

EXPERIMENTAL OBSERVATIONS ON IRRADIATED, NORMAL, AND PARTIALLY PARATHYROID-ECTOMIZED RABBITS.

I. THE EFFECTS OF PARTIAL PARATHYROIDECTOMY.

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Observations on the endocrine tissues of rabbits irradiated with a quartz mercury arc lamp have disclosed the striking hypertrophy of the parathyroid glands produced by the irradiation (1). Histologically, this hypertrophy was found to be a true hyperplasia of the endocrine tissue, but a study of the calcium and inorganic phosphate content of the blood of these rabbits failed to reveal any significant changes from the normal levels (2).

That parathyroid integrity is essential to the maintenance of a normal blood calcium level has been established by the extirpation experiments of many investigators, but the upper limit of calcium concentration seems to be determined by factors independent of parathyroid control. Hence, in the normal animal, parathyroid hypertrophy due to an external stimulus, such as light, appears to result only in an increase in the factor of safety, and to be unaccompanied by a recognizable increase in functional activity for which there is no physiological demand.

On the other hand, the presence of an increased factor of safety might be revealed by an unusual functional strain or emergency, and a study of such conditions, experimentally produced, might throw further light upon normal parathyroid function. Having at hand methods of producing a hypertrophic condition of the parathyroid glands by means of ultra-violet light, and a condition of reduced function by partial extirpation, we undertook a series of experimental comparisons of groups of rabbits with enlarged, normal, or partially extirpated parathyroids.

In preparation for these studies, partial parathyroidectomies were performed on a number of rabbits, some normal, some after irradiation with ultra-violet light. The effects of the operation itself upon the calcium and the inorganic phosphate level of the blood of these animals form the subject matter of this paper.

Experimental Procedure.

Since the partially parathyroidectomized rabbits were to be used subsequently in other experiments, the operations were performed on small groups of animals and at various seasons of the year. In some instances only the two external parathyroids were removed; in others, the thyroid gland was divided at the isthmus, and one lobe, with its internal parathyroid gland, was removed also. The animals were all healthy, adult, albino males, and the glands were removed aseptically, under ether anesthesia, after a median incision and blunt dissection of the tissues of the neck. The operative wounds healed without infection. At intervals after the operation, small samples of blood were taken from the marginal ear vein and allowed to clot, and calcium (3) and inorganic phosphorus (4) determinations were made on the serum.

Preliminary observations on two normal rabbits, after removal of the two external parathyroid glands, showed that the operation was followed by a sharp drop in blood calcium to a low level on the 2nd or 3rd day, and a more gradual increase toward the normal over a period of days or even weeks. No signs of tetany appeared in these two animals.

Experiment 1.—The next groups of six and four normal rabbits respectively were deprived of both external parathyroid glands and one internal gland also. In spite of individual variations in reaction, the trends of the blood calcium curves of these rabbits were essentially similar. Immediately after operation, the blood calcium of all ten rabbits dropped from normal levels, between 11.6 and 13.0 mg. per 100 cc. of serum, to 6.2 to 10.2 mg. (37 to 88 per cent of the control figures) by the 2nd day. The rabbit with 6.2 mg. of calcium per 100 cc. of blood serum succumbed on that day in severe tetany. From this point the blood calcium of seven of the remaining nine rabbits began to rise towards normal, so that figures of 10.8 mg. to 12.6 mg. were obtained on the 4th to the 6th days, and these levels were usually maintained thereafter (Chart 1). The other two rabbits had persistently lower blood calcium levels, and one of them showed a drop from 8.5 mg. per 100 cc. of serum on the 14th day after operation, to 4.8 mg. on the 17th day, when it had a typical tetanic seizure and died. The other rabbit maintained a serum calcium level between 8 and 10 mg. for 35 days. 2 days later it was transferred to a fasting

experiment, in which it succumbed on the 5th day. On the day before death its blood calcium was 5.65 mg. per 100 cc. of serum.

