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STUDIES ON THE DIFFERENCES IN ACTIVITY OF LUNG AND BRAIN THROMBOPLASTIN

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Recent reports have suggested that thromboplastin (Tpl) prepared from lung may reflect more accurately than does brain Tpl the changes produced in the prothrombin complex by coumarin derivatives (1, 2). As evidence for this, it has been observed that as factor VII concentration decreases in the plasma of Dicumarol¹-treated patients, the prothrombin time obtained with lung Tpl becomes disproportionately longer than that obtained with brain Tpl (1). The inference from this observation has been that brain Tpl contains a factor VII-like activity, not possessed by lung Tpl. This activity would mask a lowered plasma concentration of factor VII and would result in spuriously short prothrombin times. Verstraete, Clark and Wright (1, 2) concluded that brain thromboplastins possess, although to a lesser degree, the disadvantage previously observed for Russell's viper venom (3, 4). The latter, by virtue of its intrinsic convertin-like activity, is insensitive to changes in factor VII concentration and is, therefore, no longer used in following anti-coagulated patients.

Other observations, however, suggest that brain Tpl does not contain more factor VII activity than does lung and may, in fact, contain less. Mann and Hurn (5) have noted that contamination of the Tpl with small quantities of blood may result in marked enhancement of activity when tested on the plasma of patients receiving coumarin derivatives. If the factor VII-like activity of tissue Tpl was due to contamination by blood and not to intrinsic properties of Tpl itself, one would expect brain Tpl to show less such activity than lung Tpl, inasmuch as lung preparations are more heavily contaminated by blood during their preparation. In addition, Verstraete and associates (1) found that when Tpl solutions were adsorbed with BaSO₄, lung Tpl showed a pronounced decrease

in activity when tested on the plasma of patients receiving Dicumarol, whereas brain Tpl showed virtually no change.

An alternative explanation for the longer prothrombin times obtained with lung Tpl is suggested by the observations of Conley, Morse and Stewart (6). They showed that, if plasma was diluted with saline, the prothrombin times obtained with lung Tpl became shorter than those obtained with the corresponding dilutions in BaSO₄-adsorbed plasma. This would suggest the presence in plasma of an inhibitor of lung Tpl.

The purpose of the present investigation was to study further the differences in activity of lung and brain Tpl.

MATERIALS AND METHODS

Collection of plasma. Nine parts of blood were mixed with one part of 0.1 M sodium oxalate and the plasma separated after centrifuging at 3,000 rpm for 10 minutes. Human plasma was used for all experiments. Normal plasmas were pooled by mixing equal parts from 5 donors.

BaSO₄-adsorbed plasma. Plasma was incubated with BaSO₄ (100 mg per ml of plasma) at 37° C for 10 minutes and then removed after centrifuging.

Commercial thromboplastins. Alban Permaplastin (AP) was used as the source of rabbit brain extract Tpl, and Difco Aplastin (DA) as the source of rabbit lung Tpl. The latter, a dry material, was reconstituted each day by extracting 100 mg of the powder with 4 ml of saline at 50° C for 10 minutes. The extracts were not frozen, as this resulted in considerable loss of activity.

Prepared thromboplastins. Brain and lung Tpl's were prepared from normal rabbits and from Dicumarol-poisoned rabbits. Brain Tpl's were prepared by acetone dehydration (7) and were stored in air-evacuated glass ampuls. Aliquots were reconstituted on the day of use by extracting 100 mg with 4 ml of normal saline at 50° C for 10 minutes. Lung Tpl was prepared as described by Johnson and Seegers (8). The first 24-hour saline extract of the ground lung was discarded. The second 48-hour extract was used as the source of thromboplastin. Rabbits that received Dicumarol were given

¹ Bishydroxycoumarin.

5 mg per kg of body weight each day for 4 days. When sacrificed, their prothrombin times (Permaplastin) were 2 minutes or longer.

Plasma prothrombin conversion inhibitor. To 50 ml of BaSO₄-adsorbed plasma was added an equal volume of saturated ammonium sulfate solution. After standing at 4° C for 10 hours, the material was centrifuged and the supernatant was removed. Saturated ammonium sulfate was then added to bring the final concentration to 80 per cent saturation. The precipitate which formed during 4 hours at 4° C was removed by filtration, dissolved in 5 ml of water and dialyzed until free of the sulfate.

"Albumin-free" plasma fraction. Equal volumes of BaSO₄-adsorbed plasma and saturated ammonium sulfate were mixed and allowed to stand 3 hours at 4° C. After centrifuging, the precipitate was washed twice with cold 50 per cent ammonium sulfate. It was dissolved in saline (one-half the original plasma volume) and dialyzed against normal saline until free of the sulfate.

Plasma eluate. Twenty ml of plasma was incubated with BaSO₄ as described. The BaSO₄ was washed with cold saline and eluted with 2 ml of 0.2 M sodium citrate.

Serum eluate. Blood was allowed to clot and after 6 hours at 37° C the serum was removed by centrifugation. This was adsorbed with BaSO₄ and citrate elution was carried out as for plasma.

