

THE EFFECT ON THE EXTERNAL PARATHYROID  
GLANDS OF THE EXPOSURE OF RABBITS TO  
ULTRA-VIOLET LIGHT.

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PLATE 2.

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Because many instances have been observed of the effects of light on metabolic processes, and because in the higher animals the glands of internal secretion act in part as chemical regulators of metabolism, we are undertaking certain studies on the effects on the endocrine tissues of exposure of the skin to ultra-violet light. In preparation for these studies it seemed desirable to make preliminary observations on the reactions of all the ductless glands available for examination, and to note such changes in weight and microscopic appearance as might indicate abnormal activity. Such a study is the more necessary because of various interrelationships among the endocrine glands that make isolated observations on a single tissue of doubtful value.

Dr. Wade H. Brown and Dr. Louise Pearce have directed attention to this interdependence among certain of the glands of internal secretion in connection with their extensive studies on experimental syphilis and carcinoma in the rabbit. During the past 2 years they have collected data on individual and seasonal variations in the size, appearance, and functional activity of the endocrine tissues of a large number of normal animals.<sup>1</sup> These valuable records, mostly unpublished, they have kindly placed at our disposal. It would have been fruitless to make the observations on which this paper is based without the extensive control material thus afforded us. Even during the period covered by the experiments—November to February—there occurred in certain of the endocrine tissues a change, regarded as

<sup>1</sup> Brown, W. H., Pearce, L., and Van Allen, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1923-24, xxi, 373.

seasonal, of as much as one-fourth in their weight. Individual differences were even greater. It was essential, therefore, to establish a concurrent base line with a considerable number of normal rabbits in order to determine those differences from the normal that could be ascribed to the experimental procedures. This explains why the rabbit was chosen for our study.

A summary of the results of this preliminary study of the effects of ultra-violet light on normal rabbits has already been published.<sup>2</sup> Among the changes found in various endocrine glands, those involving the external parathyroids arrested our attention and seem worthy of more extended consideration. The present paper deals mainly with these secondary effects of ultra-violet light on the parathyroid glands and only a brief reference will be made to the observations on the other glands of internal secretion in order to correct and extend the earlier data in the light of subsequent observations.

#### *Experimental Procedure.*

*Experiment 1.*—Albino or light colored adult male rabbits weighing more than 1,600 gm. were selected from healthy stock and caged separately in a well lighted room under the usual conditions of ventilation and diet maintained for normal animals. Ears were shaved and backs closely clipped before exposure and at intervals during the experiment. In groups of six these rabbits were exposed for 30 minutes daily over periods of 3 to 7 weeks to the unfiltered radiations of a quartz mercury arc lamp (110 volts, 4 amperes) at a distance of 1 meter. This arbitrary exposure caused at first a marked hyperemia of the exposed skin followed by a persisting erythema and some desquamation, especially of the ears. The erythema was followed by a leathery thickening of the skin, notably of the back, and moderate pigmentation. During the first few days certain of the rabbits were drowsy, refused some of their food, and lost weight. The feces were often soft and three animals had a transient diarrhea. One of these three died of undetermined cause on the 5th day of exposure. The other rabbits soon regained their normal weight and appetite and appeared to be in good condition throughout the experimental period. Two rabbits, one showing a severe coccidiosis, the other a carcinoma of the liver with metastases, were discarded at autopsy. The dose chosen, while somewhat toxic at first, apparently was relatively ineffective during the final periods when the thickening and pigmentation of the skin had occurred.

At weekly intervals from the 3rd to the 7th week, five or six of the exposed

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<sup>2</sup> Grant, J. H. B., and Gates, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1923-24, xxi, 230.

rabbits and two normal controls were etherized and bled from the aorta. The following glands were then weighed and examined in gross and microscopically: pineal, hypophysis, external parathyroids, thyroid, thymus, adrenals, and testicles. The weights of these glands were recorded in mg. per kilo net body weight after removal of the gastrointestinal tract and its contents and the expression of bladder urine.

