The Treatment of Anemia in the Tumor-bearing Hamster with Cortisone and Sodium Salicylate*

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SUMMARY

Both cortisone acetate and sodium salicylate administered from the time of, or 1 week after, tumor transplantation inhibited the growth of a transplantable fibrosarcoma. Hamsters in these two treatment groups did not develop the anemia and splenomegaly which characteristically accompany the growth of this tumor in untreated animals. Their failure to develop anemia was attributed primarily to inhibition of tumor growth.

In anemic hamsters bearing large, advanced fibrosarcomas, however, the administration of cortisone prevented profound splenic changes and restored the hemoglobin to normal levels. Tumor necrosis was increased somewhat in these animals, but the abnormal splenic hemolysis produced by the necrotic tissue was thought to have been inhibited by the cortisone. Sodium salicylate was of transient therapeutic benefit in correcting the hemolytic anemia of the tumor-bearing hamster, and the morphology of the spleen was less abnormal than in the untreated tumor-bearing animal.

Previous studies have shown that hamsters with transplanted fibrosarcomas develop anemia and splenomegaly after 30 days of tumor growth (28). The exact etiology of this anemia is unknown, but the presence of necrotic tumor tissue and the associated splenomegaly are apparently important factors in its pathogenesis (12, 26, 28, 29). Recent studies have indicated a definite hemolytic component to this anemia, since shortened erythrocyte survival times have been found in tumor-bearing animals (23, 25). In addition, a modified antiglobulin (Coombs-like) or erythrocyte agglutination reaction can be demonstrated in these anemic, sarcoma-bearing hamsters (1).

In view of the beneficial effect of cortisone and ACTH in the therapy of certain hemolytic anemias in man (7, 8), it was decided to study the effect of cortisone on the anemia of the tumor-bearing hamster. At the same time, the therapeutic effect of sodium salicylate on the hemolytic anemia of malignancy was investigated. The latter drug was used because of the often reported similarity in effect of cortisone and salicylate in various human diseases (4, 5, 17) and experimental situations (30). Moreover, each of these compounds supported the growth of a transplantable human, malignant, melanoma in the hamster, suggesting that salicylate as well as cortisone interfered in some way with the "immunological response" of this animal (27). The assumption was not made, however, that salicylate and cortisone achieve their effect through similar mechanisms. Rather it was hoped that, with the sarcoma-bearing hamster as a model, some differences in mode of action between the two drugs would be revealed.

MATERIAL AND METHODS

One hundred seventy-seven golden hamsters (Mesocricetus auratus) of both sexes, 8–10 weeks of age, fed Purina Laboratory Chow and water ad libitum, were used. The tumor employed is a 100 per cent transplantable fibrosarcoma. The method of tumor transplantation, observation and measurement, and the characteristics of the tumor have been previously reported (18). All the hamsters

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received cheek pouch transplants of the sarcoma. Prior to transplantation, all animals were weighed. Hemoglobins were determined by standard hematologic technic before implantation of any tumor. Thirty untreated, tumor-bearing hamsters were used as controls.

**Experiment I.**—Forty hamsters were given injections subcutaneously of 0.1 cc. of cortisone acetate (Cortone Acetate, Merck, Sharp & Dohme, 2.5 mg/cc) on the day of tumor implantation and then twice weekly for 7 weeks. Forty other hamsters were given 0.15 cc. of sodium salicylate (10 per cent solution) intraperitoneally on the day of tumor transplantation, and then twice weekly for 7 weeks. The 80 animals in this experimental group had hemoglobin determinations on days 30, 40, and 50 following tumor transplantation. The animals were sacrificed on day 50, and autopsies were performed.

**RESULTS**

**Experiment I.**—Tumors transplanted to hamsters treated with cortisone acetate from the day of tumor transplantation grew very poorly. The animals did not become anemic, and they failed to develop splenomegaly. In the group of hamsters treated with sodium salicylate, the average tumor size was greater than in the cortisone-treated group, but it was significantly less than that found in hamsters given no therapy. Neither splenomegaly nor anemia was present in this treated group (Table 1). There was no significant difference between the spleen volumes of the group treated with cortisone and that treated with salicylate. The difference between the average spleen

<table>
<thead>
<tr>
<th>Condition of hamster</th>
<th>Hemoglobin (gm/100 ml of blood)</th>
<th>Spleen volume (cu. mm.)</th>
<th>Tumor weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>10.0 ± 0.98</td>
<td>479 ± 121</td>
<td>9.3 ± 0.91</td>
</tr>
<tr>
<td>Cortisone, treated (0.1 cc., S.C., cortisone acetate, 2.5 mg/cc)*</td>
<td>16.6 ± 1.01</td>
<td>220 ± 89</td>
<td>0.81 ± 0.58</td>
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<tr>
<td>Sodium salicylate, treated (0.15 cc., I.P., sodium salicylate, 10 per cent solution)*</td>
<td>16.1 ± 0.92</td>
<td>225 ± 70</td>
<td>2.86 ± 0.91</td>
</tr>
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* Drug started at the time of tumor transplantation and injected thrice weekly for 7 weeks.

**Experiment II.**—Forty-seven nonanemic hamsters with 8-day-old sarcoma transplants were divided into two groups of ten and 37 animals, respectively. In the first group, the ten hamsters were given injections subcutaneously of 0.1 cc. of cortisone acetate 3 times a week for 7 weeks. The other 37 hamsters were treated intraperitoneally with 0.15 cc. of sodium salicylate 3 times a week for 50 days. Hemoglobin values were obtained on the 40th and 50th days after therapy was started, and on the 50th day the hamsters were sacrificed and autopsied.

