Effect of Treated Polygalacturonic Acid on Blood Volume Loss in the Rat Following Traumatic Injury

By Charles E. Brambel, Ph.D. and David Murphy, B.S.

Evidence is presented that a polygalacturonic acid derivative (Stypturon) when administered intravenously to rats in an optimal dosage results in concomitant diminution of blood loss from experimental injury to the tail. Mortality rate associated with hemorrhagic shock was significantly reduced. Blood coagulation was inhibited at the site of injury by immersion of the traumatized tail in an anticoagulant solution at 37 C. for the duration of the experiment. The data for the enhancement of the normal hemostytic mechanism by a polymeric substance without the apparent mediation of the blood coagulation system is discussed.

The concept of the initiation of a thrombus or clot by platelets at the site of injury is not a new one. On the other hand, the possibility of introducing a chemical substance into the blood stream having no effect on the clotting mechanism but increasing the tendency of blood platelets to clump at the site of vascular injury is a new approach. Such a thesis predicates the exudation of tissue juice (thromboplastin) at the site of injury and platelets laden with the introduced chemical would be clumped at the site. The term "conglutination" has been used in this case. Thus, a new principle in hemostasis has been suggested: Formation of a cellular plug at the site of injury with a decrease in blood loss without involving the mediation of the blood clotting mechanism.

Hummel and Habe,

Methods

Test Animals. A total of 516 albino rats in various weight ranges including two well established strains (Sprague-Dawley and Lobund Institute) were used in this study. The distribution of these animals by sex was: 293 females and 223 males. Three hundred rats were in the weight range 180 to 210 Gm. which included 126 rats of the Sprague-Dawley strain and 174 rats of the Lobund Institute strain. Two hundred and sixteen rats of the Lobund Institute strain were in the weight range 210 to 500 Gm. All of the animals were fed the Rockland diet and were kept under uniform environmental conditions for a minimum period of 2 weeks before use.

Intravenous Hemostatic Agent—Polygalacturonic Acid Derivative. Stypturon* is a low molecular weight derivative of polygalacturonic acid. This material differs from pectin in that it does not contain methyl groups and is not metabolized in the body. In contrast to pectin, Stypturon forms soluble nondissociable complexes with calcium. Excretion of this macromolecule by the kidneys is effected in a few hours.

Injection with Agents. Under light anesthesia, the rats were injected in the jugular vein with a 25 gage needle and a tuberculin syringe. A constant injection volume (0.5 ml.) was maintained for the various dosages studied (table 1). The animals were injected very slowly and were kept under light ether anesthesia to prevent struggling and tearing of the vessels during injection. After injection the animals were allowed to recover from the anesthetic. Autopsy reports

* A product for parenteral administration was prepared by the Abbott Laboratories, North Chicago, Ill., under the trade name Stypturon. Sterile ampoules of 5.0 ml. containing 1,000 mg. of specially processed polygalacturonic acid.
POLYGALACTURONIC ACID AND BLOOD VOLUME LOSS

TABLE 1.—Relationship of Dosage, Mortality and Blood Loss in Test Animals ISO to 210 Gm.

<table>
<thead>
<tr>
<th>Dosage (mg./Kg.)</th>
<th>N</th>
<th>Mortality (per cent)</th>
<th>Mean blood loss (ml.)</th>
<th>S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>76</td>
<td>21.0</td>
<td>6.2</td>
<td>1.26</td>
</tr>
<tr>
<td>20</td>
<td>42</td>
<td>20.3</td>
<td>5.8</td>
<td>1.45</td>
</tr>
<tr>
<td>40</td>
<td>44</td>
<td>9.1</td>
<td>4.7</td>
<td>1.48</td>
</tr>
<tr>
<td>80</td>
<td>56</td>
<td>14.0</td>
<td>5.3</td>
<td>1.45</td>
</tr>
<tr>
<td>160</td>
<td>49</td>
<td>28.6</td>
<td>6.0</td>
<td>1.79</td>
</tr>
<tr>
<td>320</td>
<td>32</td>
<td>30.3</td>
<td>5.3</td>
<td>1.53</td>
</tr>
</tbody>
</table>

on 50 animals showed that the needle entered the jugular veins, and that there was no extravasation of blood in the majority of rats and a minimal amount in some, in the area of entry of the needle.

One hundred and fifty-six rats which varied in weight from 170 to 500 Grm. were controls, i.e., they were injected with 0.5 ml. of physiologic saline (table 2).

Animals in the weight range 180 to 210 Gm. constitute the main protocol. This weight range was selected on the basis of uniformity of blood volume loss (table 2). Other weight ranges were also explored as follows: 210 to 250 Gm., 26 rats as controls and 17 received 20 mg./Kg. Stypturon; 250 to 300 Gm., 19 rats as controls, 16 received 20 mg./Kg. Stypturon and 14 received 40 mg./Kg. Stypturon; 300 to 400 Gm., 21 rats as controls, and 15 received 20 mg./Kg. Stypturon.