We had, in addition to the two controls for each group of rabbits, the observations of Dr. Pearce and Dr. Van Allen on groups of twelve to twenty-four normal animals examined within 2 or 3 weeks of the time our rabbits were killed. It was found necessary thus to confine the control groups to normal rabbits killed almost coincidentally because of the apparently seasonal variations in the ratio gland : body weight during the course of the experiment. The changes in the endocrine glands, in weight per cent, compared with the figures for the normal

TABLE I.

*Effect of Daily Exposure of Normal Rabbits to Ultra-Violet Light on the Weights of Certain Endocrine Tissues.*

Glands.	Per cent change from normal.						
	Time of exposure in weeks.						
	1	2	3	4	5	6	7
Pineal.....	±0	+24	+11	-5	+9	+7	+18
Hypophysis.....	+16	+25	+19	+14	+17	+7	+8
External parathyroids.....	+10	+41	+39	+56	+26	+36	+18
Thyroid.....	+10	+10	-17	-20	-2	-11	-12
Thymus.....	+20	-12	+13	+5	+9	-14	+16
Adrenals.....	+8	-17	-3	+3	+17	+19	+10
Testicles.....	+1	-14	+18	+4	+36	-3	+2

animals, are shown in Table I. The figures in this table differ somewhat from those already published. The former figures were based on an average of the gland : body weight of all the normal animals examined during the course of the experiment. A review of these data showed that a closer approximation to the actual ratios would be obtained by comparing the glands of radiated animals only with those of normal rabbits examined during the month in which the radiated animals were killed. This has been done in Table I.

When the results of 3 to 7 weeks daily exposure to the quartz mercury arc lamp had been obtained and changes noted in some of the glands after 3 weeks radiation, other groups of rabbits were radiated in an identical manner for 1 and 2 weeks to fill the gap in the earlier observations. The resulting figures have been included in Table I, but they are not strictly comparable with those obtained earlier because they are based on February controls, whereas the earlier experiments were com-

pleted when the normal control figures for several of the glands differed considerably from those obtained in February. The high figures for the thyroid and adrenal glands after 1 week of exposure may be due to marked congestion and even gross hemorrhages sometimes found in these glands at autopsy.

*The Secondary Effects of Ultra-Violet Light on the Parathyroid Glands.*

Without a discussion of the possible significance of the changes in the other endocrine tissues, attention may be directed to the parathyroid glands. Only the two external parathyroids were considered in this study. The internal glands, buried in thyroid tissue, are inaccessible to direct determination by weight and their size must be estimated by the indirect method employed by Luce.<sup>3</sup> Hence our figures are relative rather than absolute. It seems improbable, however, that exposure of an animal to ultra-violet light would have diverse effects on the external and the internal glands, and we have assumed the changes in the external parathyroids to be an index of the secondary effects of light on the whole parathyroid apparatus. If this is the case, it appears that exposure of the skin to the radiations of a quartz mercury arc lamp caused a marked increase in the weight of the parathyroid glands. This increase began during the first few days of exposure. It was the more striking in that it occurred during a period when the parathyroids were normally undergoing an apparently seasonal diminution in weight. The average weights of the two external parathyroid glands in the normal rabbits decreased at a fairly uniform rate from 6.9 mg. per kilo net body weight in November to 5.4 mg. in February.<sup>4</sup> In the radiated rabbits not only was this normal decrease arrested but the external glands underwent an active hypertrophy. The peak in the size of the external parathyroids occurred after 4 weeks daily exposure to the quartz lamp. Then the average weight of the two glands was 10 mg. per kilo body weight, an increase of 56 per cent over the normal weight for December, when these rabbits were examined. Fig. 1 is designed to show the relation of the changes in weight of the external parathyroids of radiated

<sup>3</sup> Luce, E. M., *J. Path. and Bact.*, 1923, xxvi, 200.

