Intravenous Trypsin

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Studies were carried out regarding the effect of intravenous trypsin on rabbits. These included investigations into the toxicity and the tendency for the production of intravascular coagulation. The effect on artificial preformed thrombi was also observed. It was found that in the doses used, the intravenous administration of trypsin was not efficacious in the treatment of intravascular thrombosis. These studies failed to provide evidence to justify its use as a therapeutic agent in man.

Interest in the possible therapeutic value of crystalline trypsin, administered intravenously, has been stimulated by favorable reports by Innerfield, Schwartz and Angrist.1, 2, 3 These investigators have claimed that trypsin may be safely administered intravenously, provided the concentration and rate of administration of the solution is strictly controlled. From their animal studies with this substance they have reported lytic effects on preformed artificial thrombi and, in certain dosage, consistent “complete dissolution of the thrombus with restoration of circulatory integrity.” Recently they have reported beneficial results following its administration to 538 patients,4 but in their report an antiinflammatory effect was stressed rather than a thrombolytic one.

One of the earliest statements concerning blood coagulation and trypsin was made by Wright4 in 1915. At that time, he said: “The antitryptic power of the blood fluids represents, be it noted, much more than merely a power of inhibiting microbic growth. With the loss of antitryptic power are lost also the complementing, opsonic, bactericidal and coagulating powers of the blood.” The following year Douglas and Colebrook5 indicated that Wright’s report had influenced them in their search for an ideal blood culture medium. They reported his observations and stated: “Emery, continuing these investigations, showed that by neutralizing the antitryptic power of the serum with trypsin the bactericidal action and other bacteriotropic properties of the serum were destroyed. From the same laboratory came the fact that when sufficient trypsin was added to the blood to neutralize the antitryptic power of the blood fluids no clotting took place.” Douglas and Colebrook then carried out some investigational work of their own and reported as follows: “Further experiments were carried out to find the effect of various dilutions of trypsin on the clotting time of the blood. The result obtained was that a dilution of trypsin of about 1 in 30 completely inhibited the clotting power whereas weaker dilutions had the effect of making the blood clot very much more rapidly.”

This paradoxic action of trypsin has been repeatedly confirmed in later literature but has not as yet been adequately explained. It is more or less agreed that an excess of trypsin in the blood results in inhibition of coagulation because one or more of the blood clotting components is partly or completely digested. The fact, however, that in smaller amounts it accelerates coagulation has proved to be a more complex problem. Dale and Walpole,6 in 1916, observed that a “formation of kinase and (in the presence of calcium) thrombin occurs when fowl’s plasma is treated with an appropriate proportion of trypsin.” In 1917, Heard7 observed that trypsin solutions in certain concentrations will clot oxalated blood and offered the following explanation: “Trypsin being especially active in disturbing the P and Ca in the protein molecule it is held to be probable that the surface forces brought into action by the disturbance of these atoms are the principal cause of the clotting.” In 1928, Waldschmidt-Leitz8 suggested that trypsin

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accelerated coagulation by hastening the enzymatic hydrolysis of fibrinogen by thrombin. In 1935, Mellanby\(^9\) stated his belief that the coagulation observed by Heard was simply due to traces of calcium present in the commercial trypsin preparation. In 1937, however, Eagle and Harris,\(^10\) illustrated “the fact that coagulation is observed only within a comparatively narrow optimum zone of trypsin concentration.” They pointed out that trypsin, unlike papain, does not coagulate fibrinogen which contradicted the theory of Waldschmidt-Leitz that trypsin is analogous to thrombin. On the basis of their experimental work they came to the conclusion that trypsin activated prothrombin to thrombin and that it is therefore analogous to the physiologic system, calcium plus platelets (or calcium plus tissue extracts). They further suggested tentatively that calcium plus platelets constituted a proteolytic enzyme. The role of trypsin and of tissue proteolytic enzymes (tryptase or plasmin) on blood clotting has been studied in detail by Ferguson and co-workers,\(^11, 12\) and interesting related experiments have been conducted by Kleinfield and Habif.\(^13\)

Eagle and Harris\(^10\) then injected trypsin intravenously into rabbits and reported that it caused almost immediate death, large blood clots being found in the heart and large vessels at autopsy. They concluded that “trypsin thus seems to initiate blood coagulation in vivo as readily as it does in vitro.” This observation was confirmed by Tagnon\(^14\) and others.