The inorganic phosphorus in the blood serum of most of these rabbits was not examined at regular intervals. It was observed, however, that before death the serum phosphorus of the rabbits that succumbed on the 2nd, 17th, and 42nd days after operation was 9.4 mg., 13.1 mg., and 12.0 mg. respectively per 100 cc. of serum, as compared with a normal average of 7.4 mg.

In this experiment the capacity of the residual parathyroid tissue to respond to sudden demands is shown by the fact that in seven of ten rabbits, after the initial drop, blood calcium levels only 5 to 10 per cent below normal were attained within a week, and these levels were usually maintained thereafter. If, after partial para-

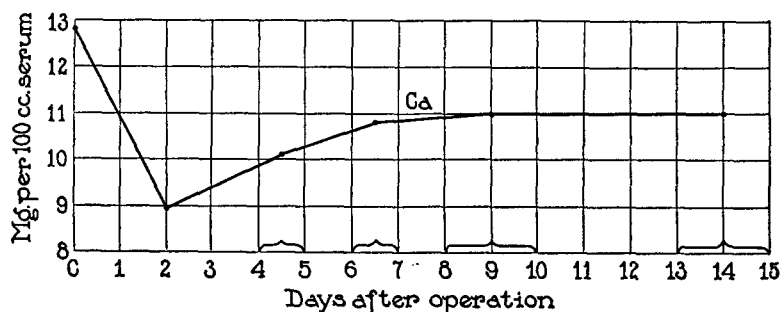


CHART 1. Experiment 1. Serum Ca levels after partial parathyroidectomy.

thyroidectomy in normal rabbits, the remaining tissue is usually able to restore the blood calcium level nearly to normal in 4 to 6 days, this is the interval in which evidence of parathyroid hyperplasia and consequent increase in the factor of safety must be sought in irradiated rabbits.

In the following experiment, normal and irradiated rabbits were partially parathyroidectomized and observed during the ensuing period.

Experiment 2.—A group of ten adult albino rabbits had both external parathyroids removed. Five of these animals, with backs clipped and ears shaved, had been exposed for 30 minutes a day, 6 days a week, for 3 weeks, at 1 meter distance, to a quartz mercury arc lamp (67 volts, 5.5 amperes). These rabbits presumably had hypertrophic parathyroid glands (1). The exposures were continued for the further period of observation. The five other rabbits served as controls for the

irradiated group. All were bled for a serum sample before operation, on the 1st, 2nd, and 4th days following, and then every 2 or 3 days to the 15th or 16th day.

A difference in the reaction of the irradiated and control rabbits to the loss of their external parathyroids became apparent within 24 hours after operation. None of the irradiated rabbits showed signs of tetany, but three of the controls were observed in acute attacks, characterized by gross and fibrillar twitchings of the voluntary muscles,—notably those of the jaw,—tonic and clonic convulsions, opisthotonos, deep, heaving respiration, venous engorgement, and

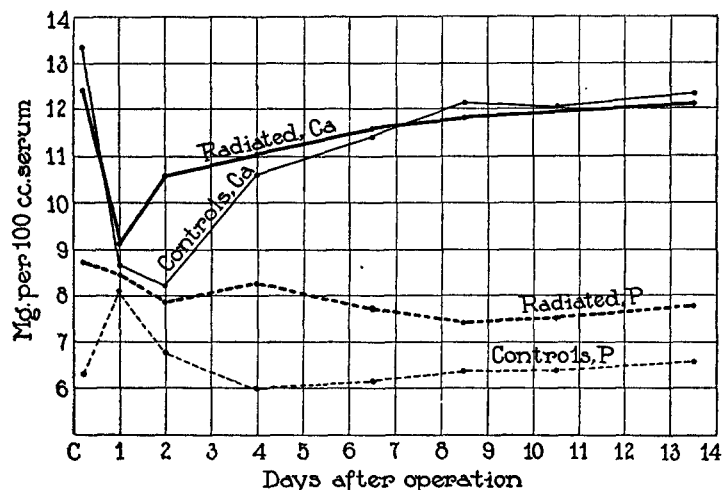


CHART 2. Experiment 2. Comparison of serum Ca and P in irradiated and control rabbits after partial parathyroidectomy.

excessive salivation. In two of these rabbits an intravenous injection of 10 cc. of $\frac{M}{8}$ CaCl_2 relieved the signs immediately, but one of them succumbed during the following night. The third rabbit recovered spontaneously.

