SCIENTIFIC SECTION

THE COMPARATIVE PHARMACOGNOSY OF THE ANTERIOR AND POSTERIOR LOBES OF THE PITUITARY OF CATTLE.*

BY HEBER W. YOUNGKEN.[1]

The Pituitary body or *Hypophysis cerebri* is a small, somewhat ovoid endocrine gland found within the skull of vertebrates firmly lodged in a depression known as the sella tursica of the sphenoid bone. It is encased by a fibrous capsule of connective tissue representing chiefly dura mater which lines the cranium. It consists of a large anterior lobe, a small posterior lobe and a *pars intermedia*. The anterior lobe is epithelial in origin and represents an offshoot from the primitive buccal cavity of the embryo. It is hollowed out behind so as to form a cavity for the lodgment of the posterior lobe. The *pars intermedia* is fused with the posterior lobe and lies behind the hypophyseal cleft which separates the two lobes. It is more pronounced in cattle, sheep and hogs than in man. It is epithelial in origin being derived from the posterior wall of Rathke's pouch.

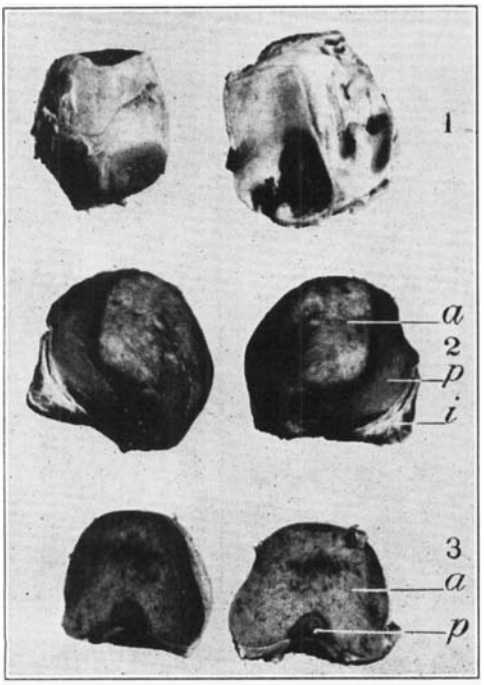

Fig. 1.—Pituitary glands of cattle (*Bos taurus*). 1. To left, convex surface; to right, concave, infundibular surface and lateral surface of gland. 2. Lateral views of gland. 3. Gland halved to show internal structure. *a*, anterior lobe; *p*, posterior lobe; *i*, infundibulum.

The purpose of this paper is to render a report on the pharmacognostical and histological differences as found by the writer between the anterior and the posterior lobes of the pituitary of cattle and their powdered, desiccated glands.

In a previous paper (1) the writer published the results of his early studies made upon the powdered, desiccated whole pituitary, anterior pituitary and posterior pituitary. These studies have been continued with the aim of finding methods which would simplify the problem of distinguishing these powdered endocrine products.

The materials studied consisted of preserved and desiccated whole pitui-

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taries, preserved and desiccated anterior and posterior lobes and powdered, desiccated, anterior and posterior lobes.

Celloidin and paraffin sections of the whole pituitary of cattle and of both of its lobes were cut in vertical and horizontal planes and variously stained, mounted and examined under low and high power magnifications under the compound microscope. The desiccated gland and its separate lobes were macerated in warm water, dissected under a dissecting binocular microscope and representative portions transferred to microslides, teased apart with dissecting needles and examined separately in water and various stains. The powdered, desiccated materials were mounted directly in water and in various stains and also fixed to slides with diluted Mayer's albumin fixative (1:20), allowed to dry over night and subsequently stained in staining wells, washed, dehydrated, cleared in xylol and mounted in balsam. The filter and funnel method was also used, especially for a modification of the Penfield-Hortega silver carbonate method of preparation and proved satisfactory. The chief objection to this method is the occurrence of vegetable fibers from the filter paper in some of the stained powder but these and other débris can readily be distinguished from the natural elements of the pituitary by a skilled microscopist.

The stained sections and fragments of the gland were compared under the compound microscope with similarly stained and mounted powders representing powdered, desiccated anterior and posterior lobes of the pituitary of cattle, and the elements found in the powdered products thus identified.

The stains used included the following: Weigert's hematoxylin with and without copper acetate solution as a mordant, Delafield's hematoxylin, alcoholic eosin, aqueous eosin, Mallory's stain, a mixture of Mallory's stain with 1 per cent phosphotungstic acid, Mann's stain, aqueous solution of methylene blue and eosin (1 per cent of each), a 1 per cent solution of acid fuchsin with 0.1 cc. of dilute hydrochloric acid added to each 100 cc. of solution, 1 per cent osmic acid solution, gold chloride test solution, 3 per cent aqueous solution of silver nitrate. Penfield's modification of Del Rio-Hortega's silver carbonate method was employed in the examination of the pituicytes found only in the posterior lobe.

