of cellular origin.

3. It appears to be protein in nature, possibly a lipo-protein.

4. It can be partially purified by ammonium sulfate precipitation and dialysis followed by desiccation at room temperature, in which condition it is fairly stable.

REFERENCES

(1) Bellis, C. J., W. Birnbaum and F. H. Scott. Proc. Soc. Exper. Biol. and Med. **29**: 1107, 1932.
(2) Bellis, C. J. and F. H. Scott. Proc. Soc. Exper. Biol. and Med. **30**: 1373, 1933.
(3) Eagle, H. J. Exper. Med. **65**: 613, 1937.
(4) Eagle, H. and T. N. Harris. J. Gen. Physiol. **20**: 543, 1937.
(5) Ferguson, J. H. This Journal **117**: 587, 1936.
(6) Ferguson, J. H. This Journal **123**: 341, 1938.
(7) Hunter, J. B. Brit. J. Surgery **16**: 203, 1928.
(8) Mellanby, J. Proc. Roy. Soc. (London) **B107**: 271, 1931.
(9) Quick, A. J. This Journal **115**: 317, 1936.

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