Purified prothrombin. This preparation, containing Stuart factor but essentially free of factor VII activity, was kindly supplied by Dr. Sandor Shapiro of the National Institutes of Health.

One-stage prothrombin time (Quick). Equal parts of Tpl extract and 0.025 M CaCl₂ were mixed together and 0.2 ml of this mixture was added to 0.1 ml of plasma.

RESULTS

1. *Differences in activity between brain and lung Tpl.* Prothrombin times were determined using normal plasma diluted with saline and with BaSO₄-adsorbed plasma (Figure 1). At higher dilutions with BaSO₄ plasma, the lung Tpl gave increasingly longer prothrombin times than did brain Tpl, resulting in a wide divergence of the curves. When saline was used as a diluent, the difference between the two Tpl's was considerably less and the curves obtained were almost parallel. Moreover, with brain Tpl the dilution of plasma with saline resulted in considerably longer prothrombin times than did comparable dilution with BaSO₄-adsorbed plasma. When lung Tpl was used the curves obtained with both diluents were similar, and at high dilutions the values obtained with saline were even shorter than those with adsorbed plasma. These findings essentially confirm the observations of Conley and

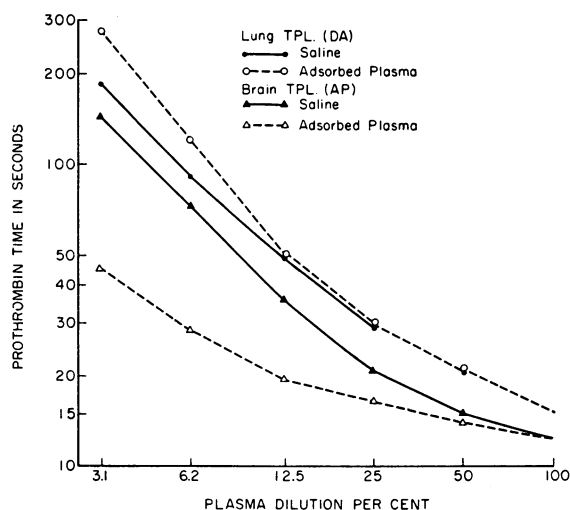


FIG. 1. THE EFFECT OF THE DILUENT ON THE PROTHROMBIN TIMES OBTAINED WITH BRAIN (AP) AND LUNG (DA) TPL. Pooled, normal plasma was diluted with normal saline and with BaSO₄-adsorbed plasma.

associates (6) and Verstraete and co-workers (1), and strongly suggest the presence in BaSO₄-adsorbed plasma of an inhibitor of lung Tpl that becomes more effective at increasing dilutions of plasma.

Further evidence for the presence of an inhibitor was obtained by diluting plasma with "albumin-free" plasma fraction. The curves obtained with

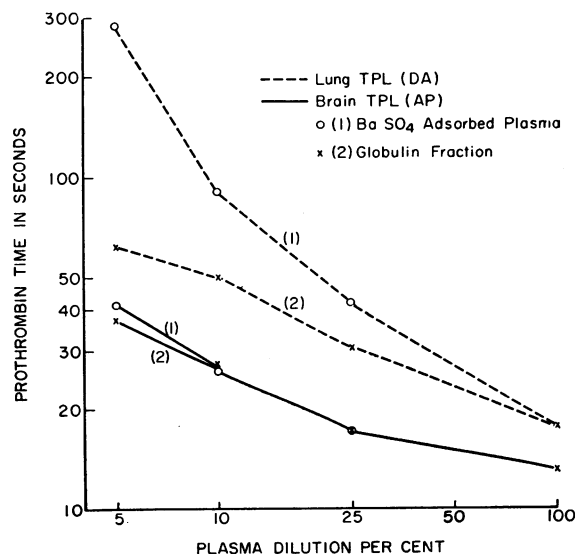


FIG. 2. PROTHROMBIN TIMES OF POOLED, NORMAL PLASMA DILUTED IN 1) BaSO₄-ADSORBED PLASMA, 2) "ALBUMIN-FREE" PLASMA FRACTION PREPARED FROM BaSO₄-ADSORBED PLASMA.

the two Tpl's were compared with those using the ordinary adsorbed plasma as diluent (Figure 2). When brain Tpl was used there was virtually no difference between the two diluents. With lung Tpl, however, the curve obtained with "albumin-free" plasma was much flatter and was now parallel to the curve obtained with brain Tpl.

2. Evidence that brain thromboplastin does not possess a greater factor VII-like activity than lung. It has been observed that Tpl obtained from the tissues of Dicumarol-poisoned animals is less active with the plasma of coumarin-treated patients than is Tpl from normal animals (9-12). According to Mann and Hurn (5), this difference reflects the contamination of tissue Tpl with blood. If the blood trapped in the tissues has been depleted of coagulation factors, the Tpl extracted from these tissues would be correspondingly less active. If brain Tpl contains more factor VII activity than lung, then Dicumarol poisoning would cause a greater change in the activity of brain Tpl. To test this, plasma was diluted with BaSO₄-adsorbed plasma and tested with brain and lung Tpl obtained from normal and Dicumarol-treated rabbits (Figure 3). The results show that pretreating animals with Dicumarol had a greater effect in decreasing the activity of lung Tpl, suggesting that

