HYPOPHYSEAL ADENOMAS IN AGED RATS OF A WISTAR STRAIN

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ABSTRACT

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IN AGED RATS OF A WISTAR STRAIN

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The pituitaries of 56 female and 52 male modified pathogen-free Wistar rats of the Upjohn strain were examined for the presence of hypophyseal adenomas. Sixty-six per cent of the females and 44% of the males had adenomas. The trichrome-periodic acid-Schiff staining technique was used to facilitate identification of the hypophyseal cells. Of the adenomas found 86.7% were chromophobe adenomas, 10% acidophilic adenomas, and 3.3% basophilic adenomas. The adenomas tended to occur in older rats. Nineteen per cent of the rats less than 600 days of age and 62% of the rats older than 600 days had adenomas. The pituitaries containing adenomas varied in weight from the normal of 12 mg. to 547 mg., with an average weight of 155 mg. Acidophils were present in 98%, and basophils in 46%, of the chromophobe adenomas studied. Together acidophils and basophils made up less than 25% of the cells present. Areas of hemorrhage with hemosiderin were present in 81% of the chromophobe adenomas, 100% of the acidophilic adenomas and 50% of the basophilic adenomas. The hemorrhage was generally greater in the larger adenomas. The majority of the adenomas had no discernible architectural arrangement.

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By

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INTRODUCTION

The albino rat is the most widely used laboratory animal (Griffith and Farris, 1942) and is a useful experimental animal for an enormously wide variety of laboratory purposes (Robinson, 1965). With the increased use of the rat it becomes important to recognize spontaneously occurring pathologic processes and to be aware of the incidences and characteristics of these processes in order to evaluate accurately the effects of experimental procedures.

The study of spontaneously occurring pituitary adenomas in rats is important because they must be differentiated from induced pituitary changes that may be brought about by techniques or drugs which have an effect upon the endocrine system. Secondly, because of the complex interrelationships of the endocrine system, the presence of an adenoma of the pituitary can be expected to cause secondary changes in other endocrine glands. The study of hypophyseal adenomas in connection with other endocrine changes can thus be useful to elucidate relationships between endocrine glands in pathologic conditions.

This study was an attempt to identify, classify, and describe the spontaneous pituitary adenomas in 108 Wistar rats of the Upjohn strain and to tabulate the incidence of these adenomas with respect to age and sex.

REVIEW OF THE LITERATURE

Although adenomas of the anterior lobe of the pituitary were reported as early as 1903 (Erdheim) and 1907 (Löwenstein), they continued to be considered rare until 1933 when Roussy and Oberling stated that adenomatous foci occurred in 10% of the human pituitary glands they examined at autopsy. Costello (1936) demonstrated that, with careful and routine examination of the hypophysis, the incidence of adenomas in man approached 25%.

Pituitary adenomas or foci of anterior-lobe cells considered to have early adenomatous changes were described in rats by Bryan, Klinck, and Wolfe (1938) in approximately 10% of the females of the Albany strain. Wolfe, Bryan, and Wright (1938), in a subsequent paper, reported a high incidence in 2 other strains, the Vanderbilt strain (males 11.8% and females 29%) and the Wistar strain (females 68.2%; males of this strain were not studied). Saxton (1941) and Saxton and Graham (1944) found pituitary adenomas with increasing frequency at older ages in the Yale strain of rats (older than 600 days; 60% of males and 30% of females had adenomas). In the Sherman and the Wistar strains, in which they studied the males only, there were 3.6% and 11.1% adenomas, respectively.

Furth (1955) stated that chromophobe pituitary adenomas were common in the Wistar strain of rats examined by him. Crain (1958) completed a comprehensive study of spontaneous tumors in 786 Wistar rats of the Rochester strain in which he found 11 adenomas of the hypophysis.

Griesbach, Nichols, and Chaikoff (1966) reported a 46% incidence of pituitary adenomas in a control group of female Long-Evans rats 2 years of age.

In most studies, chromophobe adenomas made up over half of all pituitary adenomas and in some strains of rats this figure approached 100% (Wolfe et al., 1938; Saxton, 1941; Saxton and Graham, 1944; Furth, 1955; and Crain, 1958).

Nurnberger and Korey (1953) described 3 architectural arrangements of chromophobe adenomas; diffuse, sinusoidal, and papillary. The architecture of the sinusoidal type of chromophobe adenoma was similar to that of the normal pituitary gland but there were much less stroma and more cells in the so-called sinuses than in the normal gland. Many septa of fine connective tissue, containing blood vessels, divided the adenoma into sinusoids. Many of these spaces had incomplete septa, and the sinuses had much more variation in size and shape than did the spaces of the normal pituitary gland.

In the papillary type of chromophobe adenoma a blood vessel with a slight amount of adventitia was located centrally and the cells of the adenoma radiated from it, usually to a depth of 4 or more cells. Kernohan and Sayre (1956) found the connective tissue to be excessive in some adenomas of this type.

The outstanding characteristic of the diffuse type of chromophobe adenoma was the complete lack of any architectural arrangement of the cells; it was simply a mass of cells without any regular or specific spatial relationship to each other.

Kernohan and Sayre (1956), in a study on a series of pituitary adenomas in man, found that the diffuse type was the most common, the sinusoidal next in order of frequency, and the papillary type the rarest.

Wolfe and Wright (1947) described 3 types of adenomatous changes in the pituitaries of rats: hemorrhagic chromophobe adenomas, adenomatous foci of chromophobes, and large chromophobe adenomas.

