Effect of Pituitary Hormones on Carcinogenesis with 9,10-Dimethyl-1,2-dibenzanthracene in Hypophysectomized Rats*

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The suppression of carcinogenesis in hypophysectomized animals has been reported by several investigators (11, 23, 25). We have previously reported on this phenomenon in rats with neoplasms developing during chronic injection of growth hormone (21), with spontaneous tumors (21), and with sarcomas induced with methylcholanthrene (19, 20). The present studies were concerned with the effects of crude pituitary extract, growth hormone, and adrenocorticotrophic hormone on the carcinogenic response of hypophysectomized rats to 9,10-dimethyl-1,2-dibenzanthracene.

MATERIALS AND METHODS

Two series of experiments were conducted. In Series I, female rats of the Long-Evans strain, 5-7 months old, were used. Three groups of fifteen rats each were selected and treated as follows: (a) Intact, normal rats were given intramuscular injections of 2 mg. of 9,10-dimethyl-1,2-dibenzanthracene (4) dissolved in 0.25 ml. tricaprylin. The animals were maintained without further treatment. (b) Hypophysectomized rats were also injected with 2 mg. of the carcinogen 21 days postoperatively and maintained without further treatment, as in the preceding group. (c) Hypophysectomized rats were treated with 2 mg of the carcinogen 21 days postoperatively, following which treatment pituitary extract 1 was given intraperitoneally 6 days weekly.

1 The completeness of hypophysectomy was determined as accurately as possible during the period between hypophysectomy and administration of the carcinogen. Animals which showed significant increases in body weight, external evidence of sexual maturation or activity, or presence of adult pellage were discarded. At autopsy the completeness of hypophysectomy was confirmed by examination of the pituitary gland and the endocrine organs.

1 The pituitary preparation was an aqueous alkaline extract of acetone-dried beef anterior lobes. The daily dose contained the extractable material from 5 mg. and, later in the experiment, from 7.5 mg. of acetone powder. This extract was rich in growth, thyrotrophic, adrenocorticotrophic, lactogenic, and interstitial cell-stimulating hormones.

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In Series II, four groups of immature female rats of the Long-Evans strain, 26-28 days of age, were used. Three of the groups were hypophysectomized at this age. At 54-56 days of age, 1 month after hypophysectomy, all rats were given intramuscular injections of 2 mg. of 9,10-dimethyl-1,2-dibenzanthracene dissolved in 0.25 ml. tricaprylin. The animals were divided into the following groups: (a) fifteen rats with intact pituitary glands, given no further treatment following the carcinogen; (b) fifteen hypophysectomized rats given no further treatment; (c) hypophysectomized rats given a daily intraperitoneal injection of growth hormone (12) 6 days weekly; the initial daily dose of growth hormone was 100 μg., and this was increased progressively to a maximum of 500 μg.; (d) 90 hypophysectomized rats given a daily intraperitoneal injection, 6 days weekly, of adrenocorticotrophic hormone. The daily dose of this hormone varied between 50 and 60 μg. (It was occasionally necessary to withhold the adrenocorticotrophic hormone for short intervals because of undue loss of weight.)

1 The adrenocorticotrophic hormone employed was Fraction F, described in a recent publication by Li et al. (18), and containing 45 USP units/mg. It was dissolved in gelatin solution.

The rats in all groups were weighed every 3 days and examined for lesions at the site of injection. The animals which developed rapidly growing, incapacitating neoplasms during the course of the experiments were sacrificed and autopsied. The study in older rats, Series I, was concluded 380 days after the injection of 9,10-dimethyl-1,2-dibenzanthracene. The experiment in the younger rats, Series II, was terminated 183 days after injection of the carcinogen.

At autopsy the site of injection of the carcinogen and representative portions of all organs were fixed in formalin for histological study.
microscopic sections. Paraffin sections, 7 μ in thickness, were prepared and stained with hematoxylin and eosin and silver impregnation counterstained with van Gieson.

RESULTS

Series I

Growth response (Chart 1).—The group of rats with intact pituitary glands showed a slow and progressive increase in body weight. The group of hypophysectomized rats which received no replacement therapy initially lost considerable weight and did not regain their pre-operative weight.

![Chart 1](chart1.png)

*Chart 1.*—Body weights of normal and hypophysectomized rats and of hypophysectomized rats treated with pituitary extract, during the year following a single intramuscular injection of 9,10-dimethyl-1,2-dibenzanthracene.

The hypophysectomized rats which were injected with the pituitary extract fluctuated considerably in their growth response during the early phases of the experiment, but subsequently, as the dose was adjusted, these animals grew rapidly and eventually greatly exceeded even the normal group in size.

Neoplastic response (Chart 2).—Intact rats given injections of 9,10-dimethyl-1,2-dibenzanthracene, with two exceptions, developed malignant tumors at the site of injection. One of the rats which did not develop a tumor died immediately after the onset of the experiment. The other died 168 days after injection of the carcinogen. A severe inflammatory reaction occurred in all animals at the site of injection, and this subsided gradually. Approximately 155 days after injection of the carcinogen, seven of fourteen surviving rats developed small firm nodules at the site of inoculation, with subsequent rapid, progressive growth of the lesions. Therefore, the appearance of nodules was designated as the "onset of tumor." The remaining six animals developed tumors at later periods, the last neoplasm occurring at 295 days.