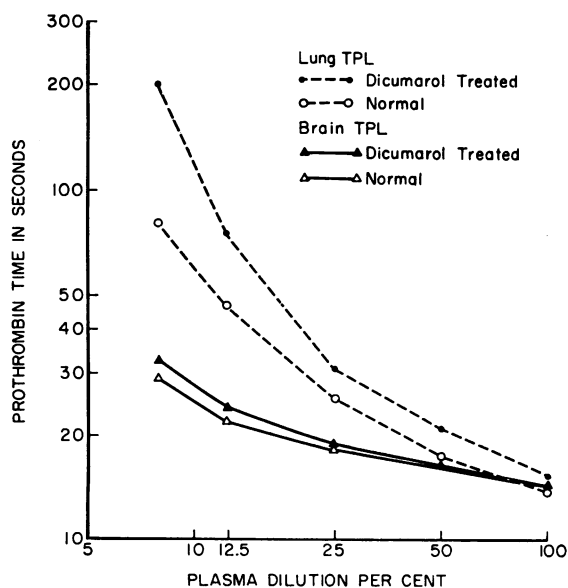


FIG. 3. PROTHROMBIN TIMES OF NORMAL PLASMA DILUTED WITH BaSO₄-ADSORBED PLASMA, USING BRAIN AND LUNG TPL FROM NORMAL AND DICUMAROL-TREATED RABBITS.

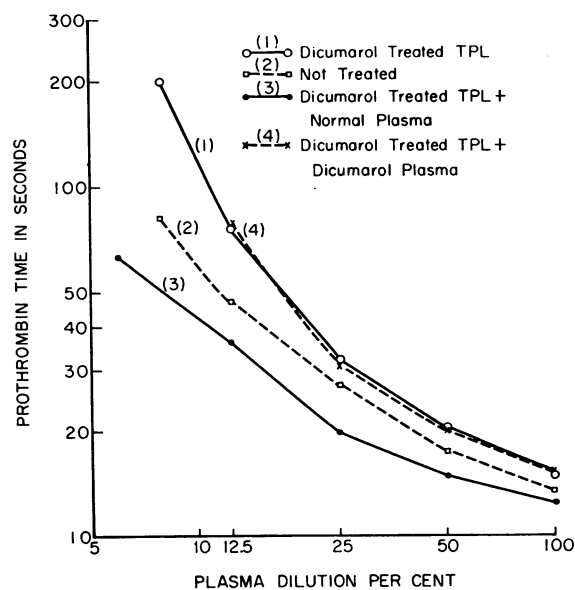


FIG. 4. PROTHROMBIN TIMES OF NORMAL PLASMA DILUTED WITH BaSO₄-ADSORBED PLASMA USING LUNG TPL 1) FROM RABBITS WHICH HAD RECEIVED DICUMAROL AND 2) FROM NORMAL RABBITS. Hemoglobin concentration of these extracts was 75 mg per 100 ml. Curve 3 shows the result of adding to the "Dicumarol-treated" Tpl 0.0025 ml of normal rabbit plasma per ml of Tpl solution, which corresponds roughly to a hemoglobin contamination of 50 mg per 100 ml. In curve 4 the same amount of plasma from a Dicumarol-treated rabbit was used.

factor VII activity is lower in brain than in lung Tpl.

The hemoglobin concentration in the brain Tpl solution was 1.0 mg per 100 ml, and in the lung Tpl, 80 mg per 100 ml. It was estimated that a hemoglobin concentration of 50 mg per 100 ml corresponded to a plasma contamination of 0.0025 ml per ml of thromboplastin extract. Therefore, this amount of plasma was added to the Dicumarol-treated lung thromboplastin and the effect on its activity was observed (Figure 4). The addition of normal rabbit plasma resulted in a markedly enhanced activity, while the addition of an equivalent amount of plasma from a Dicumarol-treated animal had no effect.

It is concluded that contamination of Tpl by blood alters the activity considerably and that, because of its greater contamination, lung is more affected than brain Tpl. The hemoglobin concentrations in the commercial Tpl's used throughout this study were 2 mg per 100 ml for brain, and 37 mg for lung Tpl.

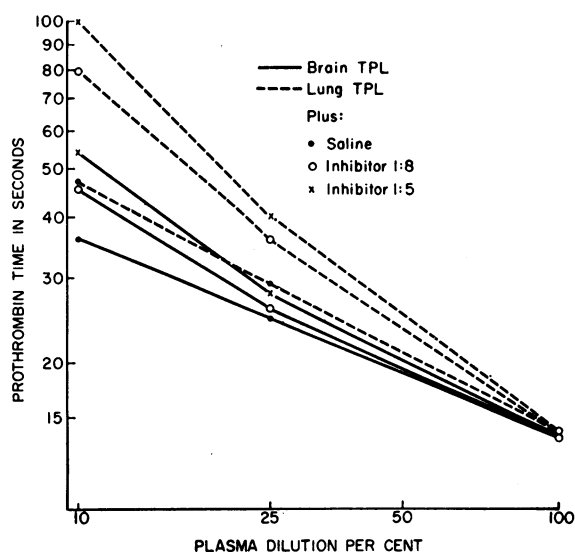


FIG. 5. EFFECT OF ADDING INHIBITOR TO NORMAL PLASMA DILUTED WITH SALINE: 0.2 ML TPL + 0.1 ML PLASMA + 0.02 ML OF NORMAL SALINE OR INHIBITOR.