Hemorrhagic chromophobe adenomas were characterized by wide blood spaces which separated the adenomatous cells. These blood spaces did not contain endothelial linings and numerous hemosiderin-filled reticuloendothelial cells were present throughout the lesion. Many mitotic figures were found in approximately half of this type of adenoma. The adenomatous chromophobes in these tumors were made up of cells with varying size. The nuclei and nucleoli were usually enlarged. In fact, nucleolar hypertrophy and, to a lesser degree, nuclear enlargement were the outstanding features of the adenomatous chromophobes and often occurred in cells in which there was no enlargement of the cell itself. The nuclei often tended to be markedly distorted and the nuclear membrane was often wrinkled and indented. In some of the nuclei 2 large, prominent, and eosinophilic nucleoli were present. In more than half of the hemorrhagic adenomas the cytoplasm of the chromophobes contained fat vacuoles. In many of the enlarged adenomatous chromophobes the negative image of the Golgi body was markedly hypertrophied. Saxton and Graham (1944), in their studies on this type of adenoma, described similar changes except that they usually found the nuclei to be oval rather than distorted and indented. They also noted that the chromatin in the nuclei was usually in the form of coarse granules.

In the second type of adenoma described by Wolfe and Wright (1947), the adenomatous nodules, the cells were essentially the same as those in the hemorrhagic adenomas. There was, however, no evidence of hemorrhage and acidophils was found intermingled with the chromophobes in 20% of the cases. Mitoses were rare, fat vacuoles were present in most chromophobes, and there was no compression of the surrounding normal cells.

In the third type, the chromophobe adenomas, the tumors were usually well demarcated from the surrounding anterior lobe and in most cases there was an actual compression of the bordering anterior lobe cells. The cells composing this type of adenoma were similar to those described for the first 2 groups. In most of these chromophobes mitotic figures were numerous and fat droplets were present, but no hemorrhagic clefts were present. The characteristics of this type corresponded to the diffuse chromophobe adenomas described by Nurnberger and Korey (1953).

Although chromphobe adenomas are by far the most common type of pituitary adenoma, the chromophil cells of the adenohypophysis, the acidophil and basophil, may also undergo adenomatous changes resulting in acidophilic and basophilic adenomas. In addition, mixed pituitary adenomas, those which contain 2 or even all 3 cell types, are found in a small percentage of cases (Kernohan and Sayre, 1956).

Bielschowsky (1954) reported 3 functional acidophilic adenomas in Wistar rats. These consisted of predominantly small cells which were about the size of normal chromophobes. The majority of these cells were free of chromophilic granules, but among them were larger cells with acidophilic granules. Large sinuses filled with erythrocytes and hemosiderin-containing macrophages were present.

Basophilic adenomas of the pituitary in the rat are rare. Griesbach _et al_. (1966) described these as consisting of cells containing PAS-positive granules in the cytoplasm, often having vesicular nuclei, and sometimes having an angular shape.

Adenomas of the pars intermedia, described as not uncommon in some species of animals by Jubb and Kennedy (1963), have been described in 2 rats by Wolfe and Wright (1947). Griesbach _et al_. (1966) reported 3 adenomas of this type in a group of Long-Evans rats.

Adamantinomas have been found commonly in pituitaries in man (Kernohan and Sayre, 1956), but no reports have been made of their occurrence in pituitaries of rats.

The question often arises as to whether pituitary adenomas are neoplastic or just hyperplastic and where to draw the line between hyperplasia and neoplasia of the pituitary. Clifton and Meyer (1956) set arbitrary weight limits of 12 mg. as the maximal weight of a normal rat pituitary and a minimal weight of 30 mg. for a tumor. The pituitaries weighing between 12 mg. and 30 mg. were considered hyperplastic.

Saxton and Graham (1944), by using homologous intraocular transplants, have shown that chromophobe adenomas in the rat hypophysis were probably more often neoplastic than hyperplastic. Normal hypophyseal tissue persisted for a considerable period of time after intraocular transplantation, whereas adenomatous tissue grew slowly and could be serially carried into second and third intraocular generations. The growth rate of the transplants was independent of the growth rate of tissues of the recipients and the ability of the transplants to grow was independent of the age or strain of the recipients.

Giok (1961) summed up the results of his research on this question by stating,

> "Evidently putitary tumours are in a borderland between 'normal' hyperplasia and neoplasia and it therefore depends on one's definitions of these terms, whether these tumours be considered neoplastic."

One of the main difficulties in the classification of pituitary cell types, and thus in effect, pituitary adenomas, on a morphological basis has centered about the problem of whether the cells called acidophils and basophils are truly acidophilic and basophilic, respectively.

Peterson and Weiss (1955) have shown that with controlled pH staining, using acidic and basic dyes, the 3 main types of anterior pituitary cells, acidophils, basophils, and chromophobes, can be distinguished. However, with staining techniques other than those based on pH, densely granulated chromophil cells were often seen in the hypophysis (Golden, 1959). Pearse (1952) and Russfield (1957) suggested that these densely granulated cells represented a storage phase and that active secretion could be attributed to generally larger cells with prominent nuclei, whose cytoplasm was more delicately stippled with chromogenic material. Golden (1959), in addition to describing storage and secretory chromophils, also described what he called "hypersecretory cells". These were cells with large and often vesicular nuclei containing dense nuclear chromatin and a variable cytoplasmic mass containing only occasional PAS-positive or orangophilic (PAS-negative, stained by the counterstain orange G) granules.

The difficulties encountered in identifying the cell types of the hypophysis, especially when there are adenomatous changes present, without the application of newer staining techniques and histochemistry, thus become evident.