In view of these early reports the intravenous administration of trypsin had seemed inadvisable, but following the reports of Innerfield and co-workers, it was decided to carry out independent animal experiments in our laboratory. The results of some of our work are here described briefly and our conclusions presented. The lyophilized crystalline trypsin (Armour Enzar) used in these experiments has been stated to be composed of 90 per cent pure trypsin, 5 per cent magnesium ash, 4 per cent traces of ribonuclease plus cathepsin and alpha, beta and gamma chymotrypsin, and 1 per cent undetermined matter. Two thousand Armour units of trypsin (Enzar) correspond approximately to 1.1 mg. by weight.

**Results of Animal Experiments**

I. **Toxicity**

Twenty mg. of trypsin in 10 ml. of saline were administered intravenously over a period of 45 seconds to each of six 3 Kg. rabbits. All six animals died almost immediately after a few brief convulsions. Autopsy examination revealed large adherent blood clots in the chambers of the right side of the heart in every case.

It was then found that if the trypsin solution (20 mg. per 10 ml. of saline) was administered more slowly, over a period of 15 to 20 minutes, the mortality rate was reduced to approximately 50 per cent. The concentration of the solution also appeared to be a factor involved in the mortality rate, and it was soon found to be possible to administer 100 mg. of trypsin in 200 ml. of saline at an approximate rate of 15 drops per minute with the same approximate mortality. Local phlebothrombosis, cold cyanosed ears and dyspnea were frequent complications noted during administration. Soybean trypsin inhibitor administered to the animal immediately prior to the trypsin (in doses equivalent in milligrams to the trypsin dosage about to be given) eliminated the mortality rate. The use of a trypsin solution buffered to the pH of rabbit blood was no less lethal than the unbuffered solution.

II. **Results of Intravenous Administration**

It was found that the intravenous administration of small amounts of trypsin (5 to 15 mg.) had no detectable effect whatever on the blood clotting mechanism, whereas large amounts had a marked effect on the blood clotting mechanism.

III. **Results of in Vitro Studies**

A. It was confirmed that there is a narrow optimum zone in which small amounts of trypsin will accelerate blood coagulation. In large amounts blood coagulation is completely inhibited. Soybean trypsin inhibitor when added to the trypsin solution in various
amounts resulted in inhibition of the trypsin effect on the blood coagulation mechanism.

B. Using oxalated blood, results of a similar nature were obtained but clotting times were less rapid. The addition of calcium along with the trypsin, however, resulted in accelerated clotting times which approached those obtained with trypsin and unoxalated blood.

C. Oxalated rabbit blood was treated with barium carbonate to remove the prothrombin. It was then found that the blood could still be clotted by the addition of thrombin but not by the addition of trypsin.

D. It was confirmed that, when trypsin is brought into contact with fibrinogen or thrombin, changes result which prevent coagulation. It is believed that this inhibition of coagulation is the result of partial or complete digestion of the thrombin or fibrinogen, but this has not been finally determined.

E. Using fibrin plates prepared according to the method of Astrup and Müllertz it was found that no evidence of fibrinolytic activity of rabbit plasma could be demonstrated during a trypsin infusion until approximately 100 mg. had been administered. At this stage there was free trypsin in the plasma as it could be used to clot oxalated rabbit plasma and could be inhibited by soybean antitrypsin. It was also demonstrated by the use of these plates that normal rabbit plasma has considerable antitryptic properties, as it can protect any portion of the fibrin on which it is spread from proteolysis by a trypsin solution which has dissolved the unprotected portion of the plate.

