

# The Nonessentiality of the Hypophysis for the Induction of Tumors with 3,4-Benzpyrene\*

FREDERIC J. AGATE, JR., WILLIAM ANTOPOL, SUSI GLAUBACH,  
FAY AGATE, AND SAMUEL GRAFF

(Departments of Anatomy and Biochemistry, Columbia University; and Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, New York, N.Y.)

The report by Moon, Simpson, and Evans (6) that "of fifteen hypophysectomized rats treated with methylcholanthrene, only one developed a sarcoma at the site of implantation" has revived interest in the possible influence of the hypophysis on the induction and growth of neoplasms.

In 1932 Ball *et al.* (1) showed that the growth rate of 1,2,5,6-dibenzanthracene-induced tumors was slowed by hypophysectomy, their findings indicating that the reduced growth rate was specifically due to the absence of the hypophysis. McEuen and Thomson (5), however, concluded that the retardation of Walker rat carcinoma following hypophysectomy was a consequence of cachexia. Korteweg and Thomas (4) applied 3,4-benzpyrene to the skin of hypophysectomized mice. In one series 40 per cent, twelve out of 30, of their hypophysectomized animals died without tumors. The remaining 60 per cent all developed skin tumors. The authors suggested that the 10-12-month survival time following the operation was too short for maximum tumor development and that the difference in response to carcinogens between intact and hypophysectomized animals was entirely quantitative rather than qualitative. Most recently the work of the Stanford group (2, 3, 7) indicated that hypophysectomy prevented the occurrence of cirrhosis and of liver tumors in rats following administration of 3'-methyl-4-dimethylaminoazobenzene.

The inference of the experiments of Moon *et al.* was that hypophysectomy abolished the primary malignant transformation, quite apart from non-specific metabolic effects. The choice of carcinogen would appear to be relatively unimportant for this

generalization, which it was desirable to test further. An experiment wherein 3,4-benzpyrene was administered to both intact and hypophysectomized rats in order to observe the effect of hypophysectomy on tumor induction is reported here. The procedure differs from that of Moon, Simpson, and Evans in the choice of carcinogen and in the postoperative care of the animals. The latter appears to be of some consequence.

The influence of inanition and cachexia on tumor *growth* is well documented in the literature. These considerations may not necessarily apply to tumor *induction*. Nevertheless, an effort was made in these experiments to minimize their effects in so far as possible by supplementation of the diet and by heating of the cages. Hypophysectomized animals suffer from disturbances of heat-producing mechanisms. Other experiments under way in these laboratories suggest a temperature influence on the growth rate of tumors even apart from nutritional inanition.

Initially, a series of rats were hypophysectomized in the Anatomy Department laboratory and moved to animal quarters in another laboratory, where they were given injections of 3,4-benzpyrene; a group of intact controls was treated similarly. In these quarters conditions proved unsuitable for the long-term maintenance of hypophysectomized animals, and an inordinately high mortality was encountered. Only seven of the fourteen hypophysectomized animals survived until the first intact controls developed tumors. All these surviving animals developed tumors. As a result of this trial experiment it seemed advisable to repeat the work under more suitable conditions.

## MATERIALS AND METHODS

Twenty Long-Evans strain rats (about 100 days of age) of both sexes were hypophysectomized. Twelve rats of approximately the same age and weight were employed as controls. Between 14 and

\* Aided by a grant from the American Cancer Society. Some of this material was presented at the annual meeting of the American Association of Anatomists, Galveston, Texas, and published in abstract form in *Anat. Rec.*, **118** (2): 276, 1954.

Received for publication July 15, 1954.

30 days after hypophysectomy each operated rat and each unoperated control animal was injected with 1 cc. of sesame oil containing 10 mg. of 3,4-benzpyrene. The carcinogen was injected subcutaneously in the dorsal midline at a point about 3 cm. caudad to the shoulder girdle.

All animals in this series were maintained in the Anatomy Department animal colony in which over a period of 25 years the maintenance of hypophysectomized animals has been a routine procedure, and the facilities and technical assistance are particularly adapted to this purpose. Under these conditions it is not uncommon to maintain complete hypophysectomized rats for 18 months and more.

All the rats were maintained on a dry mixed diet composed of:

|                     |                                     |
|---------------------|-------------------------------------|
| 15 lb. ground corn  | 10 lb. meat scraps                  |
| 15 lb. ground wheat | 5 lb. whole dried milk              |
| 15 lb. barley       | 5 lb. skim milk                     |
| 15 lb. rolled oats  | 2 lb. sodium chloride               |
| 15 lb. soybean meal | $\frac{1}{2}$ lb. CaCO <sub>3</sub> |

This mixture was fed *ad libitum* to all animals, together with a daily supplement of fresh lettuce and Rockland Farm pellets.