The chemical composition of at least 6 hormones of the anterior hypophysis is fairly well established (Li and Evans, 1948). Somatotrophin, adrenocorticotrophic hormone, and prolactin are simple proteins, whereas the gonadotrophins (FSH and LH) and thyrotrophin are mucoproteins containing appreciable amounts of carbohydrates (Wilson and Ezrin, 1954). These latter hormones, or their precursors, should therefore be demonstrable by histochemical techniques that are specific for carbohydrates (Catchpole, 1949). The periodic acid-Schiff (PAS) technique, introduced independently by McManus (1946) and Hotchkiss (1948), is a histochemical method which stains carbohydrates in fixed tissues. Pearse (1949, 1952) has done extensive work on the human and on various animal pituitary glands using a modification of the PAS technique, the trichrome-PAS method, to localize the mucoprotein hormones. He found that none of the acidophilic granules contained any demonstrable carbohydrate, and that all of the basophils and some of the chromophobes contained granules which stain red (PAS positive) following the PAS reaction.

Rinehart and Farquhar (1953) demonstrated by electron microscopy that pituitary chromophobes usually contained enough granules to be placed in the category of acidophils or basophils. Such granules were either too small or too few to be resolved using conventional staining procedures and light microscopes.

It has been shown that the basophils are the site of production of the carbohydrate-containing hormones, FSH, LH, and thyrotrophin (Halmi, 1950; Purves and Griesbach, 1951, 1954), and that the acidophils are the site of production of the simple protein hormones, somatotrophin and prolactin (Giok, 1961). The exact source of ACTH has not been firmly established (Giok, 1961).

It is evident from this literature review that chromophobe adenomas are common in the pituitaries of aged rats. Several types of adenomas and several subtypes of chromophobe adenomas occur in rats and special staining techniques are often needed to determine the cell type. The exact nature of pituitary adenomas, whether hyperplastic or neoplastic, has yet to be established.

MATERIALS AND METHODS

The pituitary glands of 52 male and 56 female Wistar rats of the Upjohn strain were studied. The rats used were born in a pathogen-free environment and were removed at the time of weaning to a conventional environment where they were raised (modified pathogen-free rats). These rats have been shown to live longer than conventionally reared rats and are more desirable for long term studies and especially for work with aged rats.

After their removal to a conventional environment the rats were caged separately and were not used as breeders. They were fed a commercial laboratory rat diet* and water ad libitum. The rats which became moribund and those alive at the termination of the study were killed by electrocution. The rats dying spontaneously were necropsied as soon as their deaths were discovered and those euthanatized were necropsied immediately.

At necropsy the pituitary gland of each rat was removed, weighed, and fixed immediately in 10% buffered formalin. It was later embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E) (Armed Forces Institute of Pathology, 1960). Each enlarged pituitary was also stained by the trichrome-periodic acid-Schiff (trichrome-PAS) technique (Pearse, 1950). Gomori's iron stain (A.F.I.P., 1960), was applied to selected sections.

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*Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.

Histologic descriptions were made of each pituitary and photomicrographs were taken of representative conditions. Age, sex, and incidence and types of adenomas were recorded.

RESULTS

Of the 108 rat pituitaries studied, 48 were nonadenomatous, 52 were considered to be chromophobe adenomas, 6 acidophilic adenomas, and 2 basophilic adenomas. These findings are summarized with respect to age and sex (TABLE 1).

The adenomas tended to occur more frequently in older rats. The pituitaries of 19% of the rats younger than 600 days were adenomatous and 62% of those older than 600 days were adenomatous.

Chromophobe adenomas. Chromophobe adenomas, found in 48% of the pituitaries examined, occurred with greater incidence in females than males (TABLE 1). The average age of the rats in which chromophobe adenomas were found was significantly greater than that of rats with nonadenomatous pituitaries (TABLE 1). The pituitaries containing chromophobe adenomas varied in weight from 12 mg. to 547 mg., with an average weight of 155 mg. There was little difference in average weight of chromophobe adenomas between the 2 sexes (males 151 mg. and females 158 mg.). Some histologic observations in the chromophobe adenomas that were studied are summarized (TABLE 2).

The chromophobes comprising the chromophobe adenomas varied greatly from one adenoma to the next and there was often much variation of cells within an adenoma. The nuclei of most chromophobes were round to oval with some variation in size (Figures 1 and 2). The larger nuclei were more often variable in shape. Large nuclei were often indented and sometimes distorted. These large distorted nuclei were not numerous in any

TABLE 1. Incidence of hypophyseal adenomas in relation to age and sex in 108 Upjohn-strain Wistar rats (52 males and 56 females)

Condition of pituitary	Number M	Number F	% incidence M	% incidence F	Average age in days M	Average age in days F
Nonadenomatous	29	19	56	34	709	618
Chromophobe adenoma	20	32	38	57	746	719
Acidophilic adenoma	2	4	4	7	722	706
Basophilic adenoma	1	1	2	2	843	731

TABLE 2. Histologic characteristics of chromophobe adenomas in various weight ranges

Weight range of pituitary	Number of pituitaries	Number containing				
		Hemorrhage and hemosiderin	Numerous mitotic figures	Prominent nucleoli	Acido-phils	Baso-phils
12-25 mg.	10	3	2	3	9	1
26-100 mg.	18	16	9	6	18	9
101-250 mg.	9	8	3	5	9	4
over 250 mg.	15	15	8	6	15	10

of the chromophobe adenomas studied. The chromatin of adenomatous chromophobes varied from a fine network in some nuclei to a clumped state in others. About 2/3 of the adenomas contained mainly nuclei with clumped chromatin. The remaining adenomas had unclumped chromatin or a mixture of the 2 types. Prominent eosinophilic nucleoli were seen in 20 chromophobe adenomas (TABLE 2). Numerous mitotic figures were seen in 22 chromophobe adenomas and were seen in association with prominent nucleoli in 12 of the adenomas.

The cytoplasm of the chromophobes in chromophobe adenomas varied in amount from scant to abundant, with most adenomas containing cells which had an intermediate amount. It was usually foamy in appearance (Figure 1), sometimes homogeneous, and it occasionally contained vacuoles that appeared to be fat on the basis of H and E stained sections.

