Pituitary Siderosis

A Histologic, Immunocytologic, and Ultrastructural Study

C. Bergeron, MD, and K. Kovacs, MD, PhD

Pituitaries of 6 patients with various iron overload states have been studied by morphologic techniques, including immunostaining and electron microscopy. The immunoperoxidase technique combined with the prussian blue reaction revealed iron deposition in all five adenohypophysial cell types, indicating that iron uptake per se does not entirely block hormone storage. Iron distribution was uneven; more iron was demonstrated in PAS-positive cells than in orangeophils, and a preferential localization was disclosed in the gonadotrophs. In 2 cases of hemochromatosis, reduction of pituitary gonadotrophs was implicated in the genesis of hypogonadism. By electron microscopy, iron particles were noted in the cytoplasm of various adenohypophysiocytes, partly incorporated into lysosomes. Some adenohypophysiocytes with iron accumulation showed degranulation by light and electron microscopy and decreased hormone storage by the immunoperoxidase technique. Although these changes may be causally related to iron deposition, more work is required to prove that iron has a direct toxic effect on adenohypophysial cells. (Am J Pathol 93:295–310, 1978)

ALTHOUGH SIDEROSIS occurs frequently in the human adenohypophysis with iron overload states,[1] the cytologic changes in the anterior lobe are not sufficiently explored, and the role of the pituitary in the pathogenesis of the endocrine disturbances often associated with disorders of iron metabolism is unresolved.

To obtain a deeper insight into adenohypophysial iron storage and correlate it with hormone content, a new procedure has been developed in our laboratory, which combines the immunoperoxidase technique with the prussian blue reaction and demonstrates various adenohypophysial hormones and iron pigment on the same section. By using this method, adenohypophysial cells taking up iron can clearly be defined and answers can be provided as to whether iron deposition interferes with hormone storage. Attempts were also made to investigate adenohypophysial siderosis by electron microscopy. To our knowledge, the ultrastructural changes of the pituitary in iron overload states have not been previously reported.

From the Department of Pathology, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada.

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Address reprint requests to C. Bergeron, MD, c o Dr. N. B. Rewcastle, Department of Neuropathology, Banting Institute, 100 College Street, Toronto, Ontario M5G 1L5, Canada.

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Materials and Methods

The clinical data and histologic findings in 6 patients (4 males, 2 females) with iron overload were reviewed. The ages of the patients ranged between 32 and 82 years. Autopsies were performed at St. Michael's Hospital. The cases included idiopathic hemochromatosis (Cases 1 and 2), transfusional hemosiderosis (Case 3), and micronodular liver cirrhosis with iron overload (Cases 4 through 6).

The organs examined histologically are listed in Table 1. After fixation in 10% buffered formalin and paraffin embedding, 4- to 6-μ-thick sections were stained with hematoxylin–phloxine–saffron and the prussian blue technique. The pituitaries, in addition, were stained with the PAS method and subsequently with the prussian blue technique and orange G.

A semiquantitative estimation of iron content was made according to the following scale: trace, rare prussian blue granules under high magnification; +, rare prussian blue granules at low magnification; ++, moderate siderosis; +++, severe siderosis; ++++, massive siderosis.

For immunocytologic localization of adenohypophysial hormones, the immunoperoxidase technique was used. Details of the method were reported elsewhere.[2] From the paraffin blocks, 4- to 6-μ-thick sections were cut and immunostained using antibodies raised in rabbits against growth hormone (Imperial Cancer Research Foundation, London, England), prolactin (donated by Dr. H. Friesen, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada),[1-39]ACTH (Wellcome Reagents Ltd., Beckenham, England), TSH (Imperial Cancer Research Foundation, London, England), FSH (β subunit), and LH (β subunit) (donated by the National Pituitary Agency, University of Maryland, School of Medicine, National Institute of Arthritis, Metabolism and Digestive Diseases, Baltimore). Binding sites of immunologic reaction were demonstrated by 3,3′-diaminobenzidine. The specificity of immunostainings was verified by replacing the antibodies with normal rabbit serum as well as phosphate-buffered saline.

To demonstrate immunoreactive hormones and iron deposits on the same section, a combined staining technique has been developed. Sections were first immunostained for various adenohypophysial hormones as described above. After treatment with 3,3′-diaminobenzidine and subsequent osmication, they were washed in tap water for 5 minutes and then stained for 20 minutes in a solution containing equal volumes of 2% hydrochloric acid and 2% potassium ferrocyanide. After rinsing in distilled water and ethanol, they were cleared in toluene and mounted.

