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

SAUL HERTZ, M.D.* AND ALFRED KRANES, M.D.†
From the Medical Service and Metabolism Laboratory,
Massachusetts General Hospital
BOSTON

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-

*Dalton Scholar and Graduate Assistant in the Department of Medicine, Massachusetts General Hospital.
†Assistant Resident in the Department of Medicine, Massachusetts General Hospital.

ally equivalent to the tissue obtained from half of an anterior lobe. This was then drawn into a syringe and injected into the thigh muscle of the rabbits.

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 injections or of the untreated animals. Figures 1A and 1B illustrate two litter-mate 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

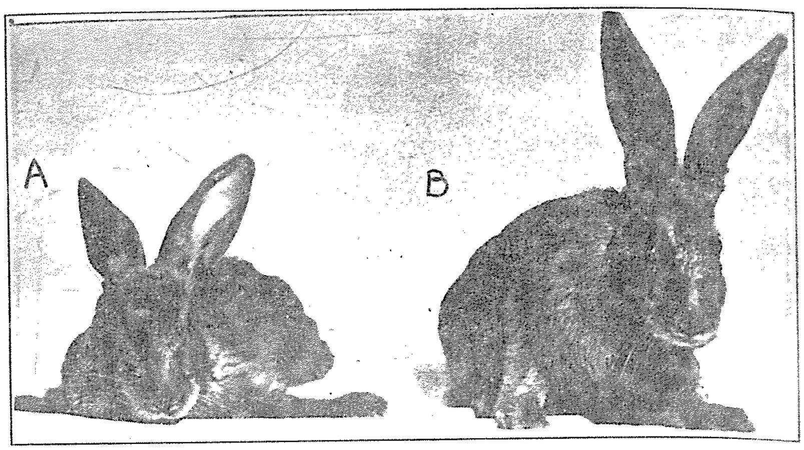

Figure 1. A—Photograph of rabbit No. 22, showing typical prostration and muscular atony of an animal which received injections of emulsions of fresh bovine anterior pituitary glands. B—Photograph of rabbit No. 9, an animal which received injections of heated emulsions of bovine anterior pituitary glands.

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 evaporation as well as the inevitable differences in the nutritional state of the animals.

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

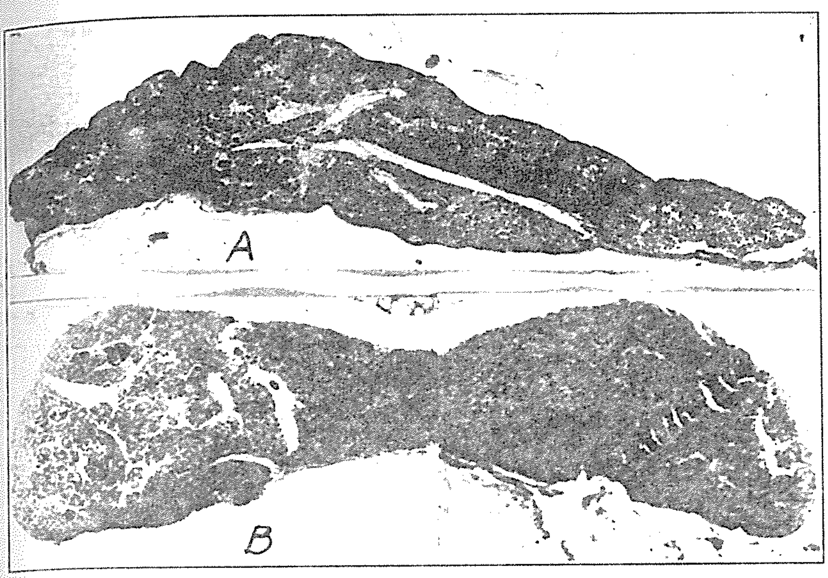

Figure 2. A—Photomicrograph (x 100) of the parathyroid gland of a normal untreated animal (No. 622). B—Photomicrograph (x 100) of the parathyroid gland of an animal which had received 5 daily injections of an acid extract of bovine anterior pituitary glands (No. 699).

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 vari-

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

ous types of anterior pituitary preparations were examined. The details of extractions employed and of dosages used are indicated below.

