Cytology of Spontaneous Adenomas in the Pituitary Gland of the Rat*

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(Received for publication June 11, 1947)

In recent years, the spontaneous appearance of adenomas in the anterior hypophyses of rats has been described by Wolfe, Bryan, and Wright (39), by Oberling and his associates (24), by Saxton (28) and by Saxton and Graham (29). These adenomatous growths have been found almost entirely in the anterior lobes of old rats and their presence is seemingly a manifestation of advancing age.

The size, appearance and cellular constituents of these adenomatous lesions is variable, Wolfe, Bryan and Wright (39) having described three types. The first type was characterized by varying amounts of hemorrhage and was classified as a hemorrhagic adenoma. These growths ranged in size from small lesions of 0.4 X 0.5 mm., which could not be recognized grossly, to large swollen tumors involving practically the entire anterior lobe. Histologically they were composed of chromophobes although small numbers of acidophiles were present in some instances. The chromophobe cells in these lesions generally, but not invariably, showed various degrees of hypertrophy, some being of enormous size. The adenomatous acidophiles were invariably enlarged. Capsules were not present about these lesions although the neighboring anterior lobe tissue was often compressed.

The second type of lesion was designated as an adenomatous nodule. They were made up of small diffuse masses of enlarged chromophobes or acidophiles and sometimes both. The smallest of these nodules contained only a few cells while the largest measured only 0.4 X 0.6 mm. Hemorrhages were not present and there was no compression of the surrounding tissue.

The third type of adenoma described by these authors was large, occupying practically the entire anterior lobe, and was made up entirely of chromophobic cells of varying size. There was no evidence of hemorrhage.

In all three types of lesions, there was a marked tendency for the nuclei and nucleoli of the adenomatous cells to be enlarged and often there was hypertrophy of the negative image of the Golgi apparatus. Many of the adenomas contained mitoses; in contrast, mitotic figures were rare or absent in the non-adenomatous tissue.

The lesions described by Saxton (28) and later by Saxton and Graham (29) appear to be quite similar to those described above except that adenomatous acidophiles were not described. In many of their animals the lesions were multiple. In preparations stained with Sudan III many adenomatous cells were found to contain fat which was not present in the normal anterior lobe cells. These authors also made intraocular transplants of adenomatous and non-adenomatous anterior lobe tissue. On the basis of the behavior of the transplants of the adenomatous tissue, it was concluded that these growths represented true neoplasms.

Anterior lobe adenomas have now been described in old rats, both male and female, of several strains, and although the data are still rather scanty, it seems clear, as Saxton and Graham (29) have already pointed out, that there are both strain and sex differences in the incidence of these tumors.

The studies mentioned above have furnished considerable information concerning the general histologic structure of these tumors. The purpose of the present communication is to supplement our previous description of these lesions, placing special emphasis on certain cytological aspects not considered previously. In addition, two spontaneously occurring adenomas of the intermediate lobe of the pituitary gland of the rat are described.

MATERIAL AND METHODS

These observations are based on the study of 44 anterior lobe and 2 intermediate lobe adenomas found in the pituitary glands of 24 rats of the Vanderbilt strain and 7 from the Albany strain. The incidence of anterior lobe lesions in old female rats of these strains is 29 per cent in Vanderbilt breeding females (39), 27 per cent in Vanderbilt non-breeding females (36) and 11 per cent in Albany non-breeding females (41). No data are yet available on the incidence of these lesions in the breeding females of the Albany strain.

At autopsy the hypophyses were usually divided into two sagittal halves, fixed in Regaud’s fluid, cut serially at 3 microns and stained by the basic fuchsin mitochondrial technic of Fain and Wolfe (13).

* The studies were aided by a grant from The Donner Foundation, Cancer Research Division, and by the Winthrop Research Fund.
Fairly often, however, the glands were preserved in 2 fixatives, one half being fixed in Regaud's fluid and the second half in Champy's fluid. The Champy-fixed tissue was postchromatized according to the modified Champy-Kull method of Severinghaus (31) and stained by the mitochondrial method mentioned above. With both Regaud- and Champy-fixed tissue this technic stains mitochondria a brilliant fuchsin, acidophilic granules pinkish, orange or orange-red and the basophilic granules or secretion deep blue. Connective tissue fibers stain blue and red blood cells red or orange red.

**OBSERVATIONS**

**CYTOLOGICAL STUDIES OF SPONTANEOUSLY OCCURRING ANTERIOR LOBE ADENOMAS**

During the course of this study, we have observed the three types of adenomatous lesions in the anterior lobe described by us previously; i.e., hemorrhagic adenomas, adenomatous nodules, and chromophobic adenomas (39). Although the three separate types of lesions were easily differentiated from each other, the cytologic appearance of the adenomatous cells constituting the various tumors was similar, i.e., the characteristics of the cells present in adenomatous nodules, in hemorrhagic adenomas and in chromophone adenomas were generally the same. Therefore, the descriptions of certain adenomatous cells in one type of lesion are usually applicable to the cells of the other varieties.

The drawings of cells shown in Figs. 1 to 27 will, therefore, be used to illustrate similar cells found in all types of lesions.

**Hemorrhagic adenomas.**—Eleven hemorrhagic adenomas were found. These varied in size from a small lesion measuring 0.8 x 0.8 mm. to a large adenoma involving practically all of the anterior lobe. In most instances the normal anterior lobe tissue surrounding the tumors was compressed. Eight of the lesions were purely chromophobic, the remainder contained, in addition to chromophobes, a few acidophiles. As we have pointed out previously (39) these adenomas were characterized by variable amounts of hemorrhage. Scattered throughout the tumors were blood-filled spaces of variable size which apparently did not possess an endothelial lining, their contents appearing to be in direct contact with the adenomatous anterior lobe cells. These interstitial hemorrhages have been more fully described previously (39).

Generally, the adenomatous chromophobes in these tumors were made up of cells showing varying degrees of hypertrophy but it should be emphasized that many adenomatous chromophobes were not increased in size. Numerous cells showed only slight hypertrophy whereas in others the hypertrophy was pronounced; cells measuring approximately 30 x 30 microns were regularly observed.