Microscopically, the tumors were sarcomas arising in muscle and were essentially similar to those observed with methylcholanthrene pellets (19).

In the single rat in which no tumor developed, there was an area of necrosis surrounded by granulation tissue at the site of injection of the carcinogen.

*Hypophysectomized rats given injections of 9,10-dimethyl-1,2-dibenzanthracene.*—Three animals in this group died soon after injections of the carcinogen. An additional three animals died before the time of 50 per cent incidence of sarcomas in the intact rats, 155 days after the injection of the carcinogen. None of these were included in the analysis of incidence of tumors.

The inflammatory reaction at the site of injection of the carcinogen was less severe than in the intact rats.

Sarcomas gradually appeared in five of the nine survivors between 182 and 379 days. The hypophysectomized rats which did not develop sarcomas were sacrificed 234–379 days after injection of the carcinogen.

The neoplasms in this group grew more slowly than those in the intact rats. Histologically, they were similar to those in the intact rats.

*Hypophysectomized rats treated with 9,10-dimethyl-1,2-dibenzanthracene and pituitary extract.*—Nine survived beyond the time of 50 per cent incidence of tumors in the intact rats. Eight of the survivors developed sarcomas at the site of injection, the latent period of the first tumor being 165
days. Thereafter, the tumors developed in rapid succession and, by 240 days, almost equaled the incidence in intact rats. The rate of growth of tumors was comparable to that observed in intact rats.

Therefore, in Series I, the results show a lower incidence and a delayed appearance of sarcomas in hypophysectomized rats. The administration of crude pituitary extract reinstated the neoplastic response.

**SERIES II**

**Body weights (Chart 3).**—At the beginning of the experiment the body weights of all rats were between 60 and 70 gm. The intact rats grew rapidly and attained a maximum weight of 90 gm. The hypophysectomized controls attained a maximum weight of 85 gm. The hypophysectomized rats given injections of growth hormone responded by growing at a rate which compared favorably with that of the intact rats, and attained a weight of 265 gm. The hypophysectomized rats injected with adrenocorticotropic hormone consistently weighed less than the untreated hypophysectomized rats.

**Thyroids, adrenals, and ovaries.**—The hypophysectomized controls had atrophic thyroids, adrenals, and ovaries. In the hypophysectomized rats which received growth hormone, these endocrine organs were similar to those of the hypophysectomized controls, showing no evidence of stimulation. The adrenal cortices of the hypophysectomized rats given injections of adrenocorticotropic hormone showed stimulation, whereas the thyroids and ovaries were atrophic.

**Neoplastic response (Chart 4).**—Intact rats: Fourteen of fifteen intact rats (93 per cent) developed sarcomas at the site of injection of 9,10-dimethyl-1,2-dibenzanthracene. Fifty-five days after injection of the carcinogen, small nodules, less than 1 cm. in diameter, were detected in six of the animals. These lesions showed a progressive and usually rapid increase in size. The longest latent period between injection of the carcinogen and the detection of a neoplastic lesion was 155 days; the study was terminated at 188 days. The time of 50 per cent incidence in this group was 60 days. In the one animal without a tumor there was fibrosis, vascular thickening and hyalinization, and lymphocytic infiltration at the site of injection.

**Hypophysectomized rats without hormonal treatment.**—Three of the rats in this group died without developing tumors prior to the time of 50 per cent incidence of sarcomas in the intact rats. For this reason, they were not included in evaluating the effect of hypophysectomy on carcinogenesis. Seven of the twelve surviving rats (58 per cent) developed sarcomas after latent periods ranging from 85 to 160 days. The time of 50 per cent incidence in hypophysectomized rats was 156 days, in contrast to 60 days for the intact animals. The neoplasms grew more slowly than those in the intact rats. The tumors were similar in appearance to those in rats with pituitary glands.
Of the five animals which did not develop sarcomas, two survived for the experimental period of 188 days; in one of these a small fibroma was present at the site of injection. In animals which did not develop neoplasms, the site of injection of the carcinogen showed degeneration of skeletal muscle, fibrosis, and chronic inflammation.

**Hypophysectomized rats injected with growth hormone.**—The entire group of fifteen hypophysectomized rats receiving growth hormone survived beyond the time of 50 per cent incidence of sarcomas in the intact rats. Four were sacrificed 63–108 days after the injection of the carcinogen, because of their poor condition. These animals had not developed tumors, but, because they had survived beyond the time of 50 per cent incidence of sarcomas in intact animals, they were included in the calculation of incidence. Eleven rats (73 per cent) developed sarcomas at the site of injection of the carcinogen after latent periods ranging from 60 to 90 days. These tumors were similar in appearance to those developing in the intact rats. The time of 50 per cent incidence of neoplasms for this group receiving growth hormone was 65 days, virtually the same as that for the intact rats and in marked contrast to the 156 days for the hypophysectomized controls. In the rats which did not develop neoplasms, the site of injection was characterized by degeneration of muscle, fibrosis, and infiltration by lymphocytes.

