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PARATHYREOTROPIC ACTION OF THE ANTERIOR PITUITARY: HISTOLOGIC EVIDENCE IN THE RABBIT

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In December, 1932, we received a preliminary impression that intramuscular injections of saline emulsions of anterior lobes of beef pituitary glands gave rise to enlargement and increased vascularity of the parathyroid glands of rabbits. As far as we could discover at the time, no previous reference had been made to such an effect of anterior lobe substances. We, therefore, undertook more extensive experiments to collect data on the gross and microscopic anatomy of the parathyroid glands of rabbits under treatment with various anterior lobe substances, pregnancy urine and control injections of brain tissue emulsions.

PLAN OF EXPERIMENTS AND METHODS

Rabbits of the same age, sex and strain (blue bevern) were used in each of our early experiments. These factors were later varied singly; and since it was found that neither sex, age nor strain had any modifying effect, females of mixed strains were used in later experiments. Rabbits were segregated as to sex in order to avoid the complicating factor of pregnancy. Animals used in individual experiments ranged from 10 to 22 weeks of age. They were fed on rations of oats, greens, fresh carrots and occasional additions of cow's liver. No cod-liver oil was added. Normal control animals (untreated) were healthy and survived in excellent condition in the hygienic atmosphere of the animal farm except for a rare attack of diarrhoea in the younger members of the colony.

Intramuscular injections were carried out with sterile precautions after preparation of the skin with alcohol. The materials listed below were assayed for a qualitative effect on the parathyroid glands. Animals were kept under the conditions of the laboratory for a preliminary control period before any injections were given.

Fourteen animals received saline suspensions of fresh bovine anterior lobes which were obtained daily from the slaughter house directly after the death of the cows. The pituitary glands were kept cold and the anterior lobes were dissected free within one to three hours. These were then ground in saline with mortar and pestle. Care was taken to have all the utensils sterile. When the material was ground to a consistency fine enough to pass through a large bore needle it was diluted with saline so that 5 cc. was usu-

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Three animals received heated suspension of this material (boiling water for 5 to 10 minutes). Two animals received emulsions of brain tissue prepared in a similar fashion. Twelve animals were kept under the same housing conditions, but received no injections.

The above materials were coarse and not sterile. They frequently caused local abscesses. In subsequent experiments, sterile extracts were used. They were prepared as follows:

1. Saline emulsion of anterior lobe was passed through a Seitz filter for sterilization. Two animals were treated with this extract in daily dosage equivalent to 1.5 grams of tissue, for five days.

2. Acid extraction of anterior lobe was carried out with acetic acid (pH 4.8 to 5.0) and six animals received daily injections of 1 gram equivalent for from three to 28 days.

3. Alkaline extraction was carried out with sodium hydroxide at pH 8.0 to 8.5, and sterilization was obtained by passage through a Seitz filter. Six animals received the equivalent of 1 gram of tissue of this material in daily dosages for four to six days.

4. Acid extraction was carried out following preliminary alkaline extraction and used in two animals in dosage of 1 gram equivalent of tissue, for four days.

5. Alkaline extraction, following preliminary acid extraction, was performed and this material was administered to one animal in dosage of 1 gram equivalent for six days.

The above sterile filtrates were kept on ice for a period of seven to eight weeks (at pH 7.4) and used for the injection of four additional animals, as an assay of stability under these conditions.

Progynon, Antophysin, Thyreotropic Hormone and Parke-Davis Growth Hormone were injected into a group of seven animals. Pregnancy urine, prepared as for the Friedman test, was injected into five animals for one to four days. Urine of two non-pregnant individuals was similarly injected into control animals.

Animals were killed by bleeding, pithing or etherization. The parathyroid glands were removed immediately after death of the animals and fixed in Zenker's solution and 10 per cent alcoholic formalin. The usual methods of dehydration and paraffin imbedding were used and sections were cut to a thickness of 5 to 7μ. Haematoxylin-phloxin and phloxin-methylene blue stains were employed.

RESULTS

The clinical picture of the rabbits which received active pituitary materials was strikingly different from that of animals receiving control injec-

tions or of the untreated animals. Figures 1A and 1B illustrate two littermate animals which were treated respectively with identical dosages of fresh and heated saline emulsions of beef pituitary glands. The loss of weight, muscular weakness and atonia, which the animal in Fgure 1A showed, were seen in varying degrees in all animals which received injections of active pituitary extracts.

Notes were made as to the size and gross appearance of the parathyroids. Microscopic descriptions were made of each gland sectioned but will not be included in this report. They are, however, the basis for the following descriptions.