\*\*These cells were drawn by Miss Alice Pauline Schafer.\*\*

**DESCRIPTION OF FIGURES 1 TO 15**

All cells are shown at a magnification of x 1440. The acidophilic granules are shown by small circles. Mitochondria are shown in various shades of black which in some cells shade into dark gray. The granular cytoplasm of the anterior lobe basophile and the intermediate lobe cell are grayish. All cells are from the anterior lobe except those specially designated.

**Fig. 1.**—A small acidophile from the normal portion of the anterior lobe.

**Fig. 2.**—A moderately, enlarged adenomatous acidophile. Note the very large nucleolus and nucleolar vacuoles. The Golgi apparatus was quite hypertrophied.

**Fig. 3.**—A much enlarged adenomatous acidophile. There were abundant fine mitochondria and hypertrophy of the Golgi apparatus. The nucleus showed evidence of lobulation.

**Fig. 4.**—An enlarged adenomatous chromophobe. The large nucleolus contained vacuoles. The mitochondria were quite variable in size and some were vesicular in nature.

**Fig. 5.**—A small chromophobe from the normal portion of the anterior lobe.

**Fig. 6.**—A small chromophobe from the normal portion of the anterior lobe. Note enlarged Golgi apparatus and abundant mitochondria.

**Fig. 7.**—A small adenomatous chromophobe. Note the large nucleolus, the nucleolar vacuole and the nucleolar protrusion. The Golgi apparatus was much enlarged.

**Fig. 8.**—A chromophile of moderate size from the normal portion of the anterior lobe. The Golgi apparatus was enlarged and there were abundant mitochondria.

**Fig. 9.**—Granular basophile from the normal portion of the anterior lobe.

**Fig. 10.**—Nongranular basophile from the normal portion of the anterior lobe.

**Fig. 11.**—Large adenomatous chromophobe with many mitochondria, a few of which were quite large.

**Fig. 12.**—Large adenomatous chromophobe which measured approximately 55 x 35 microns. The nucleus measured 30 x 20 microns and the largest nucleolus 6 x 6 microns. Note the nucleolar vacuoles and the invagination of the cytoplasm into the nucleus. The mitochondria were variable in size and in the intensity of their staining reaction.

**Fig. 13.**—A normal intermediate lobe cell.

**Fig. 14.**—A moderately enlarged adenomatous intermediate lobe cell which had dense blue cytoplasm. It is thought that the scattered canals represented a diffuse Golgi apparatus.

**Fig. 15.**—A very large adenomatous intermediate lobe cell. The mitochondria were large and vesicular, the center portions took a light fuchsin and the outer borders a darker fuchsin stain. The very large nucleolus contained many vacuoles.
FIGS. 16-27
and cells as large as 50 × 40 microns were seen fairly frequently (compare the normal and adenomatous chromophobes in Figs. 1 to 27). The nuclei and nucleoli were also often but not invariably enlarged, nuclei measuring up to 20 × 28 microns and nucleoli as large as 5 × 5 microns being observed. In fact, nucleolar hypertrophy and to a lesser degree nuclear enlargement were outstanding features of the adenomatous chromophobes and often occurred in cells in which there was no increase in size of the cell itself.

The nuclei of the adenomatous chromophobes were of unusual interest. They tended to be markedly distorted in shape and often the nuclear membrane was wrinkled and indented (Figs. 12, 18, 22, 24 and 27); in many cells the nuclei were lobulated and in some there was evidence of nuclear budding (Fig. 19). Frequently cells containing 2 or more nuclei were seen (Figs. 21 and 25), indicating the possibility of direct cell division. As a rule the nuclei were stained pale blue and the nucleoplasm was homogenous in appearance. In some instances, however, small irregular bodies, stained a dull blue, were scattered throughout the nucleus (Figs. 4, 17, 22 and 24) and often they were more numerous. The relation of these small bodies was generally similar to that of the nucleoli (see below).

In most of the nuclei, one or two large and prominent nucleoli were seen (Figs. 4, 17, 22 and 23) and often they were more numerous. The nucleoli also showed varying degrees of hypertrophy; in a few instances they reached a diameter of 5 × 5 microns (Fig. 12). Usually, they were stained varying shades of blue but in some cells they were deep red and not infrequently pale red or grayish-orange in the center with a narrow band of blue on the periphery.

As a rule, the nucleoli were more or less rounded in shape (Figs. 4 and 25) but reference to Figs. 7, 16 and 20 will indicate that more irregular forms were often encountered. Sometimes small protrusions of various shapes extending from the main body of the nucleolus into the surrounding nucleoplasm were observed (Fig. 7). Often on the surface of the nucleolus small spherical bodies, apparently made up of material identical to that of the nucleolus, could be seen. Sometimes these bodies could be shown to be continuous with the nucleolus (Fig. 20); sometimes they appeared separate. In addition to these small bodies, which appeared to be actually continuous with the nucleoli or at least very close to them, other small structures were sometimes found scattered throughout the nucleus (Fig. 22). Their staining reaction was identical to that of the nucleoli. The relation of the nuclear structures mentioned above to each other and to the nucleolus is vague and should be the subject of further study.  

The staining technic used in this study did not differentiate the nucleoli from the adjoining nucleolus-associated chromatin which has been described in many cells (Hyden, H. Protein metabolism in the nerve cell during growth and function. Acta Physiol. Scandinav., 6 (Suppl.): 1-136. 1943; Caspersson, T. The relations between nucleic acid and protein synthesis. Symposium Soc. Exper. Biol., No. 1. 1947, pp. 127-143). In recent studies (38) made on non-adenomatous anterior lobe cells of the rat, one or more

DESCRIPTION OF FIGURES 16 TO 27

All cells showed at a magnification of ×1440. Mitochondria are shown in various shades of black which in some cells shade into dark gray.

Fig. 16.—A slightly enlarged adenomatous chromophobe. The nucleolar mass appeared to be made up of several nucleoli closely packed together.

