Effect of Heparin upon Various Forms of the Thrombohemorrhagic Phenomenon (THP)

By HANS SELYE, BEATRIZ TUCHWEBER and GIULIO GABBIANI

In earlier communications we have described a thrombohemorrhagic phenomenon (THP) which can readily be elicited in rats by the intravenous injection of various metal combinations, reticuloendothelial blocking agents and certain agar preparations, especially if these are given in conjunction with catecholamines (epinephrine, norepinephrine) or severe systemic stressors. This syndrome is characterized by multiple hemorrhages and microthromboses located mainly in the kidneys, duodenum, heart and lung. In addition, very severe and sometimes necrotizing thrombohemorrhagic lesions are observed at the site of catecholamine injection. In its morphologic manifestations, the THP resembles the generalized and local forms of the Schwartzman-Sanarelli phenomenon; however, unlike the latter, the THP does not depend upon the properly spaced administration of a "preparatory" and a "provocative" treatment, one of which at least must be a bacterial endotoxin.14

It had been observed, furthermore, that if an anaphylactoid inflammation is produced in rats by dextran, dextrin, egg white or other similarly acting mast-cell dischargers, the concurrent intravenous administration of agar induces a type of anaphylactoid purpura. Here, the thrombohemorrhagic lesions affect particularly the anaphylactoid shock organs—namely the snout, paws and sometimes the root of the tail.5 Cyproheptadine (an antiserotonin and anti-histaminic agent), which prevents the ordinary anaphylactoid inflammation, also blocks the production of this thrombohemorrhagic variant. At the same time, however, it aggravates the accompanying thrombohemorrhagic lesions in internal organs and the mortality rate.6

In view of the prominence of fibrin thrombi among the lesions characteristic of the THP, the question arose whether this syndrome could be prevented by heparin. It will be recalled that both the classical Schwartzman-Sanarelli phenomenon in the rabbit7,8 and several apparently related diseases of man—e.g., the thrombohemorrhagic phenomena of septic abortion9 and purpura fulminans10—are favorably influenced by heparinization.

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MATERIALS AND METHODS

One hundred and twenty female albino rats of the Holtzman strain with a mean initial body weight of 100 Gm. (range 90–110 Gm.) were subdivided into 12 equal groups and treated as indicated in tables 1 and 2. The first experiment was designed to examine the effect of heparin upon the simple THP, while the second was to show the effect of this agent upon the anaphylactoid form of the THP induced by agar in combination with dextran or egg white.

The optimal dosages and timing of the agents used for the production of the THP and of their prevention by heparin have been established by a series of preliminary experiments which need not be reported here in detail. The procedures given in the text were those found to be most effective. The agents used were agar (Agar U.S.P., Brickman & Co., Montreal, Canada); dextran (Abbott Laboratories, North Chicago, Ill.). 6 per cent solution; ovalbumen (50 per cent aqueous solution of chicken egg white filtered once through two layers of Kleenex tissue); Cerium chloride (CeCl₃·7H₂O, Fisher Scientific Co., Fair Lawn, N. J.); Ferric chloride (FeCl₃·6H₂O, Fisher Scientific Co.); lead acetate [Pb(C₂H₃O₂)₂·3H₂O, Fisher Scientific Co.]; Higgins American India Ink (30 per cent aqueous solution); norepinephrine (1-Arterenol bitartrate, Winthrop Laboratories, New York, N. Y.); heparin (Liquaemin Sodium, Organon, West Orange, N. J.); and Depo-Heparin Sodium (Upjohn, Kalamazoo, Mich.).

The experiments were terminated on the day after injection of the evocative agents by killing the survivors with chloroform. The organs were inspected with a dissecting loupe and the degree of thrombohemorrhagic lesions was estimated in terms of an arbitrary scale in which 0 = no lesion, 1 = just visible, 2 = moderate, 3 = most pronounced lesions, as previously described. The means of these lesions are listed in the tables. The readings for the internal organs correspond to the mean of the macroscopically visible lesions observed in the kidney, heart, lung and duodenum. Immediately after autopsy, specimens of the organs were fixed in Susa solution saturated with picric acid and subsequently stained with the PAS-technic and with the multipurpose polychrome stain.

RESULTS

First Experiment: Effect of Heparin upon the Simple THP Induced by Various Agents

To elicit the THP, the following agents were used: agar (6 mg.); CeCl₃ (5 mg.); FeCl₃ (in Groups 3 and 4, 2 mg.; in Groups 7 and 8, 3.5 mg.); lead acetate (5 mg.); and india ink (30 per cent). All of the substances were administered in 1 ml. of distilled water under light ether anesthesia into the exposed jugular vein. In addition, norepinephrine (250 μg.) was injected once in 0.1 ml. water under the plantar aponeurosis of the right hind paw in Groups 3, 4, 5, 6, 7 and 8, while Groups 3 and 4 received an additional injection of 250 μg. of norepinephrine in 0.2 ml. water subcutaneously on the back 30 minutes earlier. To prevent the THP, we injected 1 mg. of heparin in 0.2 ml. water subcutaneously 1 hour before, and 0.5 mg. in 0.2 ml. water simultaneously with the agents employed to produce the THP.