In the hypophysectomized animals the diet was supplemented by a mixture of:

|                     |          |
|---------------------|----------|
| Whole wheat         | 67.5 gm. |
| Casein              | 15.0 gm. |
| Powdered whole milk | 10.0 gm. |
| Vegetable oil       | 4.0 gm.  |
| Cod liver oil       | 1.0 gm.  |
| Sodium chloride     | 1.0 gm.  |
| Calcium carbonate   | 1.5 gm.  |

This was mixed with water to the consistency of a soft paste and fed daily. Unpublished data show that hypophysectomized animals fed on this supplement lose less weight than those fed on stock diet alone, and the percentage of animals surviving is higher. On the other hand, if this supplement is fed to the intact controls a considerable obesity ensues. It was felt that using this supplement in the hypophysectomized rats and not in the controls tended to decrease the nonspecific effects of cachexia and inanition on tumor growth and to produce a higher rate of survival.

In ten of the hypophysectomized animals this soft diet was supplemented further for a time by the addition to the mixture of a whole liver extract. However, this was discontinued about 50 days after injection of the carcinogen, because the animals appeared reluctant to eat the supplement containing liver.

The hypophysectomized animals were kept in

cages in which the back walls were electrically warmed. We have found that the body temperature of hypophysectomized rats in these cages averages between 37 $\frac{1}{2}$ ° and 38 $\frac{1}{2}$ ° C. at 2 months after hypophysectomy, whereas hypophysectomized rats kept in unheated cages in our colony display a rather varying body temperature but may average at least 2 degrees lower. The possible significance of the body temperature is under further investigation.

## RESULTS

Of the twenty hypophysectomized animals injected with benzpyrene, eight died with no tumor on the 37th, 38th, 41st, 44th, 46th, 75th, 92d, and 117th day after injection of the carcinogen. Nine animals died with tumors on days 180, 182, 183, 184, 189, 189, 196, 208, and 231.

Three animals with tumors were sacrificed while still in good health on the 106th, 175th, and 180th day. Small fragments of tumor from each of these three animals were transplanted subcutaneously into nine normal intact rats. The transplants took and continued to grow in all cases. Three benzpyrene-injected control rats were sacrificed at the same time as the hypophysectomized animals, and similar test transplants were made with the same results.

Of the twelve controls, two died with tumors on days 184 and 195. Three with tumors were killed and autopsied on days 106, 175, and 180. The remaining seven, all with tumors, were autopsied on the 204th day.

During the 28 days prior to injection with benzpyrene the controls gained an average of 35 gm., from 194 to 229, whereas the hypophysectomized animals lost 32 gm., from 194 to 162 (Chart 1). At autopsy of the controls, on the 204th day after the injection of benzpyrene, the average total weight had increased further by 123 gm., of which 108 gm. was tumor. No remnant of hypophyseal tissue could be found at autopsy of the operated animals. The average time of death of the hypophysectomized animals with tumors was 195 days, at which time these rats had an average body weight less tumor weight of 110 gm., a loss of 52 gm. from the average weight at time of injection with benzpyrene, and a weight loss from the body weight at hypophysectomy of 84 gm. The weight of the tumors in these animals averaged 28.2 gm.

The first tumor appeared in the controls at 99 days, and the first tumor was clearly detectable in the hypophysectomized animals 7 days later. However, by 174 days, three surviving hypophysectomized animals still showed no tumors, while all surviving controls had large tumors. All surviv-

ing hypophysectomized rats had tumors by the 190th day.

The tumors were spindle-cell sarcomas with varying amounts of pleomorphism. Frequently, necrosis was present.

The hypophysectomized animals which received the diet supplemented with liver extract for 50 days showed no significant difference in tumor weight or incidence as compared with those hypophysectomized animals which received no liver extract.