For electron microscopy, formalin-fixed pituitaries were used in 2 cases (Cases 4 and 5) and a paraffin-embedded pituitary was used in 1 case (Case 3). From the formalin-fixed pituitaries, small pieces of anterior lobe were postfixed in 2.5% glutaraldehyde in Sorensen's buffer, osmicated in 1% osmium tetroxide in Millonig's buffer, dehydrated in graded ethanol, processed through propylene oxide, and embedded in an Epon–araldite mixture. From the paraffin-embedded pituitary, a small piece of anterior lobe was removed; the paraffin was then dissolved with toluene and the tissue was osmicated and reembedded via graded ethanol and propylene oxide in an Epon–araldite mixture. Semithin sections were stained with toluidine blue and appropriate areas were selected for fine structural study. Thin sections were cut with a Porter Blum MT-2 ultramicrotome and investigated with a Philips 300 electron microscope, either as unstained specimens or after uranyl acetate and lead citrate staining.

Results

Clinical Data and Autopsy Findings

Case 1. A 32-year-old woman with a 4-year history of amenorrhea and hyperpigmentation presented 2 months prior to death with severe con-

Table 1—Iron Content in Various Organs

	Liver	Pituitary	Adrenal	Thyroid	Para-thyroid	Ovary/ testis	Pancreas	Spleen	Bone marrow	Myo-cardium	Kidney
Case 1	++++	+++	++	+++	NE	++	+++	+	+	+++	+
Case 2	++++	++	+	++	NE	+	++	+	trace	+	+
Case 3	++++	+++	+++	+++	+++	+	+++	++	++	++	++
Case 4	++	+	+	+	NE	−	++	+	−	trace	+
Case 5	+++	+	+	+	NE	trace	++	NE	+	trace	+
Case 6	+++	+	++	++	NE	+	++	++	+	trace	+

−, no iron detected; trace, rare prussian blue granules under high magnification; +, rare prussian blue granules at low magnification; ++, moderate siderosis; +++, severe siderosis; ++++, massive siderosis; NE, not examined

gestive heart failure and recurrent arrythmias. Axillary and pubic hair was sparse, and hepatomegaly with a modest elevation of SGOT blood levels was noted. The PBI and blood sugar were normal. Major autopsy findings included pigmentary cirrhosis of the liver and marked cardiomegaly. The degree of siderosis found in various organs is discussed in a subsequent section, and the findings are listed in Table 1.

Case 2. A 63-year-old man was found to have hemochromatosis 2 years prior to death. He had been impotent for 5 years at the time of diagnosis and had noted a decreased need for shaving. On examination, hyperpigmentation, sparse body hair, and atrophic external genitalia and prostate were noted. Hepatomegaly with mild elevation of SGOT blood levels was present, and liver biopsy revealed a typical pigmentary cirrhosis. The serum iron was 221 μg/dl with a TIBC of 283 μg/dl saturated at 78%. Serum T_4, ACTH stimulation, and metopyrone tests were normal. Three months prior to death, the patient deteriorated rapidly with progressive liver failure. Autopsy findings included pigmentary cirrhosis of the liver and a hepatoma with metastases to pancreas, portal nodes, right lung, and subcutaneous tissues. In the testes, severe atrophy of Leydig cells and seminiferous tubules was found.

Case 3. An 80-year-old woman developed a refractory anemia and received more than 200 units of blood over 3 years. Three months prior to death, she was found to have cardiomegaly, atrial fibrillation, and, ultimately, intractable pulmonary edema. No endocrine abnormalities other than mild diabetes mellitus of 6 years' duration were noted. Autopsy findings included moderate cardiomegaly, extensive hemosiderosis, and fibrosis of the liver.

Case 4. A 66-year-old severely hypertensive man was admitted 6 years before death with gray-brown hyperpigmentation, mild diabetes mellitus, and hepatomegaly. Liver function tests were severely disturbed. The serum iron was 208 μg/dl with a TIBC of 202 μg/dl saturated at 51%. Needle biopsy of the liver revealed micronodular cirrhosis without stainable iron. Three months prior to death, he presented with severe liver failure. At that time, testicular atrophy and gynecomastia were noted. Autopsy findings included micronodular cirrhosis, marked cardiomegaly, and severe coronary atherosclerosis. The testes revealed atrophy of Leydig cells and seminiferous tubules.

