The Relation of Age to the Occurrence of Adenoma-Like Lesions in the Rat Hypophysis and to Their Growth After Transplantation*

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The spontaneous occurrence of chromophobe adenomas and focal hyperplasia in the rat hypophysis has been reported by Wolfe, Bryan, and Wright (18), and by Oberling, Sannié, and the Guérins (13). Hyperplasia of chromophobic cells and the development of chromophobe adenomas in the rat hypophysis following injection of estrogens have been described by Wolfe and Wright (19), by Zondek (20), and by McEuen, Selye, and Collip (11). References to tumors of the rat hypophysis, either spontaneous or induced, are not frequent in the literature, and no reference to attempts at transplantation could be found.

The purposes of this paper are to report the high incidence with advancing age of adenoma-like lesions of chromophobe cells in a strain of albino rats, to describe an experiment in which successful growth was obtained by homologous transplantation, and to discuss the possible factors necessary for growth following transplantation. The results of assays of adenoma-like tissue for hypophyseal hormones are also described.

Materials and Methods

A total of 107 male and 20 female albino rats, ranging in age from 1 month to 30 months, were examined during the course of this study. These animals were derived from the Yale strain and represented normal stock rats from the colony maintained by the Department of Animal Nutrition at Cornell University. The pathological findings in other organs of this strain of rat maintained upon various experimental regimens are described elsewhere by McCay and others (10), and by Saxton and Kimball (16). Nodular hyperplasia of the hypophysis has been noted occasionally during routine autopsies on animals of this colony, but the incidence has not been tabulated for the entire group. The animals described here may be considered an adequate cross section of the colony with respect to age.

In each case the hypophysis was examined grossly and microscopic sections were prepared from glands showing gross evidence of abnormality. When the high incidence of these lesions became apparent toward the end of the study, routine sections were prepared from all hypophyses examined.

The terms nodular hyperplasia and adenoma-like lesions of chromophobe cells are used in this report because it is often impossible to differentiate on morphological grounds between a nodule of hyperplastic tissue and a true adenoma.

Incidence and Morphology

In Table I is given the observed incidence of nodular hyperplasia of chromophobe cells in relation to age. Since microscopic examination of hypophyses was made largely in cases suspected of containing lesions,

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Number of rats examined (sex)</th>
<th>Number of rats with hypophyseal lesions (sex)</th>
<th>Incidence (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-12</td>
<td>84 (77 M, 7 F)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13-30</td>
<td>43 (30 M, 13 F)</td>
<td>17 (16 M, 1 F)</td>
<td>39</td>
</tr>
</tbody>
</table>

the true incidence in older animals is probably greater than indicated. No lesion was observed in 84 animals up to 13 months of age. The youngest rat to bear either a gross or a microscopic nodule was 15 months of age. The incidence in rats of 13 months of age and over was 39 per cent, lesions of this sort having been found in 17 of 43 rats of this age group. Most of the animals examined were males, and 16 of the 17 lesions were found in this sex. One typical adenoma-like growth was found in a female. The hypophysis in this case weighed 37.5 mgm.

The gross appearances varied from small yellow nodules 1 mm. in diameter seen on the under surface of the hypophysis or in its lateral part, to a large nodular mass of soft pinkish yellow tissue measuring 5 mm. in its greatest diameter and compressing the posterior lobe and adjacent normal areas of hypophysis. The weights of hypophyses bearing nodules of hyperplasia ranged from 14.7 mgm., where only a single microscopic nodule was found, to 159 mgm. In one

* This investigation was aided by a grant from the Josiah Macy, Jr., Foundation.
hypophysis which weighed 16.7 mgm., a symmetrical enlargement of the lateral parts was present, and in section two apparently unjoined adenoma-like masses were found, one on either side of the midline. Asymmetrical enlargement was more common.

Microscopically, all of the lesions observed were similar in most respects and resembled either the nodular foci of chromophobes or chromophobe adenomas described by Wolfe, Bryan, and Wright (18). However, one lesion resembled the hemorrhagic or cavernous type of growth described in the experiments cited (11, 19). The nodules with few exceptions were composed of groups or cords of slightly enlarged chromophobes associated with abundant vascular channels. The nuclei were usually large. The cytoplasm was usually pale and agranular, and contained numerous fat vacuoles of various sizes. When stained with Sudan III, the nodules stood out in sharp contrast to the normal hypophyseal tissue, in which no fat was demonstrable. The adenoma-like areas were not encapsulated or sharply delimited from the adjacent normal tissue, but in every case examined the adjacent tissue was compressed in some degree. Invasion of adjacent structures was never seen, and in all glands bearing lesions some normal hypophyseal tissue was present. In the majority of cases the growths seemed to have multicentric origins. Usually a nodule was found in each lateral part of the hypophysis, but not always in the same relative position. In several hypophyses three or more apparently separate nodules were observed (Fig. 1). Mitotic figures were often abundant even in the smallest lesions. Differences in size of nodules within the same pituitary suggested variations either in time of origin or in rate of growth. In the largest adenoma-like growth, which was used for assay and transfer, the chromophobe cells assumed a character not seen in the other cases. At the borders of the lesion a regular arrangement of vacuolated chromophobes was seen; but near the center were numerous large cells with pale cytoplasm and hyperchromatic nuclei. These nuclei assumed bizarre shapes and multinucleated cells were not uncommon. Mitotic figures were present in this area, as well as in the peripheral regions. No necrosis or hemorrhage was found in a section through the longest diameter of the lesion (Fig. 2).