Acidophils were found intermingled with chromophobes (Figure 2) in all the chromophobe adenomas except one. The cytoplasm of the acidophils stained orange with the trichrome-periodic acid-Schiff stain and eosinophilic with the hematoxylin and eosin stain. The number of acidophils present in a chromophobe adenoma varied from a few up to about 25% of the cells present. The acidophils often had smaller and denser nuclei than the chromophobes, sometimes had nuclei similar to those of the chromophobes, and in several instances had larger nuclei.

Basophils were present within 46% of the chromophobe adenomas studied. They generally comprised a very small percentage of the cells present. The cytoplasm of the basophils stained red with the trichrome-PAS stain and blue with H & E. With the H & E stain the basophils could not be differentiated from the chromophobes.

Areas of hemorrhage and hemosiderin were present in 81% of the chromophobe adenomas studied (Figures 5 and 6), and occurred with greater incidence in the larger tumors. The average weight of hypophyses with chromophobe adenomas without hemorrhage and hemosiderin was 43 mg. and the average weight of all hypophyses with chromophobe adenomas was 155 mg. Although the amount of hemorrhage varied, it was usually more abundant in the larger adenomas and was undoubtedly a factor which added to their weight. The hemorrhage often occurred in clefts among the adenomatous cells and appeared to be compressing the cells. In several chromophobe adenomas an incomplete endothelial lining was seen in some of the blood spaces. Hemosiderin was found in association with hemorrhage except in 2 adenomas. The hemosiderin was present both in macrophages and in intercellular clumps. The hemosiderin reacted positively to the Gomori iron reaction and stained PAS positively with the trichrome-PAS method. The hemosiderin was often concentrated in the areas between the chromophobe adenomas and the compressed nonadenomatous cells (Figure 6). In several chromophobe adenomas, yellow crystalline material, considered to be hematoidin, was observed in association with hemorrhage and hemosiderin. Macrophages were observed in most chromophobe adenomas; neutrophils and lymphocytes were seen in varying numbers and were especially prevalent in some of the large chromophobe adenomas with much hemorrhage.

The chromophobe adenomas appeared to have grown by expansion. They had compressed the nonadenomatous cells of the hypophysis so that, in the larger tumors, there were only thin rims of normal tissue remaining (Figure 5). The adenomas appeared encapsulated in some areas where the expansive growth caused the stroma of the pituitary to become condensed around them.

Of the chromophobe adenomas studied, 65% had no discernible pattern of arrangement of the chromophobes (Figures 1, 2, 3, 5, and 6). According to the architectural classification of Nurnberger and Korey (1953), these were classified as the diffuse type. The remaining 35% of the chromophobe adenomas had a diffuse architecture in part of the adenoma, but also present were parts that could be classified as sinusoidal or papillary in arrangement. Areas of sinusoidal arrangement were observed in 21% of the chromophobe adenomas and were characterized by fine connective tissue which separated the adenomatous chromophobes (Figure 7). Areas of papillary arrangement in which the adenomatous cells radiated away from the blood vessels were present in 14% of the chromophobe adenomas (Figure 8). No adenomas were observed which were totally sinusoidal or papillary in arrangement and none were seen with all 3 types of architecture.

Acidophilic adenomas. Acidophilic adenomas were found in 6% of the rat pituitaries examined. Four were found in females and 2 in males (TABLE 1). The average age of rats in which acidophilic adenomas were found was greater than that of rats with nonadenomatous pituitaries and less than that of rats with chromophobe adenomas (TABLE 1). The weight of the pituitaries containing acidophilic adenomas ranged from 33 mg. to 477 mg., with an average weight of 230 mg.

The criterion used to justify a diagnosis of acidophilic adenoma was a preponderance of acidophils, or cells which were eosinophilic with H & E and orangophilic with the trichrome-PAS stain (Figure 3). Although acidophils were found in 98% of the chromophobe adenomas, the acidophils comprised less than 25% of the cells in the tumor. In acidophilic adenomas over 75% of the cells present were acidophils. No adenomas were found with an intermediate number of acidophils.

Hemorrhage and hemosiderin, similar to that described in the chromophobe adenomas, were present in all 6 of the acidophilic adenomas. Numerous mitotic figures were present in 3 of the 6 acidophilic adenomas and prominent nucleoli were seen in only one. A few basophils and some chromophobes were present in all of the acidophilic adenomas. A few inflammatory cells were also observed in the adenomas: macrophages, lymphocytes, and neutrophils. The cytoplasm varied from moderate to abundant. The cytoplasm appeared to be more abundant than that in many of the chromophobes in chromophobe adenomas. The cytoplasm was homogeneous in one acidophilic adenoma but foamy in the others.

All of the acidophilic adenomas had a diffuse form of architecture with no orderly arrangement of the cells.

Basophilic adenomas. Basophilic adenomas were found in 2 of the 108 pituitaries examined. One weighing 428 mg. was found in a 731-day-old female rat and the other weighed 71 mg. and was in an 843-day-old male. The cytoplasm of the basophils stained blue with H & E and red with the trichrome-PAS stain (Figure 4). Difficulty was encountered in classifying cells as basophils because macrophages containing hemosiderin were also PAS positive and had to be differentiated from the basophils. The cytoplasm of basophils had fine PAS-positive granules and the PAS-positive material in the macrophages was usually present as large granules or clumps. Also, the nuclei of macrophages were generally smaller than those of basophils.

The nuclei of the basophils were round to oval and generally smaller than those of chromophobes in chromophobe adenomas (Figure 4). Mitotic figures were rarely seen and nucleoli were not observed. The cytoplasm was moderate in amount, foamy in appearance and, in the larger basophilic adenoma, vacuoles were present. Hemorrhage and hemosiderin were present in the smaller adenoma only. Acidophils and chromophobes were present in both basophilic adenomas and together comprised about 30% of the cells. A small area of sinusoidal arrangement was present in the large adenoma but, otherwise, both adenomas were diffuse in architecture.

