SECTIONS

A PHARMACOGNOSTICAL STUDY OF THE PARATHYROID. I.*

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The parathyroid glands occur in all vertebrates except fishes. Those of cattle, sheep, hogs, horses and some other mammals including man have been found to secrete a water-soluble, thermostable hormone. This has been extracted from the parathyroids by Hanson and Collip as a preparation containing the hormone and is found to relieve the symptoms of parathyroid tetany and to increase the calcium content of the blood serum in man and laboratory animals.

Two common forms of preparations of the parathyroid occur in drug channels to-day, namely Solution of Parathyroid (Parathyroid Extract) and Powdered Desiccated Parathyroid (Parathyroid Powder). Both of these have been used in medicine in conditions in which the blood calcium is below normal, such as tetany in infants, certain cases of malnutrition, paralysis agitans, etc., their administration being controlled by frequent examination of the blood for calcium. The solution is largely preferred.

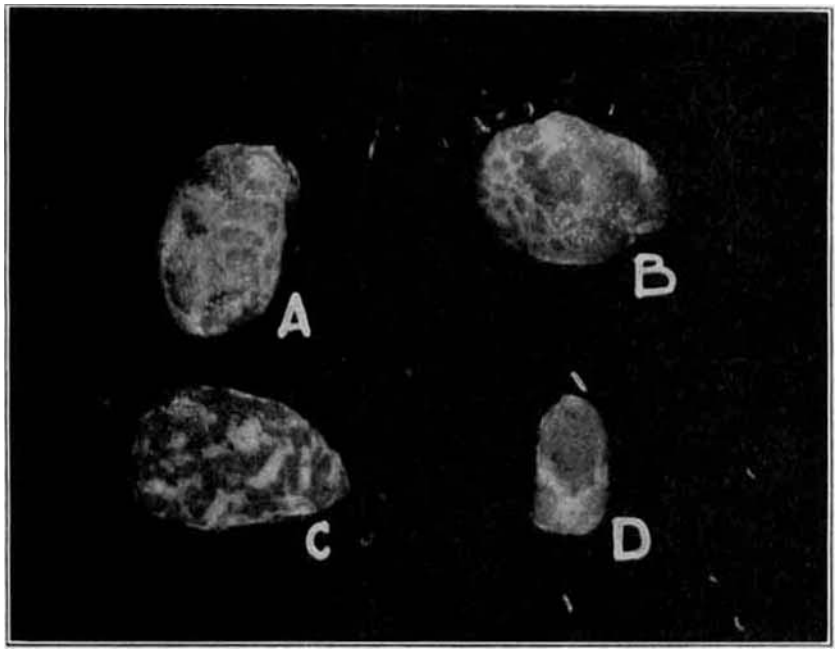

Fig. 1.—Parathyroid Glands of Cattle, × 1 1/5. A, Gland showing inner surface. B, Gland showing outer surface. C, Longitudinally halved gland showing cut surface. D, Transversely cut section of gland. The white areas in C and D represent connective tissue trabeculæ which divide the parenchyma of the gland into somewhat poorly defined lobules.

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The raw material from which these medicaments are prepared consists of the fresh parathyroids of healthy domesticated animals used for food by man. At the present time cattle appear to yield most of it.

Owing to certain similarities existing between powdered desiccated parathyroid and thyroid and the relatively higher cost of production of the former, and also because of the close anatomical relationship of these glands in animals, it is conceivable that powdered parathyroid could readily be adulterated with thyroid and that either product might be admixed with the other without detection unless some means of microscopical differentiation were available.

The chief aim of this investigation, therefore, was to endeavor to find a method whereby parathyroid powder might be distinguished from powdered thyroid microscopically or microchemically. Other objectives were to describe the whole and powdered desiccated parathyroids of cattle.

The literature on the pharmacognosy of the parathyroid is very meager. Considerable, however, has been written on the action and uses of extracts of this gland.