No consistent change was found in other endocrine organs of rats bearing these hypophyseal lesions, but in several instances the prostate and seminal vesicles were enlarged.

**Assay of Adenoma-Like Tissue for Hypophyseal Hormones**

Adenoma-like tissue from the largest hypophysis encountered was subjected to assay for hypophyseal hormones and also was used for transplantation. The hypophysis bearing this lesion weighed 159 mgm. and was found in a rat 17 months old which had been killed with chloroform. Microscopic sections of this gland showed that the normal hypophyseal tissue had been pushed far to one side by the growth of chromophobe cells. The tissue used for assay and for transfer was taken from the central portion of the growth. No normal hypophyseal structure was recognized microscopically in sections contiguous with that selected. It seemed reasonably certain, therefore, that normal hypophyseal tissue was excluded both from the tissue used for assay and from the tissue used for transfer.

The method of assay is described in detail elsewhere (15). Two immature female guinea pigs were used as test animals. One received a total of 40 mgm. of fresh adenoma-like tissue in divided doses subcutaneously daily for 4 days, and was examined on the succeeding day. The thyroid, ovaries, and adrenal glands were examined grossly and by microscopic section. The same procedure was carried out with the second test animal, using 20 mgm. of tissue. Control assays were carried out using comparable amounts of normal hypophyseal tissue from rats of the same age and strain.

The ovaries, thyroids, and adrenals of the 2 guinea pigs receiving 40 and 20 mgm. of adenoma-like tissue respectively, showed no evidence of stimulation. Similar amounts of hypophyseal tissue from normal rats produced in the controls a moderate hypertrophy of the thyroid, follicular growth and maturation, as well as conspicuous luteinization in the ovaries, and

**FIGURES 1 TO 5**

Fig. 1.—Cross section of hypophysis of male rat 15 months old, showing 5 separate adenoma-like nodules of chromophobe cells. X 17.

Fig. 2.—Representative area of chromophobe adenoma-like lesion used for transplantation and bioassay, showing variation in size of cells, moderate vacuolization of cytoplasm, and mitotic figures. X 400.

Fig. 3.—Representative area of successful transplant of adenoma-like tissue, shown in Fig. 2, removed 10 months after transplantation. Several mitotic figures are present and the cytoplasm of many cells is vacuolated. X 400.

Fig. 4.—Photograph of rat eye containing transplant of adenoma-like lesion shown in Fig. 2. The photograph was taken 15 months after transplantation. The transplant is seen as a rounded mass in the anterior chamber nearest the posterior palpebral commissure. X 3.

Fig. 5.—Same eye as shown in Fig. 4, photographed 19 months after transplantation. The transplant has approximately doubled in size during the 4-month period between photographs. X 3.
enlargement of the adrenals with an increase in mitotic figures in the cortex.

**Transplantation**

Small pieces of the largest adenoma-like lesion were transplanted into the anterior chamber of one eye of each of 9 male rats of the same strain, 2 to 3 months of age. In each instance the tissue was visible for a short period, but in 6 of the rats it had disappeared completely after 5½ months had elapsed. The remaining 3 rats were observed at short intervals over the course of more than a year and the observations in each of these 3 cases are described separately below.

**Rat No. 1.**—The transplant remained visible as a small pinkish-yellow fleck for several months, gradually assuming a rounded shape, but not perceptibly increasing in size. From the color it was considered that a vascular supply had become established. After 7 months the tissue was noticeably enlarging. The mass became bilobate, with rounded margins, and at the end of 10 months occupied about one quarter of the anterior chamber. At this time the eye was enucleated and the tissue used for section and second serial transfer. Section revealed a well vascularized nodule composed of chromophobe cells similar to those of the original lesion. A thin capsule of fibrous tissue surrounded the transplant. Cytoplasmic vacuoles were present, and nuclei were relatively large; but no bizarre forms were seen. The entire transplant appeared viable, and contained many mitotic figures (Fig. 3).