WHOLE BEEF PITUITARIES.

The beef pituitaries studied were preserved in part in Zenker's fluid and in part in alcohol and formalin. They were irregularly oval, from 2 to 2.3 cm. in length, 1.5 to 1.8 cm. in width and 1.3 to 1.5 cm. in vertical height and showed a circular to lens-shaped scar on one surface, marking the place of severance from the infundibulum. The whole gland excepting the scar was enveloped by a tough, fibrous capsule composed of meningeal tissues. About three-fourths the weight of the gland consists of anterior lobe.

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Covering the entire gland is a capsule composed for the greater part of wavy fibers, and separating the two lobes from each other is a fibrous lamella of connective tissue. The fibers stain bluish with Delafield's hematoxylin and blue to greenish blue with a mixture of Mallory's connective tissue stain and 1 per cent phosphotungstic acid solution. Mann's stain imparted a purple color while copper-hematoxylin stained the fibers a pinkish purple. Sections of blood vessels occurred imbedded in the fibrous capsule.

Anterior Lobe.—The anterior lobe was found to be composed for the most part of polygonal epithelial cells of two types, chromophobe or chief cells and chromophile cells. These polygonal cells are arranged in solid branching cords and alveoli, each surrounded by a connective tissue capsule. The two types of epithelial cells can be excellently differentiated through the use of copper hematoxylin and eosin, using a differentiator containing potassium ferricyanide and borax. With this combination of stains the chromophobe cells show a clear, non-glandular, pink cytoplasm. The chromophile cells studied were of two types, *viz.:* (*a*) those containing eosinophilic or acidophilic granules and (*b*) those containing basophilic granules or, according to Bailey and

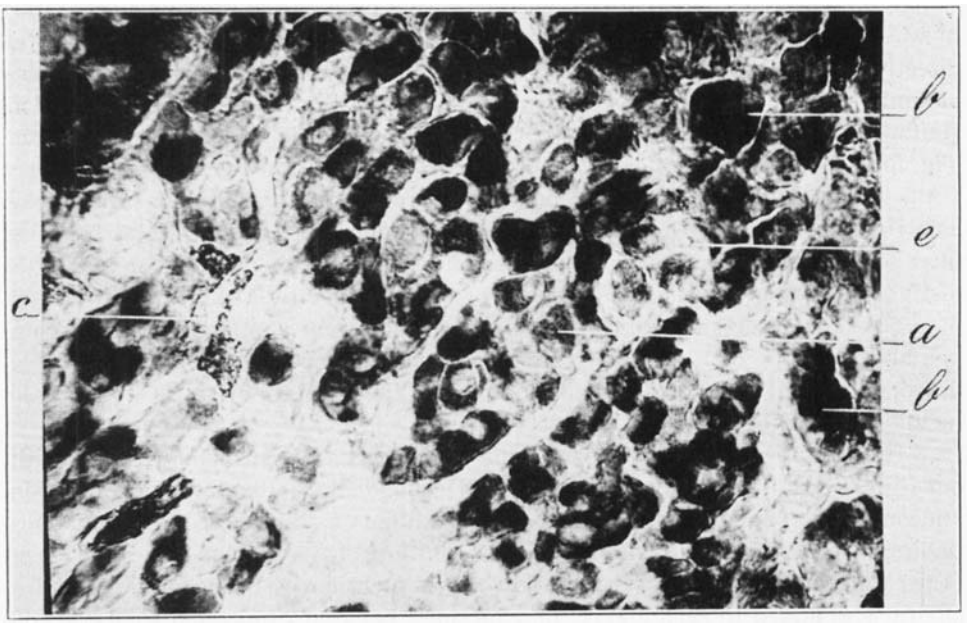

Fig. 2.—Sagittal section of the Anterior Pituitary of Beef, stained by the copper-hematoxylin method followed by treatment with Weigert's differentiator. *a*, Chromophile cells with alpha granules; *b*, chromophile cells with beta granules; *e*, chromophobe cells; *c*, capillary × 400.

Davidoff (2) chromophile cells containing alpha granules and chromophile cells containing beta granules. Both the alpha- and beta-granule containing cells are arranged without any definite order. The alpha-granule containing cells showed a blue-stained nucleus and a cytoplasm possessing distinct, small, rounded, blue-stained bodies while the beta-granule containing cells showed a similar nucleus with much finer, less distinct blue-stained granules which tend to form a homogeneous mass. Some lipoid granules colored brown to black with osmic acid solution also occurred in both chromophobe and chromophile cells.