It will be seen from table 1 that, irrespective of the agents used for the production of the THP, heparin exerted a protective effect; it inhibited the development of thrombohemorrhagic lesions, not only in the internal organs, but also at the site of norepinephrine injection into the paws. (When given subcutaneously, norepinephrine rarely produced any topical thrombohemorrhagic lesions so that their inhibition by heparin was not significant and, hence, these
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Table 1.—Effect of Heparin upon Simple THP Induced by Various Agents

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lesions (Scale 0–3)*</th>
<th>Paws</th>
<th>Internal Organs</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agar</td>
<td>0.9</td>
<td></td>
<td>Trace</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>As Group 1 + Heparin</td>
<td>Trace</td>
<td></td>
<td>Trace</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>CeCl₃ + FeCl₂ + Norepinephrine</td>
<td>1.2</td>
<td>0.6</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>As Group 3 + Heparin</td>
<td>Trace</td>
<td>Trace</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CeCl₃ + India Ink + Norepinephrine</td>
<td>1.7</td>
<td>0.8</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>As Group 5 + Heparin</td>
<td>0</td>
<td>Trace</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ph-acetate + FeCl₂ + Norepinephrine</td>
<td>0.7</td>
<td>0.4</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>As Group 7 + Heparin</td>
<td>0</td>
<td>Trace</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

*Means of 0.3 or less are listed as "Trace."

findings are not listed in the table.) The mortality rate was also markedly inhibited and, with the exception of Group 8, it was totally abolished by heparin.

Histologically, the affected organs showed essentially the same lesions throughout. These were characterized by the formation of fibrin thrombi in the arterioles, venules and capillaries, with hemorrhagic infiltration of the vascular walls and their surroundings. In the damaged regions, polymorphonuclear leukocytes were plentiful, not only in the hemorrhagic tissues, but also within the lumina of larger arteries whose walls remained essentially normal. The most severely affected internal organ was the kidney. It exhibited widespread hyaline thrombi in the glomerular capillaries, although some of the loops revealed only thickening and hyalinization of their walls ("wire loop" capillaries). The tubules showed degenerative changes, sometimes progressing to necrosis, and hyaline cast formation. In Group 1, the glomerular capillaries were almost completely filled with agar, which is readily demonstrable by its characteristic blue color in sections stained with the multipurpose polychrome technic. This agar deposition was not inhibited by heparin (Group 2), although the associated vascular lesions and the hemorrhages were almost totally prevented here as in Groups 4, 6 and 8 (fig. 1).

Second Experiment: Effect of Heparin upon the Anaphylactoid THP Induced by Agar Plus Dextran or Egg White

The agents used to produce an anaphylactoid type of THP were agar (5 mg. in 1 ml. water) and dextran (6 per cent, 0.5 ml.) or egg white (50 per cent, 0.5 ml.), the agar always being given just before the anaphylactoid agent. All compounds were injected intravenously as in the first experiment. For the inhibition of this syndrome, we employed Depo-Heparin at the dose of 1 mg. in 0.2 ml. water, given subcutaneously 1 hour before, and again 30 minutes before the intravenous injections.

The hemorrhagic lesions were essentially the same in all the anaphylactoid shock organs (snout, forepaws and hindpaws, root of the tail). However, in table 2, only the readings of lesions in the hindpaws are listed, since these are comparable to those produced in the first experiment by the direct injection of norepinephrine into the hindpaws. These changes were completely prevented in most of the heparin-treated animals and greatly diminished in the
Fig. 1.—Inhibition by heparin of the renal lesions characteristic of the simple THP. A: THP produced by agar. The wall of the arcuate artery (long arrow) is infiltrated and surrounded by erythrocytes. The adjacent arcuate vein is essentially normal but densely packed with red corpuscles. A smaller arteriole (upper right corner) is completely thrombosed. The capillaries of the glomerulus (small arrows) are filled with agar, readily recognizable on the slide by its blue coloration. Adjacent tubules toward the right contain hyalin casts (multipurpose polychrome stain, × 460). B: Similar region from the kidney of the rat treated with agar plus heparin. The arcuate artery and vein are normal and the tubules contain no casts, but the glomerular capillaries are virtually free of blood being filled completely with homogeneous agar masses. C: Renal glomerulus of a rat treated with CeCl₃, India ink and norepinephrine. A PAS-positive hyaline thrombus completely fills the lumen of a capillary loop which is cut crosswise (arrow). Just above it is a longitudinally sectioned loop; PAS-positive material is attached only to the wall, while the lumen still contains erythrocytes. There is precipitated protein in Bowman's capsule (PAS, × 1,000). D: Glomerulus of a rat which was given heparin in addition to the treatments administered to C. Normal glomerular structure (PAS, × 1,000).
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Table 2.—Effect of Heparin upon Anaphylactoid THP Induced by Agar plus Dextran or Egg White

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Lesions (Scale 0–3)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paws</td>
<td>Internal Organs</td>
</tr>
<tr>
<td>1</td>
<td>Agar + Dextran</td>
<td>1.7</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>As Group 1 + Heparin</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Agar + Egg white</td>
<td>2.6</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>As Group 3 + Heparin</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>

remainder, irrespective of whether dextran or egg white was administered in conjunction with the agar (fig. 2). Agar plus dextran caused only minimal lesions in the internal organs and no mortality; hence, in this respect, the inhibitory effect of heparin cannot be assessed. However, the more pronounced internal lesions and the 40 per cent mortality induced by agar plus egg white were completely abolished by heparin.