In 1855, Robert Remak (1), a German physiologist and embryologist, first discovered the parathyroids. In 1880, Ivar Sandström (2), a Swedish anatomist, established their occurrence in the dog, cat, ox, horse, rabbit and man and published a description of them limiting a detailed description to those of man. He believed them to represent displaced, undeveloped fragments of thyroid tissue. In 1891, Gley (3) demonstrated that they functioned independently.

In 1897, E. Gley (4) published a paper on the effects of extirpating the thyroid glands in the dog and rabbit wherein the animals were shown to develop the symptoms of parathyroid tetany, a condition marked by violent convulsions. This condition was shown to be relieved temporarily by the intravenous injection of calcium salts.

Until the early part of the twentieth century, however, only very brief consideration of these glands was given in the anatomical works. One of the earliest anatomical textbooks to give a good description of the human parathyroid glands including their histology was Piersol's "Human Anatomy" (5) published in 1907 which contains photomicrographs illustrating their inner structure. More extensive treatment of the histology of the human parathyroid has been given in later works on histology, especially those of Maximow and Bloom (6) and Bremer (7).

In 1932, R. Wasicky (8) briefly described the parathyroid glands and the preparation of the extract and stated their location in the horse, cattle, sheep and hogs. The same author in his "Leitfaden für die Pharmakognostischen Untersuchungen" briefly described and figured a teased-out preparation of the fresh parathyroid (9).

MATERIALS AND METHODS.

The materials studied included whole fresh and desiccated parathyroid glands of beef, and powdered desiccated parathyroid and thyroid glands of beef. The whole glands were examined *in situ*. A number of whole glands were fixed and preserved in formol-alcohol and in formol-acetic-alcohol. Segments of these were later dehydrated and imbedded in paraffin, and later sectioned, stained and mounted in balsam for microscopical examination. A variety of single and double stains were employed as well as chemical reagents which will be discussed later in this paper under the description of the sections and the powdered materials.

The powdered desiccated glands were variously treated with a number of single and double stains on slides, in watch glasses and by the centrifuge method, all of the stains excepting Mallory's

and phosphotungstic acid being diluted with water. In the watch-glass method, the powder was placed in a Syracuse watch glass, the diluted stain introduced and allowed to act for a variable length of time depending upon the stain or reagent used. By means of an eye dropper, the stain was removed and any excess washed out of the powdered fragments by water or alcohol. It was necessary to place the watch glass containing the stained powders on the stage of the microscope in some instances and examine the effect of the stains in order to prevent over- or under-staining. After staining, the powder was either transferred to a clean slide and mounted for microscopic examination in water or glycerin solution or run through 50%, 70% and 95% alcohols, followed by clove oil and xylol and mounted in balsam as a permanent mount.

By the centrifuge method, a portion of powder was placed in a clean centrifuge tube, the diluted stain introduced and the mixture centrifuged for several minutes. The tube was then removed from the centrifuge, and the stain decanted from the powder packed at the bottom, water introduced into the tube and the contents again centrifuged in order to wash out excess of stain, and the washing decanted. With some stains it was necessary to employ several washings of water for the removal of excess stain. The powder was then centrifuged successively with ascending grades of alcohol through absolute alcohol and xylol, the xylol mostly decanted and powder removed by a pipette and mounted in balsam for microscopic examination. The stained sections and powders were studied under a biological microscope equipped with a 10 × ocular and with 16 mm., 4 mm. and oil-immersion objectives.

The glandular materials used in this investigation were supplied generously by Armour & Co., the Wilson Laboratories and Parke, Davis & Co., to whom my thanks are due.