Fig. 17.—A moderately enlarged adenomatous chromophobe containing 3 large droplets of lipoid (grayish masses surrounded by light areas). The generally large mitochondria in this cell took a pale fuchsin stain. Note the cytoplasmic filaments and two nebenkern. Note the practical absence of mitochondria in the region of the filaments. The nuclear membrane was considerably wrinkled, the nucleolus large and vacuolated.

Fig. 18.—A large adenomatous chromophobe with scant numbers of mitochondria, some of which stained very lightly. The nucleus showed a wrinkled membrane and some evidence of budding.

Fig. 19.—A large adenomatous chromophobe with a large nucleus, a small nucleolus and many smaller bodies which stained identically to the nucleolus. Similar staining but smaller bodies were found in the cytoplasm. Only scant numbers of mitochondria were present.

Fig. 20.—A small adenomatous chromophobe. Note the relatively large nucleolus, the large nucleolar protrusion and the abundant mitochondria.

Fig. 21.—A moderately large binucleated adenomatous chromophobe.

Fig. 22.—Adenomatous chromophobe with cytoplasmic filaments and two nebenkern. Note the practical absence of mitochondria in the region of the filaments. The nuclear membrane was considerably wrinkled, the nucleolus large and vacuolated.

Fig. 23.—Adenomatous chromophobes which had deep blue cytoplasm and a large vacuolated nucleolus.

Fig. 24.—Large binucleated adenomatous chromophobe that showed an evagination of cytoplasm containing mitochondria into the nucleus. Note the cytoplasmic filaments and the two possible nebenkern.

Fig. 25.—Moderately large binucleated adenomatous chromophobe. Some of the mitochondria took a deep fuchsin stain, others were much lighter. Note the large Golgi apparatus.

Fig. 26.—Moderately large adenomatous chromophobe with an enlarged Golgi apparatus and a vacuolated nucleolus.

Fig. 27.—A small adenomatous chromophobe. Note the wrinkled nuclear membrane, the vacuolated nucleolus and the variations in the size of the mitochondria.
We have reported previously (39) that the nucleoli of the adenomatous anterior lobe cells sometimes contained one or more small lightly staining vacuoles. In this study we have encountered such nucleolar vacuoles frequently in adenomatous anterior lobe cells (Figs. 4, 17, 22, 23, 26 and 27). In most of these cells, only 1 or 2 were present but sometimes there were several (Fig. 17). Most often the vacuoles were found well in the interior of the nucleolus (Figs. 12 and 23) but fairly frequently they were on the periphery (Figs. 26 and 27) and sometimes even seemed to lie on its surface.

In those nucleoli which were stained deep blue, the material in the vacuoles was usually pale orange-red in color but sometimes it was merely lighter blue, grayish or colorless. In the nucleoli that stained red, the vacuolar substance assumed a lighter red. In those nucleoli that were stained pale orange-red or grayish-orange in the center and had a deep blue periphery, vacuoles were not observed.

In the normal anterior lobe cells (Figs. 1, 5, 6, 8, 9 and 10), the nucleoli were much smaller than in the adenomatous cells. While they also contained nucleolar vacuoles these were smaller and far less abundant.

The cytoplasm of the enlarged adenomatous chromophobes generally stained a light to moderately deep blue but in many cells it was stained intensely by aniline blue (Figs. 23 and 27). Fairly often, the amount of cytoplasm was small and it appeared fragmented, being pulled away from the nuclear membrane or the cell membrane or both. In most of the hemorrhagic adenomas varying numbers of cells contained rounded cytoplasmic vacuoles of variable size. These are believed to be due to the dissolving out of lipid droplets which, as will be shown later, can be demonstrated in Champy-fixed tissue.

In most cells mitochondria were moderately numerous (Figs. 7, 16, 21 and 26) but sometimes they were either extremely abundant (Fig. 11) or relatively few in number (Figs. 18, 19, 22 and 24). In shape they were usually spheroidal but some-

small nodular masses of chromatin were found to be in contact with or close to the nucleolus and in some instances the nucleolus was completely surrounded by a narrow band of chromatin. Both the nodular masses and the perinucleolar chromatin gave a positive test for desoxyribosel acid when stained by the Feulgen technic. We think it quite likely that the small protrusions which extended from the nucleolus and the small bodies lying close to the nucleolus, which we have described in this paper, were actually nucleolus-associated chromatin.

In the present report the observations on the Golgi apparatus are limited to a study of its negative image. While we fully realize that such a study cannot furnish precise information concerning its structure, it does give some idea of its size and general shape in the adenomatous cells. In many of the enlarged adenomatous chromophobes the negative image of the Golgi body was markedly hypertrophied, being made up of a system of irregularly anastomosing colorless canals (Figs. 21, 23, 25 and 26). However, in others, the negative image could not be detected, even though serial sections of the cells were studied. Generally speaking, the negative image of the Golgi body was seen more frequently in the slightly or moderately enlarged adenomatous chromophobes, in which it was generally hypertrophied than in the largest cells (compare Figs. 12 and 25).

Since the changes in the Golgi apparatus have generally been correlated with the functional activity of cells, a consideration of this structure in the adenomatous anterior lobe cells is of considerable interest. In several papers (32, 33 and 34) dealing with the cytology of anterior lobe cells, Severinghaus has associated hypertrophy of the Golgi apparatus with elaboration of secretion by the cell and an increase in the number of mitochondria with the release of the secretion. Wolfe and Brown (40) have confirmed the findings of Severinghaus which associated hypertrophy of the Golgi apparatus in the anterior lobe cells with elaboration of secretion and have found a certain amount of evidence which associated an increase in the number of mitochondria with release of secretion from the
cell. In a later paper Wolfe (37) was unable to associate conclusively an increase of mitochondria with the release of secretion although it was not doubted that an increase of mitochondria indicated increased functional activity of the cell.