F. When a trypsin solution is added to a solution of Overman's thromboplastic inhibitor in various amounts, a mixture results which causes marked acceleration of the clotting time of rabbit blood.

G. Heparinized blood can be clotted by trypsin solutions within a very narrow concentration range. This finding is contradictory to the findings of Tyson and West.

IV. Inhibition of Trypsin-Induced Intravascular Coagulation

Experiments were then carried out to determine whether any of the anticoagulants could prevent intravascular coagulation and death by trypsin. Heparin was found to be such a substance, thus confirming the reports by Tagnon, by Schultze and Schwick, and by Kleinfeld and Habif. The exact mechanism of its protective effect is uncertain. The last named authors feel that it acts by increasing plasma antithrombic activity. Dicumarol in doses which resulted in a prothrombin time four times greater than normal did not protect the animals but in two rabbits 150 mg. of cyclocumarol resulted in a prothrombin time greater than three minutes, a potentially dangerous level. In these two cases trypsin was administered rapidly without death occurring. A thrombin solution administered slowly until the blood was incoagulable also resulted in a considerable reduction in the death rate due to intravenous trypsin, the only deaths in this particular series being due to hemorrhages.

V. Potentiation of Trypsin-induced Intravascular Coagulation

In view of the fact that trypsin and thromboplastic inhibitor together form an extremely active coagulating agent in vitro it was decided to determine whether it would result in intravascular coagulation and death when

### Table 1.—Effect of Small and Large Amounts of Intravenous Trypsin on the Blood Coagulation Mechanism

<table>
<thead>
<tr>
<th>Trypsin Level</th>
<th>Clotting Time (min.)</th>
<th>Prothrombin Time (sec.)</th>
<th>Calcium Chloride Time (sec.)</th>
<th>Antithrombin clotting Time (Quick's Method) (sec.)</th>
<th>Proconvertin Time (Owen's Method) (sec.)</th>
<th>Fibrinogen mg.%</th>
<th>Total Protein Gm.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.5</td>
<td>8.5</td>
<td>45</td>
<td>27</td>
<td>58</td>
<td>270</td>
<td>5.1</td>
</tr>
<tr>
<td>15 mg.</td>
<td>5</td>
<td>8.2</td>
<td>48</td>
<td>28</td>
<td>58</td>
<td>270</td>
<td>5.1</td>
</tr>
<tr>
<td>120 mg.</td>
<td>∞</td>
<td>∞</td>
<td>∞</td>
<td>16</td>
<td>57</td>
<td>Absent</td>
<td>4.8</td>
</tr>
</tbody>
</table>

∞ Represents infinity.
administered intravenously. This was proven to be the case, a mortality rate resulting which was even higher than that due to trypsin alone. At autopsy large adherent blood clots were found in the right side of the heart, and also in the great veins and the pulmonary arteries.

VI. Pathology

Almost all animals dying in the early stages of a trypsin infusion exhibit large adherent blood clots in the right auricles and ventricles at autopsy. Fairly frequently clots were also found in the large veins and in the pulmonary arteries. Hemorrhages into the lungs and myocardium were occasionally noted in the early stages, but these were the commonest lesions found at autopsy when death occurred during the latter part of the infusion, particularly after 35 mg. per kilogram had been administered and the blood was incoagulable. Retroperitoneal hemorrhages were also occasionally noted at this time.

A number of animals which survived the intravenous administration of varying doses of trypsin (from 8 to 110 mg.) were sacrificed 48 hours after the infusion. Microscopic examination of the tissues of these animals by Kellner revealed the presence of numerous isolated areas of necrosis in the myocardium and in the musculature of the diaphragm.

These lesions were unrelated to areas of hemorrhage, or to thrombosis or emboli.