From this transplant a second series of transfers was made into 6 young rats. In only 2 of these did the tissue persist, and these rats are at present under observation. After 8 months each transplant shows slight evidence of growth.

The rat from which the eye with its transplant was removed was killed, 4 months later, when it had reached the age of 17 months. The pituitary was grossly normal. On section several small foci of chromophobe cells with vacuolated cytoplasm were found. There was no adenoma-like lesion. A mitotic figure was seen in one of the foci.

**Rat No. 2.**—Growth of the transplant was not evident until 10 months after inoculation. During the ensuing 3 months a relatively rapid growth took place. In gross appearance the transplant was identical with that already described. This rat was found dead at the age of 16 months, or 3 months after growth was first noted. The transplant in the eye was a well rounded, orange-yellow mass, weighing 9.7 mgm. and measuring 4 by 3 mm., as seen through the cornea. Microscopic section resembled in all respects the transplant previously described, except that an occasional large hyperchromatic nucleus was seen. There was no necrosis in the transplant. The pituitary of this rat weighed 14.7 mgm. and was of normal gross appearance. On section, however, it contained a small, sharply outlined nodule of hyperplasia of chromophobe cells, measuring 1.5 by 1 mm. The cytoplasm of many of the cells contained vacuoles. This nodule is included in the tabulated incidence of adenoma-like lesions.

**Rat No. 3.**—Slight enlargement of the transplant was noted after about 12 months. After 15 months it appeared as a rounded, orange-yellow mass 2 mm. in diameter, as seen through the cornea (Fig. 4). Between the 15th and 19th months after transfer it had approximately doubled in size (Fig. 5). The rat bearing this transplant was killed at 2 years of age, or 21 months after transplantation. The transplant was a yellow lobate nodule measuring 3.5 by 2.5 by 2 mm. It had a thin capsule and was easily shelled out of the anterior chamber. The tissue was used for section and for a second serial transfer. Section revealed a well vascularized nodule composed of chromophobe cells similar to the original lesion. In a few of them the cytoplasm contained vacuoles. An occasional mitotic figure was found. From this animal second serial transfers were made into the anterior chambers of 4 young and 4 old male rats and these rats are at present under observation.

The hypophysis of rat No. 3 was greatly enlarged and the anterior and posterior lobes appeared to be pushed forward by a relatively large hyperemic mass measuring 5 by 4 by 3 mm. and continuous with the anterior lobe. A considerable amount of fresh blood escaped during removal and the lesion then appeared sponge-like in texture. After washing in saline the weight of the hypophysis bearing this lesion was 25 mgm.

Microscopically, the normal anterior lobe tissue was slightly compressed at the margin of the nodule that has been described. This lesion had no capsule, but was sharply demarcated from the normal tissue by the character of the cells. It consisted of an adenoma-like growth of moderately enlarged chromophobe cells, which were arranged in cords and trabeculations of varying thickness. Small blood vessels were seen within the larger of the cords. Between the cords were large spaces which before removal of the hypophysis appeared to be filled with blood and now contained a few red cells. They had no endothelial lining. In one place in the stroma were several phagocytes laden with brown pigment. The lesion appears to be identical with the hemorrhagic or cavernous adenomatous growths described by several observers (11, 19).

**Discussion**

The high incidence of chromophobe hyperplasia or adenoma-like lesions in older rats suggests that ageing
processes may play a part in their development. Wolfe and Wright (19), Zondek (20), and Muens, Selye, and Collip (11) have shown that continued administration of estrogens favors development of these tumors, and the former authors (19) have suggested that an endocrine imbalance in ageing rats may induce hyperplasia of the chromophobe elements. The multicentric origin of the lesions favors this view. The high incidence in the strain here described and in the strains used in the experiments cited (13, 18) suggests that this is a relatively common lesion of old rats. It may occur in either sex. Parsons (14) and Costello (1) have independently shown that small adenoma-like lesions of various types and foci of cellular hyperplasia are frequent in the hypophysis of man, and increase in number with age.

The adenoma-like tissue that was assayed did not contain detectable amounts of gonadotropic, thyrotrropic, or adrenal cortex stimulating hormones. It is usually believed that diminution of hormones in the hypophysis indicates diminution or even absence of production of hypophysal hormones. The results of the assay are in agreement with the generally accepted opinion that chromophobe tumors in man do not produce clinical evidence of secretory function. Zondek (20), however, assayed adenomatous pituitaries of rats and found a normal level of gonadotropic hormones. The entire hypophysis was assayed, whereas in these experiments only adenoma-like tissue was used. The results of the control assays of normal hypophyses are in accord with the findings of Loeb (6).

