A FURTHER NOTE ON ANTI-COAGULANTS.

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IN a previous paper the question arose whether blood contains anti-kinase. The fact that all breakdown occurs in the organism points to a certain amount of kinase being thrown into the blood and renders it inherently probable that some mechanism exists for the destruction of this kinase, whether such destruction be brought about by an anti-kinase or by some other mechanism.

It was shown in the last communication that oxalated blood recalcified at intervals of twenty-four hours did not show any prolongation of coagulation time. Two alternate conclusions were drawn :—

1. That blood contains no anti-kinase, or, more strictly, has no power of inactivating kinase.

2. That blood oxalated immediately on shedding contains very little free kinase, hence its destruction would cause but slight alteration of coagulation time.

Further experiments have been undertaken to investigate this matter.

Firstly, blood was oxalated at short intervals after shedding, and then subsequently recalcified, and its coagulation time was estimated. The following table shows results obtained :—

- I. *Formation of kinase in shed blood.*

Blood was oxalated after an interval and recalcified subsequently.

Oxalated after intervals of	Coagulation time on recalcification
4 mins.	6 mins.
5 „	$5\frac{1}{2}$ „
6 „	$4\frac{1}{2}$ „
7 „	$3\frac{1}{4}$ „
8 „	$2\frac{3}{4}$ „

The foregoing table shows that the longer the blood is allowed to stand before oxalation the shorter was the time of coagulation on recalcification. Clearly some change occurred in the blood adjuvant to coagulation, and this change is the production of kinase. That fibrin ferment is not produced in this stage of coagulation is shown by the fact that coagulation does not occur after the oxalate is added even in the later cases. This kinase, which is produced in the pre-oxalate stage of the above experiments, is destroyed on standing, as is shown by the following results :—

II. *Destruction of kinase on standing.*

- (a) Blood was shed and oxalated after
standing - - - - 4 mins.
Coagulation time on recalcification
immediately - - - - $1\frac{3}{4}$ „
Coagulation time on recalcification
20 hours later - - - - $6\frac{3}{4}$ „
Coagulation time on recalcification +
kinase 20 hours later - - - - $1\frac{1}{4}$ „
- (b) Blood shed and oxalated after - $2\frac{1}{2}$ mins.
Coagulation time on recalcification
immediately - - - - 35 secs.

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Coagulation time on recalcification			
after 20 hours	-	-	- 2 $\frac{1}{4}$ mins.
Coagulation time on recalcification +			
kinase after 20 hours	-	-	- 40 secs.

Kinase accordingly is inactivated in oxalated blood, for the lost coagulation power is replaced to a nicety by kinase solution. Thrombogen is obviously present because the addition of kinase and calcium causes rapid coagulation; therefore, it is kinase and not thrombogen which has been destroyed.

The next point investigated was whether kinase solution was inactivated on mixing with oxalated blood.

Before this question can be considered the behaviour of kinase solutions on standing must be discussed. From observations made it appears that these solutions differ in behaviour according as to their origin; for instance, kinase solution obtained from thymus gland loses its activity rapidly on standing, whereas solution derived from the testes of a rabbit maintains its activity for fully forty-eight hours. It was the latter kinase, therefore, which was utilised in the following experiment:—

III. *Influence of oxalated blood on testicular kinase of rabbit.*

Blood was oxalated and testicular kinase solution added. Coagulation time on recalcification noted. Activity of kinase also tested.

Coagulation time on recalcification			
immediately	-	-	- 1 $\frac{1}{4}$ mins.
Coagulation time on recalcification			
after 1 $\frac{1}{2}$ hours	-	-	- 1 $\frac{1}{6}$ „
Coagulation time on recalcification			
after 5 hours	-	-	- 1 $\frac{1}{4}$ „

Coagulation time on recalcification			
after 24 hours	-	-	$2\frac{3}{4}$ mins.
Coagulation time on recalcification			
after 29 hours	-	-	$1\frac{3}{4}$ „
Coagulation time on recalcification			
after 48 hours	-	-	$2\frac{3}{4}$ „
Kinase (after 48 hours) + Blood.			
Coagulation time	-	-	$1\frac{1}{2}$ „

In this instance the testicular kinase shows but slight loss of activity when mixed with oxalated plasma and allowed to stand. The divergence of this result from that obtained when the kinase of blood was allowed to stand in oxalated plasma is possibly due to specific character of testicular kinase. (Note.—In above experiment kinase solution was strong, and so owing to excess of kinase being present partial destruction would show but slightly.) Unfortunately, so far, one cannot declare whether blood kinase free from blood has the power of maintaining its activity for several days in a similar way to that of testicular kinase.

A very interesting point arose in these investigations. It was found that when oxalated blood had stood about three days the supernatant plasma would not clot on recalcification. This coincides with the experiments of Hammarsten, who, however, kept the plasma cooled to $0^{\circ}\text{C}.$, and found that in such circumstances thrombogen and kinase had, to a certain extent, disappeared from the upper layers. The superficial explanation would be that kinase or its precursor, or possibly thrombogen, had been destroyed in the solution. That this is fallacious is shown by the fact that the corpuscular layer still clots readily on the addition of calcium. It is a legitimate conclusion that

the prolonged standing has resulted in the settlement of some suspension which is essential to coagulation.

This suspension might be thrombogen, or kinase, or a precursor of kinase. To determine the nature of the suspension the supernatant plasma was recalcified and kinase was added.

IV. Loss of coagulability in plasma on standing.

Blood oxalated and allowed to stand 72 hours.

Supernatant plasma recalcified.—No. clot in 30 mins.

Supernatant plasma recalcified + kinase.—Coagulation in 2 mins.

Kinase + blood.—Coagulation in $1\frac{3}{4}$ mins.

Corpuscular layer on recalcification.—Coagulation in 7 mins.

The result of the foregoing table shows that what was deficient in the plasma was kinase or its precursor, and that thrombogen must be present, because it clots with the mere addition of calcium and kinase. There are strong reasons for supposing that the suspension is not free kinase, for we have seen in the earlier part of the paper that kinase is inactivated in oxalated blood in twenty hours, and the above blood has stood seventy-two hours, and the corpuscular portion, which contains the suspension clots with the mere addition of calcium.

I suggest that oxalated blood maintains its power of clotting on recalcification not because it contains free kinase but because it contains a suspension which is capable of forming free kinase. As to the nature of this suspension, microscopic investigation points to its consisting of platelets. That these are the source of thrombo-kinase is a view which has been held by many observers.

GENERAL CONCLUSIONS.

1. Kinase, when present in oxalated blood, is inactivated on standing; whether that is due to an anti-kinase or to some other action is uncertain. The length of time required seems to point more to protein absorption of the kinase, as Mellanby suggests, than to an anti-body.

2. The reason why oxalated blood will clot on recalcification after a long interval is because it contains not free kinase, which would be destroyed on standing, but a precursor which is not destroyed on standing, and which precursor is associated with a suspension which probably consists of platelets. So long as the kinase is in this condition it is not destroyed when the plasma is allowed to stand.