THE EFFECT OF ANTIBIOTICS ON FIBRINOLYSIS

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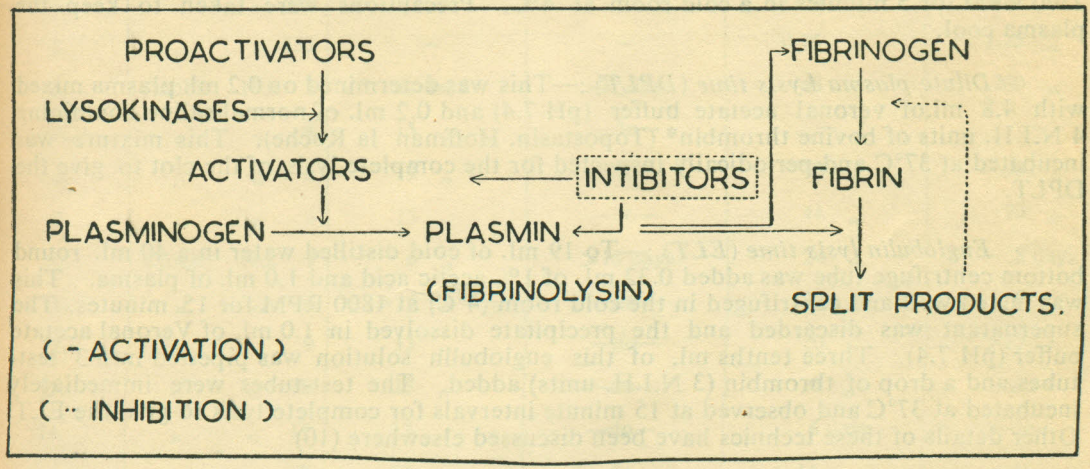
Introduction :—The fibrinolytic activity of blood in vivo, is the result of interaction of a complex series of activators, precursors and Inhibitors. This fibrinolytic system (see scheme below) is present in most vertebrates (2, 14). Differences between species in fibrinolytic activity are believed to be due to levels of proactivators (6), Hageman factor (8) and probably others.



THE FIBRINOLYTIC SYSTEM

Interest in thrombolytic therapy has given rise to a search for potent fibrinolytic substances which are safe for administration. Nicotinic acid (4) and pyrogens (12, 13, 15) have been tried in this connection. Von Kaulla, *et al.*, (16, 17) have made an intensive study in this field using several organic compounds including numerous dyes and surfactants.

In the course of an investigation of differences between species in their circulating fibrinolytic activity (11) some observations were made on the effects of antibiotics on this enzyme system. These were pursued further to result in this study.

MATERIALS AND METHODS

In the first part of the study the effects of antibiotics in vitro on blood fibrinolytic activity in different species were investigated. The subjects for this study included 18 men. 14 women, 5 monkeys, 4 rabbits, 4 rats and 6 chicks. The human subjects were young adults on their normal home diets. The animals were maintained on adequate laboratory stock diets. Blood samples were drawn in a fasting state.

In the second part of the study the effects of parenteral injections of certain antibiotics on the plasma fibrinolytic activity of 4 volunteers were investigated. Two human volunteers were employed at a time. Into one volunteer the drug (Pencillin G sodium 500,000 Units in 1 ml. of sterile normal saline or 0.5 g. each of streptomycin and dihydro-streptomycin in 2.5 ml. of sterile normal saline) was injected intramuscularly and the other person received an equal injection of the solvent (sterile normal saline). Venous blood samples were drawn from both the volunteers before injection and after the drug reached peak levels in the blood. Blood Pencillin levels have been shown to reach a peak 15 minutes after injection, the corresponding time for streptomycin being 1 hour (3). Pencillin was tested in three volunteers and streptomycin in one with simultaneous controls (volunteers injected with saline). All the volunteers were young men on normal diets in a fasting state.

Blood drawn in siliconized syringes was mixed with 1/9th volume of 3.8% sodium citrate solution and immediately chilled. Plasma was separated after centrifugation at 2300 RPM for 5 minutes in a cold room at 4°C. Precautions were taken to keep the plasma cool.