3. *Effect of plasma prothrombin-conversion inhibitor (PPCI) on brain and lung Tpl.* PPCI diluted 1:5 and 1:8 was added to plasma diluted with saline and prothrombin times were performed. The results show that the prolongation of the prothrombin times at increasing dilutions of

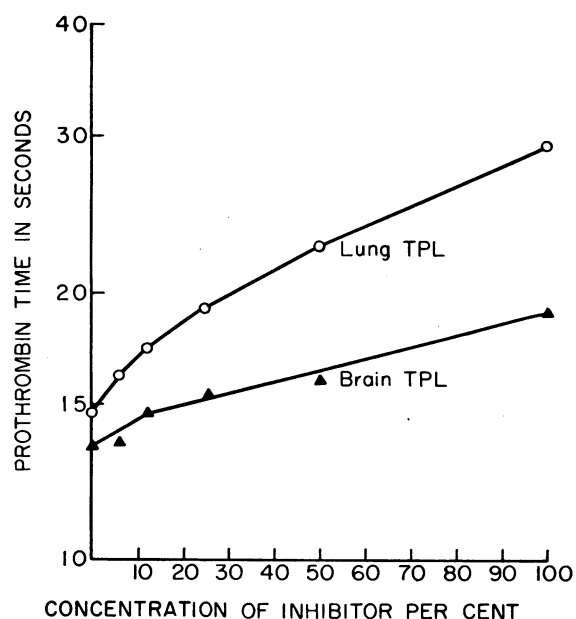


FIG. 6. EFFECT OF ADDING INHIBITOR TO NORMAL PLASMA: 0.2 ML TPL + Ca + 0.1 ML PLASMA + 0.02 ML INHIBITOR.

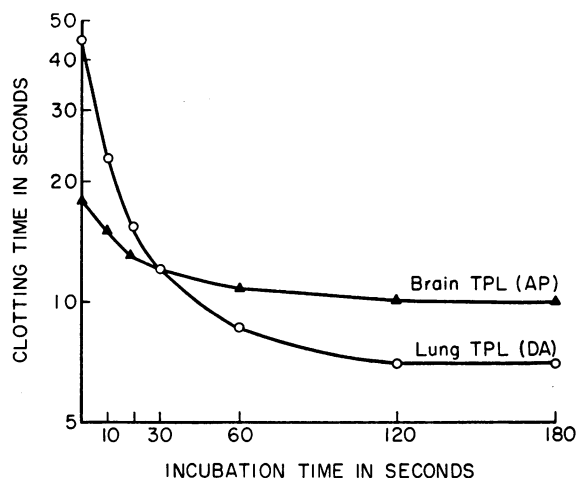


FIG. 7. EFFECT OF PRE-INCUBATING TPL + Ca WITH PLASMA ELUATE BEFORE ADDING TO BaSO_4 -ADSORBED PLASMA: 0.2 ML TPL + Ca + 0.1 ML PLASMA ELUATE (1:20) INCUBATED FOR 2 MINUTES AT 37°C AND THEN 0.1 ML BaSO_4 -ADSORBED PLASMA ADDED.

plasma was greater with lung than with brain Tpl (Figure 5).

Increasing concentrations of PPCI were added to normal plasma. The effects on the prothrombin time are shown in Figure 6. Increasing the concentration of inhibitor has a greater effect in prolonging the prothrombin time with lung than with brain Tpl.

The activity of the two types of Tpl was further studied in inhibitor-free systems. Tpl + calcium mixture was pre-incubated for varying periods of time with plasma eluate, then added to BaSO_4 -adsorbed plasma and the clotting time recorded (Figure 7). When plasma eluate diluted 1:20 was added to Tpl + Ca and the mixture was added immediately to adsorbed plasma without pre-incubation, lung Tpl gave a clotting time of 45 seconds, while brain gave 18 seconds. However, with pre-incubation, the clotting time obtained with lung Tpl sharply decreased. After 2 minutes of pre-incubation, it was shortened to 7 seconds, compared with 10 seconds for brain Tpl. When eluate dilutions of 1:40 were used, pre-incubation for 2 minutes with lung Tpl shortened the clotting time from 155 to 12 seconds, whereas with brain Tpl it was only shortened from 34 to 20 seconds. Thus, in the absence of inhibitor, lung Tpl appeared to be more active than brain Tpl.