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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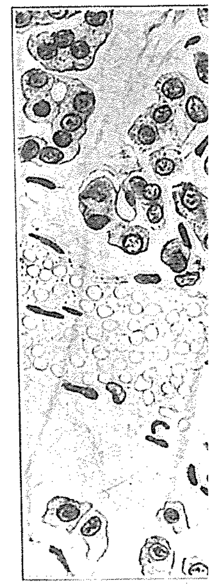
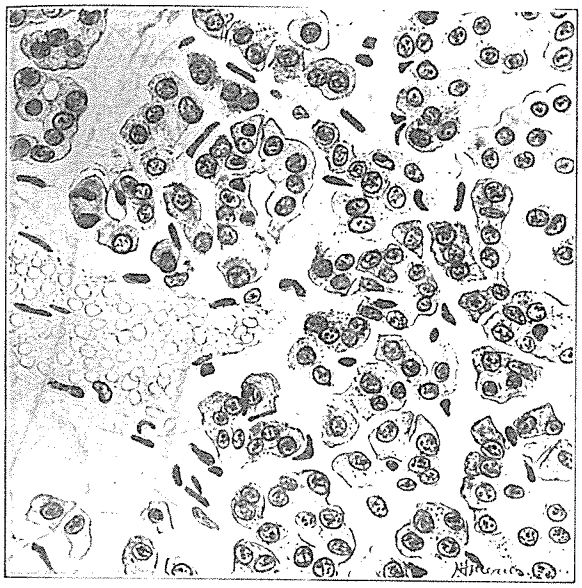


an entirely similar drawing
gland (i.e., No. 606, a refe

Figure 3. A—Camera lucida
normal untreated animal (No. 62
lucida drawing of the appearance o
699) at oil immersion magnificatio

alveolar septa and the general increase in epithelial cellularity. The appearance of this same section of parathyroid gland from Animal 699 under higher magnification is illustrated in Figure 3B, which is a camera lucida drawing under oil immersion (x 970). It is to be compared with Figure 3A,



an entirely similar drawing of a normal untreated animal's parathyroid gland (i.e., No. 606, a reference control). Figure 3B shows a marked

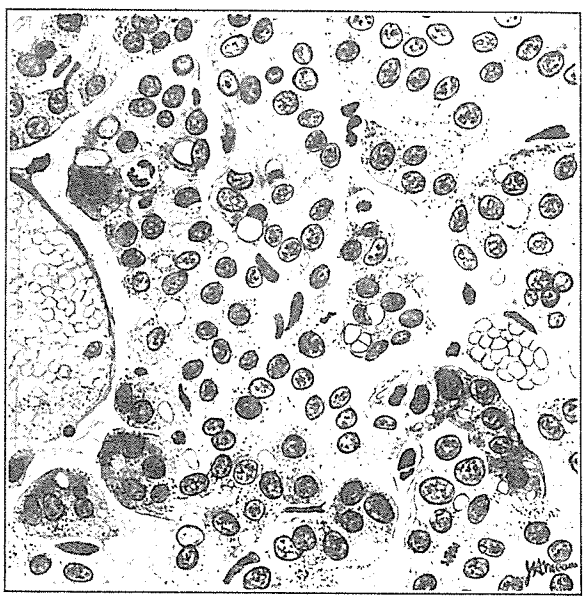


Figure 3. A—Camera lucida drawing of the appearance of the parathyroid gland of a normal untreated animal (No. 622) at oil immersion magnification (x 970). B—Camera lucida drawing of the appearance of the parathyroid gland of a pituitary treated animal (No. 699) at oil immersion magnification (x 970).

increase in cellularity, enlarged alveoli, high cells and active growth as evidenced by numerous mitotic figures (3 mitoses and 2 pyknoses in a single field). Large vacuoles in the cells indicate a probable increase in secretory activity. The blood vessels are increased in number as evidenced by the numerous endothelial cells interspersed in the perialveolar spaces as well as between the alveolar cells. The cell nuclei are lighter staining and larger than in the normal gland. Pyknoses are common. The nuclei are spaced farther apart than in the normal parathyroid because of the increased size of the cells.

It would require a description of each gland studied to give an impression of the intermediate stages between the normal parathyroid histology and that of the hyperplastic gland illustrated in 3B. At this point, however, we wish to call attention to the difference in histological changes in glands which have been rendered hyperplastic at different rates. It is our impression that animals which were subjected to large doses of active material over a short period of time showed parathyroids with marked increase in number and change in arrangement of cells without much increase in size of cells. This type we have designated as "conglomerate alveolar" or "mulberry type" of hyperplasia in our own discussions. An example of this type is given in No. 683 with measurements of the acini, cells and vacuoles (see Table I).

A more chronic treatment of the animal gave rise to the type of hyperplasia that is illustrated in Animal 699 (see Table I and Figure 3B). This type is characterized by moderately increased number of cells, but with predominant enlargement and vacuolization of cells. Mitoses were seen in both types, but more readily in this second type which we have designated "large vacuolated cell type." We have seen both of these histological pictures in tumors removed from human cases of hyperparathyroidism at the Massachusetts General Hospital.