Anatomy of the Normal Rabbit's Parathyroid Gland. The rabbit has two main parathyroid glands. They are situated lateral to the internal carotid artery and at varying distances from the inferior-lateral aspect of

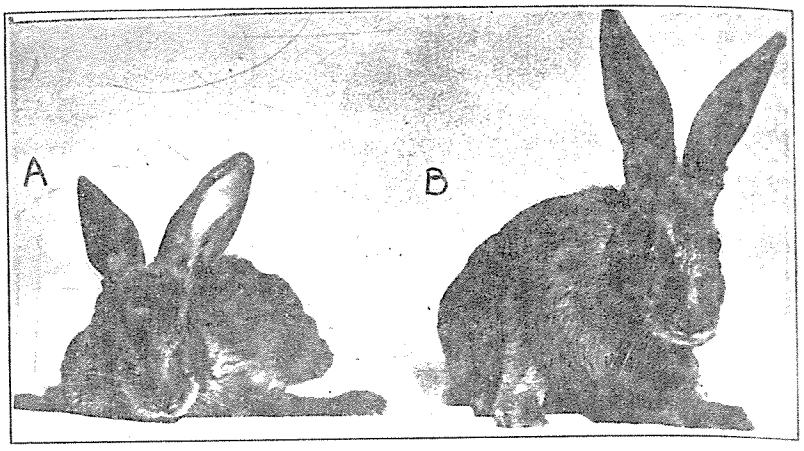

Figure 1. A—Photograph of rabbit No. 22, showing typical prostration and muscular atony of an animal which received injections of emulsions of fresh bovine anterior pituitary glands. B—Photograph of rabbit No. 9, an animal which received injections of heated emulsions of bovine anterior pituitary glands.

each thyroid lobe. There is considerable variation in their size, but their shape is quite constant. They are rounded at their upper poles and measure about 1 to 2 mm. in breadth at that, their widest point. They taper longitudinally for about 0.5 to 0.75 cm. to become attached to the carotid fascia in which they are enveloped. The main parathyroids are occasionally constricted at their mid-point, giving the impression of being bilobed. They are at times situated quite low in the carotid sheathe, close to the mediastinal space.

Glands were not weighed in our gross study because it is our belief that such determinations are subject to too great an error. Differences in amounts of capsule, fat, fascia, thyroid and muscle to various glands in microscopic sections indicated some of the sources of such error. It is further our belief that these measurements are vitiated by varying degrees of

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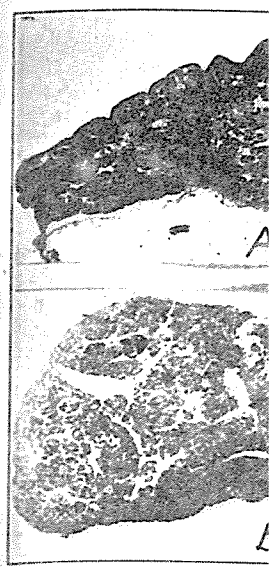

Figure 2. A—Photomicrog
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Normally, the parathyroid gland is white and glistening. It may also have a pink color, but as a rule is not injected on its flat presenting surface. The larger blood vessels may be visible.

The normal accessory parathyroid glands are inconstantly and rarely seen at post-mortem, but were found quite regularly in our miscroscopic examinations of the thyroid glands.

Microscopic Examination. The appearance of a normal rabbit's parathyroid under low magnification (x 100) is illustrated in Figure 2, Photomicrograph A, which is that of a section of the parathyroid gland of Rabbit 622, an untreated animal. The striking feature of the section is the

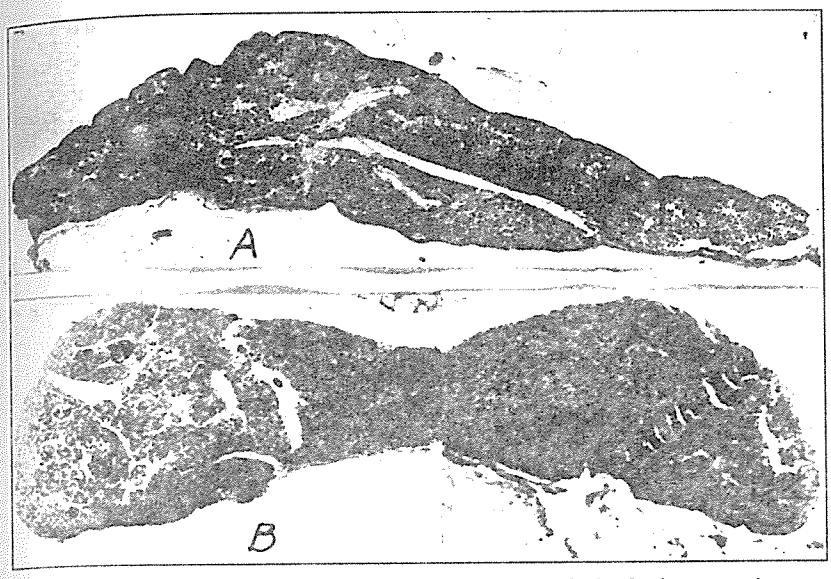

Figure 2. A—Photomicrograph (x 100) of the parathyroid gland of a normal untreated animal (No. 622). B—Photomicrograph (x 100) of the parathyroid gland of an animal which had received 5 daily injections of an acid extract of bovine anterior pituitary glands (No. 699).

prominence of the interalveolar septa. Its fenestrated structure is in distinct contrast to the solid and dense appearance of a similar section of the parathyroid gland of a pituitary treated animal 699 (see Figure 2, Photomicrograph B).