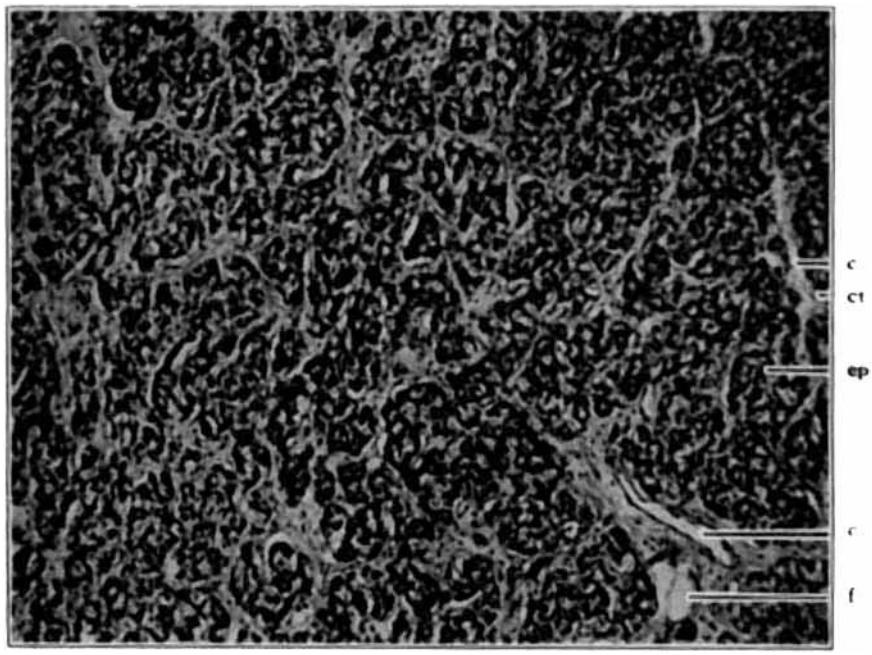

Fig. 2.—Representative Portion of a Transverse Section of the Parathyroid Gland of Cattle, ×100. Note the dark-colored anastomosing columns of epithelial cells (ep), surrounded by light-colored, reticular connective tissue (ct) containing a capillary network. c, longitudinal sections of capillaries; f, fat tissue. The section was stained with hematoxylin and eosin.

DESCRIPTION OF WHOLE PARATHYROIDS OF CATTLE.

The parathyroid glands of cattle are small, red or yellowish, oval, elliptical or pyriform bodies situated near the ventral border or toward the middle of the posterior surface of the lateral

lobes of the thyroid. They are claimed by embryologists to be produced by the third and fourth pharyngeal pouches as epithelial (entodermal) bodies which become attached during development to the posterior surface of the thyroid gland. Those examined by the writer were up to 2 8 cm. x 1.5 cm. x 0.8 cm. They were lying in connective tissue on the surfaces of the lobes of the thyroid, not imbedded in the surface or attached to it by pedicles as in some other animals. Each was surrounded by a fibrous capsule of connective tissue which supported blood vessels. (See Fig. 1.)

The whole desiccated parathyroids examined occurred in plano-convex pieces, representing longitudinally sliced halves, rarely as bean-shaped entire glands from 1 to 2.7 cm. in length, from 0.8 to 1.4 cm. in breadth and from 0.4 to 0.7 cm. in thickness; externally pale yellow, with brown blotches, dull and waxy and showing on the convex surfaces irregular depressions, lines or ridges, the latter on some glands appearing like blisters; texture spongy and oleaginous; internally pale yellow, waxy and oily with irregular cavities; odor suet-like; taste oily and saponaceous.

HISTOLOGY OF THE PARATHYROIDS OF CATTLE.

Transverse sections exhibited an outer zone of loose connective tissue in which were imbedded sections of blood vessels and which surrounded the main body of the gland composed of numerous epithelial cords disposed as solid masses and branching columns. Between the cords was to be noted a framework of reticular fibers and here and there sections of sinusoidal capillaries, some containing blood corpuscles. (See Fig. 2.) The capillaries form a veritable network in the parenchyma. Some of the capillaries were in close relation to the epithelial cells of the cords. Scattered through the gland were many areas of fat tissue. Fat globules were visible in many of the epithelial cells in teased-out preparations.