The effect of inhibitor on the prothrombin conversion system was then studied. Plasma eluate

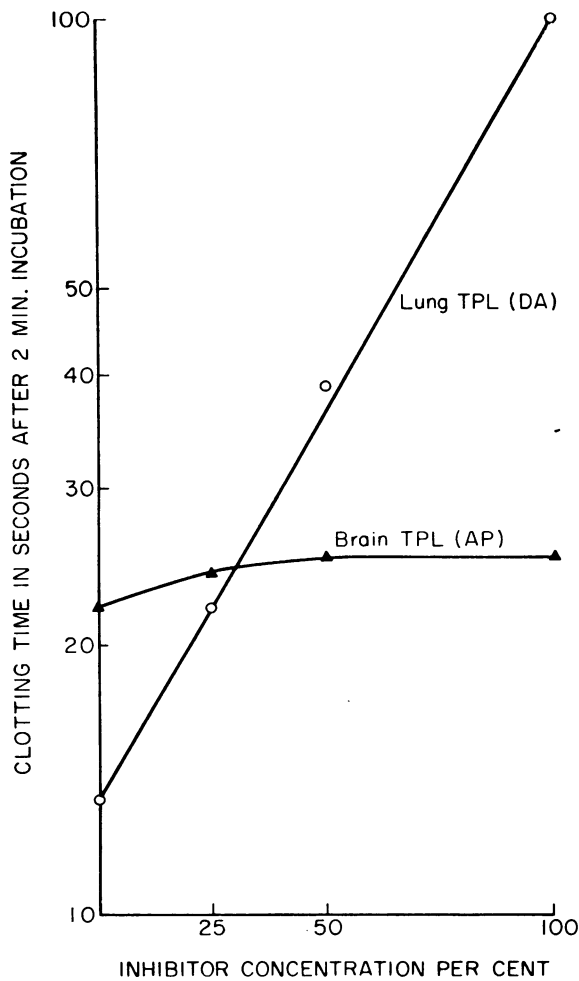


FIG. 8. EFFECT OF ADDING INHIBITOR DURING THE PRE-INCUBATION (2 MINUTES) OF TPL WITH PLASMA ELUATE: 0.2 ML TPL + Ca + 0.1 ML PLASMA ELUATE (1:40) + 0.02 ML INHIBITOR DILUTED IN SALINE INCUBATED 2 MINUTES AT 37° C AND THEN 0.1 ML BaSO₄-ADSORBED PLASMA ADDED.

and Tpl + Ca were pre-incubated in the presence of increasing amounts of inhibitor for 2 minutes, at which time BaSO₄-adsorbed plasma was added. A dilution of plasma eluate of 1:40 was used in this experiment. Figure 8 shows the marked effect of the inhibitor on lung Tpl and the negligible effect on brain Tpl.

Studies were also performed with varying concentrations of eluate and inhibitor (Figure 9). Pre-incubation of Tpl + Ca + eluate was again carried out for 2 minutes in the presence of inhibitor. The results show that with no inhibitor, lung Tpl was more active (i.e., gave shorter prothrom-

bin times) than did brain Tpl at all concentrations of plasma eluate. When inhibitor was added, there was virtually no effect on the curves obtained with brain Tpl, while the curves obtained with lung Tpl became widely divergent.

Further studies were then carried out to determine the reason for the greater activity of lung Tpl when pre-incubated with plasma eluate. During this pre-incubation, activation of at least two systems, factor VII and prothrombin (13-16), is known to occur.

To determine the effects of pre-incubation on the factor VII system, the Tpl + Ca mixtures were pre-incubated with a serum eluate for varying periods of time and then added to a mixture containing BaSO₄-adsorbed plasma and purified

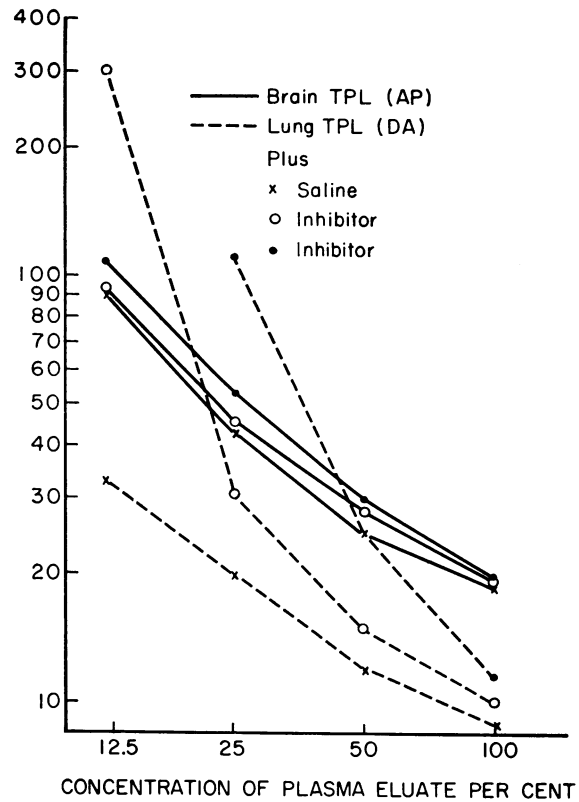


FIG. 9. EFFECT OF ADDING INHIBITOR DURING PRE-INCUBATION (2 MINUTES) OF TPL WITH PLASMA ELUATE: 0.2 ML TPL + Ca + 0.1 ML PLASMA ELUATE OF VARYING CONCENTRATION + 0.02 ML NORMAL SALINE OR INHIBITOR INCUBATED 2 MINUTES AT 37° C AND THEN 0.1 ML BaSO₄-ADSORBED PLASMA ADDED. A 1:20 eluate dilution is represented as 100 per cent. Inhibitor 1:20 is represented by solid circles. Inhibitor 1:40 is represented by open circles.

