

SCIENTIFIC SECTION

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A NEW FIELD OF INVESTIGATION IN PHARMACOGNOSY: THE MICROSCOPY OF GLANDULAR PRODUCTS.

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While numerous advances have been recorded in pharmacognostic investigation, new vistas opened and many refinements in technique developed during the past decade, a very important field, strange as it may seem, has apparently been overlooked. We refer to the microscopy of powdered, desiccated glandular products.

These substances of animal origin have within recent years increased in the favor of the medical profession and the amounts and kinds used have steadily enlarged. Competition between manufacturers has been keen and imminent danger of adulteration and substitution exists, since no means of identification of the altered tissue elements found in these products has been previously recorded.

It thus became obvious, if these products were to receive official recognition in our works on standards, some means for their adequate standardization would have to be provided. At the request of Chairman E. N. Gathercoal, of the National Formulary Revision Committee, the senior author was induced to try his hand in an attempt to solve what appeared to be an almost unsurmountable problem, *i. e.*, the description of the histological elements found in various of these products. The literature had been searched and no record of any results of such work could be found.

MATERIALS AND METHODS.

The glandular products examined consisted of fresh and preserved pituitary body, anterior pituitary, posterior pituitary, whole ovary, corpus luteum, desiccated strips of these and powdered, desiccated products, specially prepared from authentic materials without contamination and representing the powders of the aforementioned and ovarian residue. Cattle and hogs yielded the products.

Some of the glands were embedded in celloidin, stained, sectioned and studied under the compound microscope in comparison with figures in recognized texts on animal histology. This examination was of help in establishing the relationship of regions and tissues. But it early became apparent that the knowledge gained therefrom was of little value in identifying the altered elements in the powdered products. The next step consisted in separating layers and in teasing apart the various regions of the preserved glands and the macerated dried strips, examining these in water, and in other temporary mounts with various reagents and stains, and in comparing the histological elements observed with similarly mounted powdered, desiccated gland materials.

The reagents and stains employed included the following:

1. Delafield's hematoxylin.
2. Borrel's methylene blue.
3. Mallory's connective tissue stain.
4. Eosin and hematoxylin (1% aqueous solution of each mixed and filtered).
5. Eosin (1% aqueous solution).
6. Osmic acid (0.5% and 1% solution in distilled water).
7. Silver nitrate (1% and 3% solution in distilled water).
8. Picric acid (1% solution in distilled water).
9. Gold chloride (1 Gm. in 35 cc. distilled water).
10. Acid fuchsin (1% aqueous solution with 0.1 cc. diluted HCl added to each 100 cc. of solution).
11. Phosphotungstic acid (1% aqueous solution).
12. Hematoxylin and alum solution.
13. Acetic acid (15% aqueous solution).
14. Iodine water (ss iodine crystals in distilled water).
15. Eosin and methylene blue (1% aqueous solution of each, mixed and filtered).
16. Van Gieson's connective tissue stain (Curtis' modified method).
17. Sulphuric acid (10% and concentrated).

Both the compound and binocular types of microscopes were used in the microscopic examination of the materials in sections and powder. The dissecting microscope was employed in the separation of the layers and in teasing apart the tissues, preparatory to mounting and later study under the compound microscopes.

POWDERED DESICCATED WHOLE PITUITARY.

This occurred as a gray to yellowish gray amorphous powder with a characteristic odor and a saline and disagreeable taste.

The microscopical elements detected were as follows:

Numerous yellowish masses of polyhedral cells surrounded in parts by connective tissue, the latter staining blue with Mallory's stain; numerous large, polyhedral chromophile cells with central rounded nuclei and coarse cytoplasmic granules staining red with acid fuchsin, the nuclei colored blue and the cytoplasm reddish purple with eosin and methylene blue solution; numerous cubical to low columnar chromophobe cells with or without distinct cytoplasmic granules whose nuclei are stained light blue and cytoplasm paler blue with eosin and methylene blue, both chromophile and chromophobe cells frequently with minute fat droplets colored brown to black with 0.5% osmic acid solution; few cells containing a colloidal substance and appearing greenish in water mounts; few segments of blood vessels of tubular, hyaline nature, the cut ends of which showed serrated dark outlines when examined in silver nitrate solution; numerous mossy neuroglia fragments, the cells with spherical nuclei and elongated, branching processes; numerous elongated somewhat ovoid multipolar cells with few bluish black processes when viewed in phosphotungstic acid and hematoxylin reagent, a few small faintly basophilic, polyhedral cells from the pars intermedia with pale blue nuclei and a pink, granular cytoplasm when stained with hematoxylin and eosin; a number of angular hyaline fragments; fragments of nerve fibres with or without a bulbous end, the axons of which are colored mauve with eosin and hematoxylin; a few cells colored black with osmic acid solution; a number of spindle-shaped, bipolar nerve cells.

POWDERED DESICCATED ANTERIOR PITUITARY.

This occurs as a yellowish brown amorphous powder with a characteristic odor and saline taste. It is partially soluble in alcohol, ether, water and acetone.

The histological elements detected were as follows:

Numerous yellowish masses of polyhedral cells surrounded in parts by connective tissue, the latter staining blue with Mallory's stain; numerous large polyhedral chromophile cells with

central rounded nuclei and coarse cytoplasmic granules staining red with acid fuchsin, the nuclei colored blue and the cytoplasm red-purple with eosin and methylene blue solution; numerous cubical to low columnar chromophobe cells with or without distinct cytoplasmic granules whose nuclei are stained light blue and cytoplasm paler blue with eosin and methylene blue, both chromophile and chromophobe cells frequently with fat droplets colored brown to black with 0.5% osmic acid solution; colloidal material occurring between certain of these cells appearing greenish in water mounts; a few scattered cylindrical nerve fibres often attached to fragments of blood vessels, appearing hyaline in water mounts, their axis cylinders staining a mauve color with eosin and hematoxylin solution; few segments of blood vessels with cut ends showing crenate inner endothelial margins and best seen in silver nitrate solution; a few spheroidal cells with reddish brown lipid content colored black with 0.5% osmic acid solution; a few fragments of colloidal substance of greenish aspect in water mounts.

POWDERED POSTERIOR PITUITARY.

This occurs as a yellowish or grayish amorphous powder with a characteristic odor and a saline, disagreeable taste. It is partially soluble in water, alcohol, ether and chloroform.

The following histological elements were detected:

Numerous fragments of neuroglia tissue with spheroidal nuclei and long, slender, branching processes best distinguished with 1% phosphotungstic acid and hematoxylin which stains the nuclei blue and the processes bluish black; numerous spindle-shaped bipolar nerve cells; a number of ovoid, multipolar nerve cells whose cell bodies sometimes contain pigment granules and whose several processes appear bluish black in phosphotungstic acid and hematoxylin mounts; fragments of nerve fibres with or without a bulbous end, some of the bulbous ends of which are surrounded by cells containing a greenish yellow colloidal substance; the axons of the nerve fibres colored mauve with eosin and hematoxylin solution; a few amyloid bodies of ovoid or crescent shape staining a deep purple with iodine water; many irregular hyaline fragments.

POWDERED DESICCATED WHOLE OVARY.

This product occurs as a pale buff to yellowish brown, amorphous powder, the predominance of yellow or brown in the color combination depending upon the ratio of corpus luteum to other ovarian substance, the species of the animal yielding the product and the period at which the ovaries were removed from the animal. It possesses an odor resembling ground mash and a salty, disagreeable taste. It is slightly soluble in water, alcohol, ether and petroleic ether.

The following histological elements were observed:

Numerous young Graafian follicles and fragments of older Graafian follicles, the young follicles appearing as spherical to oval-shaped bodies containing a central cell or oocyte which is colored deep blue with hematoxylin and alum solution; surrounding the oocyte occurs a single layer of flattened follicular cells whose nuclei are stained deep blue with Delafield's hematoxylin, while attached to parts of the follicle is a small amount of connective tissue which is colored pink with eosin solution and blue with Mallory's stain; a few scattered cubical to low columnar and transitional germinal epithelial cells occurring singly or in groups with a round central nucleus and granular cytoplasm, the granules glistening in water mounts, the nucleus staining a deep blue with Delafield's hematoxylin; a number of small, compact masses of dense, white fibrous connective tissue consisting of white collagenous fibres and fibrocytes, the fibres appearing long, narrow, transparent, with distinct, pointed ends, difficult to discern in water mounts but staining a brilliant red in acid fuchsin, the bundles of fibres showing numerous fibrillæ which exhibit a dark outline when mounted in 3% aqueous solution of silver nitrate, the fibrocytes appearing irregularly polygonal to slightly elongated, usually forked at one end in surface view and spindle-shaped in profile view, the nuclei staining a deep blue and the cytoplasm a pale blue to purplish blue with Delafield's hematoxylin; few scattered spindle-shaped, smooth muscle fibres with

centrally placed nucleus clearly visible in gold chloride T.S.; few capillaries of tubular, hyaline nature, occasionally branched and grayish to grayish black in outline with 1% silver nitrate solution; fragments of larger blood vessels with circular to oval cut ends, their endothelial layer at ends being serrated and taking a pink color with eosin and hematoxylin; occasional large spherical cells containing globules of lipoid substance which stains black with 1% osmic acid solution; scattered segments of non-medullated nerve fibres of cylindrical form and consisting of neuraxon and neurolemma, the neuraxon taking a blue color with Delafield's hematoxylin, a mauve color with hematoxylin and eosin solution and a deep red with acid fuchsin; numerous interstitial cells of rounded to ovate form, some of them slightly beaked, containing granules and shining fat globules, their nuclei staining a deep blue and their cytoplasm a pink color with hematoxylin and eosin; numerous lutein cells appearing yellow in water mounts; when the material contains corpora lutea, the cells large, polyhedral to oval, often in masses, each containing a central nucleus, lutein granules and fat droplets.

When 1 Gm. of powdered desiccated whole ovary is mixed with water to form a smooth paste and about 0.5 cc. of sulphuric acid is added to this paste, a reddish brown to deep red color is produced within 15 seconds.

POWDERED DESICCATED OVARIAN RESIDUE.

This represents the whole ovary from which the corpus luteum has been separated by means of a scalpel, ground, dried, powdered and sifted. But the perfect separation of corpus luteum is not always carried out in practice.

The powder, therefore, contains the same elements as that of powdered, desiccated whole ovary but only a relatively small number of lutein cells.

It may also be distinguished from powdered, desiccated whole ovary by the following test:

When 1 Gm. of ovarian residue is mixed with 1 cc. of sulphuric acid, a yellowish green or fig color is produced.

Upon the addition of about 0.5 cc. of old ammonium polysulphide to this mixture, a yellow color is produced which immediately changes to white.

POWDERED DESICCATED CORPUS LUTEUM.

A yellow- to buff-colored amorphous powder with a characteristic, malt-like odor and a saline taste. It is partly soluble in water, alcohol, petroleic ether and ether. The color varies with the stage of pregnancy of the animal and with its age.

The histological features of this product are as follows:

Numerous hypertrophied, yellowish lutein cells occurring singly or in small groups or irregular masses, the lutein cells polyhedral, ovoid, oblong to irregularly elongated with a rounded, central nucleus staining deep blue and cytoplasm staining purple with Delafield's hematoxylin, many of these cells containing fat globules and lutein granules which take a black color with 0.5% osmic acid, the lutein cells and granules staining greenish to greenish blue in 10% sulphuric acid; between various lutein cells in a clump occur connective tissue septa which are colored deep red with acid fuchsin and bluish with Mallory's stain; occasional capillaries of cylindrical shape, sometimes branched, hyaline, and showing black outlines in 1% silver nitrate solution; occasional fragments of large blood vessels whose severed ends exhibit a serrate or crenate endothelium; very few non-medullated nerve fibres, the axon-colored mauve with eosin and hematoxylin, the fibrillæ clearly seen in phosphotungstic acid and Mallory's stain; a few spindle-shaped, smooth muscle fibres with central nucleus colored deep blue with hematoxylin; a number of amyloid bodies colored purple to violet with iodine water; a faint yellow, colorless crystalline substance.

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