Using these cytologic criteria as a basis, our observations indicate that many of the adenomatous chromophobes were in an active secretory state since they showed pronounced enlargement of the Golgi apparatus and contained numerous mitochondria. Since they showed cytologic evidence of the elaboration of secretion and yet no storage of it in the form of resolvable granules, it is considered likely that the secretion was being released into the blood stream as rapidly as it was formed. Although many adenomatous chromophobes did show undoubted cytologic evidence of the formation and release of secretion, our observations do not necessarily indicate that excessive amounts of secretion were being formed and it should be stated that any evidence other than the cytological findings just referred to, that these adenomas were producing and releasing hormones, was not found.

Not infrequently, in some of the most noticeably hypertrophied chromophobes a negative image of the Golgi apparatus was not observed. However, examination of cells such as that shown in figures 4 and 18 suggests that the Golgi material occurred in the form of fragments scattered throughout the cytoplasm. These cells contained many small clear vacuoles and careful microscopic examination revealed that the vacuoles were arranged in a pattern suggestive of a markedly hypertrophied Golgi body in which the material composing it had become quite diffuse. If in some cells the Golgi material became even more diffuse, perhaps being scattered through the cytoplasm in the form of isolated fragments, it seems possible that the negative image of such bodies, which would appear as small vacuoles or clear spaces in the cytoplasm, would be difficult and perhaps impossible to recognize with any degree of certainty. Although we know of no definite instance where the Golgi body of anterior lobe cells has been found in such a diffuse and fragmented state, it does not seem inconceivable that this actually was the case in the adenomatous cells considered here. On the other hand, it might also be possible that in many of these enlarged adenomatous cells, for reasons as yet unknown, the Golgi material was contracted or compressed into such small structures that the negative image was overlooked. It is thus quite clear that a study of the Golgi apparatus by these methods is less satisfactory than by methods that color this body (osmic or silver impregnation). Only by such a positive method will be obtained a satisfactory knowledge of the structure and behavior of this body in adenomatous cells of the anterior hypophysis.

In many of the hemorrhagic adenomas, the enlarged adenomatous cells were often irregular or unusual in shape (Figs. 17, 23 and 24). The reason for this irregularity is believed to be due to the fact that the adenomatous cells were tightly packed together and were presumably exerting pressure on each other. Certainly, they compressed the normal anterior lobe tissue surrounding them.

As previously noted, 3 of the hemorrhagic adenomas also contained adenomatous acidophiles. These cells showed varying degrees of hypertrophy (Figs. 1-3), the largest found measuring 25 × 25 microns. The hypertrophied acidophiles exhibited the same nuclear hypertrophy and the same enlargement in size of the nucleoli as were noted in the chromophobes, although the degree of hypertrophy, lobulation, wrinkling of the nuclear membranes and enlargement of the nucleoli were not as prominent. The acidophiles were almost always well filled with granules, the negative image of the Golgi body usually was markedly hypertrophied (Figs. 2 and 3) and mitochondria, while moderately abundant (Figs. 2 and 3), were never pronouncedly so. Since hypertrophy of the Golgi apparatus is usually associated with elaboration of secretion, it would seem that these adenomatous acidophiles had produced large amounts of secretion which was stored in the cells as granules. On the other hand, the stimulus which ordinarily induces the acidophiles to release their secretion into the blood stream was apparently nonoperative; at least there was no cytologic evidence that the adenomatous acidophiles were releasing secretion. In these adenomatous acidophiles, therefore, we encounter a cytologic condition which suggests an imbalance between the processes of the elaboration and the release of secretion; these cells had the capacity to elaborate secretion but not to release it in detectable degree.

In one of the hemorrhagic adenomas, a cytologic condition which we have never noted before in anterior lobe cells of any type, was observed. In the enlarged nuclei of a considerable number of hypertrophied chromophobes, round bodies surrounded by a deep blue staining membrane were found. These bodies were made up of a substance that resembled the cytoplasm of the cell and even contained red spherical bodies identical in appearance to mitochondria; in other words, it was as if masses of cytoplasm were being encountered within
the nuclei. A study of serial sections of individual cells showing this phenomenon has led us to conclude that these curious bodies were formed as a result of the inclusion within the nucleus of small polyoid masses of cytoplasm which had formed nuclear invaginations (Figs. 12 and 14). These invaginations were usually enlarged at their terminal end but were reduced in diameter as they approached the nuclear membrane (Figs. 12 and 24). The cause of such cytoplasmic invaginations is, of course, unknown, but it seems possible that the pressure of the anterior lobe cells on each other—and in this adenoma there was definite evidence of such pressure—may have played a role. As stated previously, the nuclei of these adenomatous cells were often wrinkled, indented and lobulated. It seems reasonable to conclude that the same abnormality in the structure of the nuclear membrane which made these conditions possible might also have been responsible in whole or in part, for the cytoplasmic invaginations into the nucleus.

Two hemorrhagic adenomas were of particular interest. Both were fairly large (one measured 1.2 X 1.9 mm. and the other 1.5 X 1.6 mm.). They were made up almost entirely of small, closely packed chromophobes whose nuclei were only slightly enlarged and contained 1 or 2 moderately hypertrophied nucleoli (Fig. 27), which contained variable numbers of nucleolar vacuoles. These cells had scant amounts of rather dense blue cytoplasm and often the cell membranes were too indefinite to be seen so that the cells resembled a syncytial mass. As a result of the reduced amounts of cytoplasm present in these adenomatous cells, many more nuclei were seen in a single microscopic field than in a field of similar size in the normal portion of the gland. Although numerous and fairly large blood-filled spaces were scattered throughout the tumors, there were large foci of adenomatous tissue where capillaries were not visible, so that many cells seemed far removed from a visible source of blood supply. Mitochondria were usually quite abundant in these small adenomatous cells, and in many the negative image of the Golgi apparatus was visible and often moderately enlarged. In one of these adenomas a few fairly large chromophobes, measuring up to 40 X 50 microns with nuclei of 25 X 15 microns and nucleoli 4 X 4 microns, were found scattered among the smaller cells. The outstanding structural characteristics of these adenomas, however, were not cell hypertrophy but a slight and fairly constant nuclear enlargement and a more definite nucleolar hypertrophy.