Dilute plasma Lysis time (*DPLT*) :—This was determined on 0.2 ml. plasma mixed with 4.8 ml.of veronal acetate buffer (pH 7.4) and 0.2 ml. of normal saline containing 4 N.I.H. units of bovine thrombin* (Topostasin, Hoffman la Roche). This mixture was incubated at 37°C and periodically inspected for the complete lysis of the clot to give the DPLT.

Euglobulin lysis time (*ELT*) :—To 19 ml. of cold distilled water in a 40 ml. round bottom centrifuge tube was added 0.32 ml. of 1% acetic acid and 1.0 ml. of plasma. This was mixed well and centrifuged in the cold room (4°C) at 1800 RPM for 15 minutes. The supernatant was discarded and the precipitate dissolved in 1.0 ml. of Veronal acetate buffer (pH 7.4). Three tenths ml. of this euglobulin solution was pipetted into 3 test-tubes and a drop of thrombin (3 N.I.H. units) added. The test-tubes were immediately incubated at 37°C and observed at 15 minute intervals for complete lysis to give the ELT. Other details of these technics have been discussed elsewhere (10).

The effects of antibiotics in vitro were studied by the addition to the incubation mixture of 0.05 ml. of normal saline only (control), or 0.05 ml. of normal saline containing 50 units of Penicillin G sodium, or 1 mg. of streptomycin, or both in the same quantities.

RESULTS

Table 1 shows that in rabbits and chicks, the addition of antibiotics to diluted plasma inhibited fibrinolysis. The effect of antibiotics on diluted plasma of monkey, rat or man were inconsistent and the differences were negligible.

Euglobulin lysis in monkey and rat was found to be marginally and inconsistently affected by antibiotics while in rabbits and chicks there was an apparent inhibition. The inhibition of dilute plasma lysis as well as euglobulin lysis in the case of rabbits and chicks would appear to be not a direct effect of antibiotics but an effect mediated through the prevention of bacterial contamination incidental to prolonged duration of incubation

*Obtained through the courtesy of Dr. G. S. Hattiangdi of Hindustan Lever Ltd., Bombay.

TABLE I

Experiment I : Effect of Antibiotics on Fibrinolysis time

Sl. No.	Subject	Dilute Plasma Lysis Time		Euglobulin Lysis Time	
		Without antibiotics	With antibiotic	Without antibiotic	With antibiotic
1.	Monkey No. 550	—	—	2.75 hrs.	2.75 hrs.
2.	„ „ 551	—	—	2.50 „	2.75 „
3.	„ „ 541	—	—	3.75 „	4.25 „
4.	„ „ 544	—	—	1.50 „	1.50 „
5.	„ „ 553	—	—	0.75 „	1.25 „
6.	Rabbit No. 3	21 days	>37 days	48 „	200 „
7.	„ „ 4	15 „	—do—	—	—
8.	„ „ 5	19 „	—do—	70 „	90 „
9.	„ „ 6	15 „	—do—	44 „	70 „
10.	Chick No. 7	6 „	>15 days	24 „	8 days
11.	„ „ 8	>15 „	—do—	27 „	8 „
12.	„ „ 9	13 „	—do—	20 „	>15 „
13.	„ „ 10	15 „	—do—	20 „	—do—
14.	„ „ 11	13 „	—do—	6 days	—do—
15.	„ „ 12	13 „	—do—	>15 „	—do—
16.	Rat No. 25A	—	—	6 hrs.	6 hrs.
17.	„ „ 27B	—	—	8 days	8 days
18.	„ „ 30A	—	—	100 hrs.	10.75 hrs.
19.	„ „ 30B	—	—	100 „	>16 days
20—61	18 + 14 (Men) (Women)	—	—	9.42±0.916* hrs.	7.88±0.758* hrs.

*Mean ±S. E.

>: Denotes the time of last observation at which lysis was not complete.

needed in these species. The interesting finding was that in man there was actually an acceleration of fibrinolysis. In 50 experiments with 42 samples of human clotted euglobulins, tubes to which antibiotics were added lysed distinctly earlier in 37 cases. In

49 instances the antibiotics reduced lysis times by 0 to 10 hours. In only one instance was the lysis time enhanced by 1 hour. The mean and standard error for euglobulin lysis times without antibiotics was 9.42 ± 0.916 which was reduced by the addition of antibiotics to 7.88 ± 0.758. However, statistical analysis revealed that these differences were not significant.