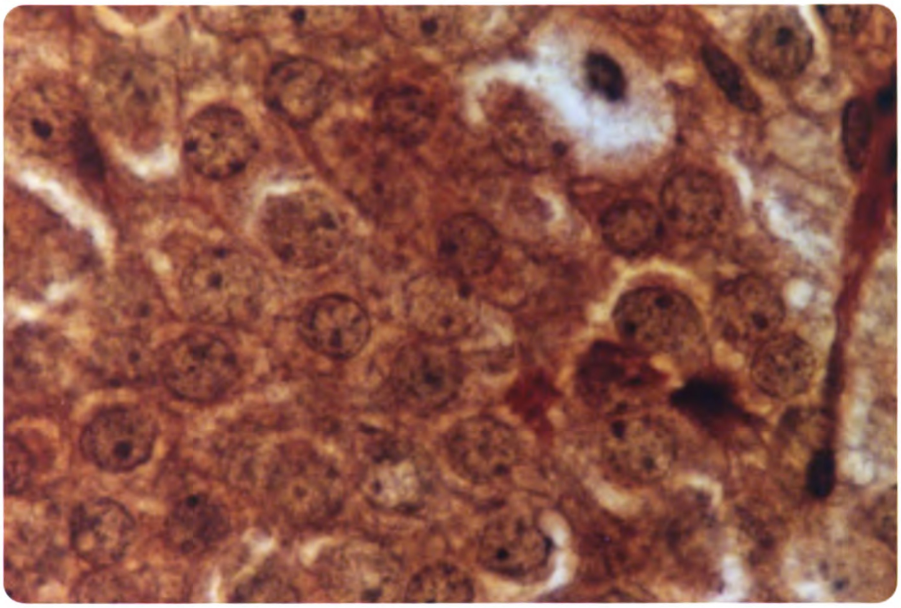

Figure 1. Chromophobe adenoma. Note foamy cytoplasm and round nuclei. Periodic acid-Schiff-trichrome. x 750.

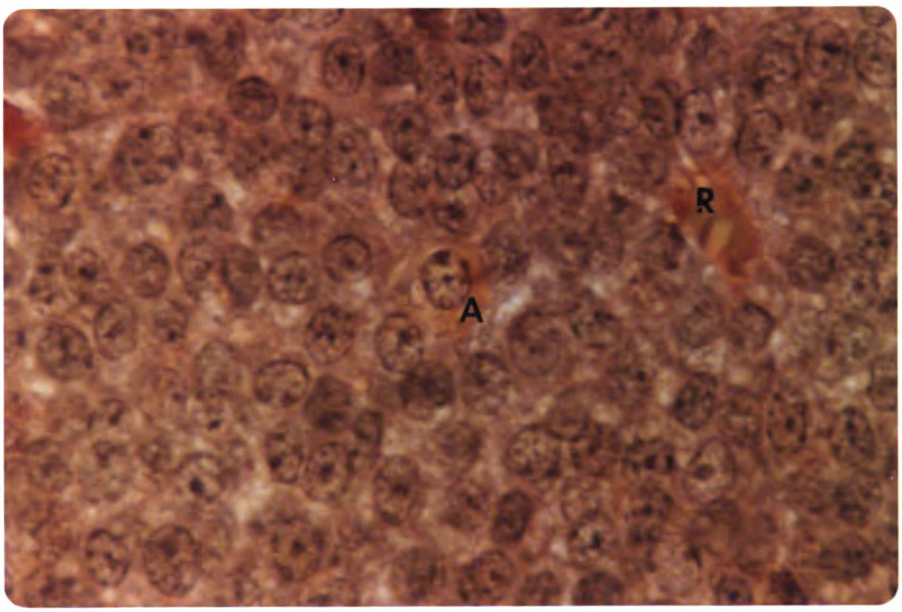

Figure 2. Chromophobe adenoma with an acidophil present. Note the staining characteristics of the acidophil (A) and the red blood cells (R). Periodic acid-Schiff-trichrome. x 750.

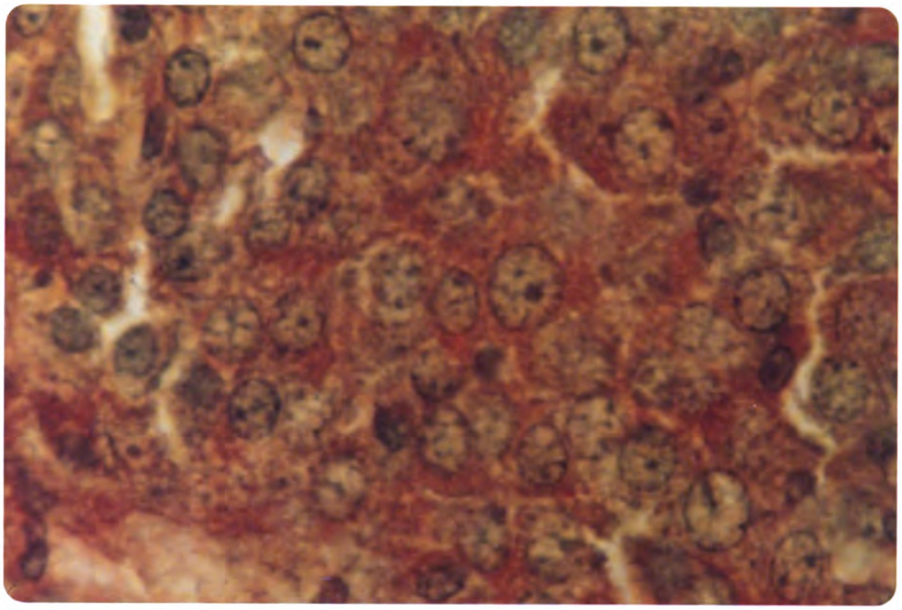

Figure 3. Acidophilic adenoma. Note the orangophilic cytoplasm and the variation in size of the nuclei. Periodic acid-Schiff-trichrome. x 750.

