Prothrombin Activity in Rats with Hepatic and Other Tumors*

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It is generally believed that the synthesis of prothrombin occurs in the liver. This has been demonstrated in several ways. The prothrombin activity of plasma is low in experimental animals after partial (17) or total (19) hepatectomy. Furthermore, when the hepatotoxins chloroform and carbon tetrachloride are administered to dogs and rats, hypoprothrombinemia results (3, 14, 18). Clinical investigators have indicated that a prothrombin deficiency arises in intrahepatic diseases such as obstructive jaundice, Laennec's cirrhosis, and acute yellow atrophy (1-3, 20). The question therefore arises, whether hepatic tumors can alter the animal's capacity to synthesize prothrombin. It is conceivable that the tumor itself alters prothrombin and thereby tend to induce a state of hyperprothrombinemia. On the other hand, the presence of the tumor might interfere with the synthesis of prothrombin by the remaining normal tissue of the liver. These possibilities were tested in rats with large primary hepatic tumors induced by the ingestion of p-dimethylaminoazobenzene. Determinations of the prothrombin activity of the plasma from such animals were made both before and after the administration of 3,3'-methylenebis(4-hydroxycoumarin), an anticoagulant that specifically lowers the prothrombin activity of the plasma from such animals were made both before and after the administration of 3,3'-methylenebis(4-hydroxycoumarin), an anticoagulant that specifically lowers the prothrombin activity of the blood (4, 15).1 Prothrombin determinations were also made on the blood from normal rats and from rats bearing tumors in other parts of the body, before and after feeding the anticoagulant.

METHODS

The method of administering 3,3'-methylenebis(4-hydroxycoumarin) and of determining the prothrombin time of the plasma was essentially that used in our previous studies (12). The rats were fed a semisynthetic diet (casein 18, yeast 8, salts 4, cod liver oil 2, and dextrin 68) for at least a week prior to the initial determination of the prothrombin activity. They were then starved for 12 hours and fed 2.5 mgm. of 3,3'-methylenebis(4-hydroxycoumarin) contained in 2 gm. of food. This was consumed within 30 minutes, and 4 hours later the rats were allowed access to larger amounts of the basal ration. Twenty-four hours after the anticoagulant was consumed, blood samples were taken by heart puncture under light ether anesthesia. The clotting time of the 12.5 per cent plasma was then determined by our standard procedure (4). Determinations of prothrombin activity were made on whole plasma also, but the presentation of the data is restricted to 12.5 per cent of plasma for reasons previously stated (4, 12).

Types of tumors.—A hypoprothrombinemia was induced in rats with the following types of tumors: spontaneous mammary adenofibromas, primary skin tumors on the head due to methylcholanthrene, primary hepatic tumors due to p-dimethylaminoazobenzene, and transplanted Flexner-Jobling tumors. The spontaneous adenofibromas were round and discrete, and varied in diameter from 2 to 5 cm. Characteristically they were encapsulated and contained much connective tissue.

The tumors due to methylcholanthrene varied in diameter from 1.5 to 5 cm. They were rough, irregular, and papillomatous, and spread along the surface of the head and neck. The large tumors showed definite evidence of penetration into the subcutaneous tissues. These tumors had been induced by the application of a benzene solution of methylcholanthrene thrice weekly for 10 months (7), with subsequent periods of 1 to 4 months to allow the tumors to grow to a large size.

The hepatic tumors were induced by the feeding of synthetic diets containing 0.06 per cent of p-dimethylaminoazobenzene. The diets varied in the source of the vitamin B complex and in the nature of the fat of diet (8, 10), but all were low in vitamin K. The
azo dye was fed for 4 months, when the livers were examined for tumors by laparotomy (9). The rats were then fed the same diet as before, but not the azo dye. The individual rats selected for studies of prothrombin level all had hepatic tumors at least 1.5 cm. in diameter. Most of them had multiple tumors, some of which were several centimeters in diameter. The types of hepatic tumors encountered have been adequately described by others (5, 6, 11, 16). In some of the rats the major part of the liver appeared quite normal except for a fair-sized tumor attached to a single lobe. The opposite type of liver was that involved throughout by tumors, with a little or no grossly normal liver tissue remaining. Many livers represented gradations between these two extremes.