Case 5. A 46-year-old man, a chronic alcoholic, was admitted stuporous after a craniocerebral injury. A large subdural hematoma was evacuated without neurologic improvement, and he died shortly after. The liver function tests were mildly abnormal. Autopsy findings included a massive cerebral infarct with transtentorial herniation. The liver showed micronodular cirrhosis and alcoholic hepatitis. In the testes, slight atrophy of

Leydig cells, arrest of spermatogenesis, and interstitial fibrosis were noted.

Case 6. An 82-year-old man, a reformed alcoholic, was admitted 48 hours before death; he was confused and had severe pneumonia. Autopsy findings included extensive bronchopneumonia and micronodular cirrhosis. The testes revealed normal Leydig cells and atrophy of seminiferous tubules.

Pituitary Morphology

Light Microscopic Findings

The architecture of the pituitary was preserved in all 6 cases, and no necrosis, fibrosis, or atrophy was evident. As shown in Table 1, the extent of iron deposition varied from case to case. Iron particles were irregularly distributed; by using the PAS–prussian-blue–orange-G staining technique, iron granules appeared to be more numerous in the cytoplasm of PAS-positive cells compared with orangeophils (Figure 3), with the exception of Case 3 in which deposition of iron seemed to be equal in both cell types. Considerable quantities of iron were demonstrated in poorly staining "chromophobic" cells. Some of these cells were so extensively degranulated that they could not be identified, whereas others contained a few PAS-positive or orangeophil granules, permitting the disclosure of their tinctorial characteristics.

Absence of iron storage in the posterior lobe was a constant finding, except in Case 3 in which a few iron-positive granules were scattered among the nerve fibers. In Case 2, groups of PAS-positive cells extending deeply into the posterior lobe (basophil invasion) contained numerous iron-positive granules in their cytoplasm in contrast to the adjacent neurohypophysial tissue in which no iron was demonstrated.

Immunocytologic Findings

Five distinct cell types can be distinguished in the human adenohypophysis by the immunoperoxidase technique: somatotrophs, lactotrophs, corticotrophs, thyrotrophs, and gonadotrophs. Although some gonadotrophs seemed to contain only FSH while others contained only LH, recent evidence indicates that both FSH and LH are secreted by a single cell type.[3,4] The question of whether one or two gonadotroph cell populations exist is beyond the scope of our study and will not be dealt with here. Since FSH- as well as LH-containing cells reacted similarly in the course of the present investigation, the term "gonadotroph" has been adopted to designate those adenohypophysiocytes which show positive immunostaining for FSH and/or LH.

By combining the immunoperoxidase technique with the prussian blue

reaction, immunoreactive hormones were demonstrated as brown deposits, while iron particles exhibited a blue color on the same section (Figures 4 and 5). Thus, the advantage of this staining procedure is self-evident: it permits the disclosure of the cellular sites of iron storage. No cell counts were made, however, because the distribution of various cell types was uneven.

As shown in Table 2, iron storage was conclusively demonstrated in all five adenohypophysial cell types. Somatotrophs and lactotrophs seemed to contain similar amounts of iron as did corticotrophs and thyrotrophs (Figure 4). In Cases 1 and 3, however, iron accumulation in lactotrophs was more extensive than in the other cases. In Case 2, adenohypophysial cells spreading into the posterior lobe showed positive immunostaining for ACTH and were regarded as corticotrophs. Iron deposition was especially marked in the cytoplasm of these cells. An unexplained hyperplasia of thyrotrophs was noted in Case 1. Accumulation of iron was more pronounced in gonadotrophs than in other cell types, and this preferential localization seemed to be especially conspicuous in sections immunostained for LH (Figure 5). Our material is, however, too small and heterogeneous to analyze the underlying mechanisms accounting for this difference. In Cases 1 and 2, the number of gonadotrophs was strikingly reduced and virtually all the remaining gonadotrophs showed marked iron storage (Figure 6). Pituitary siderosis was only slight in Cases 5 and 6; the proportion of iron-containing gonadotrophs was, however, remarkably high compared with that in other cell types. In Case 6, although only a few gonadotrophs contained prussian-blue–positive granules, this accounted for most of the iron present, with only rare granules seen outside these cells. Iron particles were frequently noted in adenohypophysiocytes containing small amounts or no immunoreactive hormones, indicating that excessive iron deposition can be associated with a decrease in hormone content. This finding was, however, not generalized, since many adenohypophysial cells exhibited strong immunoreactivity despite marked iron storage.