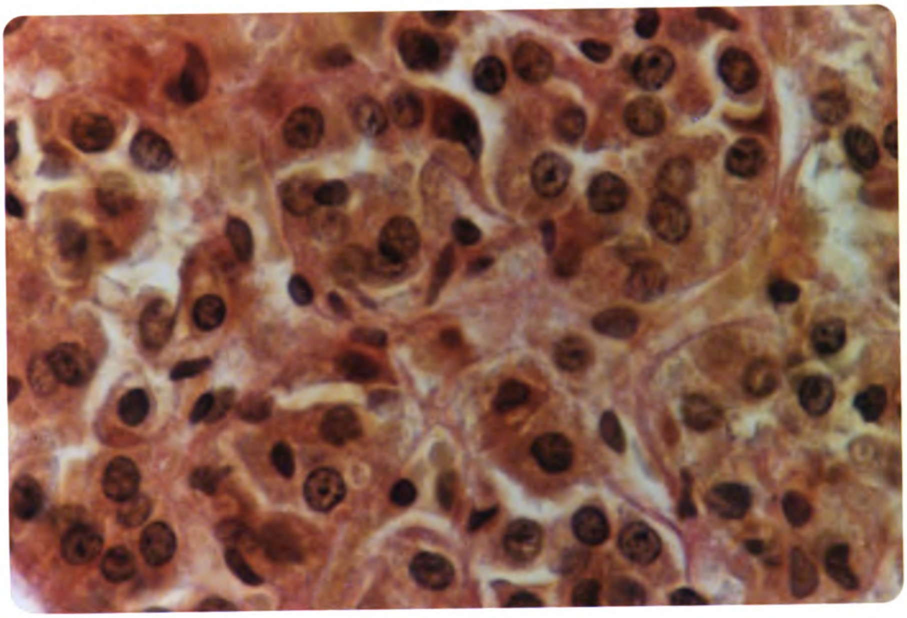

Figure 4. Basophilic adenoma. Note the reddish cytoplasm and the sinusoidal arrangement of the cells. Periodic acid-Schiff-trichrome. x 750.

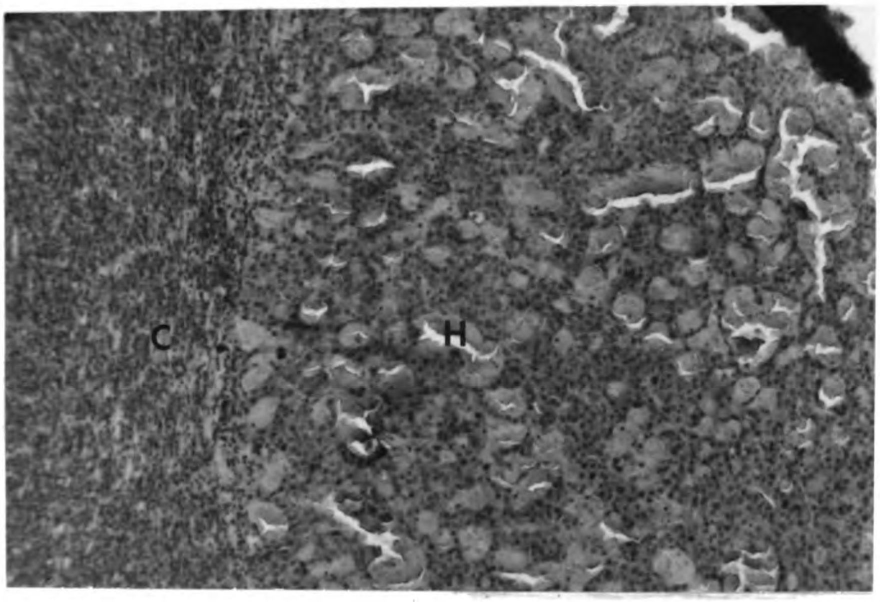

Figure 5. Hemorrhagic chromophobe adenoma. Note the compression of the nonadenomatous portion of the pituitary (C) by the hemorrhagic chromophobe adenoma (H). Hematoxylin and eosin. x 75.

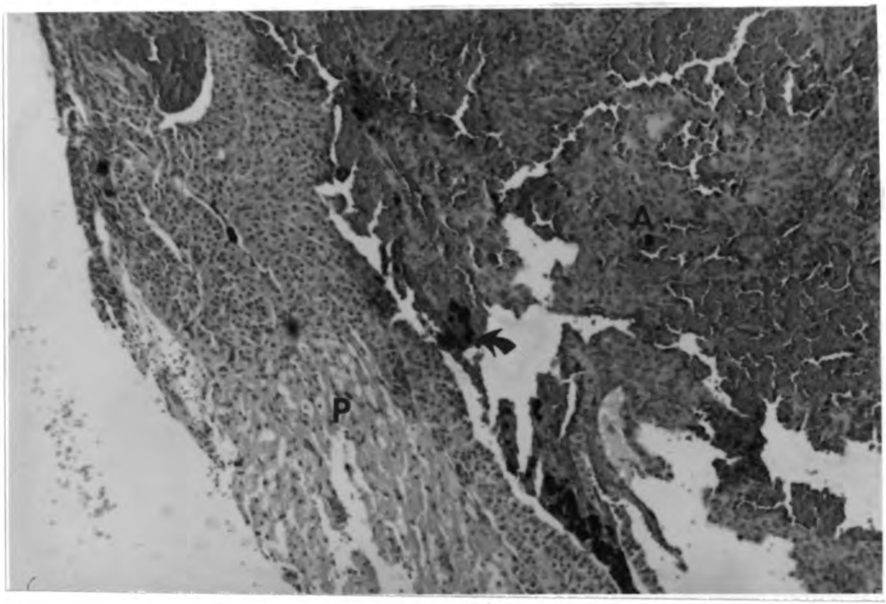

Figure 6. Hemosiderin in chromophobe adenoma. Note the hemosiderin (arrow) between the adenoma (A) and the posterior pituitary (P). Hematoxylin and eosin. x 75.