Saline suspensions, acid and alkaline extracts, pregnancy urine, Antophysin (Winthrop Company), Progynon, Parke-Davis Alkaline Growth Hormone, and Thyreotropic Hormone (Schering and Kahlbaum) were found to produce varying degrees of parathyroid hyperplasia. Brain tissue emulsions, heated pituitary suspensions and urine of non-pregnant individuals had no effect on the parathyroid histology. The control animals which received no injections showed none of the changes described above. Occasional glands in the control group showed increased vacuolization, but did not satisfy the other criteria for hyperplasia. Extracts which were assayed after a lapse of seven to eight weeks showed that the parathyreotropic effect was retained to some extent at ice box temperature and pH 7.4.

DISCUSSION

The extensive literature which has accumulated in recent years on the relationships of the anterior pituitary to the other endocrine organs contains but meagre clinical and experimental reference to the parathyroid glands.

In his monograph on the pituitary body Cushing (1) called attention to a group of cases showing primary hypophyseal disease with associated alterations in other members of the endocrine series. In his more recent writings (2a, 2b), he has delineated a clinical syndrome clearly enough to enable the prediction of the port-mortem finding of an hypophyseal basophilic adenoma.

In 1912, Schmorl (3) mentioned a case of typical osteitis fibrosa cystica in which he found the association of a large basophilic adenoma of the hypophysis with diffuse adenomatous hyperplasia of the parathyroid glands. Schmorl did not suspect a relationship between the two adenomatous growths, but Molineus (4), in reporting the same case in greater detail, made the suggestion of such a possible relationship. He quoted Fischer's contention that "acromegaly was absent in this case because of the disease in other glands (i.e., parathyroids)." The frequent association of acromegaly with eosinophilic tumors of the pituitary was not clear at that time.

Erdheim (5) stated that he had seen the association of osteitis fibrosa cystica and basophilic adenoma of the pituitary in two necropsied cases.

Lloyd (6), in 1929, reported a case from the Johns Hopkins Hospital Department of Pathology, which presented the post-mortem findings of tumor-like enlargements of the parathyroids and islets of Langerhans in association with hypophyseal tumor (type not given). No clinical data were available in that case from which to decide whether the parathyroid or islet tissue had had excessive functional activity during life.

In Cushing's descriptions of patients having pituitary basophilism, terms such as kyphosis, lordosis, multiple fractures, marked weakness, polyuria, decrease in height, renal change, albuminuria and so forth are frequently found. On the pathological side, "bones soft enough to cut with a knife," osteoporosis, compression of vertebrae and healed fractures were commonly encountered. All these things are now known to be among the classic manifestations of hyperparathyroidism.

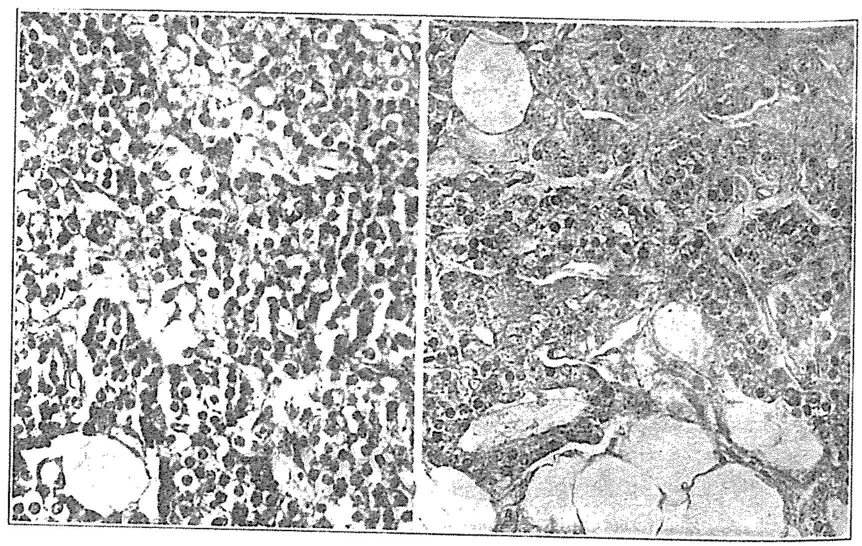
In Case No. 9 of Cushing's series (2a), a single parathyroid was examined and was reported as showing "an increase of fat and connective tissue." However, in a personally autopsied case, Dr. Cushing reported two parathyroid glands as being slightly enlarged (measured 7x5x2 mm. and 10x5x3 mm.). Histological examination was reported as follows: "Essentially normal apart from the fact that two-thirds of the gland is composed of fat" (2b). On review of a section with Dr. Cushing, we were convinced that the gland in question was truly hyperplastic and satisfied the criteria of Erdheim, which were used throughout our study of animal parathyroid glands.