<sup>4</sup> This gradual decrease in the normal size of the parathyroids from November to February may be significant in relation to the seasonal incidence of such diseases as rickets and tetany and to the distribution of sunlight through the year.

rabbits to the changes occurring in normal animals during the same season of the year.

The probable errors in the calculation of the weights of the parathyroid glands in the normal rabbits and in each experimental group have been calculated by Peter's approximation formula and recorded in the chart. The chances are even that the correct values for each determination lie within the limits defined by the probable error.

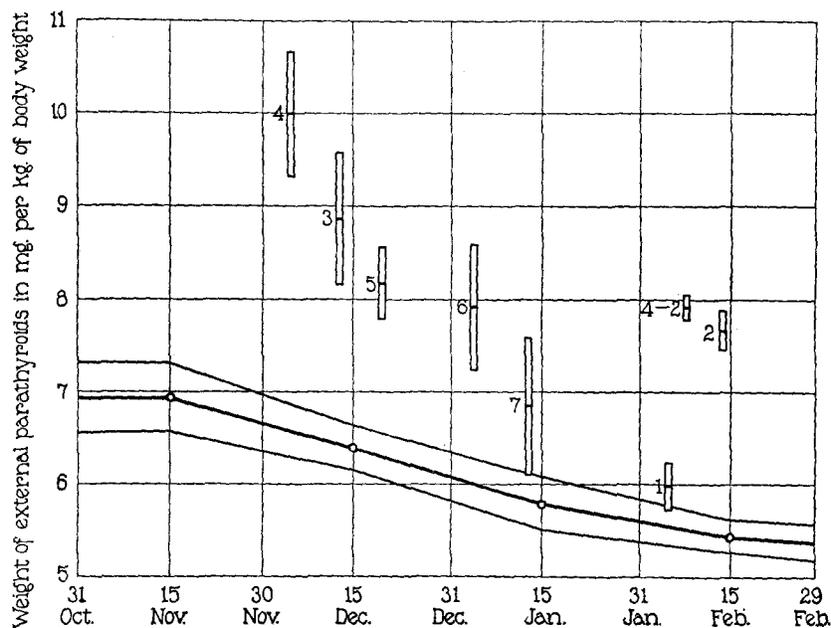


FIG. 1. The average weight of the external parathyroid glands in normal and radiated rabbits.

Solid line with circles. Weight of glands of normal rabbits examined during each month grouped on the 15th of the month. The corresponding lines above and below the normal indicate the *probable error* in these determinations.

Vertical rectangles. Average weights of glands and *probable error* for each group of radiated rabbits. Figures indicate weeks of daily exposure.

4-2 refers to Experiment 2.

Fig. 1 is based on the average figures for all the rabbits in each group. The probable errors indicate that wide individual differences were found in the ratio gland : body weight of the animals in both the normal and the radiated groups. There was, indeed, some overlapping of the groups, so that some of the normal

rabbits had larger parathyroid glands than certain of the radiated animals. This fact may be interpreted in either of two ways. It is possible that some of the radiated rabbits did not react to the light treatment by parathyroid hypertrophy. On the other hand, the smallest glands found in normal animals weighed 40 per cent less than the smallest glands found in radiated rabbits. The parathyroid glands in these radiated rabbits, though small, may nevertheless have undergone a considerable hypertrophy.

On account of the arbitrary dosage (30 minutes daily) used throughout these experiments, the results do not indicate whether the parathyroid glands can be maintained at a relatively high weight level over a considerable period of time by radiation with increasing doses of ultra-violet light. In the present instance the average size of the external parathyroids after 5, 6, and 7 weeks of daily light treatment was progressively less than after the shorter periods. Indeed, on the assumption that the glands in these animals underwent an hypertrophy comparable to that found in the rabbits examined after the 3rd and 4th weeks, the external parathyroids were decreasing in size at a much more rapid rate than in the normal controls. We attribute this relative decrease in size to the protection afforded by pigmentation of the skin, which developed gradually to a maximum during the 3rd and 4th weeks and was maintained in the later groups to the end of the experiment. Under this protection the stimulus to hypertrophy must have been removed, and the glands tended to revert rapidly to normal.