VII. Electrocardiography

An electrocardiographic study of animals receiving intravenous trypsin was then carried out. In a controlled study tracings taken by Lieberman and colleagues showed that changes in the record frequently occurred when 20 mg. and over were administered in a fairly concentrated solution, (1 mg. trypsin per milliliter saline). These changes were not observed during similar infusions with saline nor with solutions of the same pH as the trypsin solution. Similar changes were, however, seen when a thrombin solution (1000 units per 100 ml. saline) was slowly infused. When some of the animals which exhibited electrocardiographic changes following the trypsin infusion were sacrificed and autopsied 48 hours later, small isolated areas of necrosis were noted in the myocardium and diaphragm.

VIII. Effect on Artificial Preformed Thrombi

An attempt was then made to determine the effect of intravenous trypsin infusions on preformed artificial thrombi. Sections of the peripheral ear veins of many rabbits were thrombosed using thrombin (200 units) or a sclerosing agent known as sodium Sotradecol (brand of sodium tetradecyl sulfate). Within
eight hours of the thrombosing process in each case, the trypsin infusion was begun and doses varied from 10 mg. on five successive days to 150 mg. on four successive days. Single doses of 100 mg. and successive doses of 50 mg. were also administered. The trypsin solution in every case contained approximately 0.55 mg. trypsin per milliliter saline. During these infusions 40 per cent of the animals died and 35 per cent developed a thrombosis of the ear vein into which the trypsin was being administered. In no single animal was any reduction noted in the size of the thrombus. The vein remained palpable and on translumination no return of blood flow was noted. In several animals an actual extension of the thrombus was evident.

**DISCUSSION**

On the basis of these animal experiments, using a wide range of dosage, it would appear that the intravenous administration of trypsin is of doubtful value in the treatment of intravascular thrombosis. We have been unable to confirm the observation that in similar experiments with rabbits “complete clot dissolution and restoration of circulatory integrity consistently occurred at a range beyond 200,000 units of trypsin.” Indeed, extension of the preformed thrombi was noted in many animals.

In a recent report Innerfield and associates have described the results of intravenous trypsin therapy with 538 patients. They stated that intravascular coagulation did not occur in these cases. An antiinflammatory effect, however, was emphasized rather than the thrombolytic one which they stressed in their earlier reports. The dosage of trypsin used in the latter study was markedly reduced as compared with earlier reports. Formerly 100,000 to 250,000 Armour units of trypsin diluted in 250 ml. of saline were administered once or twice a day, but recently they have recommended 50,000 to 100,000 units twice daily in 100 ml. of saline. Intramuscular administration has also been suggested, using doses as low as 20,000 units. There is, however, no conclusive evidence that trypsin thus administered has any physiologic or therapeutic effect.

Independent clinical experiments carried out by Laufman and Roach, using 250,000 units dissolved in 500 ml. of isotonic saline, did not result in any evidence that intravenous trypsin therapy caused the disappearance of a palpable thrombus any more rapidly than did conventional treatment. Their animal experiments indicated that “one cannot expect to dissolve a preformed thrombus with intravenous trypsin administration almost regardless of dosage.” The clinical experience of Ormond and Knight has been somewhat similar and multiple thromboses at the sites of injection have been encountered by them and by others.

The process by which trypsin coagulates blood within a narrow concentration range is an interesting and as yet unexplained phenomenon. It is apparent on the basis of our work and on that of others that prothrombin must be present in the blood if trypsin is to clot it. Trypsin itself will not coagulate fibrinogen, and therefore it would appear fairly certain that trypsin results in the conversion of prothrombin to thrombin and that this latter agent then coagulates the fibrinogen. As demonstrated by Kleinfeld and Habif, calcium and accessory factors are not essential to this action, but calcium will accelerate the rate of thrombin formation without affecting the thrombin yield. Conversion of prothrombin by trypsin is apparently incomplete.

