Experimental Studies of Factors Influencing Hepatic Metastases

V. Effect of Cortisone and Adrenalectomy*

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SUMMARY

The incidence of hepatic metastases in rats subjected to intraportal injection of 5,000 Walker 256 carcinosarcoma cells was similar in rats receiving cortisone and their pair-fed controls. This result was obtained whether cortisone was given prior to, after, or preceding and following the injection of tumor cells. Both cortisone-treated and pair-fed control rats exhibited significantly fewer metastases than did controls allowed food ad libitum and demonstrating a gain in body weight. The number of metastatic lesions in each positive instance was similar in each group. The relationship of these findings to liver composition following cortisone administration is discussed. The increase of hepatic glycogen in cortisone-treated rats (when compared with pair-fed controls) was apparently without effect on the incidence of such metastases.

Adrenalectomy similarly failed to influence the incidence of hepatic metastases as compared with that observed in controls. The increased incidence of hepatic metastases observed following hepatic manipulation was not dependent upon intact adrenals.

It was concluded that adrenal function played little specific role in the development of hepatic metastases in the experimental model employed.

It has been demonstrated that cortisone exerts an inhibitory effect on the local growth of some experimental tumors (1, 4, 13). In other instances this agent promotes their homologous (5, 9, 12) and heterologous (20) transplantation. Divergent results are apparent from investigations concerning the effect of this agent on the phenomenon of metastasis. An increase of pulmonary metastases has been noted in transplantable and methylcholanthrene-induced tumors by Baserga and Shubik (2). A similar effect was observed by Agosin et al. (1), Duran-Reynals (4), and Molomut et al. (16), who utilized tumors which failed to produce metastases spontaneously. Iverssen (13) recorded an increase and earlier appearance of pulmonary metastases following the subcutaneous implantation of chondrosarcoma in cortisol-treated mice. However, six other neoplasms similarly investigated did not exhibit such an effect. Wood et al. (22) and Pomeroy (17) also observed increased numbers of metastases in cortisol-treated animals following the intravenous and intracardiac injection of tumor cells. The former also noted this effect following the administration of corticosterone, fluorohydrocortisone, adrenocorticotrophin, formalin, or cold stress. Gasic and Gasic (10) concluded that cortisone administration resulted in an increase of metastases only in those tumors with the natural capacity of delivering emboli into the circulation. Kaliss et al. (14) contended from their investigations that the evidence that cortisone produced an increase in metastases was inconclusive. Schatten and Kramer (18) similarly found cortisone as well as stress ineffective in enhancing tumor metastases. Martinez and Bittner (15) actually observed fewer metastases following the administration of cortisone than were found in control animals.

Details of the above studies reveal differences in types of tumor investigated, technics employed, dosage of cortisone utilized, and the duration of its administration. These factors have been considered as perhaps the reason for the varied results obtained.

The purpose of this study was to further investigate the role of cortisone on the development of hepatic metastases following the direct intraportal injection of known numbers of Walker 256

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carcinosarcoma cells. Particular attention was directed to certain parameters of cortisone effect which have not heretofore been investigated in relationship to this problem. Further, it was considered that the technic employed might provide pertinent information relative to some of the concepts proposed in explaining the effect of cortisone on tumor metastases in the experimental animal. Also included, and heretofore not concomitantly investigated, was the effect of adrenalectomy on such artificially induced metastases.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats, weighing 150–170 gm., were utilized in all experiments. Group A consisted of 121 animals that received an intramuscular injection of 2 mg. of cortisone (Cortone acetate \(^{®}\); Merck & Co.) daily for 14 days prior to and 14 days subsequent to the intraportal injection of tumor cells of a Walker 256 carcinosarcoma ((infra vide)). Sixty-one rats received an intraportal injection of tumor cells but no cortisone and were pair-fed with members of Group A1 and designated as A2. An additional 61 were injected with tumor cells without cortisone, but were allowed food ad libitum and designated as A3. Group B1 consisted of 57 rats that received 2 mg. of cortisone daily for 14 days prior to the injection of tumor cells, at which time this agent was discontinued. A similar number of pair-fed controls (B2) and 55 rats fed ad libitum (B3) were utilized as controls for this group. There were 19 rats in Group C1 that received 2 mg. of cortisone daily for 14 days subsequent to the injection of tumor cells. A similar number of pair-fed controls (C2) and 20 controls allowed food ad libitum were included in this group (C3). Group D1 consisted of 25 rats that received 2 mg. of cortisone daily for only 4 days prior to the injection of tumor cells. There were 20 pair-fed controls (D2) and 23 controls allowed food ad libitum in this group (D3). Group E1 consisted of 40 animals that received 10 mg. of cortisone daily for 14 days prior to and subsequent to the injection of tumor cells. This group included 40 pair-fed controls (E2) and 48 controls allowed food ad libitum (E3).