As mentioned previously, Saxton (28) has recently reported that cells in anterior adenomas in rats contain fat while normal cells do not. Our findings confirm his observation. In this study anterior lobe tissue containing 6 of the hemorrhagic adenomas were fixed in Champy’s fluid for 24 hours. In 4 of these lesions variable numbers of the adenomatous cells contained brownish-yellow droplets (Fig. 17), which varied in size from small structures not much larger than mitochondria to larger bodies measuring as much as 10 X 10 microns. In some cells only 1 or 2 of these droplets were seen; in other cells they were much more numerous and often occupied the greater part of the cytoplasm. These inclusions we have presumed to be lipoid in nature. The fact that they were not stained black we think was due to the fact that they were subjected only to the relatively small amounts of osmic acid in the Champy’s fluid for only 24 hours. That they were lipoid in nature seems to be evidenced also by the fact that they were identical in appearance to inclusions found in the pituicytes observed in the same sections in which there happened to be some posterior lobe present. Gersh (16) has demonstrated that the inclusions in such posterior lobe cells are lipoidal in nature. The lipoid droplets were found chiefly in the adenomatous chromophobes but sometimes they were also seen in the greatly hypertrophied acidophiles. In some instances small lipoid droplets have been observed in occasional cells of the normal portions of the gland, and in 1 or 2 cases they were quite numerous. The significance of the lipoid inclusions in either the adenomatous or normal cells is unknown but it seems obvious that their abundance in many of the adenomatous cells must be considered indicative of an altered cellular metabolism.

In 3 of the hemorrhagic adenomas cytoplasmic filaments were found in many of the chromophobes (Figs. 22 and 24). Sometimes only 1 or 2 were seen in a cell; other cells were nearly filled with them (Fig. 22). Often the filaments occurred in the form of rounded masses (Fig. 22), forming the structures known as “nebenkern.” Cytoplasmic filaments and nebenkern have been described in several varieties of cells previously (3) and in the anterior lobe they are in no sense restricted to adenomatous cells since they have been described by Kirkman (18) in the normal anterior hypophysis of the guinea pig, by Desclin (12), in the chromophobe cells of the rat hypophysis during pregnancy, lactation and after estrone administration and by Wolfe and Brown (40) in the anterior lobe of the...
rat after injections of estrogen. The significance of these structures is not known. By many they have been regarded as fixation artefacts (3) and our experience would support this viewpoint, since in the present study they have been found much more frequently in Champy-fixed tissue than in that fixed in Regaud's fluid. In the normal anterior lobe, they have been observed most often in chromophobes in glands in an active secretory state, i.e., during pregnancy, lactation and treatment with estrogen. Presumably under these conditions the cytoplasm of the chromophobes is in a physical or chemical state which makes the formation of the structures possible. The fact that we found similar cytoplasmic filaments and nebenkern in adenomatous cells would suggest that the cytoplasm of these cells was in a somewhat similar state. These structures were not found in the normal chromophobes of the glands used in this study. However, it should be pointed out that most of these cells were small and inactive.

In 6 (approximately 55 per cent) of the hemorrhagic adenomas numerous mitotic figures were found; in the normal tissue of only 1 gland a single mitosis was observed in a chromophobe.

Adenomatous nodules.—Twenty-seven adenomatous nodules were examined. These ranged in size from groups of only a few cells to masses measuring 0.9 × 0.9 mm. in diameter. Twenty nodules contained only chromophobes, 5 contained both chromophobes and acidophiles, while 2 were composed only of acidophiles. These nodules were generally diffuse, the adenomatous cells and the normal cells of the surrounding tissue intermingling freely at the periphery of the lesion. There was no compression of the surrounding normal anterior lobe cells and no microscopic evidence of hemorrhage.

Most of the nodules were made up of chromophobes showing varying degrees of hypertrophy although in a few instances the adenomatous cells were quite small (see various normal and adenomatous chromophobes already referred to in Figs. 1-27). In the lesions containing enlarged chromophobes, the largest cells measured up to 50 × 50 microns in diameter while cells measuring 30 × 30 microns in diameter were common. Nuclei and nucleoli were also enlarged, the former measuring up to 10 × 15 microns and the latter to 5 × 5 microns in diameter. The nucleoli often contained vacuoles. The nuclei in the enlarged chromophobes resembled those already described in the hemorrhagic adenomas.

The adenomatous acidophiles varied in size from cells slightly larger than normal to those measuring approximately 30 × 30 microns, with nuclei measuring up to 10 × 15 microns and nucleoli 3 × 3 microns. Generally, the negative image of the Golgi apparatus was markedly enlarged and the cells were well filled with granules. Mitochondria were moderately abundant (see Figs. 1, 2 and 3).

Four adenomatous nodules were of unusual interest. They were made up entirely of small chromophobes (Figs. 7 and 20), no larger in size than many of the chromophobes in the non-adenomatous regions of the gland. The cells had scanty light blue cytoplasm, were closely packed together, and often had indiscernible boundaries. In many cells, mitochondria were abundant; in others they were not. In the majority of the cells the negative image of the Golgi apparatus could not be seen but in some it was observed. The nuclei of the vast majority of the cells were normal in size although in a few cells they were slightly enlarged. The nuclei, however, were definitely enlarged and were more numerous than usual. They were stained deep blue and were usually more or less rounded but in some cells they were drawn out on one side and appeared club shaped. In many instances they contained vacuoles. The outstanding and only constant structural abnormalities found in these four lesions, therefore, were the changes in the nuclei. Considering our material as a whole, we are inclined to the view that as anterior lobe cells undergo adenomatous changes, the alterations in the size and appearance of the nuclei are among the first discernible changes.

Mitoses were found in only 4, or approximately 15 per cent, of the 27 adenomatous nodules observed, a much lower percentage than was found in the hemorrhagic lesions. Only 2 hypophyses containing adenomatous nodules had been fixed in Champy's fluid and in each of these, adenomatous chromophobes containing lipoid droplets were found.

