ACUTE THROMBOCYTOPENIA INDUCED IN DOGS BY ADMINISTRATION OF URETHANE (ETHYL CARBAMATE)

By W. O. Cruz, M.D.,* and H. Moussatché, M.D.

The substances inducing thrombocytopenia can be divided into two categories: those which act exclusively on the platelets and those which, in addition to this action, act also on the leukocytes and red blood cells.

True thrombocytopenia, with no alteration of leukocytes and red blood cells, has been described in man as caused by the administration of organic substances, such as arsenic salts (arsphenamine, neoarsphenamine, etc.), gold salts, and benzene. Physical agents, such as x-rays and emanations of radioactive substances, cause thrombocytopenia, always accompanied, however, by leukopenia and anemia.

Descriptions have already been made of experimental thrombocytopenia and anemia in dogs, induced by the administration of estradiol benzoate in heavy doses and by injection of antithromplatelet serum. In previous publications,1-5 one of us (W. O. C.) has studied the relation between the decrease of the number of circulating platelets and the occurrence of anemia.

This article describes the thrombocytopenic action of ethyl urethane administered to dogs in heavy doses. Other blood changes resulting from urethane administration are also described.

METHODS

The volume of the platelets was determined by the Van Allen thrombocytocrit and the results were expressed in ml. platelets per 100 ml. of blood. Details of the hematologic technic used for leukocyte and red blood cell counts are described in a previous paper.6 Urethane was used in a 60 per cent solution in distilled water.

EXPERIMENTAL RESULTS

Preliminary trials with various dosages and routes indicated that the drug exerted some effect on the platelets.

<table>
<thead>
<tr>
<th>Dog, 393, 8 Kg.</th>
<th>Leukocytes 10⁹/mm.³</th>
<th>Platelets ml./100ml. Blood</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of Experiment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10.4</td>
<td>0.38</td>
<td>Daily administration of 3 Gm. of urethane (0.38 Gm. p/Kg.) by mouth.</td>
</tr>
<tr>
<td>2</td>
<td>17.8</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18.4</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16.0</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>12.1</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.0</td>
<td>0.38</td>
<td>Administration of urethane by mouth discontinued.</td>
</tr>
<tr>
<td>34</td>
<td>0.8</td>
<td></td>
<td>Administration of the same dose of urethane subcutaneously.</td>
</tr>
<tr>
<td>36</td>
<td>0.4</td>
<td></td>
<td>Killed by injection of air intravenously. Post-mortem examination: Hemorrhages in the epiplon and mediastinum. No lesions in intestine.</td>
</tr>
</tbody>
</table>

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ADMINISTRATION OF URETHANE

_Dog 5, 7.8 Kg._

<table>
<thead>
<tr>
<th>Day of Experiment</th>
<th>Leukocytes (10^3/mm^3)</th>
<th>Platelets ml./100 ml. blood</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Daily subcutaneous injection of 3 Gm. of urethane (0.38 Gm. p/Kg.).

Injection area swollen, sensitive to touch, hindering the animal's walk.

Purpura in areas of injection and other regions of the skin.

Red blood cells, \(4.2 \times 10^6/mm^3\).

Hemoglobin, 10.8 Gm. 100 ml. of blood.

Hematocrit, 33°/c.

Found dead in the morning. Postmortem examination: Medium sized petechiae throughout the intestine, with no preferential zone. Profuse hemorrhage in injection site.

_Dog 6, 6.1 Kg., Male_

Daily subcutaneous injection (0.1 Gm. per Kg.) for twenty-two days. On thirteenth day, hemorrhagic zone was noted in inguinal region around one of injection areas; hematologic data showed 6,100 leukocytes per mm. and 0.10 ml. platelets/100 ml. blood. The purpura receded and no hemorrhagic spots were observed.

On the twenty-third day of experiment, urethane was increased to 0.4 Gm. per Kg.

On the twenty-eighth day of experiment, cutaneous petechiae were observed.