Bleeding by Tail Section. The procedure outlined by Hengge* was used with slight modification. One hour after injection, the rats were again anesthetized lightly with ether. A clean, quick, guillotine cut 1 inch from the tip of the tail was made with a sharp blow on an unused razor blade. The cut end of the tail was immediately immersed in a 25 ml. graduate cylinder containing 15 ml. of citrated saline (1.0 per cent Na citrate in 0.85 per cent NaCl) placed in water bath at 37 C. Blood was collected for 40 min. in this warm anticoagulant solution in order to block the blood clotting mechanism at the site of injury. The volume of blood loss was determined by the increase in the fluid volume in the graduate cylinder. The rats were kept under light anesthesia for the duration of the blood collection period. The animals were kept under observation for at least 72 hours after bleeding.

RESULTS

Bleeding Time. Over 95 per cent of the animals continued to bleed for the 40 min. period during which time the injured tail was immersed in the warm anticoagulant solution. Bleeding from the blood vessels continued beyond this period as a thready intermittent stream as long as the cut end of the tail was immersed in the citrated saline. Un-

TABLE 2.—Blood Loss in Controls

<table>
<thead>
<tr>
<th>Weight range (Gm.)</th>
<th>N</th>
<th>Mean blood loss (ml.)</th>
<th>S. D.</th>
<th>Range blood loss (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>170-180</td>
<td>7</td>
<td>4.8</td>
<td>—</td>
<td>3.5-9.0</td>
</tr>
<tr>
<td>180-210</td>
<td>76</td>
<td>6.2</td>
<td>1.26</td>
<td>3.5-9.0</td>
</tr>
<tr>
<td>210-250</td>
<td>22</td>
<td>7.1</td>
<td>6.93</td>
<td>2.5-10.0</td>
</tr>
<tr>
<td>250-300</td>
<td>23</td>
<td>7.5</td>
<td>7.15</td>
<td>1.0-10.0</td>
</tr>
<tr>
<td>300-400</td>
<td>19</td>
<td>7.5</td>
<td>8.95</td>
<td>1.5-13.0</td>
</tr>
<tr>
<td>400-500</td>
<td>7</td>
<td>13.0</td>
<td>—</td>
<td>8.0-15.0</td>
</tr>
</tbody>
</table>

quency distribution of the rats that received Stypturon toward less bleeding. At a dose of 40 mg./Kg. over 30 per cent of the animals lost less than 4.0 ml. of blood, while only 3 per cent of the controls bled that small an amount. At the same dosage only 5 per cent as opposed to 22 per cent of the controls bled over 7.0 ml. The controls bled a mean volume of 6.2 ml. and the group receiving stypturon lost a mean of 4.7 ml. Thus, the blood loss was decreased 23 per cent in those rats that received the polymeric material.

In all the weight ranges that were tested the animals that received the treated polygalacturonic acid derivative showed a shift in frequency distribution toward less bleeding. For example, in the weight range 210 to 250 Gm. the blood loss was decreased following the administration of 20 mg./Kg. of Stypturon. Almost 50 per cent of the rats at this dosage bled less than 5.0 ml. while only 10 per cent of the controls lost that amount of blood. In the same groups, 20 per cent of the experimental animals and almost 50 per cent of the controls bled more than 7.0 ml. The mean was decreased from 7.1 ml. in the controls to 5.4 ml. in the experimental group. Thus, in this weight range the animals that received 20 mg./Kg. lost 24 per cent less blood than the corresponding controls.
der these conditions the volume of blood lost was negligible (small fraction of a milliliter). No difference was observed in bleeding time between the controls and the animals that were injected with various dosages of Stypturon.

The injured surface of the tail did not show any accumulation of fibrin at any time during the experiment. Blood clotting on the surface had been inhibited by the citrated saline as far as it could be ascertained by gross examination. On the other hand, cut rat tails immersed in saline without citrate showed gross clot formation after a 20 min. period with concomitant cessation of blood flow.

Eighty per cent of the total blood loss occurred in the first 10 min. and 20 per cent in the last 30 min. This was found to be true of both control and experimental animals for all the various dosages.

**Mortality.** A number of rats did not survive the experiment. Some animals may have died from the toxicity of the test material in high dosages, for the mortality rate at 160 and 320 mg./Kg. was much higher than in the controls. The number of deaths in the controls was found to be directly associated with a large blood loss, for every control animal that died more than the mean for the group, i.e., 6.2 ml.

In the groups that received certain dosages of the hemostatic agent the mortality rate was found to be greatly reduced (fig. 1). The mortality was decreased in the main protocol from 21 per cent in the controls to 9 per cent in those animals which received a dose of 40 mg./Kg. In the weight range 250 to 300 Gm., the mortality rate was decreased from 37 to 7 per cent at 40 mg./Kg.