Microphotographs of a normal human parathyroid and of the gland of Dr. Cushing's case are presented in Figure 4 with his kind permission.

They give additional histological evidence of a parathyreotropic effect of the anterior pituitary.

From the experimental point of view, P. E. Smith (7) described the disabilities caused by hypophysectomy in the rat. He mentioned briefly that the parathyroid glands, among others, underwent atrophy after hypophysectomy. No statement was made at that time with regard to the question of parathyroid repair after hypophyseal replacement therapy by transplantation. Schour and Van Dyke (8) described shrinkage of epiphyseal cartilages and gradual obliteration of the pulp cavities of the incisors by dentin, as a result of hypophysectomy in the rat (1931).

Evans, in a recent review of anterior pituitary function (9) summarized the situation with regard to the parathyroid as follows: "There seems to be no question but that we have to do with a disturbance of calcium



A B

Figure 4. Photomicrograph of a normal human parathyroid gland (x 400). B—Photomicograph of the parathyroid gland of Dr. Cushing's case of pituitary basophilism (x 400).

metabolism after hypophysectomy as reflected in the decreased calcium content in the blood; (no data or reference given) whether this is due to the correlated subnormality of the parathyroid glands remains to be established. Changes in the parathyroids as a result of hypophysectomy have not been adequately studied as yet. It is difficult to view the cessation of growth of the skeletal system after hypophysectomy as essentially due to disturbance in the inorganic salt metabolism; it is more justifiable to refer it to a singular failure in cartilage proliferation in the epiphyseal discs and, of course, the dwarfism is participated in by all the organs and tissues of the body."

Our present study indicates that in the intact rabbit, histological evidences of hyperplasia and overactivity of the parathyroids can be induced by injection of emulsions or extracts of the anterior pituitary of beef. Whether this effect of such substances is an independent one and separate from the other known anterior pituitary effects or "hormones," such as Prolan A-B complex, thyreotropic or the crude growth extract of Evans, will be of considerable interest. That it may be an independent effect is indicated by the following points in our protocols. It was noted that animals 710, 712, 715 and 716 had resting ovaries, but showed definite parathyroid hyperplasia. Likewise, rabbits 265, 219, 226, 214 and 251 showed positive parathyreotropic effects when treated with pregnancy urine with no demonstrable effect in their thyroid glands Pregnancy urine has been repeatedly investigated and found negative for thyreotropic effect by several authors (10). It can be argued, therefore, that the parathyreotropic effect is at least separate from the gonadotropic and thyreotropic effects. Its independence from Evan's purified growth hormone is less clearly amenable to proof since it is conceivable that the skeletal growth obtained by pituitary products depends on the stimulation of epiphyseal cartilage growth and calcification, two phenomena intimately bound up with parathyroid activity.

To prove that the histological evidences which we have adduced in favor of a parathyreotropic effect of the anterior pituitary have functional significance, we are now engaged in metabolic studies of calcium, phosphorus and phosphatase in dogs subjected to treatment with our pituitary extracts. An anatomical study of the structure of the bones and epiphyseal cartilages of animals subjected to pituitary extract treatment is also under way.

SUMMARY AND CONCLUSIONS

In a series of seven experiments, the parathyroid glands of 48 rabbits subjected to injections of anterior lobe extracts and of pregnancy urine, as well as of several proprietary preparations from these sources, were grossly and microscopically compared with those of 17 reference control animals. The latter received either no injections or were given injections of heated pituitary tissue, normal urine, or emulsions of fresh brain substance of cows.

It was found that those animals which had been treated with unheated pituitary products and extracts of pregnancy urine exhibited parathyroids which were, as a rule, grossly larger and more vascular than those of the control group. They showed histological changes consistent with hypertrophy and hyperplasia. The criteria for the latter were described by Erdheim for the rat and were found remarkably applicable to the rabbit. Photomicrographs, drawings and measurements of representative normal and hyperplastic glands are presented to illustrate the type of changes encountered.

The degree to which these changes occurred in the individual animals varied according to the dosage and duration of treatment.

Control animals showed minor or no changes from normal in the gross and microscopic structure of their parathyroid glands.

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After the completion of the manuscript for this report and following its public presentation before the Harvard Biological Society (November 18, 1933) and the Harvard Medical Society (December 12, 1933) (see report of latter meeting in New England Medical Journal), there appeared a short report in the December 16, 1933, issue of the Klinische Wochenschrift (Vol. 12; No. 50; p. 1944) entitled: "Parathyreotrope Wirkung von Hypophysenvorderlappenextracten." We are pleased to call attention to it.