Accompanying each group of animals that received cortisone were five rats of similar strain, weight, and sex that received comparable doses of cortisone but no injection of tumor cells. These were sacrificed at the time rats treated with cortisone received an injection of tumor cells or at the completion of the experiment. Their livers were quickly removed and subjected to analysis for glycerogen according to the method of Good, Kramer, and Somogyi (11); total fatty acids by alcoholic potassium hydroxide saponification and petroleum ether extraction of a suitable aliquot of liver homogenate; and total nitrogen and protein nitrogen by the Kjeldahl method. The protein nitrogen was converted to liver protein by multiplying the former by the factor 6.25.

One hundred and seven Sprague-Dawley rats of similar weight and sex were subjected to bilateral adrenalectomy performed under ether anesthesia with a dorsolateral approach. One week following adrenalectomy, each received an intraportal injection of 5,000 tumor cells. One hundred and sixteen intact rats of similar weight were utilized as controls. All adrenalectomized rats and 86 controls were subjected to laparotomy 2 weeks following injection of tumor cells. Those considered negative by careful macroscopic examination were resutured and sacrificed 1 week later. Thirty controls were sacrificed at 3 weeks following injection of tumor cells.

All animals were maintained on Purina chow as indicated. Rats receiving cortisone and their controls were given drinking water containing 5 per cent glucose ad libitum. Those subjected to adrenalectomy and their controls received saline ad libitum.

The methods of preparation and injection of tumor cells of the Walker 256 carcinosarcoma have been described in detail previously (8). They essentially consist of mincing a subcutaneous growth in equal parts of saline and plasma, counting the cells as in a leukocyte determination, and diluting the number of cells observed with a saline-plasma mixture to the number desired for injection. All animals subjected to the latter procedure received 5,000 cells injected into a large mesenteric branch of the portal vein. In all instances tumor cells were injected alternately into treated and untreated controls so as to insure uniformity of the tumor utilized.

All animals were sacrificed 14 days following the injection of tumor cells. Their livers were examined for metastases, and the number of such lesions in each positive instance was arbitrarily judged as 1+ to 3+. At least 5 blocks were taken for histologic study. These were fixed in 10 per cent neutral formalin and processed and embedded in paraffin in the usual manner and stained with hematoxylin and eosin, periodic acid-Schiff, Rinehart-Abul-Haj, and Alcian blue technics, and with thionin, 1:10,000 for ½ hour. Adrenal glands from all animals were weighed after removal of the pericapsular adipose tissue.
RESULTS

As indicated in Table 1, animals receiving cortisone exhibited either an absolute loss of body weight or failed to gain weight comparable to controls fed ad libitum. No significant difference in the incidence of hepatic metastases was evident in animals receiving cortisone when compared with their pair-fed controls in any group studied. The variation in incidence of hepatic metastases following the various dosage schedules apparent in Table 1 is attributed to differences in the tumors utilized rather than effect of cortisone, because of the close similarity in incidence of metastases in cortisone-treated and pair-fed controls. On the other hand, the incidence of such lesions was greater in controls allowed food ad libitum and exhibiting a gain in body weight. There was no significant difference apparent in the number of hepatic metastases in livers found to be positive in cortisone-treated or control animals.

Similarly, no apparent difference in the incidence of hepatic metastases was observed in adrenalectomized animals from that noted in their controls (Table 2). Body weights were similar in both. Control and adrenalectomized animals which were considered free of tumor at 2 weeks disclosed marked increases in hepatic metastases when examined 1 week later, whereas the incidence in controls sacrificed at 3 weeks was similar to that noted in the former group of controls.

The effect of cortisone upon liver composition is indicated in Table 3. Hepatic glycogen appeared to be increased in rats receiving cortisone when compared with the values obtained with their pair-fed controls. Similarly, an increase in hepatic lipide was observed in Groups A, D, and E. On the other hand, a slight depletion of protein was noted in animals receiving cortisone as compared with their pair-fed controls.

The adrenal weights noted in Table 1 reveal a decreased weight of these organs following cortisone administration in all groups except Group C.
in which this agent was administered for only 4 days prior to the injection of tumor cells. The weights of the adrenals of pair-fed controls failed to differ statistically from those of controls allowed food ad libitum when expressed as absolute weight or per gram of body weight.