*Experiment 2.*—Because the dose of 30 minutes daily had caused signs of intoxication and an initial loss of weight in some of the rabbits, another group of six animals was exposed to the quartz lamp for 10 minutes on the 1st day and the exposure was increased 2.5 minutes each day for a period of 4 weeks. These rabbits also exhibited some degree of erythema but showed no toxic symptoms or loss in weight. At autopsy the average weight of their external parathyroid glands was 7.92 mg. per kilo net body weight, an increase of 45 per cent over the average normal weight for February. In the preliminary report<sup>1</sup> this increase is given as 32 per cent. At that time the February control statistics had not been completed and this figure was based on the average normal weight for January.

#### *Microscopic Examinations.*

The external parathyroid glands from some of the rabbits in each group were fixed in Zenker's solution and sectioned for microscopic

study. These sections showed that the increases in weight were due to a true hyperplasia of the parenchymatous tissue. There was no appreciable hyperemia, edema, or fibrosis. The mass of the hyperplastic glands was made up of closely packed cells and cell columns, with large oval or rounded nuclei and finely granular cytoplasm, like those in the normal glands. Occasional mitotic figures were found in some sections of the hyperplastic tissue. In other sections some pycnosis and some vacuolation of cytoplasm were seen. Such changes were also found in sections from normal rabbits, however, and in view of the inevitable handling the glands underwent in preparation for weighing, we feel that no definite evidence of structural changes, except hyperplasia, is to be obtained from the microscopic study of the glands. Photomicrographs of external parathyroid glands from a normal and a radiated rabbit are shown in Plate 2, Figs. 1 to 4.

#### *Blood Calcium.*

The study of the effects of ultra-violet light on the endocrine tissues of normal rabbits was undertaken without any preconception of the probable effect of light upon the parathyroid glands. Hence no preliminary blood calcium determinations were made on the radiated rabbits. When the increase in the size of the external glands was observed after 3 and 4 weeks daily exposure, the calcium content of the blood serum was determined by the method of Kramer and Tisdall<sup>5</sup> on the rabbits of subsequent groups. As controls, similar determinations were made on the sera of 60 normal rabbits. These control determinations gave an average value of 11.9 mg. of Ca per 100 ml. of serum. The limits of variation among the normal rabbits were wide, namely 14.1 and 9.4 mg. The average calcium content of the serum of the radiated rabbits in the 5th, 6th, and 7th week groups was 12.7; 12.9; and 12.6 mg. per 100 ml. of serum.

Repeated blood calcium determinations on the rabbits of subsequent groups and on normal rabbits caged and fed under similar conditions have since led us to question the extent to which these relatively slight increases in the serum calcium of radiated animals can be attributed to the hypertrophy of the parathyroid glands. The

<sup>5</sup> Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, *xlvi*, 475.

60 determinations on normal rabbits which make up the control average of 11.9 mg. Ca per 100 ml. of serum were made mostly on animals from different sources recently put in stock, whereas the radiated animals had been caged for 5 to 7 weeks before the serum calcium was determined. Now it appears that the serum calcium of normal rabbits rises during the first weeks of caging to figures comparable to those for the radiated animals. This is shown in Table II in which the results of serum calcium determinations on Groups 1

TABLE II.  
*Comparison of Serum-Calcium Determinations in Normal and Radiated Rabbits.*

	Mg. of Ca per 100 ml. of serum.				
	Before radiation.	Time in weeks.			
		1	2	3	4
Normal control rabbits.....	11.9	12.8	13.2	13.1	12.2
Radiated rabbits, Groups 1 and 2, Experiment 1; and Experiment 2.....	11.9	13.6	13.2	13.5	12.9

and 2 of Experiment 1, before and after radiation, have been combined with those of successive weekly determinations on the rabbits of Experiment 2, and these figures compared with similar figures for the control rabbits of these groups.