**Experiment III.**—Twenty anemic, tumor-bearing hamsters with hemoglobins ranging from 7.0 to 13.3 gm/100 ml of blood on the 49th day post-transplantation were divided into two groups of ten each. Ten hamsters were treated with 0.1 cc. of subcutaneous cortisone acetate 3 times daily for 5 days, and ten were given 0.15 cc. of sodium salicylate intraperitoneally 3 times daily for 3 days. Hemoglobins were determined 24 hours, 4 days, and 2 weeks after cessation of therapy, and at the end of 2 weeks all the hamsters were autopsied.

**TABLE 1**

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ml of blood) on the 43d post-transplantation day and were then treated with cortisone acetate 3 times a day for 3 days showed an increase in the average hemoglobin value (12.6 ± 0.90 gm/100 ml of blood) in 24 hours. The hemoglobin was maintained in the normal range, and on the 57th day post-transplantation (14 days following the cessation of therapy) the average hemoglobin was 15.6 ± 1.1 gm/100 ml of blood. This value is significantly greater (P < 0.01) than the hemoglobin value in untreated tumor-bearing (10.0 ± 0.98 gm/100 ml of blood) or salicylate-treated hamsters. The ten anemic (average hemoglobin, 11.7 ± 1.4 gm/100 ml of blood) tumor-bearing hamsters treated with sodium salicylate 3 times a day for 3 days showed an average increase in hemoglobin in 24 hours (12.2 ± 1.8 gm/100 ml of blood), but this was not maintained, and after 57 days of tumor growth the average hemoglobin was 8.9 ± 2.2 gm/100 ml of blood.

The animals in Experiment III, treated with large doses of cortisone acetate, had an average spleen volume of 254 ± 188 cu. mm. on day 57, whereas the salicylate-treated animals had an average spleen volume of 917 ± 285 cu. mm. This difference in spleen size is statistically significant (P < 0.01).

Hamsters treated with cortisone acetate either for 7 weeks or for the 3-day period showed a decrease in size of Malpighian corpuscles and no reticuloendothelial cell hyperplasia, plasma cell proliferation, hemosiderosis, or extramedullary hematopoiesis of their spleens—changes that are noted in untreated tumor-bearing animals. Nonanemic hamsters treated for 7 weeks with sodium salicylate likewise failed to show splenic changes of the type observed in untreated tumor-bearing animals. Anemic hamsters treated with sodium salicylate 3 times a day for 3 days had some degree of splenic reticuloendothelial cell hyperplasia, plasma cell proliferation and hemosiderosis, but this was distinctly less marked than that which was seen in the spleens of the control animals.

DISCUSSION

The results show both cortisone acetate and sodium salicylate to be capable of inhibiting the growth of the hamster fibrosarcoma used in these studies, with the former a more effective agent than the latter. The antitumor effect of cortisone was not surprising, since this has previously been shown by Kelsall and Crabb (6, 10) using a benzanthracene-induced sarcoma in the hamster and by others using tumors in mice (2, 10, 14, 31), rats (31, 32), and man (26). The fact that sodium salicylate also had an inhibitory effect on the hamster sarcoma was of great interest to us and, to our knowledge, has not been reported elsewhere.

The lack of development of anemia and splenomegaly in the animals treated with cortisone acetate or sodium salicylate for 7 weeks can no doubt be attributed, at least in part, to the failure of the tumors in these animals to reach the size which this type of tumor attains in untreated hamsters. Our previous work (12, 26, 28) suggests that appreciable amounts of both viable and necrotic tumor tissue acting upon the reticuloendothelial system are necessary for the development of anemia, and, insofar as both cortisone and salicylate inhibited tumor growth, they may perhaps be considered as "anti-anemic" agents.

The beneficial effect of cortisone on the anemia of animals bearing large, well established sarcomas, however, is obviously on a different basis and may represent the effect of this hormone on the reticuloendothelial system. Cortisone may inhibit the abnormal hemolysis which is apparently provoked in the spleen and other parts of the reticuloendothelial system by the necrotic tumor tissues (26). The relatively small splenic volume in the animals treated with cortisone despite the presence of large necrotic tumors, and the morphologic appearance of these spleens when compared with the spleens of untreated tumor-bearing hamsters, suggest that this is, in fact, the case and that the anti-anemic effect of cortisone is due, at least in part, to its known effect on cellular elements in the spleen. Since plasma-cell proliferation was much less marked in the cortisone-treated animals than in untreated tumor-bearing animals, some inhibition of antibody production in the treated animals is also probably suggested.

The slight rise in hemoglobin following salicylate administration in animals with large tumors may also be due to interference with abnormal splenic hemolysis and/or antibody production. If this is true, then the lack of sustained response after the 3 days of therapy were concluded may indicate that the dose of salicylate was inadequate and perhaps prolonged administration might have resulted in a sustained remission. It is also possible, however, that the brief rise in hemoglobin was the result of splenic contraction and "autotransfusion." In this event, salicylate cannot be considered to be "anti-anemic" as is cortisone. Further studies are in progress to evaluate the possible usefulness of sodium salicylate in the treatment of auto-immune hemolytic anemia. Salicylates have been shown to block the immunological or host response in anaphylactic shock (3), vasculitis (24,
33), serum sickness (9, 11), acute allergic encephalomyelitis (13), typhoid vaccine injections (15, 21), and hemolytic streptococcal sore throat (22).

REFERENCES

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