Distribution of Iron in Other Organs and Correlation With Pituitary Iron Storage

The degree of siderosis in the pituitary correlated best with that found in zona glomerulosa of the adrenal cortex, in thyroid follicular cells, as well as in the islets of Langerhans (Table 1). In the adrenals, iron was predominantly stored in zona glomerulosa cells. The exocrine pancreas consistently revealed slightly more iron than the endocrine portion. In all 6 cases, the predilection site of iron storage was in the liver, and the

Table 2—Distribution of Iron in Adenohypophysial Cells

	Somato-trophs	Lacto-trophs	Cortico-trophs	Thyro-trophs	FSH-containing cells	LH-containing cells	Endocrine status
Case 1	+	++++	+	+↑	+↓	++++↓	Amenorrhea and sparse body hair
Case 2	+	+	+	+	+↓	++++↓	Severe hypogonadism, ACTH and metopyrone tests normal, CHO intolerance
Case 3	+	++	+	+	++	++++	Diabetes mellitus, postmenopausal
Case 4	+	+	+	–	+	++	Severe hypogonadism, diabetes mellitus, gynecomastia
Case 5	+	+	+	+	+	++	No abnormalities noted
Case 6	+	+	+	+	+	+	No abnormalities noted

–, no iron detected; +, minority of cells; ++, approximately 30 to 50% of cells; ++++, most or all cells; ↑, increase in number of cells; ↓, decrease in number of cells

amount of iron failed to correlate with that of the pituitary. Iron deposition in testes was slight and was found predominantly in the blood vessel walls but occasionally in the Leydig cells, tubular basement membrane, and interstitium. Siderosis in the kidney was chiefly limited to the tubular cells.

Ultrastructural Findings

In unstained preparations, aggregates of granules with an average diameter of 50 Å and with a regular tetragonal arrangement and large irregular dense bodies containing closely packed electron dense cores measuring 25 to 30 Å in diameter were randomly scattered throughout the hyaloplasm (Figure 1). The number of particles varied from cell to cell; they were especially numerous in Case 3. Their fine structural features were identical to those described in siderotic liver [5] and bone marrow [6] and were regarded as representing aggregates of ferritin and clumps of hemosiderin, respectively. Definite membranes surrounding the dense bodies could not be found with certainty. Absence of limiting membranes, however, might have been due to postmortem autolysis.

In preparations stained with uranyl acetate and lead citrate, the electron-dense bodies were easily noticeable. Since autolytic changes were advanced, no attempts were made to separate various adenohypophysial cell types; to identify abnormalities in the nucleus, cytoplasmic organelles, and cell membranes; or to localize precisely the iron particles. It appeared, however, that at least some iron particles were incorporated into lysosomes and definitely in the easily recognized "enigmatic bodies."[7] The number of secretory granules showed a marked decrease in some cells which contained large amounts of iron particles (Figure 2), while other adenohypophysiocytes with extensive iron deposition were fully granulated. Thus, no close correlation was established between loss of secretory granules and extent of iron storage.

Discussion

In 6 patients with various iron overload states, the study of the pituitary showed iron deposition in all five adenohypophysial cell types (somatotrophs, lactotrophs, corticotrophs, thyrotrophs, and gonadotrophs), indicating that uptake of iron per se did not block hormone storage, not even in those cells in which iron accumulation was pronounced. Although a considerable variation existed from case to case, it was evident that iron deposition was uneven and more iron was stored in PAS-positive cells than in orangeophils. The immunoperoxidase technique combined with the prussian blue reaction revealed a preferential localization of iron in gonadotrophs compared with other cell types, thus confirming the results

of Sheldon,[8] who found more iron pigment in pituitary basophils than in eosinophils in patients with hemochromatosis, and those of Peillon and Racadot,[9] who, by using a tetrachrome stain, noted a preferential uptake of iron by gonadotrophs. In our work, however, the various adenohypophysial cell types were identified by the immunoperoxidase technique, which is more reliable and sensitive than previously applied staining procedures and permits the specific localization of immunoreactive hormones in the cell cytoplasm.