The absence of all signs of tetany in the five irradiated rabbits led us to infer that the hyperplasia of the internal glands produced by the radiations had increased their functional capacity, *i.e.*, their factor of safety, to a degree sufficient to protect the animals. This increase in the factor of safety in the irradiated rabbits is reflected in a comparison of their blood calcium and inorganic phosphorus

curves. In both groups the fall in calcium was immediate, so that 24 hours after operation the average for the irradiated rabbits had dropped from 12.4 to 9.1 mg. (73 per cent of normal), and for the controls from 13.4 to 8.7 mg. (65 per cent of normal) per 100 cc. of serum. But the following day the blood calcium average in the irradiated group rose to 10.6 mg. per 100 cc. of serum, while the four survivors of the control group suffered a further loss to 8.2 mg. By the 7th day this difference had been overcome, and both groups then maintained calcium levels approaching normal (Chart 2). Similarly, due to sharp rises in the blood phosphorus of the three control rabbits

TABLE I.

Blood Calcium and Inorganic Phosphorus 1 Day after Partial Parathyroidectomy.

Normal rabbits				Irradiated rabbits			
No.	Calcium	Phosphorus	Ratio $\frac{\text{Ca}}{\text{P}}$	No.	Calcium	Phosphorus	Ratio $\frac{\text{Ca}}{\text{P}}$
	Mg. per 100 cc. serum				Mg. per 100 cc. serum		
18	10.9	6.0	1.82	13	10.3	8.5	1.21
19	9.5	4.8	1.96	14	10.1	7.2	1.40
20*	8.5	8.7	.98	15	8.9	8.6	1.03
21†	8.0	11.0	.73	16	8.5	8.7	.98
22*	6.0	9.9	.61	17	7.8	9.2	.85

* Observed in tetany.

† Died.

that had outspoken tetany, the average for the group was carried upward on the 1st day from 6.3 mg. to 8.1 mg. per 100 cc. of serum. Meanwhile, in the irradiated group, with a high average blood phosphorus to start with (2), only one of the rabbits showed a further rise in blood phosphorus following the operation. This rabbit had the greatest drop in calcium of any in the irradiated group.

Such a reciprocal relationship between blood calcium and phosphorus, pointed out by Howland and Kramer (5), and recently observed in normal dogs by Mayerson, Gunther, and Laurens (6), is illustrated in this experiment by arranging the rabbits of each group in the order of their blood calcium levels on the day following partial parathyroidectomy (Table I). In both groups the rabbits

with the lower calcium values after partial parathyroidectomy had the higher figures for blood phosphorus.

A final group of five normal rabbits, whose external parathyroid glands were extirpated, confirmed the earlier observations by exhibiting an average fall of blood calcium from 12.0 mg. to 8.8 mg. per 100 cc. of serum, 24 hours after operation. In three of these rabbits the calcium level was restored to normal in 7 to 9 days. The other two had persistently lowered blood calcium, and one rabbit, after transfer to a fasting experiment, on the 9th day, suffered a further drop in Ca to 4.9 mg. and died on the 16th day after the partial parathyroidectomy. 2 days before death this rabbit's blood

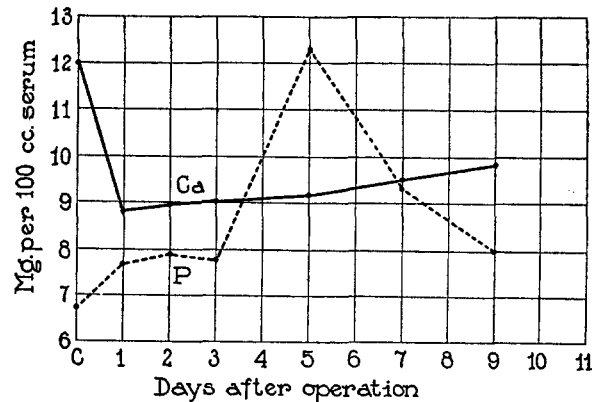


CHART 3. Serum Ca and P levels after partial parathyroidectomy.