Chromophobe adenomas.—Four of the adenomatous lesions were classified as chromophobe adenomas. The smallest of these measured 0.9 × 1 mm. while the largest occupied practically the entire anterior lobe. These growths were usually well demarcated from the surrounding anterior lobe tissue and in most instances there was an actual compression of the bordering anterior lobe cells. With one exception they were made up of chromophobes which showed varying degrees of hypertrophy and were in every way similar to those already described in the hemorrhagic adenomas and the adenomatous nodules. No further description therefore seems necessary except to point out that
the 2 of these lesions which had been fixed in Champy's fluid were found to have lipoid droplets in the adenomatous cells. Mitotic figures were present in 3 (75 per cent) of the growths; none were present in the surrounding normal tissue.

Cytologic Studies of Spontaneously Occurring Intermediate Lobe Adenomas

In 2 glands adenomas of the intermediate lobe were found. One of these lesions measured 0.4 × 0.6 mm and was well demarcated from the adjoining anterior and posterior lobes. The other was roughly conical in shape, its apex being in the dorsal region of the intermediate lobe and its larger base in the ventral portion; its greatest diameter was 1 × 1.3 mm. As the lesion extended ventrally it encroached on the anterior lobe, causing slight compression of these cells.

Both these adenomas were made up of intermediate lobe cells which, together with their nuclei and nucleoli, showed varying degrees of hypertrophy (Figs. 13-15). In the smaller growth the largest cells measured 45 × 30 microns, the largest nuclei 18 × 14 microns and the largest nucleoli 3.5 × 3.5 microns. Between these very large cells and the ordinary intermediate lobe cells, all gradations in size could be found. The smaller adenomatous cells stained deep blue, approximately of the same intensity as the normal intermediate lobe cells. As their size increased, however, there was a noticeable tendency for the adenomatous cells to stain more lightly, although there was considerable variation. Many of them were stained light blue while others were almost colorless. In many of the cells the negative image of the Golgi apparatus was noticeably hypertrophied (Fig. 14) but it was also noticed that often, in the largest cells, the Golgi body could not be seen at all, a situation previously encountered in the anterior lobe tumors. As a rule mitochondria were fairly abundant (Fig. 14); were generally spheroidal in shape, and most often showed no tendency to be hypertrophied. The nuclei, like the cells, showed varying degrees of hypertrophy (Fig. 14). They exhibited, but to a lesser degree, the same type of structural abnormalities found in the nuclei of anterior lobe adenomatous cells. The nucleoli took a moderately intense red stain and in a few instances contained vacuoles. Mitotic figures were found occasionally while none were present in the normal intermediate lobe tissue.

The larger growth differed in certain respects from the smaller one. Cell hypertrophy together with nuclear and nucleolar enlargement were more generalized although the limit of cell size was no greater than in the smaller adenomas. As a whole, the cells took a lighter blue stain, the negative image of the Golgi apparatus was seen less often and the cells contained fewer mitochondria, although in a few cells the mitochondria were hypertrophied and occasionally were vesicular (Fig. 15). The nucleoli were generally stained red and nucleolar vacuoles were quite abundant (Fig. 15). The cells in this larger intermediate lobe adenoma often contained cytoplasmic filaments or fibrillae, although nebenkern were not observed. Mitotic figures were present but were not found in the adjoining normal intermediate lobe tissue.

Discussion

This study raises several questions that seem worthy of discussion. First, what is the relation of the various types of lesions in the anterior lobe to each other? Do the hemorrhagic and the chromophobe adenomas arise as such or do they result from structural modifications in an earlier lesion, such as the adenomatous nodule? The present findings seem to support the second possibility. Although there was some overlap in size between the largest adenomatous nodules and smaller lesions of the two other varieties, the former were generally much more minute and involved a far smaller number of cells. No hemorrhagic or chromophobe adenomas as small as the smaller adenomatous nodules were found.

Considering the structural characteristics of the lesions, it seems to us that the two larger types developed as a result of progressive changes in the smaller adenomatous nodules. In the case of the hemorrhagic adenomas, it is likely that these changes included dilatation of the sinusoids and associated hemorrhage, a more definite demarcation from the surrounding normal anterior lobe cells, and often actual compression of these cells. These two latter changes could be expected to occur as a result of the growth of the adenomatous nodule. Presumably the chromophobe adenomas developed in the same fashion except that hemorrhage was absent. It appears equally clear that hemorrhagic adenomas could also be formed as the result of hemorrhage in pre-existent chromophobe adenomas. If we accept the assumption that the hemorrhagic and the chromophobe adenomas were formed as the result of structural changes in the adenomatous nodules, the question arises as to what percentage of the adenomatous nodules actually underwent such modification and what factors were involved. Unfortunately these are questions for which answers are not available.

Second, is there a relationship between the struc-
ture of the various adenomatous lesions and that of
the surrounding normal tissue? It has already been
mentioned, for example, that many of the adenoma-
tous cells presented cytological evidence of func-
tional activity. Were such adenomatous cells most
likely to occur in anterior lobes in which the nor-
mal cells also show evidence of secretory activity?
Our findings indicate that they were not. Adeno-
mas containing functionally active cells were found
in anterior lobes in which the normal cells showed
cytologic evidence either of little or no secretory
activity, of mild secretory activity, and in only 1
case of pronounced secretory activity. We have re-
ported previously (36) that the anterior lobes of
old female rats generally present cytological evi-
dence of decreased secretory activity. Since this
is the phase of life in which spontaneous anterior
lobe adenomas usually appear there would seem to
be some sort of relationship between decreased se-
cretory activity of the anterior lobe cells and the
occurrence of adenomatous lesions. The adenoma-
tous cells, therefore, appear to be at least partially
independent of the factors that regulate the activi-
ties of normal anterior lobe cells. The relative
abundance of mitosis in the adenomatous cells and
their almost complete absence in the normal cells
would point to the same conclusion. Our findings,
therefore, support the views of Saxton and Graham
(29) who concluded that anterior lobe adenomas
were true neoplasms.