The epithelial cells were polygonal to rounded polygonal and varied considerably in size. There appeared to be several types of these, the principal, oxyphil and intermediate types. The principal type possessed large nuclei of varying shape, chiefly spheroidal, oval, oblong or irregularly fusiform and pale clear cytoplasm. The oxyphil type differed from these in being somewhat larger and in possessing numerous granules in their cytoplasm which were stained red in sections stained with hematoxylin and acid fuchsin. They resembled the oxyphil cells depicted by Maximow and Bloom (6). Other epithelial cells of the cords showed characters intermediate between the principal and oxyphil types. The epithelial cells formed dense masses and anastomosing columns. Occasional acini (follicles) were observed in some of the sections, but only in one out of a large number of glands sectioned by the writer was colloid observable and this was not abundant. Colloid has been reported in the acini or follicles of the human parathyroid by Maximow and Bloom (6) and by Bremer (7). It appears to be the result of degenerative changes in the organ and not a normal constituent of it.

With equal parts of Mallory's stain and 1% phosphotungstic acid solution the epithelial cells were stained brown, the connective tissue fibrils a deep blue and the other connective tissue elements a bluish green to blue, the walls of the arterioles blue.

With Delafield's hematoxylin and eosin the nuclei of the epithelial cells were colored blue, the cytoplasm of these purple to pinkish-purple, the connective tissue, including the reticular fibers, faintly pink and the nuclei of the connective tissue cells, blue.

With copper hematoxylin and eosin the effect of the staining was similar, but sharper.

With Delafield's hematoxylin and acid fuchsin, the nuclei of the epithelial cells were colored blue to purple, the cytoplasm red to deep pink and the cytoplasmic granules of the oxyphil cells and intermediate cells (containing smaller granules) appeared pink and refractile.

With Weigert's hematoxylin and acid fuchsin, the nuclei of the epithelial cells were colored blue, the cytoplasm purple and the cytoplasmic granules of the oxyphil and intermediate types, purple to red. The reticular fibers were colored pinkish-purple to purple, the nuclei of connective tissue cells, blue.

An examination of sections of the thyroid gland of cattle stained with Delafield's hematoxylin and eosin and with Mallory's stain and phosphotungstic acid showed striking differences from the parathyroids of the same animal. In the thyroid numerous follicles containing colloid and lined by epithelial cells of one type and separated from each other by narrow strands of reticular connective tissue were apparent. This contrasted sharply with the solid masses and anastomosing columns of epithelial cells and the total absence of follicles containing colloid in most of the sec-

tions of parathyroid. Moreover, the reticular tissue between the follicles of the thyroid appeared averagely less abundant than between the masses and columns of epithelial cells in the parathyroid. Further, at least three types of epithelial cells were observed in the sections of parathyroid, whereas only one type of these was clearly discerned in the thyroid.

POWDERED DESICCATED PARATHYROID OF CATTLE.

The samples of powders examined varied in color from pale yellow to light buff. When treated with Mallory's stain and 1% phosphotungstic acid solution, equal parts, the epithelial cells were colored yellow through orange to brown, depending upon the thickness of the fragments, the connective tissue blue to greenish-blue. Reticular fibers were noted in some of the mounts projecting from fragments of epithelial cells and appearing curved.

With Bismark Brown, 0.5% sol. in 50% alcohol, diluted with an equal quantity of water, the cytoplasm of the epithelial cells was stained varying shades of yellow and brown while the cytoplasmic granules appeared globular and refractile. The connective tissue appeared yellow to brown in this stain.