Examination at higher magnification (x 430) reveals that the normal parathyroid is not uniform in its structure. The peripheral zone of alveoli is densely packed beneath the fibrous capsule of the gland. In this area the alveoli are smaller than in the remainder of the gland, but the individual cells are larger. Vacuolization and a rare mitotic figure, when found in the glands of normal animals, are situated in this zone, the "Randpartie" of Erdheim's descriptions. The centrally located alveoli vary in size and shape. They contain from one or two to ten cells per alveolus. They are

crescentic in shape and are made of one or, at most, two cell layers. The range of alveolar size under normal conditions is given in Table I, which also presents measurements of cell, nuclear and vacuolar diameters in these same normal parathyroid glands (Animals 622, 700 and 606). These and all measurements included in this report were made with graduated ocular at oil immersion magnification (x 970).

Figure 3A is a camera lucida drawing, illustrating a normal parathyroid gland as seen under oil immersion magnification (x 970). It shows widely separated alveoli of small, though not uniform size. The cells are small and mitoses, pyknoses and vacuoles are absent or rare. The alveoli are one cell deep and infolding of the epithelium is not seen. The stroma is fairly prominent. (Compare with 3B.)

Anatomy of the Parathyroid Glands of Animals Treated with Active Pituitary Substances. Parathyroid glands from animals treated with various types of anterior pituitary preparations were examined. The details of extractions employed and of dosages used are indicated below.

TABLE I

RANGE OF ALVEOLAR, CELL AND NUCLEAR SIZE IN THE PARATHYROID GLANDS IN THREE NORMAL RABBITS AND IN THREE REPRESENTATIVE RABBITS TREATED WITH ACTIVE ANTERIOR PITUITARY SUBSTANCES

Rabbit Number	Alveolar Range mm/100	Cell Range mm/100	Nuclear Range mm/100
	NORMALS		
606	7 x 7 to 70 x 77	4.2 x 5.6 to 7.0 x 8.4	2.8 x 2.8 to 4.2 x 5.6
700	7 x 28 to 56 x 70	5.6 x 7. to 5.6 x 9.8	1.4 x 1.4 to 4.2 x 5.6
622	7 x 14 to 42 x 49	4.2 x 8.4 to 5.6 x 9.8	4.2 x 4.2 to 4.2 x 6.3
	TREATED		
699	28 x 35 to 42 x560±	8.4 x14. to 9.1 x16.8	2.8 x 5.6 to 5.6 x 5.6
624	11 x 14 to140 x238±	5.6 x12.6 to 7. x14.	4.2 x 4.2 to 5.6 x 7.
683	21 x 21 to196 x350±	7. x 7. to 5.6 x14.	4.2 x 4.2 to 5.6 x 8.4

On gross examination it was found that the parathyroids of pituitary treated animals were larger than those of the reference controls. This was, however, not invariably true. More constant than the increase in size was the finding of vascular engorgement in the glands of injected animals. For the most part, there existed considerable correlation between the degree of vascularity, size, color and the microscopic appearance of the glands.

The most significant differences between the normal and injected glands were found on microscopic examination. Figure 2B is a photomicrograph (x 100) of the parathyroid gland of Animal 699. This animal received five daily intramuscular injections of an acid extract of beef pituitary containing 1.3 grams of anterior lobe tissue per dose. A comparison with the normal gland at the same magnification (Figure 2A) shows the compact arrangement of the glandular elements, the disappearance of the wide inter-

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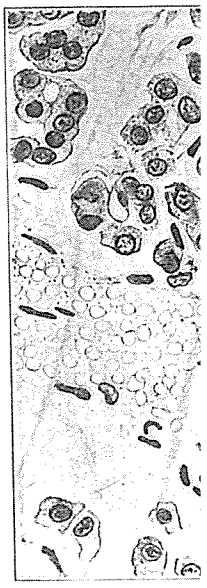

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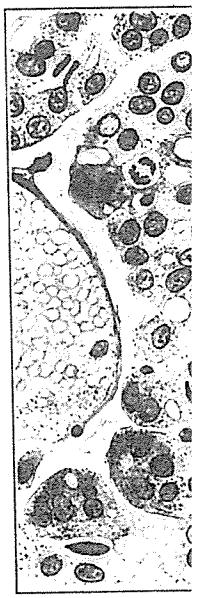

Figure 3. A—Camera lucida
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