The Flexner-Jobling tumors were inoculated subcutaneously into rats 125 to 150 gm. in weight, and at least 3 weeks elapsed between the inoculation and the first period of induced hypoprothrombinemia. The tumors varied in diameter from 2 to 5 cm. The skin above the smaller growths remained unbroken, while many of the larger ones were extensively ulcerated.

The range of 15 samples was 34 to 40 seconds, average 35. In terms of prothrombin concentration, this decrease in average clotting time probably represents an appreciable change, since prothrombin concentration varies hyperbolically with clotting time, and in this particular time range a large change in prothrombin concentration produces a relatively small change in the clotting time (4).

The plasma from rats with hepatic tumors showed a clotting time above 44 seconds (Table I). The average clotting time was also somewhat longer for the cancerous plasma than for normal plasma, 42 seconds as compared to 39 seconds. The administration of 25 mgm. of 2-methyl-1,4-naphthoquinone to rats with a high prothrombin time (average 47 seconds) failed to correct the hypoprothrombinemia. Apparently the capacity to synthesize prothrombin may be impaired in animals in which a large part of the liver has been replaced by tumor. On the other hand the mere presence of a neoplasm somewhere in the body failed to affect the prothrombin levels; normal prothrombin times were observed in the plasma of rats with large growths due either to methylcholanthrene or to the inoculation of Flexner-Jobling tumor (Table I).

Within the group bearing the Flexner-Jobling tumors it appeared that the prothrombin time decreased as the degree of ulceration of the tumor became more severe.

### Experimental

**Prothrombin activity of plasma from normal and tumor-bearing rats.**—The prothrombin time of 12.5 per cent plasma from normal rats ranged from 34 to 46 seconds, average 39 seconds (Table I). Only 9 of 360 samples required more than 44 seconds to clot. When large amounts of vitamin K were administered, the prothrombin times of the plasmas were generally lower. The range of 15 samples was 34 to 40 seconds, average 35. In terms of prothrombin concentration, this decrease in average clotting time probably represents an appreciable change, since prothrombin concentration varies hyperbolically with clotting time, and in this particular time range a large change in prothrombin concentration produces a relatively small change in the clotting time (4).

The plasma from rats with hepatic tumors showed roughly the same range in prothrombin time as plasma from normal rats. However, 13 of 49 samples showed

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1. **Table I**: Prothrombin Time of 12.5 Per Cent Plasma from Normal and Tumor-Bearing Rats

<table>
<thead>
<tr>
<th></th>
<th>Prothrombin time (in seconds)</th>
<th>Animals above normal per cent*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Normal rats</td>
<td>39</td>
<td>34-46</td>
</tr>
<tr>
<td>Hepatic tumors</td>
<td>42</td>
<td>35-50</td>
</tr>
<tr>
<td>Epithelial tumors</td>
<td>39</td>
<td>34-42</td>
</tr>
<tr>
<td>Flexner-Jobling tumors</td>
<td>36</td>
<td>32-42</td>
</tr>
</tbody>
</table>

*This upper limit of "normal" plasma was arbitrarily considered to be 44 seconds.

**Experimental**

The vitamin K (Menadione-Abbott) was kindly supplied by Dr. Carl Nielsen, Abbott Laboratories, North Chicago, Illinois.