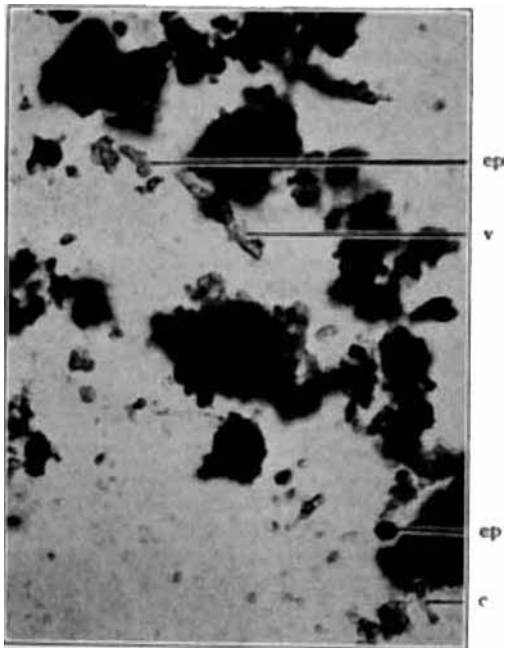

Fig. 3.—Powdered Desiccated Parathyroid. Photomicrograph, ×200. ep, epithelial cells; v, blood vessel; c, connective tissue fiber with attached epithelial cells. The dark masses represent fragments of the parenchyma of the gland, from some of which reticular fibers are seen to project. ep′, small mass of epithelial cells with two reticular fibers projecting from upper portion.

With diluted Delafield's hematoxylin the nuclei of the epithelial cells were colored blue, the cytoplasm purple, the connective tissue fibers light purple. The epithelial cells measured up to 12 microns in diameter.

With Weigert's hematoxylin and acid fuchsin, diluted with an equal amount of water, the nuclei of the epithelial and connective tissue cells were colored blue, the cytoplasm pink and the fibers red, pink or pinkish-purple.

With equal parts of a mixture of Delafield's hematoxylin, eosin and water, to which a drop of glycerin was added before applying the cover slip, the epithelial cells were colored pinkish-purple to purple in their cytoplasm and cytoplasmic granules were brought out very distinctly. The connective tissue fibers were stained pink to pinkish-purple. In only one of many mounts examined, two fragments of colloid were detected which were stained pinkish-purple. The same amount of powdered beef thyroid similarly stained and mounted showed numerous colloid fragments.

In contrasting the microscopic picture of powdered parathyroid with powdered beef thyroid, it was found that colloid fragments are common and abundant in all mounts of thyroid whereas they were unusual in parathyroid material examined and only detected once after examining a large number of mounts. Other means of distinguishing between powdered desiccated thyroid and parathyroid were also tried.

It was found that when a small amount of powdered desiccated thyroid was sprinkled on the surface of water, it almost immediately sank. When the same procedure was tried with powdered desiccated thyroid, the particles floated for a long time. This can be attributed to the fact that parathyroid contains considerable fat while thyroid does not.

The color reactions produced by treating the two powdered glands with various chemicals in white porcelain dishes proved interesting. When about 5 mg. of each drug were treated with 2

or 3 drops of a reagent consisting of equal volumes of 5% alcoholic solution of vanillin and concentrated HCl, powdered thyroid was colored brown and powdered parathyroid pinkish-brown changing to pink. Solution of NaOCl colored thyroid tan-brown and parathyroid yellow. Millon's reagent colored thyroid reddish brown and parathyroid light reddish-brown. While the color tests are helpful, they are not conclusive in determining the identity of the material. Further studies are contemplated especially to determine other microscopic differences between these powdered endocrine glands.

SUMMARY.

1. The history of the parathyroid is reviewed.

2. A description is presented of the whole fresh and whole desiccated parathyroid glands of cattle.

3. The histology of the bovine parathyroid and the appearance of its cells after treatment with various stains are described.

4. It is shown that follicles are relatively few in sections of the parathyroids of cattle and that numerous solid masses and anastomosing cords of epithelial cells separated by reticular tissue rich in capillaries make up the bulk of the parenchyma, whereas in sections of the thyroid of the same species follicles surrounded by connective tissue containing capillaries make up the greater portion of the parenchyma while occasional masses of epithelial cells (interfollicular epithelial cells) were relatively few.

5. All the follicles of the thyroid of cattle contained colloid and this material represented the most conspicuous element present, whereas only occasional colloid-containing follicles were present in the parathyroid.

6. Several types of epithelial cells occurring in the bovine parathyroid are described. Only one type was noted in the bovine thyroid.

7. The results of the study of powdered desiccated parathyroid of cattle are presented. It was found that colloid fragments were unusual and exceedingly rare in the materials examined, whereas these occurred as the most conspicuous elements in the powdered thyroid of this and other species recorded.

8. Several qualitative color tests for powdered parathyroid and thyroid are described.

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