In the second part of the study, when the conditions were standardised, it was found that in vitro addition of Penicillin enhanced activity in about 50% of the experiments, while addition of streptomycin accelerated lysis in only 25% of the experiments and a mixture of these two antibiotics actually inhibited lysis in most cases (Table II).

TABLE II

Experiment II. Effect of Antibiotics in VITRO

On addition of	Number of observations	ELT		
		Less	More	No difference
Penicillin ...	15	7	2	6
Streptomycin ...	12	3	2	7
Penicillin and streptomycin ...	5	—	4	1
Normal saline only ...	12	3	1	8

Injections of Penicillin accelerated fibrinolysis in two out of three cases. Streptomycin was effective in accelerating fibrinolysis in the one instance when it was injected. Unexpectedly, saline injections enhanced fibrinolysis in two out of four people and depressed the fibrinolytic activity in one case (Table III).

TABLE III

Experiment II. Effect of Antibiotics in VIVO

Treatment	Number of observations	ELT		
		Less	More	No difference
Injection of Penicillin ...	3	2	—	1
Injection of Streptomycin ...	1	1	—	—
Injection of Normal saline. ...	4	2	1	1

DISCUSSION

Fibrinolysis has been reported to be enhanced by several substances (2, 4, 7, 9, 14, 16). These include various organic solvents, streptokinase and staphylokinase. Trypsin and plasmin have also been tried. Other active substances include nicotinic acid, bacterial lipopolysaccharides (as pyretics) and several other synthetic organic compounds like detergents etc. (16, 17). Many of these substances are active both in vivo and in vitro. Nicotinic acid (18) and pyrogens (15) act only in the body. The synthetic organic compounds have mostly been tried in vitro (16, 17).

The activation may be achieved either by the inactivation of the inhibitors of fibrinolysis as in the case of the organic solvents or by the activation of the precursors as the case of streptokinase and urokinase (2, 14). The activation may be enzymatic as with the kinases or non-enzymatic as in the case of most organic compounds (17). Von Kaulla attributes the activity of these compounds to their chemical structure and he has found certain groups to be active (16).

So far there have been no reports of the effect of any antibiotic in therapeutic use on fibrinolytic activity. Our results in the first part of the experiment suggested such an activity with human euglobulins, but not with monkey, rabbit, rat or chick. Similar species differences between human and bovine plasmas were observed with several organic compounds in vitro (16).

We have observed no activation when antibiotics were added to plasma, but only on addition to the euglobulin fraction. The reverse was observed by Buckell (5) with sodium citrate. Anderson and Lack (1) have recently suggested that considerable variations in the fibrinolytic activity of the euglobulin fraction from human plasma could occur due to variations in the levels of circulating mucopolysaccharides as also differences in pH (to precipitate the euglobulins) and present speed of centrifugation (to collect the precipitate). In the experiments, the quantities of euglobulins precipitated as also their composition may vary between species. This may be a reason for the varying actions (in vitro) of antibiotics between the species.

The results of the second part of the experiment would, however, show that antibiotics had no consistent effect on plasma fibrinolysis. Control subjects were used to assess any changes in fibrinolytic activity due to the stress of injection or bleeding or diurnal fluctuations. When these were ruled out, it appeared that the effect of antibiotics on the fibrinolytic activity was non-specific, as even injections of normal saline were found to produce similar results.

In view of these data, it appears that penicillin and streptomycin do not have any specific action in raising the fibrinolytic activity of blood so that the way in which these agents sometimes reduce the ELT is only of academic interest.

SUMMARY

The addition of penicillin and/or streptomycin to incubation media seemed to have accelerated euglobulin lysis (but not diluted plasma clot lysis) in humans only.

However, this difference was not statistically significant. Enhancement of activity was not found with euglobulins from monkey, rabbit, rat or chick.

Injections of penicillin or streptomycin or normal saline affected the fibrinolytic activity of men in a variable manner, so that fibrinolytic activity could not be specifically attributed to either of the antibiotics.

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