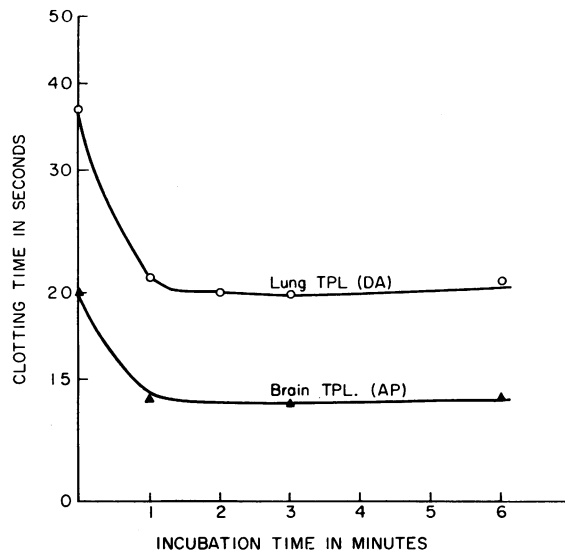


FIG. 10. EFFECT OF PRE-INCUBATING TPL WITH SERUM ELUATE: 0.2 ML TPL + CA + 0.1 ML SERUM ELUATE (1:40) INCUBATED FOR VARYING TIME AT 37° C FOLLOWED BY THE ADDITION OF 0.1 ML BaSO₄-ADSORBED PLASMA + 0.1 ML PURIFIED PROTHROMBIN (12.5 U ADDED).

prothrombin. This is, in essence, a factor VII generation test and, as may be seen in Figure 10, the rate of change of activity is the same for both types of Tpl, and the brain Tpl develops greater maximal activity.

When, however, the Tpl + Ca mixtures are pre-incubated with purified prothrombin and then added to a mixture of BaSO₄-adsorbed plasma and serum eluate (Figure 11), the lung shows a marked increase in activity while the activity of brain Tpl is virtually unchanged.

The increase in activity of lung Tpl during pre-incubation with prothrombin probably represents actual thrombin formation. When Tpl + Ca was pre-incubated with 25 U of prothrombin for 3 minutes and then added to a BaSO₄-adsorbed fibrinogen solution (0.4 per cent), a clot was obtained in 50 seconds with lung Tpl, while with brain Tpl the clotting time was 12 minutes.

The possibility of contamination of lung Tpl with factor V was studied. The markedly greater thrombin-forming capacity of lung Tpl in the presence of purified prothrombin indicated that the lung Tpl probably contained a significant amount of factor V activity. To compare this activity with that in brain Tpl, prothrombin times were performed on plasma whose factor V concentration

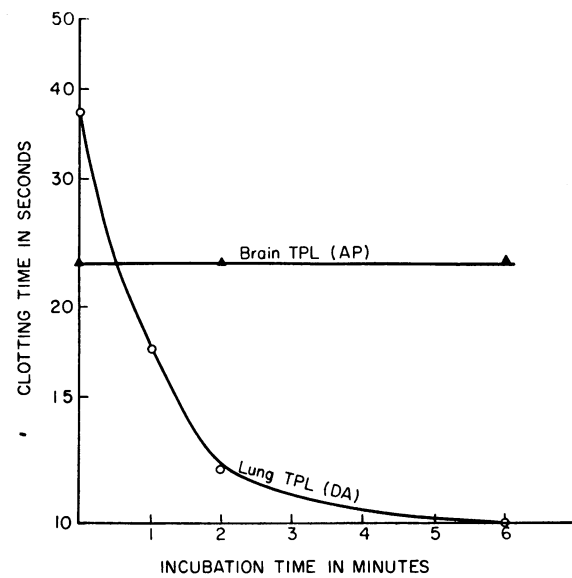


FIG. 11. EFFECT OF PRE-INCUBATING TPL WITH PURIFIED PROTHROMBIN: 0.2 ML TPL + CA + 0.1 ML PROTHROMBIN (12.5 U) INCUBATED FOR VARYING TIME AT 37° C, FOLLOWED BY THE ADDITION OF 0.1 ML BaSO₄-ADSORBED PLASMA + 0.1 ML SERUM ELUATE (1:40).

had been reduced by dilution with aged, normal plasma (Figure 12). With lung Tpl the curve was flatter, and at very low concentrations of factor V the prothrombin times were shorter than with brain Tpl. Freezing and thawing can reduce factor V activity of plasma. The reduction of Tpl activity of lung preparations after freezing may be a result of the destruction of contaminant factor V.