The fact that trypsin will coagulate heparinized blood under certain conditions, and that it will form a product with Overman’s thromboplastic inhibitor which clots blood even more rapidly than trypsin alone, lends support to Overman’s theory pertaining to thromboplastic activity. According to this theory an equilibrium reaction is postulated to explain thromboplastic activity. “This consists of a phosphatide fraction I and a protein fraction, α, which are in equilibrium with αI (thromboplastin). The activity of the thromboplastin or the rate of activation of prothrombin depends upon the concentration of (thromboplastin) in the system.” The phosphatide in-
hibitory fraction (designated as I) is a powerful anticoagulant and can produce prolonged prothrombin and clotting times. Overman does not believe that the calcium ion is necessary for the activation of prothrombin but does believe that the concentration of $\alpha I$ (thromboplastin) can be controlled by varying the calcium ion concentration. Into such a theory trypsin fits remarkably well as an "alpha-like" substance.

On the basis of this theory it may be postulated that blood will clot when "alpha" (in this case trypsin) is in equilibrium with the phosphatide thromboplastic inhibitor which is normally in excess in the blood. Heparin, we believe, may combine with alpha thus releasing the lipid coagulation inhibitor I, and therefore the heparinized blood will clot when sufficient alpha-like substance, such as trypsin, is introduced into the blood to reach an equilibrium with the inhibitor. The fact that large amounts of heparin apparently prevent trypsin-induced coagulation in vivo may be explained on the supposition that so much alpha is removed by the heparin that trypsin is unable to substitute for the missing alpha before its proteolytic effect on the blood coagulation components comes into effect. The fact that trypsin and thromboplastic inhibitor in combination form an extremely active clotting agent in vitro and a potent intravascular coagulation agent is in harmony with this hypothesis.

When trypsin is successfully administered intravenously in large doses the blood becomes incoagulable, the prothrombin and calcium chloride times are prolonged indefinitely, the factor V and antithrombic levels are reduced and the fibrinogen is eliminated. This may well be the result of enzymatic proteolysis of the protein blood coagulation components. Relatively high quantities of the amino acids, arginine and lysine, are known to be present in fibrinogen, and trypsin is known to be particularly potent in breaking the amide linkages of many arginine and lysine compounds. This would help to explain the apparent marked predilection of trypsin for fibrinogen.

CONCLUSIONS

On the basis of these experiments there appears to be grave doubt regarding the advisability of using trypsin intravenously for therapeutic purposes in man. Trypsin infusions in rabbits did not result in dissolution or lysis of preformed artificial thrombi but did result, in a high proportion of cases, in necrotic lesions in the myocardium and diaphragm and death from intravascular thrombosis or hemorrhage. Small amounts of trypsin in vivo have little or no effect on the blood clotting mechanism, whereas larger amounts frequently result in intravascular coagulation and later incoagulability of the blood. In vitro experiments confirm the earlier observation that there is a narrow zone of trypsin concentration within which blood coagulation is accelerated, whereas in larger concentrations coagulation is inhibited. While heparin in certain concentrations may inhibit the clotting effect of trypsin, the maintenance of the exact proportions for clinical purposes has not as yet been satisfactorily worked out.

SUMMARY

The literature concerned with trypsin and blood coagulation has been reviewed and earlier experiments confirmed. Experimental intravenous administration of trypsin to rabbits with artificial preformed thrombi failed to provide evidence to justify its use as a therapeutic agent in man.

SUMARIO ESPAÑOL

Se hicieron estudios relacionados al efecto de la tripsina intravenosa en conejos. Estos incluyeron investigaciones en cuanto a la toxicidad y la tendencia a la producción de coagulación intravascular. El efecto en trombos previamente formados artificialmente también se observó. Se encontró que en las dosis en que fué usada, la administración de tripsina intravenosa no fué eficaz en el tratamiento de la trombosis intravascular. Estos estudios no tuvieron éxito en proveer evidencia para justificar su uso como un agente terapéutico en el hombre.

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