The uneven distribution of iron was not limited to the pituitary. The zona glomerulosa of the adrenal cortex contained more iron than the cells of the inner cortical layers, and there was more marked siderosis in the liver than in other organs. The mechanism whereby more iron is stored in certain cell types and organs than in others is not clear.[10] In the pituitary, it cannot be attributed to circulatory factors, since blood supply to the anterior lobe is less than that to the posterior lobe [11,12]; nevertheless, iron storage was more marked in the anterior lobe. Also in areas of basophil invasion, corticotrophs located in the posterior lobe contained more iron than the adjacent neurohypophysial tissue. Transferrin-binding sites, currently being investigated, probably have an important role in the transfer of iron from the extracellular space to the cell interior.[13] The question of whether pituitary gonadotrophs have more transferrin-binding sites than other pituitary cell types has yet to be elucidated.

The pathogenesis of hypogonadism in iron overload states is unresolved. Sheldon [8] assumed that the cause of hypogonadism frequently associated with hemochromatosis was probably pituitary insufficiency. This view was supported by his finding that the testes, although often showing atrophic changes, contained only a modest amount of iron. Simon et al [14] attributed the development of hypogonadism to a general metabolic disturbance, possibly independent of iron overload, causing testicular insufficiency associated with an inadequate pituitary response. Diabetes with autonomic neuropathy, ethanol abuse, and cirrhosis of the liver itself have also been incriminated.[15,16] Cirrhotic males frequently show evidence of hypogonadism, the mechanism of which is still poorly understood.[17] Cirrhosis associated with iron overload states could certainly be an important factor contributing to hypogonadism, but, in contrast to cirrhotics, patients with hemochromatosis often display abnormal pituitary function tests in which the gonadotrophs are particularly affected, while the alterations in liver function tend to be modest.[18-21] Other investigators, however, failed to confirm these results,[22] and the pituitary function of patients in the early precirrhotic stage of hemochromatosis was found to be normal.[23]

In our study, the 2 patients with hemochromatosis had severe hypo-

gonadism (early in the disease when their liver function was minimally altered) and a marked decrease of pituitary gonadotrophs. This finding is interpreted as representing the morphologic manifestation of hypogonadotropism, since in primary gonad failure there is an increase of gonadotrophs. The causative factors accounting for the loss of gonadotroph hormones in the pituitary remain obscure.

The concept of iron cytotoxicity in iron overload states is widely accepted, and it is clear that iron storage has become a major problem in the treatment of thalassemic children with a prolonged survival, owing to transfusion programs.[24] Iron cytotoxicity has been implicated as a cause of myocardial failure,[25] while its role in the causation of diabetes mellitus is still controversial.[26-28] Convincing functional and histologic improvement is seen in the livers of patients with hemochromatosis after iron removal by phlebotomy [29-31] and in thalassemic children treated with desferrioxamine.[32] The amount of fibrosis observed in the livers of thalassemic children who have received transfusions appears to correlate with the quantity of iron in that organ as well as with the duration of siderosis.[33] HeLa cells incubated in an iron-enriched medium demonstrated a decrease in mitotic activity and evidence of cellular injury manifested by increased autophagic activity.[34]

The precise mechanism of iron toxicity is still elusive, but several hypotheses have been postulated, which include peroxidation of lipid membranes leading to destabilization of lysosomal membranes and impairment of mitochondrial function.[10,35-37] Destruction of nonspecific antioxidant vitamins C and E has also been implicated as a cause, particularly in tissues heavily dependent on these substances, even in the presence of lesser amounts of iron.[24]

Although iron cytotoxicity is a probable explanation for the decreased number of gonadotrophs in our material, further work is required to prove this assumption, and it is uncertain whether extrapolations are justified from other organs to the pituitary gland. We were unable to state whether the loss of gonadotrophs was due to their destruction or their failure to stain with the immunoperoxidase technique, because of the reduction of their hormone content and secretory granules. We favor the second possibility in the absence of any significant fibrosis or alteration in the volume of the gland. A decrease in the number of secretory granules was evident both by light and electron microscopy. In the PAS-orange-G–stained sections, the iron was most often present in cells showing only a faint PAS positivity, and some of the iron-laden cells stained more lightly than usual with the immunoperoxidase technique. Ultrastructurally, cells showing a decrease of secretory granules always contained iron, but the

amount failed to correlate with the degree of granule loss, suggesting additional contributing factors which are unknown at present. These problems require further investigation.