The possible mechanism of action of the inhibitor was next studied (Table I). When lung Tpl + Ca + serum eluate, 1:40, + prothrombin were

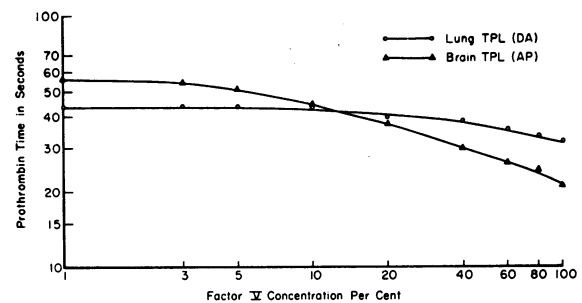


FIG. 12. EFFECT OF FACTOR V DILUTION ON PROTHROMBIN TIMES. Normal plasma was diluted with saline (1 to 100 per cent) and 0.1 ml was added to 0.9 ml of aged plasma. Prothrombin times were done on 0.1 ml of this mixture.

TABLE I
Action of inhibitors *

Experiment no.	Time added (min)				Clotting time (sec)
	0	2	4	6	
1	Ba† + Proth.‡				55
2		Ba + Proth.			21
3			Ba + Proth.		24
4		Proth.	Ba		10
5		Proth. + In.§			62
6		Proth.	In.	Ba	12
7		Proth.		Ba	13

* Two-tenths ml lung Tpl (DA) + Ca were added to 0.1 ml of serum eluate, 1:40, at zero time. The substances indicated were added at 0, 2, 4 and 6 minutes. Clotting times were obtained after the addition of BaSO₄-adsorbed plasma.

† BaSO₄-adsorbed plasma, 0.1 ml.

‡ Purified prothrombin (12.5 U).

§ Inhibitor, 0.02 ml.

mixed with BaSO₄ plasma without any pre-incubation, a clotting time of 55 seconds was obtained. If the Tpl + Ca was pre-incubated with serum eluate *before* adding the adsorbed plasma and prothrombin (thereby activating factor VII), a maximal shortening of the clotting time (21 seconds) was achieved after 2 minutes of pre-incubation (Experiments 1, 2 and 3).

Using this procedure, the experiment was conducted as follows: Tpl + Ca + serum eluate were incubated for 2 minutes, then prothrombin was added and after 2 minutes more the adsorbed plasma was added; clotting occurred at 10 seconds. If, in this sequence, inhibitor was added at the same time as the prothrombin, clotting occurred at 62 seconds.

If the addition of inhibitor was delayed until thrombin had formed, there was no inhibition. Thus, if prothrombin was added at 2 minutes, inhibitor at 4 minutes, and adsorbed plasma at 6 minutes, clotting occurred at 12 seconds. It would seem that the inhibitor acts by interfering with the conversion of prothrombin to thrombin and is effective even after factor VII has been activated.

4. *Studies of lung and brain Tpl on patients receiving coumarin drugs.* Prothrombin times were performed on an unselected group of patients who were receiving Coumadin,² using lung and brain Tpl. The prothrombin activity was calculated as percentage of normal plasmas diluted with adsorbed plasma and with saline (Figure 13). In all cases the prothrombin times obtained with lung Tpl were longer than those obtained with brain

Tpl. When prothrombin activity was calculated from the saline dilution curves, the values were always lower for lung than for brain Tpl. However, on the adsorbed plasma dilution curve, the value for lung Tpl was higher. Similar results were obtained on a patient who was followed throughout a course of therapy with Coumadin (Figure 14).

The choice of diluent markedly affected the calculation of percentage activity when brain Tpl was used; with saline, the values were considerably higher than with adsorbed plasma. With lung Tpl, the calculated activity was essentially the same for both diluents.

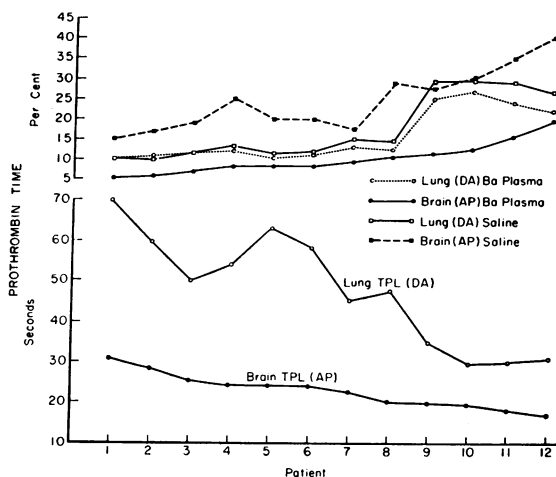


FIG. 13. PROTHROMBIN DETERMINATIONS ON 12 PATIENTS RECEIVING COUMADIN, USING LUNG AND BRAIN TPL. The results are expressed in seconds and percentage of normal activity, using reference dilution curves obtained with BaSO₄-adsorbed plasma or saline.

² Warfarin sodium.