A total of 116 control rats were run in the weight range 180 to 380 Gm. and 27 per cent of these died. A total of 54 rats in the same weight range received 40 mg./Kg. and only 8.6 per cent of these died. Therefore, the mortality rate was reduced by 68 per cent in those rats that received 40 mg./Kg.

**Toxicity.** Stypturon showed no toxic effects in dosages up to 160 mg./Kg. However, in the highest two dosages tested the mortality rate was greater than in the control group (fig. 1). At the 320 mg./Kg. dosage, convulsions and respiratory difficulties were frequently observed in the animals regardless of the rate of administration. All the rats died if the injection rate at this dosage level was rapid.

**Optimal Dosage.** The optimal dosage of Stypturon in rats was found to be 40 mg./Kg. because: (a) the mortality rate was lowest (fig. 1); (b) the blood loss after traumatic
injury was minimal (fig. 2); and (c) there were no observable toxic effects.

Differences in Response Between the Sexes. No essential difference in blood loss between control males and control females was observed (fig. 2), but in all groups that received the macromolecular substance, the females bled less than the males. A higher percentage of females died than males in the control group (fig. 1), but in the groups that received the treated polygalacturonic acid derivative a lower percentage of females died than males. An explanation for the observed differences is lacking at the present time.

DISCUSSION

Hengge injected 20 mg./Kg. of Stypturon into the tail vein of rats weighing 150 to 200 Gm. and obtained a mean blood volume loss after tail section of 0.8 ml. compared to 5.7 ml. for control animals. In contradistinction to the data presented in our report, Hengge observed a bleeding time of 532 sec. for control animals and 153 sec. for those that received 20 mg./Kg. There is no immediate explanation available at this time for this discrepancy in the observations pertaining to the difference in results obtained. In our experience, Stypturon consistently reduced bleeding volume following traumatic injury to the tail by about 25 per cent. Rats of both the Sprague-Dawley and Lobund Institute strains responded equally well to this hemostatic agent. Since the results varied somewhat from day to day, it was necessary to use a large number of rats before a statistically significant \( p < 0.001 \) decrease in bleeding volume and particularly the decrease in mortality became manifest.

Vodopivec showed that the potential bleeding tendency associated with heparin could be suppressed with Stypturon without appreciable retardation of the coagulation time. The dosage of the polymeric material in human subjects receiving heparin was 20 mg./Kg. These observations suggest that hemostasis can be accomplished notwithstanding the fact that the blood coagulation mechanism had been inhibited. The concept of labile platelets resulting in "conglutination" was used by Vodopivec as a possible explanation for the observed hemostatic effect.

The lethal dose for the polygalacturonic acid derivative has been reported to be about 900 mg./Kg. Dungemann found no toxic effects at a dose of 20 mg./Kg. in 70 patients. However, there was evidence of side effects (flushing and perspiration) associated with too rapid administration of larger dosages. In our experience, the rate of intravenous administration was a very important factor with regard to the cause of death of the animals.

The number of animals dying from hemorrhagic shock in the experimental groups was significantly diminished compared to that found for the control series. Although the decrease in blood volume loss is not as striking as that reported by other investigators, it would seem sufficient to prevent death of the rat from bleeding following severe traumatic injury to the tail. These observations suggest that Stypturon is an effective hemostytic agent. Further investigation into the details of mechanism of action and search for more effective chemical agents is in progress.

SUMMARY

The overall hemorrhage mortality rate was decreased by 70 per cent in animals that received 40 mg./Kg. of a polygalacturonic acid derivative (Stypturon) intravenously. Blood volume loss following traumatic injury was decreased by 25 per cent in the animals thereby manifesting an enhancement of the normal hemostatic mechanism.

Stypturon showed no effect on the bleeding time. All rats bled for the full 40 min. test period and lost 80 per cent of the total blood volume loss in the first 10 min. It is advisable to inject Stypturon very slowly to avert untoward side effects (convulsions and respiratory embarrassment).

SUMMARIO IN INTERLINGUA

Le mortalitate general per hemorrhagias esseva reducite per 70 pro cento in rattos recipiente per via intravenose 40 mg/kg de un derivato de acido polygalacturonic (Stypt-
turon). Le volumine del perdita de sanguine in ille animales esseva reduce per 25 pro cento. Isto esseva interpretate como manifestation de un meliorate mechanismo hemostatic.

Stypturon habeva nulle effecto super le tempore de sanguination. Omne le rattos sanguinava durante le integre periodo experimental de quaranta minutas. Illos perdeva 80 pro cento del volumine total de lor perdita de sanguine durante le prime 10 minutas. Es recommendate injicer Stypturon lentissime pro evitar effectos lateral (convulsiones e embarasso respiratori).

ACKNOWLEDGMENTS

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REFERENCES

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