Effect of Hypophysectomy upon the Leukemoid Organ Infiltrations in Walker Tumor-bearing Rats*

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In the course of experimental investigations concerning the influence of transplantable tumors upon the endocrine glands, it was noted, many years ago, that infiltrations with myeloid elements developed rather constantly in rats bearing transplants of Walker tumor 256. This leukemoid reaction was not considered to be specific for living tumor cells, because it also occurred after the injection of killed tumor cells or of necrotic non-neoplastic rat tissue (5-7).

It is also relevant that the myeloid response is not limited to the adrenals but often occurs in the livers and spleens of tumor-bearing rats, and, although pure adrenocorticotropic hormone (ACTH) does not produce this change, somatotropic hormone (STH) containing anterior pituitary extracts proved quite potent in this respect (8, 9). Lesser degrees of leukemoid organ infiltration have also been observed in animals bearing other transplantable tumors, e.g., “croton-pouch tumor No. 1” (10). However, of all agents tested until the present, the Walker tumor proved to be most effective in this respect, and, in rats bearing this neoplasm, most of the adrenal cortex is often transformed into a bone marrow-like tissue (11).

Adrenalectomy fails to prevent the leukemoid syndrome in rats maintained exclusively either with cortisol acetate (COL-Ac) or with deoxycorticosterone acetate (DOC-Ac). However, for optimal survival and for the maximal development of the leukemic infiltrates, substitution therapy with both these steroids is required. The intense, splenic atrophy, normally produced by COL-Ac in adrenalectomized rats, is not only prevented but overcompensated by the leukemogenic action of the Walker tumor (12).

The fact that STH preparations stimulate ectopic myelopoiesis (8), while hypophysectomy inhibits the proliferation of erythroblasts in the bone marrow of the rat (2, 3), suggested that perhaps the anterior pituitary participates in the pathogenesis of such lesions. STH is not necessarily the only hypophyseal hormone involved, since both ACTH preparations (4) and pituitary extracts containing the “erythropoietic factor” (1, 13) have been found to enhance myelopoiesis in the bone marrow of the rat. Furthermore (as stated above), certain nonhormonal tissue extracts possess similar actions, and it is not known whether the effects of these are mediated through the pituitary.

The object of this communication is to describe experiments designed to determine whether hypophysectomy would prevent the development of the leukemic syndrome in Walker tumor-bearing rats.

MATERIALS AND METHODS

Twenty-six female Sprague-Dawley rats, having an initial average body weight of 168 gm. (range, 155-175 gm.), were subdivided into four groups as indicated in Table 1. Groups 1 and 2 acted as controls not treated with hormone. On the first day, Group 4 was hypophysectomized, and treatment with hormones was started in Groups 3 and 4 by giving 400 fig. of cortisol acetate (COL-Ac) and 100 /ig. of deoxycorticosterone acetate (DOC-Ac) in the form of microcrystal suspensions. Each compound was injected subcutaneously, at separate sites, once daily, in 0.2 ml. of aqueous suspension fluid.

On the 10th day, the animals of Groups 2-1 were given inoculations of 0.1 gm. of a Walker-tumor suspension (prepared in a Potter homogenizing tube) in 0.5 ml. of 1 per cent NaCl, subcutaneously, under sterile conditions.

All animals were killed with chloroform on the 15th day, after the transplantation of the neoplasm. At autopsy, the tumor was carefully dissected and fixed in Susa solution, together with...
the adrenals, the kidneys, the liver, and the spleen, for subsequent weighing and histological study. All these tissues were then embedded in paraffin and stained with hematoxylin-eosin according to the Hotchkiss-McManus PAS method.

RESULTS

Our principal observations are summarized in Table 1 (Groups 3 and 4), together with the corresponding findings on untreated normal (Group 1) and untreated tumor-bearing rats (Group 2). The latter two groups are adduced, for control purposes, from an earlier experiment (12) which had been performed under identical circumstances.

In interpreting the changes observed, one must keep in mind that, obviously, hypophysectomized animals cannot long survive the toxic effects of rapidly growing Walker tumor unless they receive some substitution therapy. Our previously mentioned studies on adrenalectomized rats had shown that combined treatment with COL-Ac and DOC-Ac (at the dose levels used in the present work) afforded the best protection, without marked interfering with the growth of the tumor itself or with the development of its systemic manifestations. Significantly, the somatic growth, the growth of the tumor itself, and the weights of the organs listed in our Table for Group 3, treated with this corticoid combination, are almost exactly the same as those of the nonhormone-treated intact controls (Group 2). Apparently, at this dose level, combined treatment with COL-Ac and DOC-Ac did not change either the growth or the systemic effects of the tumor significantly, although the corticoids permitted the survival of 75 per cent of the hypophysectomized rats.

The gain in body weight (expressed here as a percentage of the initial body weight minus the weight of the previously dissected tumor) was almost normal in the intact controls of Groups 2 and 3. The hypophysectomized rats of Group 4, on the other hand, lost more than one-third of their initial body weight.

The tumor itself grew somewhat less rapidly in the hypophysectomized than in the intact controls, but only its absolute weight was subnormal; if the tumor weight is expressed as a percentage of the body weight, this difference is inconspicuous.

The adrenals were enlarged and contained numerous myeloid and lymphoid infiltrates in the intact tumor-bearing animals of Groups 2 and 3. This enlargement is due partly to the development of hemopoietic tissue (which is localized almost exclusively in the cortex) and partly to hyper trophy and hyperplasia of the cortical cell. The hemopoietic elements were rather selectively arranged along the sinusoids of the cortex, and many immature blood-forming cells were found within the lumina of the vessels, evidently being carried down with the blood into the medullary veins. In the hypophysectomized tumor-bearing rats of Group 4, the cortex was atrophic. Infiltration with hemopoietic elements, though considerably inhibited, was not totally prevented by hypophysectomy (Figs. 1-3).