The connective tissue around the alveoli was colored pink by this method and was seen to form a loose meshwork of collagenous fibers between the alveoli and cell cords, the strands being considerably detached in vertical sections. A number of brown-colored capillaries containing blood corpuscles some of which were colored red, others greenish black, were also observed between the alveoli and also thin, pink-colored nerve fibers. Here and there sinuses, containing a pink-stained colloidal substance, were noted. Many of these occurred in the center of alveoli. These colloidal areas may have been formed through the degeneration of the central cells of the original epithelial cords, as suggested by Bremer (3), or they may represent a secretion from the cord cells.

Sections of this lobe stained with Mallory's connective stain and 1 per cent solution of phosphotungstic acid showed the connective tissue to be colored blue to greenish blue and the cellular elements yellow to brown.

Posterior Lobe.—The so-called posterior lobe of the pituitary of cattle really consists of the *pars nervosa* with the adherent *pars intermedia*, the latter being attached to the front of the *pars nervosa* behind the hypophyseal cleft, and in some sections, seen to invade the *pars nervosa*.

Sections examined in Delafield's hematoxylin and eosin, copper-hematoxylin and eosin, Mann's stain, in acid fuchsin and Mallory's stain and in a mixture of Mallory's stain and 1 per cent phosphotungstic acid showed the greater portion of the *pars nervosa* to be composed of neuroglia with scattered bipolar and multipolar nerve cells, some containing pigment, and a number of non-medullated nerve fibers, some with bulbous ends. Scattered here and there occurred a few areas of colloid, a few sections of blood vessels and blood sinuses, some containing blood corpuscles.

Other sections impregnated by Penfield's modification of Del Rio-Hortega's silver carbonate method (4) showed numerous cells which P. C. Bucy has termed "pituicytes" and which possess one or more processes. Their nuclei and cytoplasm varied greatly in outline. Under oil-immersion magnification the cytoplasm in some of them was finely granular, in others the granules appeared en masse. The pituicytes varied also in the number of processes which sprang from their cell bodies. Some of them were unipolar cells, others bipolar, still others multipolar. Some of the processes were, as Bucy earlier reported in his material (5), considerably longer than processes of glial cells elsewhere observed and often divided in furcate fashion and gave rise to short branches. Some pituicytes were found with coarse granules in the cytoplasm, as also noted by Bucy. Of all the staining methods used by the writer, the Penfield modification of Hortega's silver carbonate method was found the best to distinguish the most diagnostic elements of the pituitary lobe of cattle, *i. e.*, the pituicytes. These were not found in the anterior lobe of the pituitary, nor have they been reported in any other part of the nervous system.

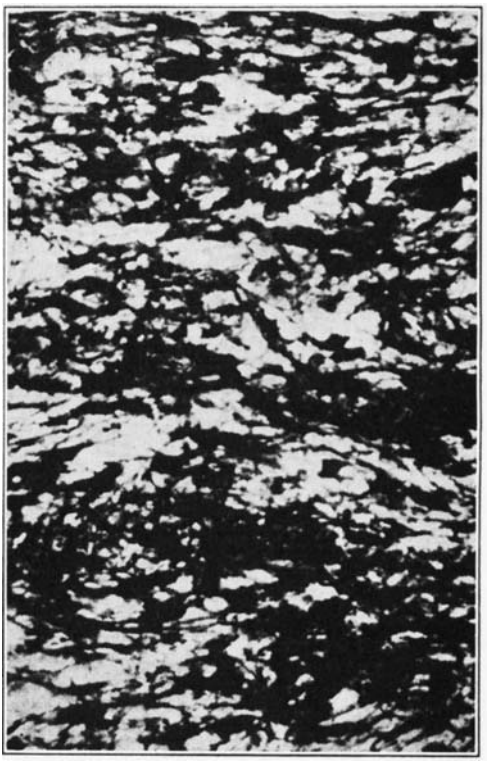

Fig. 3.—Pituicytes, dark objects, in a section of beef pituitary stained by the Penfield modification of the Hortega method. (From a Text Book of Pharmacognosy, 4th edition by the author, after Bucy in Penfield's "Cytology and Cellular Pathology of the Central Nervous System").—(*Courtesy of Paul B. Hoeber, Inc.*)