phosphorus was 12.68 mg. per 100 cc. of serum. All the rabbits in this group exhibited a high blood phosphorus at one time or another after operation, the average for the group on the 5th day being 12.3 mg. per 100 cc. of serum, compared with a control level before operation of 6.7 mg. (Chart 3).

That the ether anesthesia and the operative procedure did not contribute to the fall in serum calcium, or the rise of inorganic phosphorus, was shown by the absence of such changes in two control rabbits, in which identical operations were performed, except for the removal of the parathyroid glands.

The Relative Significance of a Fall in Blood Calcium and of a Rise in Blood Phosphorus in Parathyroid Tetany.

We have already noted the marked rise in the inorganic phosphorus of the blood serum, which often accompanied the drop in serum calcium after partial parathyroidectomy. A rise in inorganic phosphorus occurred in some rabbits which did not develop tetany, but it was never absent in those that did, and was apparently associated with the onset of the acute attack. The question arises whether it is only the fall in calcium, or in reality the accompanying change in the ratio of calcium to phosphorus in the blood serum that is more significant in tetany.

TABLE II.

Rabbit No.	Onset of tetany day after operation	Ca	P	Ratio $\frac{\text{Ca}}{\text{P}}$	Outcome
		Mg. per 100 cc. serum			
4	17th	4.8	13.1	.37	Died
6	41st	5.7	12.0	.48	Died
9	2nd	6.2	9.4	.66	Died
21	1st	7.9	11.0	.72	Given CaCl ₂ ; died
20	1st	8.5	8.7	.98	Mild attack; recovered
22	1st	6.0	9.9	.61	Given CaCl ₂ ; recovered
26	16th	4.9	12.7	.38	Died
Average.....		6.3	11.0	.60	

Normally, in rabbits, this ratio $\frac{\text{Ca}}{\text{P}}$ is not less than 1, and it usually lies between 1.3 and 2.6. Among 103 normal rabbits, with a calcium level, on admission, between 11.1 and 13.9 mg. per 100 cc. of serum, the lowest ratio $\frac{\text{Ca}}{\text{P}}$ was 1.10 and the three highest, with one exception, were 3.3. The exceptional rabbit had a very low serum phosphorus (1.8 mg.) and a unique ratio $\frac{\text{Ca}}{\text{P}}$ of 7.30. The average ratio for all the rabbits was $\frac{12.4}{6.4} = 1.9$.

In the rabbits that developed acute tetany after partial parathy-

roidectomy, on the other hand, the ratio $\frac{\text{Ca}}{\text{P}}$ was changed not only by the fall in calcium, but by an abnormal rise in phosphorus also. Table II shows the ratio $\frac{\text{Ca}}{\text{P}}$ in these rabbits, according to the last figures obtained before the attack, and it will be noted that high phosphorus figures contribute as much to change the $\frac{\text{Ca}}{\text{P}}$ ratio from normal, as do the low figures for blood calcium.

Of nine other partially parathyroidectomized rabbits, which were not observed in tetany, four at one time or another had calcium

TABLE III.

Rabbit No.	No. of days after operation	Lowest calcium level	Phosphorus level	Ratio $\frac{\text{Ca}}{\text{P}}$
		Mg. per 100 cc. serum		
3	2	8.6	7.7	1.12
5	2	7.6	8.6	.88
7	18	10.6	5.7	1.9
19	2	8.4	5.6	1.5
18	1	10.9	6.0	1.82
8	1	9.2	8.0	1.15
10	1	9.6	10.8	.89
11	5	7.3	12.2	.60
12	3	6.6	8.1	.81

and phosphorus levels comparable with some of those in the tetanic animals. The other five did not have either very low calcium or high phosphorus at any time after operation, and their $\frac{\text{Ca}}{\text{P}}$ ratios were usually more than 1 (Table III).