DISCUSSION

It is evident from the experiments reported here that heparin greatly increases resistance to the production of either the simple or the anaphylactoid form of the THP. Thus, the effect of this anticoagulant differs essentially from that of cyproheptadine, which prevents only the production of thrombohemorrhagic lesions in the anaphylactoid shock organs while it actually aggravates the internal lesions and the mortality rate in rats given agar in combination with dextran, egg white or other similar mast-cell dischargers.6 Presumably, heparin acts by preventing the formation of microthromboses, while the effect of cyproheptadine is probably due to its specific pharmacologic actions, among which the antiserotonin and antihistamine properties are most conspicuous.

The protective effect of heparin upon the THP produced by very different technics suggests that the formation of fibrin thrombi plays an essential role in the development of the hemorrhagic lesions. It also represents an additional point of similarity between the THP and the classical Shwartzman-Sanarelli phenomenon elicited by two injections of bacterial endotoxin given at an interval of 24 hours.

The histologic lesions characteristic of the anaphylactoid THP resemble those seen in certain spontaneous diseases of man, particularly the Schönlein-Henoch syndrome and thrombotic-thrombocytopenic purpura. Previous investigators had already called attention to the frequent occurrence of glomerulonephritis with "capillary occlusion by hyaline material"13 in clinical anaphylactoid purpura, and it was suggested "that both purpura fulminans and Schönlein-Henoch purpura are manifestations of the Sanarelli-Shwartzman reaction."14 The thrombohemorrhagic lesions that characterize the Waterhouse-Friderichsen syndrome,15 certain forms of septic abortion,16 abruptio placentae,17 incompatible blood transfusion,18 dermatitis nodularis necrotica,19 and many other thrombohemorrhagic diseases of man resemble the experimental changes of the THP, as regards their histologic structure. It is con-

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Fig. 2.—Inhibition of the anaphylactoid THP by heparin. *Left:* thrombohemorrhagic lesions in the right hindpaw of a rat given agar plus egg white. *Right:* inhibition of these lesions in a similarly treated rat which in addition received heparin.

It is conceivable therefore that in man, as in experimental animals, the differences in organ distribution depend upon additional conditioning factors.

Structures resembling platelet aggregates are frequently seen during the THP, especially in recent thrombi, and the blood-thrombocyte count is often considerably diminished; yet, the participation of platelets in the development of these thrombi could not be ascertained as clearly as that of fibrin. It is interesting, however, that when heparin prevented thrombus formation, vascular lesions likewise failed to develop.

**Summary**

In rats, a thrombohemorrhagic phenomenon (THP) can be elicited by the intravenous injection of various metal combinations, especially if these are given in conjunction with norepinephrine. The syndrome is characterized by multiple hemorrhages and microthromboses located mainly in the kidneys, duodenum, heart and lung. In addition, severe and sometimes necrotizing thrombohemorrhagic lesions are observed at the site of catecholamine in-
EFFECT OF HEPARIN UPON THP

Intravenous administration of agar elicits essentially the same internal changes even without the concurrent administration of norepinephrine.

An anaphylactoid form of the THP is induced by agar plus dextran or egg white. Here, the usual internal lesions are associated with thrombohemorrhagic changes in the anaphylactoid shock organs, especially the paws, snout and the root of the tail.

All these forms of the THP are inhibited by pretreatment with heparin.

SUMMARIO IN INTERLINGUA

In rattos un phenomeno thrombohemorrhagic pote esser evocate per le injection intravenose de varie combinationes de metallos, specialmente si istos es administrate in conjunction con norepinephrina. Le syndrome es caracterisate per multiple hemorrhagias e microthromboses principalmente in le renes, le duodeno, le cordo, e le pulmones. In plus, sever e occasionalmente necrotisante lesiones thrombohemorrhagic es observate al sito del injection de catecholamina. Le administration intravenose de agar evoca essentially le mesme alterationes interne, e iste effecto non depende del administration concomitante de norepinephrina.

Un forma anaphylactoide del phenomeno thrombohemorrhagic es evocate per le injection de un combination de agar e dextrano o blanco de ovo. In iste situation, le usual lesiones interne es associate con alterationes thrombohemorrhagic in le organos de choc anaphylactoide, specialmente le patas, le muso, e le radice del cauda.

Omne iste formas del phenomeno thrombohemorrhagic es inhibite per pre-tractamento con heparina.

ACKNOWLEDGMENTS

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REFERENCES


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