The *pars intermedia* which is attached to the front of the *pars nervosa* and separates with it in collecting the posterior lobe for the manufacture of pharmaceutical preparations differed from the *pars anterior* (anterior lobe) and the *pars nervosa* by showing epithelial cells arranged in incomplete alveoli. Moreover, when stained by copper-hematoxylin and eosin and differentiated with potassium ferricyanide and borax solution, these epithelial cells took the pink color, and none of the chromophile cells typical of the anterior lobe were present. However, a number of small scattered groups of small, round cells with blue-stained nucleus and cytoplasm and which may be regarded as basophiles were present. These also occur in the anterior lobe. The connective tissue between the partial alveoli was less pronounced than in the anterior lobe. With Mann's stain the connective tissue was colored blue in all parts of the gland, the colloid yellow to orange, the blood corpuscles pink to orange and the epithelial cells of the *anterior lobe* and *pars intermedia*, purple to pink.

DISTINCTIONS BETWEEN THE POWDERED, DESICCATED, ANTERIOR AND POSTERIOR PITUITARY.

The determination of the desiccated lobes afford a more difficult problem than that of distinguishing between stained sections of the parts of the pituitary. This is obviously due to the alteration of many elements in the process of preparing the products for the market, during which time a certain amount of shrinkage occurs in many cells, especially in the cytoplasm of neuroglia and glial cells including pituicytes. Nevertheless, it is not impossible for the experienced worker to quickly distinguish between the products by means of careful technique. The writer has found that a small funnel, flask and chemical filter paper are useful in some of the staining technique especially where two or more stains or solutions are employed, the powder being treated on the filter paper in position within the funnel with the staining solutions and, when dry, transferred to a slide for examination. It was often found necessary to dilute some of the stains used in order to bring out cell details in some of the elements. The personal element cannot be overlooked in this work. Usually there are a sufficient number of sufficiently thin elements present in the mounts so that the thicker, opaque masses can be passed by in making the examination, if deemed necessary. In addition to the histological characteristics previously reported by the writer for the powdered, desiccated, anterior and posterior pituitary products, the following observations may be added: Powdered, desiccated, anterior pituitary when treated with copper acetate solution as a mordant, and subsequently stained with Weigert's hematoxylin and eosin solution, and treated with Weigert's borax ferricyanide differentiator, shows polygonal to irregularly polygonal chromophile cells containing alpha and beta granules and polygonal to irregularly polygonal chromophobe cells containing clear, non-granular, pink cytoplasm, fragments of brown-stained connective tissue and nervous tissue, and occasional small circular basophiles with a blue nucleus and sky-blue cytoplasm.

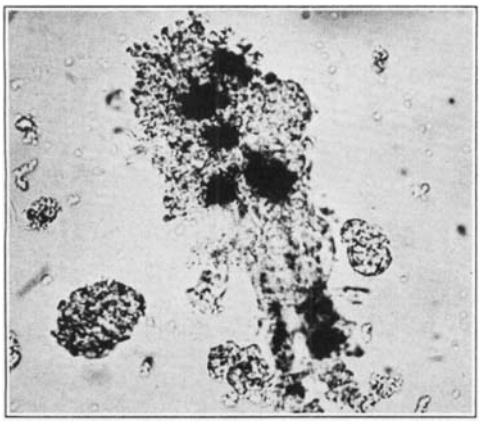

Fig. 4.—Fragments of the powdered, desiccated, pituitary, showing pituicytes as the larger black objects (× 400).

Powdered, desiccated, posterior pituitary by the aforementioned copper-hematoxylin and eosin method with Weigert's differentiator did not exhibit chromophile cells with alpha- and beta-granules, but showed mostly brownish pituicytes and pink-stained connective tissue together with a number of small rounded basophiles and a number of eosinophiles from the attached *pars intermedia.*

Pink-stained wavy connective tissue fibers, brownish capillaries and glial cells and processes occurred in the powders for both lobes.

The powders were next separately treated by a modification of Penfield's method consisting of the following procedure: They were first macerated in flasks

in 8 per cent formaldehyde, the material then repeatedly washed on a filter paper placed in a funnel with distilled water containing a few drops of stronger ammonia. The filter paper containing the powder was then immersed in 5 per cent solution of 40 per cent hydrobromic acid in distilled water at 38° C. for one hour, replaced in the funnel and washed 3 times with distilled water. The powder on the filter was then macerated in 5 per cent solution of sodium carbonate for 1 hour, the solution allowed to pass through the filter and the powder treated with Hortega's silver carbonate, weak solution (m-2) for 10 minutes. The powder was then treated with a 1 per cent solution of formalin and washed with distilled water. It was then immersed in a 1:500 solution of gold chloride at room temperature until of a bluish gray color, after which it was fixed in a 5 per cent solution of sodium hyposulfite, washed, dehydrated, cleared in xylol and mounted in balsam.