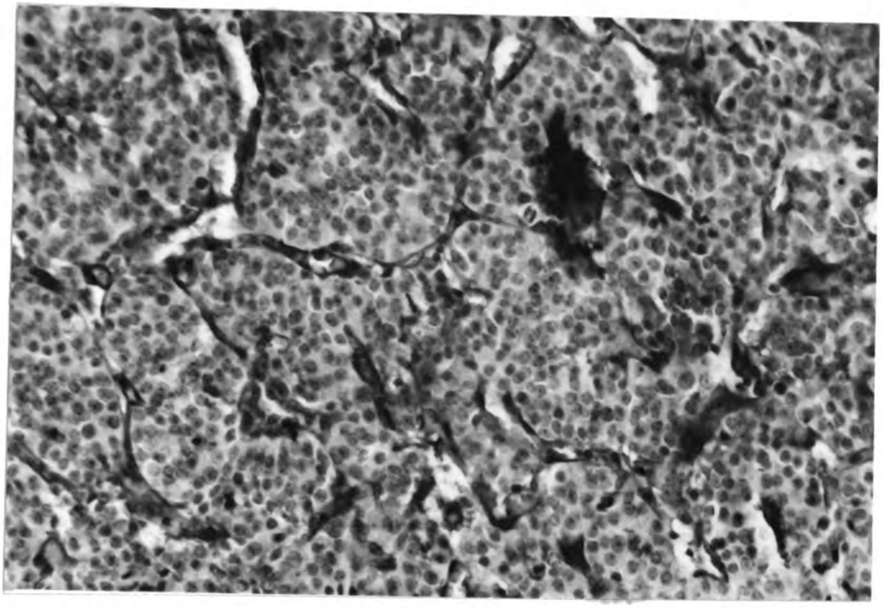

Figure 7. Sinusoidal arrangement of chromophobe adenoma. Note how connective tissue divides the adenoma into sinuses. Hematoxylin and eosin. x 188.

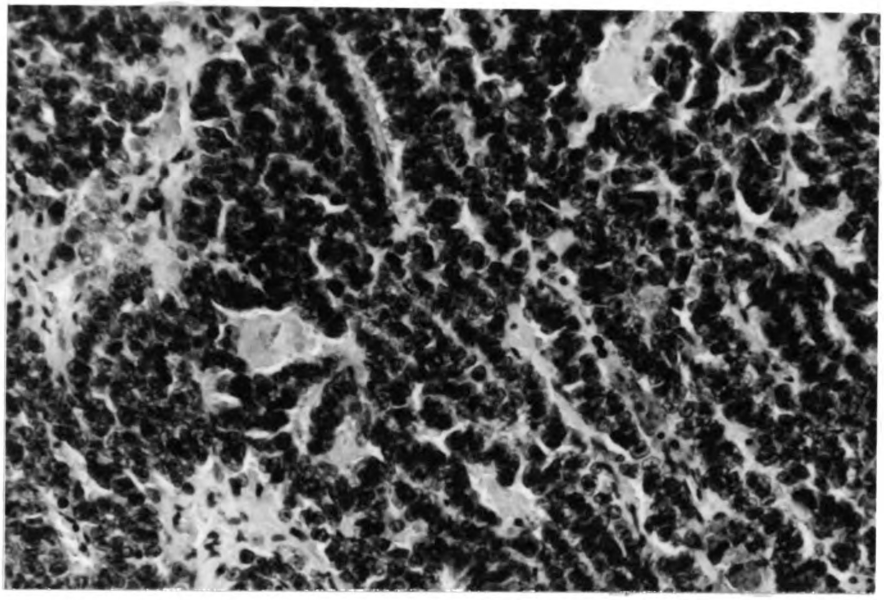

Figure 8. Papillary arrangement of chromophobe adenoma. Note how the chromophobes radiate from the blood vessels in rows. Hematoxylin and eosin. x 188.

DISCUSSION

No reports of previous work concerning hypophyseal adenomas in this particular strain of rats or in modified pathogen-free rats could be found in the literature. Because the incidence of hypophyseal adenomas in different strains of rats has been shown to be quite variable and in view of the fact that modified pathogen-free rats are becoming widely used in research, this study was conducted to determine the incidence of hypophyseal adenomas in this strain of modified pathogen-free rats. Most of the findings in this study were in agreement with earlier investigations on other strains of rats. The higher incidence in females than in males is in accord with the findings of earlier workers with the Wistar rat (Wolfe and Wright, 1947; and Saxton and Graham, 1944). The preponderance of chromophobe adenomas was reported in all of the investigations reviewed.

An unanticipated finding was the prevalence of acidophils and basophils in the chromophobe adenomas. Wolfe and Wright (1947) reported finding acidophils in 20% of one type of chromophobe adenoma. They did not mention the presence of basophils. In this study 98% of the chromophobe adenomas contained acidophils and 46% contained basophils. Kernohan and Sayre (1956) classified hypophyseal adenomas as chromophobe, acidophilic, basophilic, and mixed. Adenomas which contained 2 or all 3 types of cells they classified as mixed. If this classification had been applied to this study 98% of the adenomas would have been classified as mixed and 2% as chromophobe adenomas. The use of this classification would obviously have been misleading.