Histologic study failed to reveal any difference in the morphologic appearance or acid mucopolysaccharide content of the hepatic metastases of animals receiving cortisone from that of untreated controls.

**DISCUSSION**

The results of this study have failed to reveal any significant influence of cortisone on the incidence of artificially induced hepatic metastases. Although a significantly smaller number of treated rats revealed such lesions as compared with controls allowed food ad libitum, it is to be noted that this comparison does not take into consideration the nutritional deficit exhibited by rats receiving cortisone as employed in this investigation. Indeed, no significant difference in the incidence of hepatic metastases is apparent when the results observed in cortisone-treated animals are compared with those in pair-fed controls. The number of metastases per liver in positive instances was similar in all groups studied. These findings indicate the importance of suitable controls in experiments of this nature and may explain, at least in part, some of the diverse interpretations previously cited concerning the influence of cortisone on tumor growth and metastases. The significantly decreased incidence of hepatic metastases noted in the relatively undernourished pair-fed controls as compared with control rats allowed food ad libitum is similar to the observations of Tannenbaum and Silverstone (19), who observed fewer spontaneous mammary carcinomas and fewer pulmonary metastases in CSH mice.

The lack of effect of cortisone on the development and growth of hepatic metastases noted in these experiments tends to minimize the role of immunity in the pathogenesis of these lesions, since the administration of this agent as well as undernutrition, both associated with an inhibition of the immune response, might be expected to produce an increase in such lesions.

There can be little doubt that some of the discrepancies in results concerning the effect of cortisone on local and metastatic tumor growth is the result of the inherent nature of the type of tumor studied. Several investigators (10, 18) have noted antithetical results concerning the effect of cortisone on metastases with various tumors investigated, although the experimental model was similar in all instances. Other explanations for the different effects observed with cortisone which have been suggested are the sequence of administration of this agent as well as its dose. Our findings fail to disclose any significant relationship between these factors and the incidence of metastases observed. Coincident with lack of effect of cortisone noted herein was the similar lack of effect of adrenalectomy on the incidence of hepatic metastases. It is highly significant that the enhancing effect of hepatic manipulation on the development of hepatic metastases, demonstrated previously in our laboratory (8), is not dependent upon intact adrenals. Although it may be argued that macroscopic examination is inadequate for the detection of hepatic metastases in rats considered free of tumor at 2 weeks, it has been our experience with more than 3,000 animals that the yield on microscopic examination is less than 5 per cent greater than that obtained by gross inspection. Further, control animals not subjected to laparotomy but sacrificed at 3 weeks disclosed an incidence of metastases similar to that noted in rats subjected to laparotomy at 2 weeks. Such information indicates that adrenal function both in physiologic and pharmacologic ranges plays little role in the development of hepatic metastases.
with the Walker 256 carcinosarcoma. The concept that cortisone enhances metastatic growth by its effect on tumor emboli or the metastatic site does not appear tenable from the results of these experiments. In this regard it is of interest that tumor emboli and metastases were morphologically similar in animals receiving cortisone and in controls, and no perceptible differences in acid mucopolysaccharides, purported to play a role in the metastases from local growths (4), could be demonstrated histochemically in these lesions, or hepatic stroma containing such growths.

The results of analyses of liver composition of animals receiving cortisone appear of interest since it is well recognized that this agent may alter the composition of this organ and thus may have some bearing on the "soil" concept of hepatic metastases. The greater amount of glycogen observed in the livers of animals receiving cortisone prior to the injection of tumor cells than was found in their pair-fed controls is apparently without effect in regard to the development of hepatic metastases, although it has been frequently speculated and popularized (21) that ample stores of glycogen provide a fertile soil for the development of hepatic metastases. It appears worthy of emphasis that the glycogenic effect of cortisone, as observed in this study, is evident only when such values are compared with pair-fed controls. Little difference has been noted in the glycogen content of the livers of cortisone-treated rats and of controls allowed food ad libitum. Although we have observed a significant increase in artificially induced hepatic metastases following the administration of a high protein or fat diet (7), which results in large increases in the hepatic stores of these substances, smaller increments as noted here following the administration of cortisone are apparently without effect. It becomes apparent that the results obtained following the administration of cortisone emphasize the possibility that metastatic development may be the result of a summation of factors which inhibit metastatic growth (viz., decreased stores of hepatic protein, undernutrition) and those which might enhance tumor growth, such as elevated hepatic fat. The experimental model utilized precludes consideration of the inherent metastasizability of the experimental tumor used, a factor considered by Gasic and Gasic (10) to be significant in accounting for diverse results recorded concerning the effect of cortisone on neoplastic metastases.

REFERENCES


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