By this method the powdered, anterior lobe showed numerous microglia and oligodendroglia but no pituicytes nor bulbous ends of nerve fibers, whereas the powdered, posterior lobe showed pituicytes and non-medullated nerve fibers with bulbous ends. The non-medullated nerve fibers with bulbous ends are especially diagnostic for the posterior lobe and can also be detected with Delafield's hematoxylin and eosin and with hematoxylin and phosphotungstic acid combinations.

SUMMARY AND CONCLUSIONS.

1. A macroscopic description and illustrations are given for the whole pituitary gland of cattle (*Bos taurus* L.), the anterior lobe of which constitutes about three-fourths the weight of the entire gland.

2. The histological features of sections of beef pituitary are described and differences pointed out between the *anterior lobe*, *pars intermedia* and *pars nervosa*.

3. The *anterior lobe* was found to be composed for the most part of polygonal to irregularly polygonal epithelial cells arranged in solid, branching cords and alveoli, each of which was surrounded by a connective tissue sheath pervaded by capillaries and non-medullated nerve fibers and showing a number of microglia and oligodendroglia.

4. The epithelial cells of the *anterior lobe* are well differentiated through the use of a combination of copper-hematoxylin and eosin stains and Weigert's differentiator containing a mixture of potassium ferricyanide and borax. By this method chromophile cells with blue-colored alpha- and beta-granules can be distinguished sharply from pink-stained chromophobe cells.

5. No cells identical with the alpha- and beta-granule-containing chromophile cells of the anterior pituitary were found in sections of the posterior lobe.

6. Small, rounded basophiles occurred in little groups in the *anterior lobe*, *pars intermedia* and to a less extent in the *pars nervosa*, some sections of the last being entirely devoid of these. They possessed a deep blue nucleus and a lighter blue cytoplasm, when stained by the copper hematoxylin method.

7. The *pars intermedia* is attached to the front of the *pars nervosa* and tends to invade it in places. It differs from other regions of the pituitary by possessing incomplete alveoli of epithelial cells partially surrounded by connective tissue. Moreover, the alveolar cells are eosinophilic in character. It is totally devoid of alpha and beta chromophile cells.

8. The *posterior lobe* consists chiefly of pituicytes and ground substance.

The pituicytes are best demonstrated by the Penfield modification of the Hortega method and are chiefly of the multipolar and bipolar varieties.

9. Distinctions between the powdered, desiccated, anterior and posterior lobes of the pituitary of cattle are presented together with methods of preparing the powdered products for examination.

10. The most diagnostic elements of the powdered, desiccated, anterior lobe are the two kinds of chromophile cells with blue-stained alpha- and beta-granules as observed after treatment with copper hematoxylin and eosin and Weigert's differentiator.

11. The most diagnostic elements of the powdered, desiccated, posterior lobe are the pituicytes (formerly called mossy neuroglia), whose processes were found to be more shrunken and contracted in the powder than in the sections. The second most diagnostic element was found to be the segments of non-medullated nerve fibers with bulbous ends.

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ANTHELMINTICS II. A COMPARISON OF CERTAIN OZONIDES, CHENOPODIUM OIL AND DIHEPTANOL PEROXIDE.*,[1]

BY LEWIS W. BUTZ AND W. A. LA LANDE, JR.[2]

The major component of oil of chenopodium is ascaridole and the anthelmintic action of the oil is due chiefly to this constituent. In a previous paper (1) it was shown that hydrogen peroxide and certain oxygenated terpenes are, like the terpene peroxide ascaridole (Fig. 1), highly toxic to swine ascarids *in vitro*. Since the peroxide function was demonstrable in these oxygenated mixtures, we were tempted to ascribe the anthelmintic effect of all these substances to the peroxide grouping —O—O—. In support of such a theory is the observation (2) that *Ascaris lumbricoides* lives practically anærobically in the intestine and it is, therefore, to be expected that substances capable of supplying a large quantity of active oxygen would seriously interfere with the life processes of the parasite. However, it was shown (1) that the antiascaridic activity of the oxygenated terpenes apparently survived the disappearance of the peroxide function, as indicated by a negative vanadium pentoxide-sulfuric acid

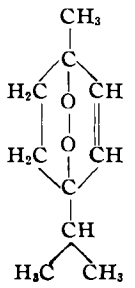

Fig. 1.—Ascaridole.

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[2] From the Laboratory of Dr. D. Jayne & Son, Inc., Philadelphia, Pa., and the Harrison Chemical Laboratory, University of Pennsylvania.