On the thirtieth day, the animal was killed, after verification of lack of platelets in circulation. Postmortem examination showed presence of many isolated hemorrhagic points throughout the intestine. Hemorrhage in intestinal lumen. Petechiae in both lungs, epiploon and hemorrhage in perirenal tissue.

_Dog 7, 5.4 Kg._

Daily subcutaneous injection of urethane (0.4 Gm. p/Kg.) for nine days. On ninth day, petechiae on skin of abdomen. On tenth day, hematologic data showed: Red blood cells 1,100,000 mm.\(^3\), Hemoglobin, 2 Gm./100 ml. blood. Hematocrit, 8 per cent. White blood cells, 3,000 ml. Platelets, 0. Animal killed. Autopsy showed in the intestine typical picture of hemorrhagic purpura. Pulmonary petechiae.

Three other animals received subcutaneously urethane in doses of 0.4 Gm./Kg. body weight for nine days, 0.2 Gm./Kg. for eighteen days and 0.4 Gm./Kg. for twelve days. They all died and showed little or no purpura. These preliminary observations led us to try experiments which would show more adequately the hematologic changes of thrombocytopenic purpura. Results are given in table 1.

DISCUSSION

The thrombocytopenic action of urethane has not been previously described. The results referred to above show that this substance has an acute thrombocytopenic action when given to dogs in sufficient quantity subcutaneously. Urethane apparently does not act specifically on platelets, but on all the cell elements of the blood. Leukopenia was also observed in the final phase, and acute anemia without regenerative phenomena; in addition, the platelets disappeared completely from the circulation. The sequence of this picture is similar to that observed when heavy doses of estradiol benzoate are administered to dogs: initial leukocytosis, disappearance of platelets, and finally acute anemia. The animals
treated with urethane present a leukopenia which may attain extremely low values in the final phase of the picture.

The fall of red blood cells coincides with the appearance of severe intestinal hemorrhages as a consequence of purpuric lesions in the mucosa of the small intestine (figs. 1 and 2). Purpuric lesions similar to those observed in the intestine are frequently found in the lung, cortical zone of kidney, heart, perirenal tissue, epiploon, and skin.

We call attention to the fact that the picture of purpura is established even

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Weight of dog Kg.</th>
<th>Day of experiment</th>
<th>Red blood cells 10^6 ml.</th>
<th>Hemoglobin Gm./100 ml</th>
<th>Hematocrit %</th>
<th>Reticulocytes %</th>
<th>Leukocytes x 10^9</th>
<th>Platelets ml./100 ml</th>
<th>Postmortem examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>407-1</td>
<td>9.2</td>
<td>0</td>
<td>5.4</td>
<td>11.6</td>
<td>42</td>
<td>0.5</td>
<td>12.4</td>
<td>0.38</td>
<td>No characteristic signs of purpura.</td>
</tr>
<tr>
<td>408-2</td>
<td>6.7</td>
<td>0</td>
<td>6.2</td>
<td>11.6</td>
<td>48</td>
<td>0.5</td>
<td>16.5</td>
<td>0.39</td>
<td>Few petechiae on intestine. Lung, heart, and skin normal.</td>
</tr>
<tr>
<td>409-3</td>
<td>7.4</td>
<td>0</td>
<td>7.5</td>
<td>15.6</td>
<td>56</td>
<td>0.1</td>
<td>10.4</td>
<td>0.48</td>
<td>Killed on 11th day of experiment. Petechiae on skin, kidney (cortical zone) lung, perirenal tissue, small intestine. Bloody stools. Hemorrhage at site of injection.</td>
</tr>
<tr>
<td>410-4</td>
<td>7.4</td>
<td>0</td>
<td>6.1</td>
<td>11.6</td>
<td>48</td>
<td>0.5</td>
<td>5.0</td>
<td>0.12</td>
<td>Petechiae on the lung. Numerous petechiae on the intestine. Large quantity blood in intestinal lumen.</td>
</tr>
<tr>
<td>411-6</td>
<td>5.3</td>
<td>0</td>
<td>6.3</td>
<td>11.6</td>
<td>48</td>
<td>0.5</td>
<td>3.2</td>
<td>0.10</td>
<td>Many petechiae of various sizes on small intestine. Petechiae on lung, heart, and kidney. Copious hemorrhage in area of injection.</td>
</tr>
</tbody>
</table>

* Urethane administration stopped.
† Coagulation time 7 minutes. Bleeding time: Higher than 70 minutes.
‡ Died.
when the administration of urethane is discontinued after the fall in the number of platelets. This was the case in dogs 409-3 and 411-6, where the administration of urethane was discontinued on the eighth day of the experiment but the blood changes continued to develop until the final stage, as can be seen in table 1.