Emphasis should be placed rather on the fact that a parathyroid hypertrophy of 20 to 50 per cent does not result in a corresponding increase in the calcium content of the blood when the calcium concentration is already at or near the normal level.

#### DISCUSSION.

So far as we are aware parathyroid hyperplasia has been demonstrated hitherto only in clinical conditions characterized by a low blood calcium level in which it might be regarded as an effort at compensation due to physiological need. Thus Luce<sup>3</sup> found a true hyperplasia of the secreting cells of the parathyroid glands in rats on a low calcium diet. Erdheim<sup>6</sup> in rats, and more recently Ritter,<sup>7</sup> and

<sup>6</sup> Erdheim, J., *Sitzungsber. k. Akad. Wissensch. Math.-naturw. Cl. Wien*, 1914, xv.

<sup>7</sup> Ritter, C., *Frankf. Z. Path.*, 1920, xxiv, 137.

Pappenheimer and Minor,<sup>8</sup> in children, observed a hyperplasia of the parathyroids in clinical conditions diagnosed as rickets. Vines<sup>9</sup> refers to a similar hyperplasia in certain cases of chronic nephritis with disturbances of the calcium balance in the blood, but notes that hyperparathyroidism is not known as a clinical entity.

In the absence of a complete understanding of parathyroid function it might be difficult to demonstrate clinical changes attributable to hyperparathyroidism. Our animals showed no variations from normal that could be so interpreted. In an effort to determine the significance of the hyperplasia produced by ultra-violet light we are therefore thrown back on present day knowledge of the relation of the parathyroid glands to calcium metabolism.

The drop in blood calcium that occurs after parathyroidectomy<sup>10-16</sup> indicates that the maintenance of the normal calcium level is dependent upon the integrity of the parathyroid function. As is the case with other essential tissues, the normal glands appear to provide the body with a wide margin of safety in this respect so that much of the parathyroid apparatus may be removed without seriously affecting the calcium level. Nevertheless it has been amply demonstrated that in the absence of sufficient parathyroid tissue the calcium level of the blood becomes permanently lowered and can be restored only temporarily by the introduction of calcium salts.<sup>16</sup> It does not follow that the converse must hold true; that an excess of parathyroid tissue must cause a rise in the calcium level above normal. Other factors, unrelated to the parathyroid function so far as our present knowledge goes, seem to define the upper limit of blood calcium concentration. The normal level is at, or near, the saturation point of the serum for calcium. Howland and Kramer,<sup>17</sup> from a review of the literature and

<sup>8</sup> Pappenheimer, A. M., and Minor, J., *J. Med. Research*, 1920-21, xlii, 391.

<sup>9</sup> Vines, H. W. C., *The parathyroid glands in relation to disease*, London, 1924.

<sup>10</sup> MacCallum, W. G., and Voegtlin, C., *J. Exp. Med.*, 1909, xi, 118.

<sup>11</sup> MacCallum, W. G., and Vogel, K. M., *J. Exp. Med.*, 1913, xviii, 618.

<sup>12</sup> Howland, J., and Marriott, W. McK., *Quart. J. Med.*, 1917-18, xi, 289.

<sup>13</sup> Hastings, A. B., and Murray, H. A., Jr., *J. Biol. Chem.*, 1921, xlvi, 233.

<sup>14</sup> Gross, E. G., and Underhill, F. P., *J. Biol. Chem.*, 1922, liv, 105.

<sup>15</sup> Salvesen, H. A., *J. Biol. Chem.*, 1923, lvi, 443.

<sup>16</sup> Salvesen, H. A., *Acta med. Scand.*, 1923, suppl. 6.

<sup>17</sup> Howland, J., and Kramer, B., *Monatschr. Kinderheilk.*, 1923, xxv, 279.

their own experiments, point out that the blood serum is normally a supersaturated solution of calcium bicarbonate and secondary calcium orthophosphate. The excess of calcium presumably is held in combination with protein. Salvesen and Linder<sup>18</sup> refer to this calcium store as a “. . . . reserve which will furnish a new supply of ionized calcium, if this important part of the blood calcium is diminished.”