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2. **Table II**: Induced Hypoprothrombinemia in Normal and Tumor-Bearing Animals

<table>
<thead>
<tr>
<th></th>
<th>Prothrombin time (in seconds)</th>
<th>Animals above normal per cent*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Normal rats</td>
<td>116</td>
<td>78-215</td>
</tr>
<tr>
<td>Hepatic tumors</td>
<td>163</td>
<td>78-313</td>
</tr>
<tr>
<td>Epithelial tumors</td>
<td>115</td>
<td>96-195</td>
</tr>
<tr>
<td>Flexner-Jobling tumors</td>
<td>106</td>
<td>89-140</td>
</tr>
</tbody>
</table>

*The upper limit for "normal animals" receiving this dosage of anticoagulant was arbitrarily considered to be 150 seconds or more.
Again, this tendency toward a more acute hypoprothrombinemia was not associated with all types of tumors. Rats bearing large primary epithelial tumors due to methylcholanthrene responded to the anticoagulant as normal rats (Table II), nor was there any evidence of a heightened susceptibility to the anticoagulant in rats bearing spontaneous mammary tumors or inoculated Flexner-Jobling tumors.

It was previously shown (12) that a single dose of 25 mgm. of 2-methyl-1,4-naphthoquinone would completely protect normal rats from the hypoprothrombinemia induced by 2.5 mgm. of the anticoagulant. However, the same dose of the naphthoquinone gave only slight protection to rats with hepatic tumors. The clotting time of 4 of these animals 24 hours after the ingestion of both the anticoagulant and naphthoquinone was 108 seconds compared to 40 seconds in normal animals (12). On the other hand preliminary experiments indicated that in rats bearing Flexner-Jobling or epithelial tumors vitamin K counteracts the anticoagulant as effectively as in normal animals. The increased sensitivity observed in rats with liver tumors was therefore attributed to changes within the liver itself rather than to the presence of tumor tissue per se.

DISCUSSION

The observations described are all in harmony with the assumption that an adequate amount of functional liver tissue is necessary for the maintenance of normal levels of plasma prothrombin. The findings indicate that hepatic tumor tissue itself does not synthesize prothrombin. In fact the reverse situation exists. Large hepatic tumors tend to replace the normal functional tissue of the liver, and a hypoprothrombinemic state results that cannot be corrected by administering high levels of vitamin K. When the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) is given to rats bearing hepatic tumors, the hypoprothrombinic response is not only of greater intensity but it persists longer.

It is not necessary to postulate any impairment in the capacity to synthesize prothrombin by those nontumor cells that still are present in the tumor-bearing liver, although such an impairment is a distinct possibility. The results observed can be attributed to a reduction in the number of normally functioning liver cells. An inadequacy in the amount of normal liver tissue would result in plasma that is borderline with respect to prothrombin activity or that is mildly hypoprothrombinemic. When the anticoagulant is given, impairment of normal prothrombin synthesis by the nontumorous liver cells is brought on, and there results the severe hypoprothrombinemia described. These observations have their counterpart in clinical reports indicating that liver cirrhosis in man produces a state of hypoprothrombinemia (1, 2, 20), which is readily augmented by administering the anticoagulant (13) and which is not corrected by vitamin K.

SUMMARY

1. The presence in the rat of spontaneous mammary tumors, of induced skin tumors, or of inoculated Flexner-Jobling tumors does not cause a prolongation of the normal prothrombin time (12.5 per cent plasma). On the other hand the presence of primary hepatic tumors due to \( p \)-dimethylaminooazobenzene may cause a mild hypoprothrombinemia.

2. A standard dose (2.5 mgm.) of 3,3'-methylenebis(4-hydroxycoumarin) usually causes a more severe hypoprothrombinemia in rats with primary hepatic tumors than in normal rats, or in those bearing tumors in other parts of the body. The extent and duration of the hypoprothrombinemia is probably influenced by the amount of normal hepatic tissue present.

3. While vitamin K protects normal rats against the hypoprothrombinemic action of a single dose of 3,3'-methylenebis(4-hydroxycoumarin), in rats with hepatic tumors this protective action is either lessened or abolished.

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