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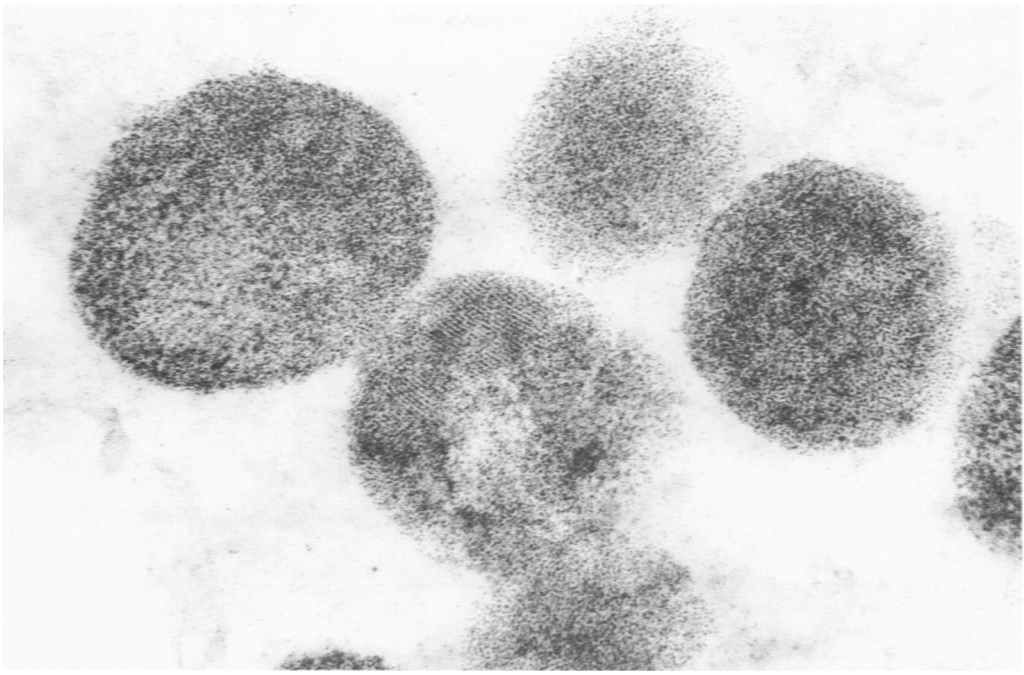

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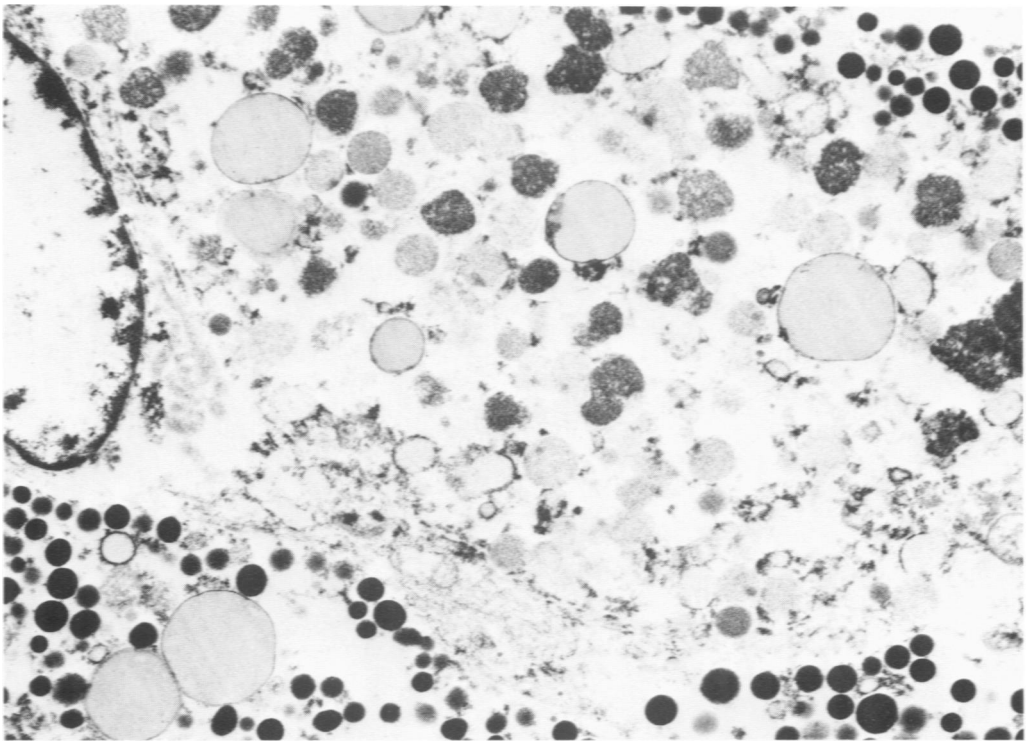