If this is so, the normal parathyroid function would seem, directly or indirectly, to maintain the calcium concentration of the blood at an upper limit determined by other, independent factors. Whereas in the absence of sufficient parathyroid tissue this limit cannot be attained, even under parathyroid hypertrophy it cannot be surpassed. The effects of ultra-violet light in our normal rabbits may, therefore, have been merely to increase the factor of safety of the parathyroid glands without producing any significant change in the blood calcium level. Experiments are in progress which may throw light upon this question.

The mechanism by which the parathyroid glands maintain the blood calcium level is not yet understood. Salvesen<sup>16</sup> found that the transient character of the rise in blood calcium caused by calcium injections in parathyroidectomized dogs was due to a rapid elimination of the injected calcium, mainly through the intestine. His observations, therefore, suggest that the parathyroid glands influence the passage of calcium through the intestinal wall.

A diminished absorption or an increased excretion of calcium *via* the intestine has been observed also in infantile tetany and certain cases of rickets in which low blood calcium values are found. This fact, in association with the well recognized effects of ultra-violet light in the treatment of rickets and tetany led Orr, Holt, Wilkins, and Boone<sup>19</sup> to ascertain whether ultra-violet light therapy promoted the absorption of calcium and phosphorus from the intestines. They found that in infants with active rickets there was little if any retention of calcium and phosphorus in the body and that most of the ingested phosphorus and almost all of the calcium was to be found in the stools.

<sup>18</sup> Salvesen, H. A., and Linder, G. C., *J. Biol. Chem.*, 1923, lviii, 635.

<sup>19</sup> Orr, W. J., Holt, L. E., Jr. Wilkins, L., and Boone, F. H., *Am. J. Dis. Child.*, 1923, xxvi, 362.

After treatment with ultra-violet light, however, coincident with the healing of the rachitic lesions there occurred a greatly increased retention of calcium and phosphorus, so that one-third to one-half of these elements was retained.

So little is known about the factors controlling phosphorus metabolism that we prefer not to include this element in this discussion. But our experiments, which demonstrate a striking secondary effect of exposure to ultra-violet light upon the parathyroid glands, considered in relation to those of Salvesen, and of Orr, Holt, Wilkins, and Boone, may provide another link in the chain of evidence to explain the effects of ultra-violet light therapy on calcium metabolism in rickets and tetany. It seems not improbable that the parathyroid glands are directly concerned in the process; that one result of exposure of the skin to ultra-violet light is some alteration in the composition of the blood to which the parathyroids are sensitive, and to which they react by hypertrophy. If this hypertrophy is accompanied by an increase in functional activity it should tend to counteract those processes which result from parathyroid dysfunction, among them the failure of the intestines in regard to the absorption and retention of calcium, with a consequent low calcium level in the blood.

#### SUMMARY.

A preliminary study has been made of the effects of radiation of normal rabbits with ultra-violet light upon the weight and microscopic appearance of certain of the endocrine tissues.

Among these tissues the external parathyroid glands showed a consistent reaction. The exposures caused an hypertrophy of the glands amounting in some instances to more than half the normal weight. The hypertrophy was a true hyperplasia of the parenchymatous tissue. It was not accompanied by any significant increase in the blood calcium above the normal level.