Hemorrhage, which was present in 81% of the chromophobe adenomas in this study, was more prevalent than previously reported for other strains. In addition to intracellular hemosiderin observed by earlier investigators, extracellular clumps of hemosiderin were observed in this study. Evidence of proliferation of endothelial cells in some of the hemorrhagic adenomas in this study, seemingly in an attempt to form a lining for the blood spaces, had not been reported in earlier studies.

Wolfe and Wright (1947) described the hypertrophy of the negative image of the Golgi body in the cytoplasm of chromophobes in chromophobe adenomas. Because of the similarity of this change to fixation artifacts and post-mortem changes which occur so swiftly in the endocrine organs, the author, in this study, ignored changes that appeared to be hypertrophy of the Golgi body. Histochemical techniques which are specific for the enzymes of the Golgi body would seem to be a more accurate measure of the hypertrophy of that organelle than the appearance of the negative image on formalin-fixed, paraffin-embedded, and routinely stained sections.

The trichrome-PAS stain has been advocated for use in studying the pituitary gland (Pearse, 1949 and 1952). In the present study it was found to be helpful in the indentification of cells. It did have several shortcomings, however. Macrophages containing hemosiderin took the PAS stain and had to be differentiated from basophils which were also PAS-positive. Acidophils stained orange, the same color as the erythrocytes (Figure 2), and in adenomas with extensive hemorrhage some difficulty was encountered in the indentification of acidophils. Most of the acidophils stained pale red with H & E but the basophils and chromophobes both stained blue and could not be differentiated with H & E alone.

From the review of the literature it is evident that the significance of chromophobes in chromophobe adenomas is not known. Three hypotheses are possible concerning the significance of the chromophobe adenoma cells: (1) the chromophobes are cells which are free of hormones and which are not secreting hormones at the present, have not done so in the past, and will not in the future; (2) the chromophobes are cells which have the potential to transform into either acidophils or basophils but which have not, as of the present, produced hormones; (3) the chromophobes are cells that have been acidophils or basophils but which have secreted all the hormones they contained or that they continue to produce them but at such a slow rate and without any storage stage in the cytoplasm so that it is not possible to recognize them as chromophils. In all 3 of these cases the cells would stain the same.

Little evidence exists to support the first hypothesis. Support for the second hypothesis can be found in the electron microscopic studies of Rinehart and Farquhar (1953) in which they found that chromophobes, at least in the normal gland, contained enough granules to be placed in the category of either acidophils or basophils.

Lending support to the third hypothesis, in this study, were the variability in nuclei, the variability in the staining of the cytoplasm, the presence of large pleomorphic cells, and the presence of acidophils and basophils in some of the chromophobe adenomas. One of the characteristics of the chromophobe adenomas studied in this series was the variability of the nuclei and cytoplasm not only between adenomas but in the same adenoma. It is reasonable to assume that, if these cells had been producing hormones and for some reason had stopped, they would have been in different stages of production and storage at the time they stopped. Pearse (1952) and Russfield (1957) described differences in the nuclei of secreting and storage cells of the pituitary gland. Golden (1959) described large pleomorphic "hypersecretory cells" in the normal gland. His description fits the cells with large vesicular nuclei seen in some of the chromophobe adenomas in this series. The interpretation of the acidophils and basophils present in the chromophobe adenomas as cells which have not yet stopped secreting hormones could also be cited as evidence for the third hypothesis. The presence of changes in other endocrine organs in rats with chromophobe adenomas (noted in this series but not tabulated or studied) indicates that there is a hormonal imbalance which could be due to secretion of hormones by the chromophobe adenoma. All this evidence, however, does not provide proof of the significance of chromophobe adenomas, but it does indicate that chromophobe adenomas have some functional significance and are not the mere proliferation of nonfunctional cells.

There have been questions raised in the literature concerning the neoplasia of hypophyseal adenomas. Runnells, Monlux, and Monlux (1965) define a neoplasm as a "growth of new cells that proliferates without control, serves no useful function, and has no orderly arrangement". The significant part of this definition, in regard to endocrine adenomas, is "new cells". It is difficult to distinguish between functional hyperplasia and neoplastic proliferation in the endocrine organs. There were no clear-cut signs of malignancy, such as metastasis or invasion of nearby structures, in this series of adenomas. The inevitable question that arises is: are hypophyseal adenomas in aged rats neoplastic processes which cause an endocrine imbalance or are they hyperplastic processes which are the result of an endocrine imbalance? Unfortunately the complex relationships of the endocrine system allow no simple answer to this question. In time, as research uncovers the exact relationships of the endocrine organs and the causes and effects of imbalances in the system, a logical deduction concerning the nature of hypophyseal adenomas will ensue.

SUMMARY

The pituitaries of 56 female and 52 male modified pathogen-free Wistar rats of the Upjohn strain were examined for the presence of hypophyseal adenomas. Sixty-six per cent of the females and 44% of the males had adenomas. The trichrome-periodic acid-Schiff staining technique was used to facilitate identification of the hypophyseal cells. Of the adenomas found 86.7% were chromophobe adenomas, 10% acidophilic adenomas, and 3.3% basophilic adenomas. The adenomas tended to occur in older rats. Nineteen per cent of the rats less than 600 days of age and 62% of the rats older than 600 days had adenomas. The pituitaries containing adenomas varied in weight from the normal of 12 mg. to 547 mg., with an average weight of 155 mg. Acidophils were present in 98%, and basophils in 46%, of the chromophobe adenomas studied. Together acidophils and basophils made up less than 25% of the cells present. Areas of hemorrhage with hemosiderin were present in 81% of the chromophobe adenomas, 100% of the acidophilic adenomas and 50% of the basophilic adenomas. The hemorrhage was generally greater in the larger adenomas. The majority of the adenomas had no discernible architectural arrangement.

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VITA

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