It is unfortunate that little fresh material was available for transplantation. Although the numbers of transplants are too small to warrant conclusions, certain inferences may perhaps be drawn from the behavior of the three successful transplants. In each instance there has been a long latent period followed by slow growth. Growth of the transplants was not noted until periods of 7 to 12 months had elapsed, and when growth did occur, the rate was considerably slower than in the case of malignant tumors transplanted to the anterior chamber (4). A slow and irregular rate of growth following transplantation of benign tumors has been found with transplanted adeno-fibromas of the rat, by Loeb and Fleisher (7) and by Heiman (5). However, in the experiment cited, the transplants grew more rapidly and attained a size greater than that of the transplants of hypophysal adenoma-like tissue. The small volume of the rat's eye does not seem to have been a limiting factor for growth, because in no instance was more than one third of the anterior chamber occupied by the transplant. It seems probable that the hypophysal lesion here studied is more benign in character than the adeno-fibromas described by Loeb and Fleisher, and that it has a lower growth energy.

The significance of the prolonged latent period, which was from 7 to 12 months, was not evident. It is possible that a long latent period following transplantation is merely an expression of the benign character of these growths. In the experiments of Loeb and Fleisher, the latent periods following the initial transfer of benign tumors ranged up to 4 months, and in Heiman's experiments there was wide fluctuation of growth energy. On the other hand, it is possible that advanced age of the host may be necessary for growth of this type of tissue following transplantation, whereas on the contrary, according to the usual experience, young animals are more suitable for the transplantation of tumors (8). In the period of several months during which the transplants persisted but did not grow, the hosts were approaching the period of life in which these nodules might develop spontaneously. It is conceivable that internal factors responsible for the spontaneous hyperplasia may then induce proliferation of cells in the transplant. It is noteworthy that two of the hosts developed in their own hypophyses similar adenoma-like growths. This is perhaps analogous in certain respects to the observation of Fleisher and Loeb (2) and of Greene (3), showing that transplantation of mammary tumors was more frequently successful in hosts with spontaneous growths.

If this interpretation is correct, the adenoma-like lesion has scant, if any, autonomy of growth, but is dependent upon a specific stimulus provided by the host. Growth may be compared to that which occurs in a specialized tissue introduced into a medium where certain stimuli act upon it. In this respect the growth resembles the physiological responses obtained in normal uterine and prostatic tissue transplanted into the anterior chamber (9, 12, 17). The static condition of the second serial transfer indicates that increased energy of growth was not developed by transplantation. Further experiments are necessary to test the validity of these inferences.

From a consideration of the morphology and observed behavior of these lesions it is difficult to draw a sharp line between focal hyperplasia and truly adenomatous proliferation. In the largest nodule encountered there were areas closely resembling neoplastic proliferation. Such areas were not observed in the smaller and presumably younger tumors, although mitotic figures were frequent. If original growth is dependent upon stimulating factors from the host, then a stronger stimulus or perhaps a longer period of stimulation may bring about changes more closely identified with those of neoplasms. The largest tumor described has the morphological characters of a chromophobe adenoma, even though the behavior of the transplants indicated little if any autonomy of growth. Whether all of the smaller growths observed...
represent adenomas in a strict sense is open to question. These observations suggest a close relationship between focal hyperplasia and growth of an adenomatous nature, and it is doubtful if this characteristic lesion of the hypophysis is analogous to adenomas of other organs. Adenoma-like hyperplasia is perhaps a more accurate designation for these lesions.

It seems unlikely that the lipoid content of the cells in the nodules is evidence of cellular degeneration. Nuclear degeneration was not observed and fat-laden cells were present in areas of active proliferation. A high lipoid content may be considered as a characteristic of the chromophobe cells comprising these lesions, being absent in only one; but its significance is not clear.

SUMMARY

Chromophobe adenoma-like lesions of the hypophysis occur frequently at an advanced age in albino rats of the Yale strain. Adenoma-like lesions were found in the hypophyses of 17 of 43 rats aged between 1 and 2 years, whereas none was found in 84 rats of less than 1 year of age. Conspicuous morphological features were frequent multicentric origin of the nodules and abundant lipid vacuoles in the chromophobe cells.

Bioassay of adenoma-like tissue failed to indicate the presence of gonadotropic, thyrotropic, or adrenocortical hormones. These hormones were demonstrated in comparable amounts of normal rat hypophyseal tissue.

Growth of homologous intra-ocular transplants of adenoma-like tissue occurred in three out of nine instances after latent periods of 7, 10, and 12 months, at a time when the recipients had reached an advanced age.

The advanced age at which adenoma-like lesions appear in the hypophysis, their multicentric origin, and the prolonged latent periods of the transplants suggest that they are dependent upon factors in the host which make their appearance at an advanced age. They have perhaps no autonomous growth and represent hyperplasia in response to conditions that appear with advancing age.

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REFERENCES

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