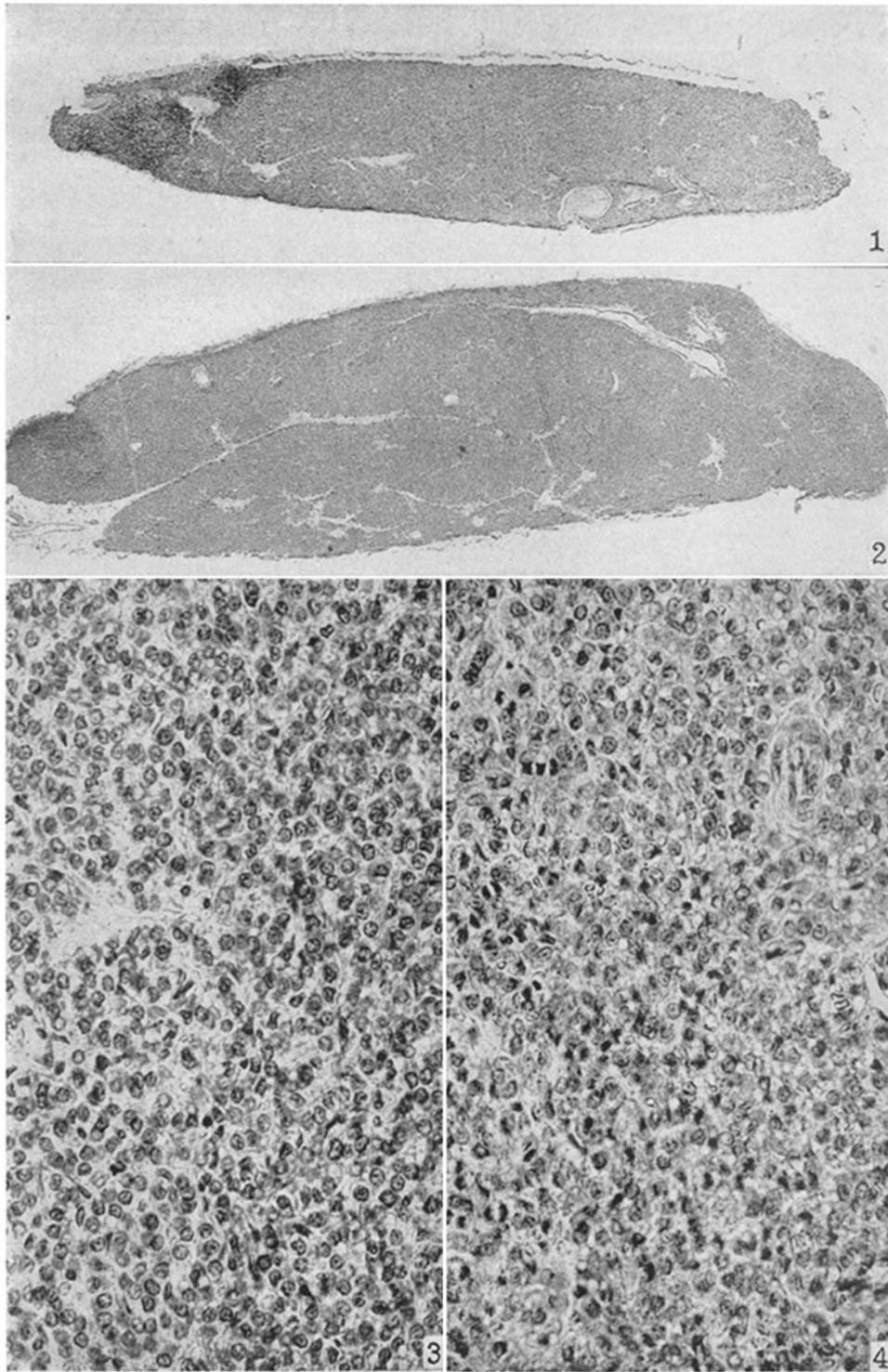
## EXPLANATION OF PLATE 2.

FIG. 1. Section of external parathyroid gland of normal rabbit.  $\times 26$ .

FIG. 2. Section of external parathyroid gland of rabbit radiated daily for 3 weeks with ultra-violet light.  $\times 26$ .

FIG. 3. Higher magnification of section shown in Fig. 1.  $\times 362$ .

FIG. 4. Higher magnification of section shown in Fig. 2.  $\times 362$ .



(Grant and Gates: Effect on parathyroid of ultra-violet light.