During the course of the present study an ob-
servation which may be of some significance in
relation to the origin of the spontaneously occurring
adenomatous lesions was made. In the anterior
lobes of old female rats, whether or not tumors
were present, we have occasionally observed widely
scattered single cells, usually chromophobes, which,
when compared with the surrounding cells, con-
tained slightly but definitely hypertrophied nuclei
and nucleoli. If such cells had been multiple,
forming small clusters or clumps, the clusters thus
formed certainly would have been classified as ade-
nomatous nodules. Whether these scattered indi-
vidual cells actually were of the same nature as
tue adenomatous cells, and really played a role in
the genesis of the adenomatous lesions, are matters
of both conjecture and interest.

It is informative to compare the cytological char-
acteristics of adenomatous and normal anterior lobe
cells. A prominent feature of the former was their
frequent enlargement. However, the degree of
hypertrophy was extremely variable and many ade-
nomatous cells were not enlarged at all. Indeed,
sometimes adenomatous chromophobes were actu-
ally smaller than the surrounding normal chromo-
phobes. Thus, although adenomatous cells tended
to be larger than normal cells, enlargement was not
constant.

The nuclei of the adenomatous cells were also
generally enlarged but the degree varied greatly.
In some lesions the enlargement was slight or ab-
sent and in practically every adenoma, cells in
which the nuclei were not enlarged at all could be
found. Other changes noted fairly frequently in
the adenomatous cells were nuclear lobulation, nu-
clear budding, wrinkling of the nuclear membrane,
and the presence of multiple nuclei. With the ex-
ception of occasional wrinkling of the nuclear mem-
brane such changes were not found in the non-
adenomatous cells. A few adenomatous cells also
showed cytoplasmic invagination into the nucleus,
a phenomenon not observed in normal cells.

Hypertrophy of the nucleoli was probably the
outstanding single cytological characteristic of the
adenomatous cells although the degree of enlarge-
ment was extremely variable. Hypertrophied nu-
cleoli were not present in all cells classified as
adenomatous but it is possible that many such cells
contained enlarged nucleoli which were not actually
detected. In many instances successive serial sec-
tions through the enlarged nuclei of adenomatous
cells were studied. When this was done there was
not much possibility that any nuclei were over-
looked. However, the study of serial sections of
single adenomatous cells was not a routine proce-
dure. It is, therefore, possible that many nuclei
contained enlarged nucleoli which went unnoticed.
Although we feel that increase in size of the nu-
cleoli was perhaps the most constant change noted
in the adenomatous cells such enlargement was not
invariably found. It should be noted, moreover,
that very considerable enlargement of the nucleolus
in non-adenomatous anterior lobe cells may be in-
duced by experimental means. It is well estab-
lished that the anterior lobes of rats which have
received sufficient amounts of estrogen contain
many chromophobes which show various degrees of
enlargement. These cells contain enlarged Golgi
bodies and abundant mitochondria (33, 34 and 40)
and usually the cytoplasm stains deep blue with
aniline blue (38). It has been suggested by Sever-
inghaus (33) and by Wolfe and Brown (40) that
these cells are in an active secretory state. Studies
which are being carried out in this laboratory indi-
cate that such cells contain nucleoli which are
moderately but definitely enlarged when compared
with those in smaller chromophobes which do not
exhibit cytological evidence of functional activity.

The presence of light staining areas, or nucleolar
vacuoles as we have called them, in the nucleoli
of the adenomatous cells was another interesting cytologic feature. Although they were also found in the nuclei of normal anterior lobe cells they were much larger and many times more numerous in the adenomatous cells.

Changes in the cytoplasm of the adenomatous cells were less conspicuous than those in the nuclei. Lipoid droplets, often large and numerous, were found in variable numbers of the cells. Smaller droplets were found occasionally in normal cells. In many adenomatous cells mitochondria were extremely numerous, much more so than in normal cells, yet such an increase was not constant and in a few of the adenomatous cells relatively few mitochondria were found. Mitochondrial hypertrophy was pronounced in a few of the adenomatous cells but not present at all in normal cells of the glands examined. However, it should be pointed out that mitochondrial hypertrophy may occur in enlarged but non-adenomatous chromophobes of rats which have received estrogen.

Our observations on the Golgi apparatus in this study are admittedly unsatisfactory since only the negative image of this body was observed. In many adenomatous cells, however, hypertrophy of the Golgi apparatus was extremely pronounced, although this finding was by no means constant. In contrast, the Golgi bodies in the cells of the normal portions of the glands were generally small or only slightly enlarged. In nonadenomatous anterior lobe cells of estrogen-injected rats, however, enlargement of the Golgi apparatus is also pronounced (33, 34 and 40). Therefore, hypertrophy of the Golgi apparatus in adenomatous cells of the anterior lobe is not only not a constant, but also not an exclusive, cytological characteristic.

Analysis of these findings indicates that within the limits to which the comparison was carried there was no constant or unique cytoplasmic or nuclear change which differentiated adenomatous from normal anterior lobe cells. The structural differences between the two types of cells generally seemed to be of degree rather than kind. It is interesting to point out that Cowdry in a recent review (8) has described the differences between cancer and normal cells as quantitative rather than qualitative. The same is true when normal and adenomatous anterior lobe cells are compared.

Although we are not dealing with cancerous cells in this consideration of anterior lobe adenomas, it is found that when adenomatous and normal anterior lobe cells are compared, many of the differences between the 2 types are the same as those which have been described as occurring between malignant and normal cells. For instance Ludford (20) has reported that cancer cells tend to be enlarged, both the nuclei and the cytoplasm being involved. More recently Cowdry and Paletta (9) in an analysis of squamous cell carcinomas induced in mice by methylcholanthrene found that the malignant cells in some growths were larger than the surrounding hyperplastic cells; in others they were smaller. The findings are similar to what we have observed in anterior lobe adenomas since cell hypertrophy was often found but it was not a constant feature.