DISCUSSION.

Our observations are in accord with those of other investigators who have studied calcium and phosphorus metabolism after parathyroidectomy. The characteristic drop in blood calcium has often been found and needs no further comment. This fall in blood calcium is certainly a primary reaction, which may or may not be followed by a

rise in phosphorus, either immediately, or later, when the blood calcium is returning toward the normal level.

A phosphorus retention, following parathyroidectomy in dogs, was observed in 1911 by Greenwald (7), who has since published a series of papers in which the work of others is reviewed. He has recently stated (8, *a*) that in over 50 parathyroidectomized dogs tetany never appeared without phosphorus retention, and conversely, a drop in phosphorus excretion after attempted parathyroidectomy was observed in only one of ten dogs that did not develop tetany. However, the increase in "acid-soluble" phosphorus in the blood of parathyroidectomized dogs in tetany, which Greenwald had observed in 1913 (8, *b*), was not so marked in his later experiments, and in 1924 (8, *c*) he appears to minimize its significance, assuming that the excess of phosphates retained is deposited in the tissues, and only reappears in the circulation under certain abnormal conditions, such as after ether anesthesia, or cerebral or general anemia. In those of our rabbits that developed tetany, an excess of phosphorus was found in the blood.

Salvesen (9) also found that the inorganic phosphorus of the blood serum of completely parathyroidectomized dogs was considerably increased, and tended to remain above the normal value. According to one protocol, a dog with a normal blood calcium of 10 mg. per 100 cc. of serum and inorganic phosphorus of 3.0 mg. ($\frac{\text{Ca}}{\text{P}} = 3.3$) developed severe tetany 2 days after complete parathyroidectomy, with a $\frac{\text{Ca}}{\text{P}}$ ratio of $\frac{3.50}{12.5} = 0.28$, and the serum phosphorus level remained above 6.0 mg. per 100 cc. of serum thereafter.

In a study on blood changes and clinical symptoms, following oral administration of phosphates in dogs, Salvesen, Hastings, and McIntosh (10) discuss the changes in blood phosphorus and calcium that result, and they conclude that the fall in calcium is the significant factor in the development of tetany.

In a recent private communication, Dr. John Howland stated his conviction that the clinical syndrome known as infantile tetany is certainly not the result of a high phosphorus content of the serum which drives down the calcium concentration (11), and observed that infantile tetany is found at times with a very low phosphorus concentration of serum. He has found that in many instances the phosphorus is high, perhaps in two-thirds of the cases, but there is the one-third which cannot be neglected.

We shall postpone further discussion of the subject until we report a series of observations on rabbits to which secondary sodium orthophosphate was given subcutaneously. The point to be noted at this time is that the calcium level itself was apparently not the sole determining factor in the development of tetanic signs in these partially

parathyroidectomized rabbits. Only in connection with an increase in blood phosphorus, which further disturbed the normal $\frac{\text{Ca}}{\text{P}}$ ratio, were signs of hyperirritability observed.

SUMMARY.

Previous irradiation of rabbits with ultra-violet light, with a consequent hypertrophy of the parathyroid glands, resulted in some degree of protection to these animals, when part of the parathyroid tissue was extirpated. This fact indicates that the remaining hyperplastic tissue was potentially active, and that the increased factor of safety represented by this condition of the remaining tissue resulted in less loss of blood calcium, a more rapid initiation of recovery, and an absence of the rise in the inorganic phosphorus of the blood serum which was a characteristic result of partial parathyroidectomy in normal rabbits. A fall in calcium in all the irradiated rabbits, without a corresponding rise in phosphorus, shows that the drop in calcium is the primary reaction, and a rise in phosphorus a secondary reaction following parathyroidectomy. But the relation of the rise in phosphorus to the development of tetany remains obscure.

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