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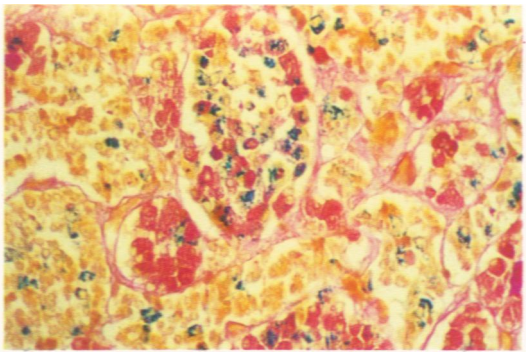
Figure 1—Aggregates of ferritin. The 50-Å granules have regular tetragonal arrangement. (Unstained, × 74,900) **Figure 2**—Adenohypophysial cell containing aggregates of ferritin and hemosiderin with a marked decrease in the number of secretory granules. Part of the cytoplasm of two adjacent cells is normally granulated. (Uranyl acetate, lead citrate, × 10,200)

Figure 3—Iron-positive granules are more abundant in PAS-positive cells. Case 2. (PAS-prussian blue–orange G stain, × 250)

Figure 4—Iron-positive granules are evident in some corticotrophs. Case 3. (Immunoperoxidase technique and prussian blue reaction, × 400)

Figure 5—Iron-positive granules are apparent in many gonadotrophs. Case 3. (Immunoperoxidase technique for β-LH and prussian blue reaction, × 400)

Figure 6—Marked decrease in gonadotrophs with virtually all residual cells containing iron. Case 2. (Immunoperoxidase technique for β-LH and prussian blue reaction, × 400)



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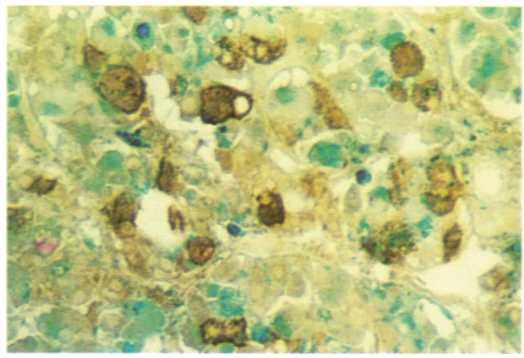

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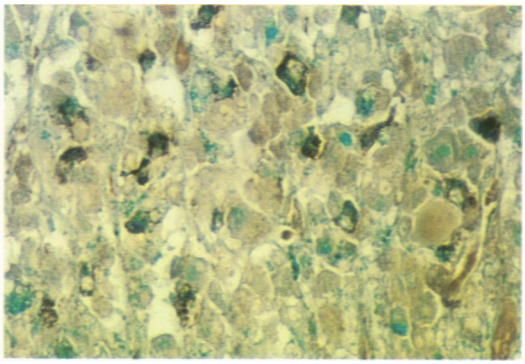

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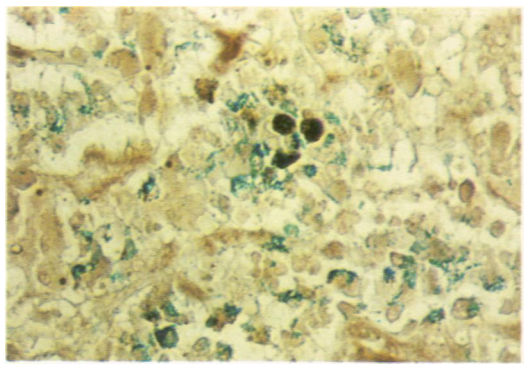

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