The liver was greatly enlarged in the tumor-bearing intact rats, while in the hypophysectomized animals the hepatic weight decreased below that of the intact nontumor-bearing controls. On the other hand, the infiltration with hemopoietic elements was approximately the same in all tumor-bearing groups (Fig. 4).

The spleen was about 4 times as large in the tumor-bearing intact rats, while in the hypophysectomized animals the hepatic weight decreased below that of the intact nontumor-bearing controls. On the other hand, the infiltration with hemopoietic elements was approximately the same in all tumor-bearing groups (Fig. 4).

The spleen was about 4 times as large in the tumor-bearing intact rats as in the normal controls, and histologic studies showed that this enlargement was due primarily to infiltrations with myeloid elements. The megakaryocytes were particularly large and numerous, a change rather characteristic of Walker tumor-bearing rats. The content of PAS-tingible material in these cells was extremely variable. In the hypophysectomized
Fig. 1.—Adrenal of an intact rat from Group 3. Marked infiltration with hemopoietic tissue, particularly along the cortical sinusoids. X 400.

Fig. 2.—Corresponding region of the adrenal cortex of a hypophysectomized rat from Group 4. The parenchymatous cells are atrophic, and there is a small islet of hemopoietic tissue near the center. X 400.

Fig. 3.—Numerous myeloid cells within the lumen of a vein in the adrenal shown in Figure 2. X 400.

Fig. 4.—Liver of the hypophysectomized rat from Group 4. Large myeloid and small lymphoid cell infiltrations near the lower right corner of the field. The venules in the left upper corner are replete with large myeloid cells. X 250.
FIG. 5.—Spleen of an intact tumor-bearing rat of Group 3. Note the diffuse infiltration with myeloid (dark) cells and the extraordinarily large number of splenic megakaryocytes.

FIG. 6.—Spleen of a hypophysectomized rat from Group 4. Neither myeloid cells nor megakaryocytes are visible. X100.

FIG. 7.—High magnification of the spleen shown in Figure 5. There are four fully formed megakaryocytes in this small field: two of them (top) stain very poorly with PAS, while two others are deeply stained. X480.

FIG. 8.—High magnification of a region from the spleen shown in Figure 6. Megakaryocytes are absent, but there are several macrophages (to the left of the small arteriole) which contain numerous deeply PAS-positive granules and hence stain darkly.
tumor-bearers, the spleen underwent considerable atrophy, and even careful search through many sections of each spleen failed to reveal a fully developed megakaryocyte (Figs. 5-8).

The weight of the kidneys is given here only for comparison, to show that the hepatomegal and splenomegal in Walker tumor-bearing rats are not merely part of a generalized enlargement of the viscera. No intense renal hypertrophy was observed in any of the animals, and the mean weight of the kidneys did not parallel that of the spleen and liver in the various groups. Hemopoietic infiltrations were not seen in any of the kidneys.

The mortality was almost the same in the intact as in the hypophysectomized hormone-treated tumor-bearers. Apparently, the corticoid substitution therapy was successful in maintaining a high degree of tumor resistance in the hypophysectomized animals.

DISCUSSION

It is evident that, under the circumstances of our experiment, hypophysectomized rats given adequate corticoid substitution therapy can withstand a developing Walker tumor almost as well as can the intact controls. On the other hand, the systemic changes produced by the Walker tumors are greatly altered in the absence of the pituitary. At first sight, one may gain the impression that this change in the systemic response is merely due to an inhibition of tumor growth. However, when expressed as a percentage of the total body weight, the tumor mass carried by the hypophysectomized and the intact animals was quite comparable; yet the development of hemopoietic elements was greatly inhibited in the adrenals of the former. It is also significant that, although several intact rats bore tumors which were smaller than those of certain hypophysectomized animals, hemopoiesis was always more pronounced in the adrenals of the former. The same considerations apply to the interpretation of the hepatic and splenic atrophy.

Apparently, hypophysectomy acts selectively on some, but not all, manifestations of the leukemic syndrome. In the presence of adequate corticoid substitution therapy, removal of the hypophysis slightly diminished the absolute (but not relative) weight of the tumor itself; it also increased the catabolic effect of the neoplasm, diminished myeloid infiltrations in the adrenals and spleen, caused total disappearance of splenic megakaryocytes, as well as pronounced atrophy of the spleen and liver. Conversely, under these circumstances, hypophysectomy did not interfere with the development of myeloid infiltrations in the liver and did not greatly increase the mortality rate, despite the extraordinarily marked tumor cachexia.

SUMMARY

Hypophysectomized rats receiving maintenance doses of cortisol acetate (COL-Ac) and deoxycorticosterone acetate (DOC-Ac) had an almost normal resistance to the lethal effect of Walker tumor grafts. In such animals, the transplanted plasmas grew nearly as well as in intact controls given the same steroid treatment.

On the other hand, the systemic effects produced by the Walker tumors were significantly altered in the absence of the pituitary:

1. The tumor cachexia was greatly increased, and the liver and spleen underwent extraordinarily severe involution.

2. The development of leukemoid tissue infiltrates in the adrenals and spleen was greatly inhibited, and the proliferation of splenic megakaryocytes (characteristic of Walker tumor-bearing rats) was totally prevented.

3. Despite the marked hepatic atrophy, hemopoietic tissue development in the liver was not inhibited by hypophysectomy.

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