The dose of urethane used in the animals listed in table 1 (0.4 Gm. per Kg.) may approach a dose which would intoxicate the animal prior to the appearance of purpura; this might explain the premature death of two dogs (3 and 407-1).

![Figure 1. Dog 409-3. Digestive tract showing extensive purpuric lesions in the small intestine, especially in the jejunum. Stomach, duodenum, and large intestine free of lesions.](image)

The administration of a smaller dose of urethane (0.2 Gm. per Kg.) for eighteen days did not reveal the typical picture of fulminating purpura. Administration by mouth (0.4 Gm. per Kg.) for thirty-six days in dog 393 did not produce purpura.

It would still be necessary to discover whether the urethane action is specific for dogs, as is the case with estradiol benzoate. In support of this idea is the fact that urethane was administered to many other species without producing a picture similar to that here reported.

With regard to the mechanism of urethane purpura, it would be well to remember the studies of Krogh and co-workers on the action of urethane on the capillaries. This author, by microscopic observation, verified an intensive dilatation of
capillary vessels of the tongue of frogs submitted to the action of urethane. Also, Landis observed, by introduction of a micropipet in a capillary, an increase of the capillary pressure and permeability in these animals. In the cases of intoxication with high doses of urethane, the capillary pressure may attain values close to arteriolar pressure; the increase in permeability would be connected with the toxic action of the urethane on the capillary wall. It may be supposed that the rupture of the capillary results from damage of the capillary wall combined with

the increase of capillary pressure. Rupture of capillaries was observed by Doljanski and Rosin in studies made on the effects of urethane on the sinusoid capillaries of rat livers.

The known toxicity of urethane for the capillaries and its capacity for inducing purpura could be interpreted as facts in support of the hypothesis of Bedson and others that the primary cause of purpura lies in a change in the capillary endothelium, and not in the thrombocytopenia. However, disappearance of the platelets of the circulating blood some days before the occurrence of purpura in the animals treated with urethane, or, as was shown in previous observations, in animals treated with estradiol benzoate, indicates that the platelets are involved in the mechanism of purpura.

It has not been possible to the present time to determine the primary cause in

Fig. 1. Dog 411-6
Extensive purpuric lesions in the small intestine, especially in the jejunum.
the mechanism of urethane purpura, but it seems to us that the whole question of purpura could be attacked with advantage by the use of urethane. Water solubility, low price, as well as easy commercial availability are some of its advantages over estradiol benzoate and antiplatelet serum for the study of experimetal purpura.

Because of its leukopenic action, urethane administered by mouth has been used recently in the therapeutic of leukemias. Despite the fact that it is necessary to administer higher doses to dogs than those administered to patients with leukemia, and taking into consideration the fact that subcutaneous injections were used in dogs, it appears to us that in patients treated with urethane it would be advisable to determine periodically the number of circulating platelets.

**Summary**

1. Dogs treated for one to two weeks with daily injections of urethane (ethyl carbamate) subcutaneously, in doses of 0.4 Gm. per Kg. of body weight, presented a typical picture of thrombocytopenic purpura.
2. The pathologic changes consisted in numerous purpuric lesions in the small intestine and a smaller number on the skin, heart, lung, cortical zone of the kidney, epiploon, and, rarely, on the stomach and large intestine.
3. The hematologic changes occurred in the following sequence: leukocytosis, leukopenia and thrombocytopenia, and finally acute anemia coinciding with severe intestinal hemorrhage. In the final phase, the coagulation time was normal and the bleeding time very much increased.

**REFERENCES**

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