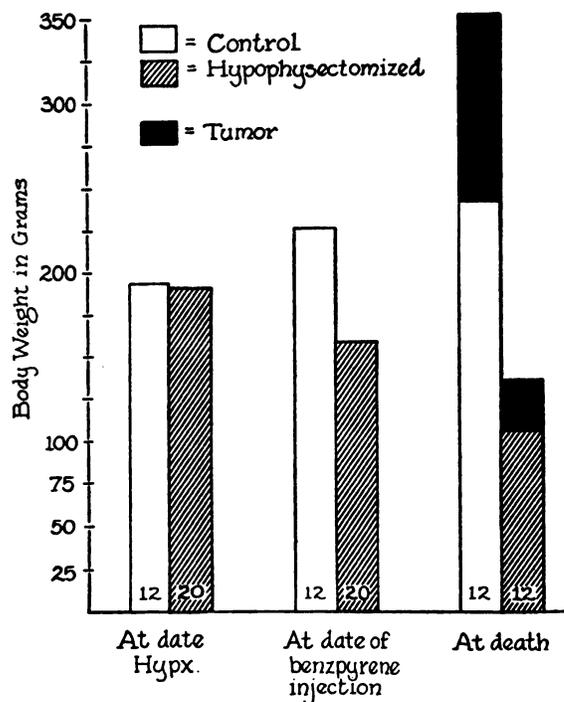


CHART 1.—Comparison of body weight and tumor weight in control and hypophysectomized rats after single injection of 3, 4-benzpyrene.

#### DISCUSSION

It will be noted that all the hypophysectomized animals which failed to develop tumors with one exception died before the 99th day or the earliest time of appearance of tumor in a control animal. The exceptional animal died 18 days later but still at a time when many of the controls showed no evidence of tumor.

The hypophysectomized animals which died before tumors appeared apparently succumbed to infection. The average time between injection with benzpyrene and death in this group was 61 days, during which time the average weight loss was 32 gm.

The data indicate that the hypophysis is non-essential for the induction of tumors of connective tissue by a single injection of benzpyrene.

It appears also that such tumors once induced grew steadily in the absence of the hypophysis. The growth rate was considerably slower than in the controls, but nevertheless growth took place in spite of metabolic deficiencies in the whole organism which were sufficient to cause complete cessation of body growth in the host (Chart 1). In fact, the autonomous nature of neoplastic growth seemed to be strikingly demonstrated by the ability of these tumors to increase in mass while the whole animal was losing weight at a rapid rate.

#### SUMMARY

1. Twenty hypophysectomized rats were given injections subcutaneously of a single dose of 3,4-benzpyrene. Twelve intact control rats were similarly treated. All the intact controls developed tumors.

2. Seven hypophysectomized rats died without tumors prior to the 93d day before any of the controls showed tumors. One hypophysectomized animal died without a tumor on the 117th day. The remaining twelve animals all developed tumors between the 106th and the 174th day.

3. Tumors grew at a considerably slower rate in the hypophysectomized animals than in the controls, averaging 28.2 gm. as against an average of 108 gm. in the controls.

4. It is concluded that the hypophysis is non-essential for the production of neoplasms with 3,4-benzpyrene.

#### REFERENCES

- BALL, H. A., and SAMUELS, L. T. The Effect of Hypophysectomy on Transplanted Mammary Carcinoma in the White Rat. *Am. J. Cancer*, **16**:351-59, 1932.
- GRIFFIN, A. C.; RINFRET, A. P.; and CORIGHIA, U. F. The Inhibition of Liver Carcinogenesis with 3'-Methyl-4-dimethylaminoazobenzene in Hypophysectomized Rats. *Cancer Research*, **13**:77-79, 1953.
- GRIFFIN, A. C.; RINFRET, A. P.; ROBERTSON, C.; and O'NEAL, M. Factors Involved in the Inhibition of Azo Dye Carcinogenesis by Hypophysectomy. *Proc. Am. Assoc. Cancer Research*, **1**:21-22, 1953.
- KORTEWEG, R., and THOMAS, F. Tumor Induction and Tumor Growth in Hypophysectomized Mice. *Am. J. Cancer*, **37**:36-44, 1939.
- McEVEN, C. S., and THOMSON, D. L. The Effect of Hypophysectomy on the Growth of the Walker Rat Tumor. *Brit. J. Exper. Path.*, **14**:384-91, 1933.
- MOON, H. D.; SIMPSON, M. E.; and EVANS, H. M. Inhibition of Methylcholanthrene Carcinogenesis by Hypophysectomy. *Science*, **116**:331, 1952.
- ROBERTSON, C. H.; O'NEAL, M. A.; GRIFFIN, A. C.; and RICHARDSON, H. L. Pituitary and Adrenal Factors Involved in Azo Dye Liver Carcinogenesis. *Cancer Research*, **13**:776-79, 1953.