**Hypophysectomized rats injected with adrenocorticotrophic hormone.**—Ten of 30 animals died without tumors prior to 60 days, the time of 50 per cent incidence of sarcomas in intact animals, and therefore were excluded in determining the incidence. Ten of the remaining 20 (50 per cent) developed sarcomas after latent periods ranging from 90 to 160 days, thus attaining an incidence of 50 per cent. The sizes attained by the tumors were smaller than those in intact animals but were not histologically different. The sites of injection of the carcinogen in the animals which did not have tumors showed necrosis. Fibrosis and chronic inflammation were minimal.

Thus, the experimental data in Series II confirmed the depression of the carcinogenic response, in hypophysectomized animals, to 9,10-dimethyl-1,2-dibenzanthracene. Robertson and his associates have likewise observed fewer sarcomas in hypophysectomized rats treated with 9,10-dimethyl-1,2-dibenzanthracene than in intact rats.

However, the studies of other investigators have also indicated that the refractory state of the hypophysectomized animal to carcinogenesis is relative and not absolute. Factors other than the pituitary gland are of unquestioned importance, namely, differences in species, age, carcinogenic stimuli, nutrition, and length of the period of observation.

The slower rate of growth of neoplasms in hypophysectomized rats observed here has been reported previously by McEuen and Thomson (17), Reiss et al. (24), and Talalay et al. (31). Ball and his associates, as early as 1932, demonstrated that the growth of transplanted, induced, and autogenous tumors was slower in hypophysectomized rats (1, 2, 27).

The reinstatement of neoplastic response in hypophysectomized rats both by crude pituitary extract and by purified growth hormone affords confirmatory evidence of the significance of the anterior pituitary in carcinogenesis. The carcinogenic response in both instances approximated that of rats with intact pituitaries.

The administration of adrenocorticotrophic hormone to hypophysectomized rats failed to restore the carcinogenic response to 9,10-dimethyl-1,2-dibenzanthracene. Robertson and his associates have reported that adrenocorticotrophic hormone, as well as growth hormone, tended to reinstate the neoplastic response of hypophysectomized rats to 3-methyl-4-dimethylaminoazobenzene. This difference in results may be due to differences in purity of the pituitary preparations, the dosages of the hormones, the characteristics of the carcinogenic stimulus, and the age and strain of rats.

Both crude pituitary extract and purified growth hormone restored body growth, and the parallelism with the carcinogenic response was striking. Neither body growth nor carcinogenesis was restored in hypophysectomized rats injected with adrenocorticotrophic hormone. In view of the parallelism between body growth and the carcinogenic response, it seems reasonable to assume that the two processes are similar affected by growth-promoting and growth-inhibiting factors. Evi-
ence supporting this concept is provided by the experiments of several investigators, including Loeb (15), Visscher et al. (35), Tannenbaum (32, 33), and Saxton and his associates (29), who have demonstrated that restriction of caloric intake sufficient to depress body growth also depresses the incidence and delays the appearance of tumors.

Inasmuch as both pituitary hormones and nutrition modify the carcinogenic response, the interrelationship of these factors is pertinent. There is considerable evidence that pituitary secretions modify the nutritional requirements and utilization of dietary constituents. Samuels et al. (38) have observed that forced feeding of young hypophysectomized rats failed to restore to normal their ability to store nitrogen. The effectiveness of pituitary growth hormone in promoting nitrogen retention and protein synthesis has been adequately demonstrated (3, 14, 30, 34, 36, 38). The anabolic effect of growth hormone is in turn dependent on adequate amounts of dietary protein (7, 8). It has been shown that pituitary function can be altered by restriction of diet (5, 29) or by changing the diet qualitatively (37). However, there are no specific data available concerning the effect of nutrition on the rates of secretion of growth hormone and of adrenocorticotropic hormone.

The inhibitory effect of adrenocorticotropic hormone on body growth is well known (5, 6, 10, 16, 18). This inhibition of growth has been shown to be due to interference with protein synthesis (9, 14). In the present experiment, the failure of adrenocorticotropic hormone to reinstate the neoplastic response of hypophysectomized rats may be explained by the same mechanism.

CONCLUSIONS

The carcinogenic response of hypophysectomized rats to 9,10-dimethyl-1,2-dibenzanthracene was significantly depressed. This altered response was manifested by a delayed appearance and lowered incidence of neoplasms. The administration of crude pituitary extract or purified growth hormone to hypophysectomized rats reinstated their carcinogenic response. Adrenocorticotropic hormone failed to restore carcinogenesis in hypophysectomized rats. The difference in the effects of growth hormone and adrenocorticotropic hormone may be attributable to the difference in their effects on protein metabolism and, hence, on growth.

REFERENCES

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