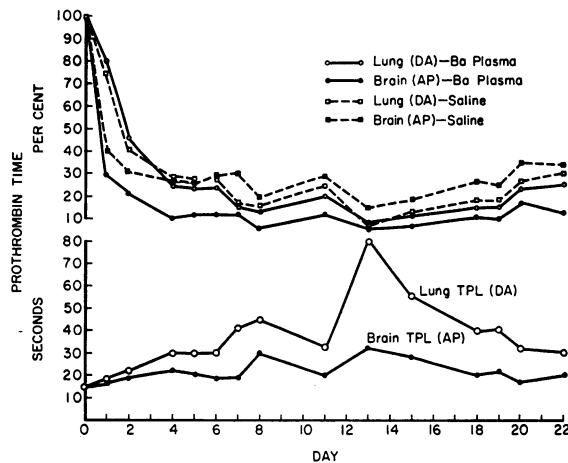


FIG. 14. PROTHROMBIN DETERMINATIONS ON A SINGLE PATIENT WHO RECEIVED COUMADIN, USING LUNG AND BRAIN TPL. Calculation of percentage was obtained from both saline and BaSO₄-adsorbed plasma dilution curves.

DISCUSSION

When different tissue Tpl's are used to perform prothrombin times on the plasma of a patient receiving coumarin derivatives, widely varying results may be obtained (1, 2, 6, 17-21). In particular, it has been noted that lung Tpl's give longer prothrombin times than do brain Tpl's. Verstraete and co-workers also showed that the disproportionately long prothrombin times obtained with lung Tpl and plasma diluted with BaSO₄-adsorbed plasma was due to a dilution not of prothrombin, but of factor VII (1). They concluded that this difference might represent a greater factor VII-like activity of brain extracts, and they suggested therefore that lung Tpl might be a more sensitive indicator of factor VII depletion. Other authors have also stressed the possibility of "activators" in tissue Tpl (19, 20). Since a reduction in factor VII appears to be a major effect of coumarin drugs (22, 23), the employment of a tissue Tpl sensitive to changes in its activity is obviously important for controlling coumarin therapy. It is for such reasons that Russell's viper venom, which apparently contains factor VII activity, is not recommended (3, 4).

However, the longer prothrombin times obtained with lung extracts do not necessarily mean that brain Tpl contains more factor VII-like activity. The previous work of Conley and associates (6) suggested that lung Tpl might be more sensitive to the effect of an inhibitor in

BaSO₄-adsorbed plasma. In view of the findings of Verstraete and co-workers (1, 2) this inhibitor might involve the factor VII system. Such plasma inhibitors of the prothrombin conversion system have been described. Wagner, Brannan and Brinkhous (24) described an antagonist of factor VII present in the fraction of canine plasma which is precipitated by ammonium sulfate between 70 and 80 per cent saturation. Schaefer, Therriault and Jensen (25) described an inhibitor in rabbit serum which precipitated between 50 and 80 per cent saturation and which inhibited the interaction of prothrombin-converting factor (presumably factor VII) and prothrombin. In addition, a lipid-extractable inhibitor in plasma with the properties of an antithromboplastin has been described (26), which may act by inhibiting the activation of factor VII (25). Other Tpl inhibitors have been reported by Overman (27) and by Lanchantin and Ware (28).

Our studies indicate that an inhibitor, precipitated by ammonium sulfate between 50 and 80 per cent saturation, is present in human plasma. This inhibitor is more active when lung extract is used as a source of tissue Tpl than when brain extract is used. The presence of such an inhibitor would explain why lung Tpl gives longer prothrombin times at increasing dilutions of plasma in BaSO₄-adsorbed plasma than does brain Tpl. It would also explain the shortening of the prothrombin time observed for lung Tpl when saline instead of BaSO₄-adsorbed plasma was used as a diluent. Therefore, the shorter prothrombin times obtained with brain Tpl on the plasma of anticoagulated patients need not mean that brain extract contains more factor VII activity, nor is it necessarily a less sensitive Tpl.

The importance of expressing prothrombin activities as percentage of normal rather than as absolute prothrombin times has been stressed (17). In this respect, the choice of diluent used in obtaining a normal curve is important. The ideal diluent would be human plasma which has been depleted of factors reduced by coumarin derivatives. Conley and associates (6) showed that BaSO₄-adsorbed plasma best approximates this. Our studies have shown that, when this diluent is used, brain Tpl, although giving a shorter prothrombin time, actually reveals a lower percentage of activity than does lung Tpl.

SUMMARY

The differences in activity between lung and brain thromboplastin (Tpl) have been studied—in particular, the greater prolongation of the prothrombin times obtained with lung Tpl at decreasing factor VII concentration. It was found that lung extract was actually more active when the prothrombin conversion system was studied in the absence of plasma inhibitors. In the presence of purified prothrombin, lung Tpl produced a greater coagulant effect than did brain thromboplastin.

The values obtained with lung Tpl are evidently subject to two uncertain variables: 1) the amount of blood remaining in the tissue extract, and 2) the susceptibility of lung Tpl to the effect of an inhibitor present in normal plasma. Thus, one variable tends to speed up the prothrombin time; the other tends to slow it down. The results obtained with lung Tpl could vary with factors other than the "prothrombin complex" in the plasma of the patient.

Brain Tpl is not subject to these variables. It contains insignificant amounts of blood and it is not affected by the inhibitor.

The practice of mixing lung and brain Tpl in unspecified proportions, as recommended by the USP (16th ed.), would seem to compound the variables and the problems of controlling anticoagulant therapy.

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