Many investigators have reported that the nuclei and nucleoli of malignant cells are enlarged; for a review of the extensive literature, see Cowdry (8), von Hamn and Alexander (35), Cowdry and Paletta (9), Biesele and his associates (2) and Caspersson and Santesson (7). MacCarty and his associates have been unusually active in this field. MacCarty, Haumeder and Berkson (23) have reported that both the nuclei and nucleoli of malignant cells are hypertrophied, the degree of increase being greater in the nucleoli. Later MacCarty (22) reported that the much larger size of the nucleolus in relation to the nucleus in malignant cells might be used as a basis for the diagnosis of malignancy, although this criterion has not been generally accepted (7-9 and 35). Cowdry and Paletta (9) in a study of squamous cell carcinoma in mice found that in many instances both the nuclei and nucleoli of the malignant cells were larger than those of the surrounding cells, but in some of the growths they were either of the same size or actually smaller. Because of this inconstancy they concluded that the size of the nuclei, or indeed of the nucleoli or the cell itself, could not be used as a single diagnostic criterion of malignancy.

Although Caspersson and Santesson (7) believe that nucleolar enlargement occurs in practically all tumor cells they do not regard it as indicative of malignancy. They point out that nucleolar hypertrophy may be found in cells of irritated tissues although the degree of enlargement is not usually as great as that found in malignant cells, and that in certain normal cells the nucleolus might sometimes be quite large. Frugoni (14), in a study of pituitary adenomas in the human being, reported that the nuclei and nucleoli were slightly enlarged in adenomas classified as simple, and markedly enlarged in those considered to be active. He associated enlargement of both the nucleus and the nucleolus with cellular activity. In the pituitary adenomas studied by us hypertrophy of the nucleus and nucleolus was often, though not constantly, found but the degree of enlargement was extremely variable and was sometimes not present.
Regan, Page and MacCarty (27) have reported the presence of unstained nucleolar bodies in both malignant and normal cells and have pointed out that such structures are larger and more numerous in the former. In a later and a more detailed study Page, Regan and MacCarty (25) described two types of intranucleolar structures, refractive bodies which occurred as unstained areas or as vacuoles, and argentophil bodies which stained with silver technics. The refractive bodies were larger and more numerous in malignant and benign tumor cells than in normal cells; while the argentophil bodies were more numerous in the neoplastic cells than in the normal. In fact, they concluded that the more malignant the neoplasm the more numerous were the intranucleolar bodies. These authors pointed out that previous to their studies intranucleolar bodies had already been found in many varieties of normal cells by numerous investigators; these studies they reviewed rather fully. It seems clear that the intranucleolar vacuoles observed by us in adenomatous and normal anterior lobe cells are similar to the refractive bodies of Page, Regan and MacCarty and our findings are similar to theirs in the sense that, in our studies, intranucleolar vacuoles are larger and occur more frequently in adenomatous than in normal cells. The significance of these intranucleolar bodies is unknown. Page and his associates (25) have suggested that they are concerned with the changed metabolism of fast growing cancer cells, a view that seems entirely logical. However, since they occur in non-malignant cells, although to a much lesser extent, it seems that they must represent the visible expression of some phase of nucleolar function in normal as well as in adenomatous cells.

The findings on the cytoplasmic structures of malignant cells have been inconstant. This question has been considered and reviewed by Ludford (20, 21) and Cowdry (8). Although variations from the normal have been described in both mitochondria and Golgi bodies, no constant or characteristic modification of these cell structures have been found in malignant cells. In anterior lobe adenomas there was marked hypertrophy of the Golgi apparatus and in some cells an increase in the number and size of the mitochondria. These changes, however, were inconstant and were in no way characteristic of the adenomatous cells.

Without further consideration of the literature, it seems legitimate to conclude that the adenomatous anterior lobe cells present many of the variations from normal which have been described for cancerous cells. The significance of these findings is difficult to evaluate at the present time.
natural to suggest that the pronounced nucleolar changes noted in many of the adenomatous anterior lobe and intermediate lobe cells might indicate some sort of dysfunction of the metabolism of nucleic acid and proteins. A further study of adenomas with this thought in mind might well yield valuable information.

SUMMARY

Three types of adenomatous lesions were found in the anterior hypophyses of old female rats, i.e., hemorrhagic adenomas, adenomatous nodules and chromophobe adenomas. The first two types of lesions contained chromohobes and in some instances acidophiles also. The last type contained only chromohobes. Although the three separate types of lesions were easily differentiated from each other, the cytological appearance of the cells constituting them were identical.

The adenomatous chromohobes usually, but not invariably, exhibited various degrees of hypertrophy when compared with normal chromohobes. Quite often, however, they were not enlarged at all and rarely they were even smaller than normal. Hypertrophy of the nucleus occurred more frequently than hypertrophy of the cell as a whole while enlargement of the nucleolus was found in a great majority of the adenomatous cells. The degree of enlargement of the adenomatous chromophobes, of their nuclei, and their nucleoli was extremely variable. The enlarged nuclei were often surrounded by wrinkled nuclear membranes, or exhibited lobulation and evidence of nuclear budding. Binucleate cells were frequently found. The nucleoli, in addition to being enlarged, often contained nucleolar vacuoles. Nucleolar vacuoles were also found in the normal chromohobes but much less frequently.

The adenomatous chromohobes often contained hypertrophied Golgi bodies. Often increased numbers of mitochondria were found and occasionally hypertrophy of these bodies occurred. Lipoid droplets were found fairly frequently in the adenomatous chromohobes and infrequently in the normal cells.

The adenomatous acidophiles constantly showed various degrees of hypertrophy. These cells were always well filled with granules. The Golgi apparatus was generally enlarged and mitochondria were generally abundant. The same nuclear and nucleolar changes which occurred in adenomatous chromohobes were also found in the adenomatous acidophiles but usually the changes were not so pronounced.

Mitoses were found frequently in the adenomatous cells; they were practically absent in the normal cells.

The cells found in the two intermediate lobe adenomas were characterized by varying degrees of enlargement. The nuclear changes were generally similar to those found in the anterior lobe lesions. In many of the adenomatous cells the Golgi apparatus was enlarged and often an increase in the numbers of mitochondria was found.

Comparison of adenomatous cells with normal cells in both the anterior and intermediate lobes indicates that adenomatous cells did not possess any distinctive cytological characteristics which would permit their differentiation from normal cells.

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Cytology of Spontaneous Adenomas in the Pituitary Gland of the Rat

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