

# THE ANTIRACHITIC ACTION OF COD LIVER OIL AND IRRADIATED ERGOSTEROL IN PARATHYROIDECTOMIZED AND THYMECTOMIZED RATS

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The purpose of this study has been to ascertain whether the anti-rachitic action of cod liver oil and of irradiated ergosterol is dependent upon the integrity of the parathyroid or thymus glands.

## I. *The Effect of Parathyroidectomy*

Several investigators have studied the effect of these agents in experimental tetany of dogs. The observations have been somewhat conflicting. Whereas Brougher (1), Wade (2), and more recently, Jones, Rapoport and Hoden (3), and Demole and Christ (4), were able to mitigate the symptoms of tetany, to prolong life, and to bring about an elevation of blood calcium after parathyroidectomy, Urechiu and Popoviciu (5), and Greenwald and Gross (6), were unable to demonstrate such effects. Wade found that the preoperative administration of cod liver oil mitigated symptoms and prolonged life, but did not prevent a fall in the blood calcium. Hess, Weinstock and Rivkin (7) produced latent tetany in a monkey by a low calcium diet, with a fall of the blood calcium to about 6 mg. The administration of a large amount of irradiated ergosterol *per os* raised the blood calcium to 11 mg. After thyroparathyroidectomy, the blood calcium could not be raised above 7 mg. In numerous further experiments upon dogs and monkeys, they were not able to raise the level of the blood calcium above normal, although administration of irradiated ergosterol for some weeks before operation resulted in a less precipitous fall in blood calcium after extirpation of the parathyroids. These experiments suggested to the authors that "a vitamine may function at least to a certain extent, through the medium of the ductless glands." In more recent experiments (8), the same authors have found that with very large doses of irradiated ergosterol (400 to 800 times the therapeutic dose), hypercalcemia could be produced even in the absence of the parathyroids.

We have found no previous work bearing directly upon the subject of this study.

*Methods*

Litters of young hooded rats of from 25 to 40 gm. in weight were used. Parathyroidectomy was performed in the usual way under amytal anaesthesia. The completeness of the extirpation was established in each case by complete serial sections of the excised tissue. The rats were placed on the Steenbock low phosphorus rickets producing diet from the onset of the experiment. Cod liver oil (Mead Johnson's tested) was administered with a pipette. For curative experiments, 5 daily doses were given after 21 days on the rickets producing diet. Radiograms were taken at the beginning and end of the treatment, the rats being killed 5 days after the last dose. Irradiated ergosterol (Mead Johnson's concentrated preparation having a potency 10,000 times that of cod liver oil), was diluted with 10,000 parts of olive oil, and administered by pipette. The ribs and tibia in each rat were decalcified in Mueller's fluid and studied histologically.

TABLE I  
*Litter A*

Rat No.	Parathyroidectomy	Days on diet	Weight	Treatment	Rickets
3	I/20/30. Complete	21	35-52	C.L.O. 4 gtt. daily	0
7	I/20/30. Complete	21	35-40	None	++++
8	I/20/30	27	62-72	C.L.O. 4 gtt. for last 5 days	++++ Healing +
9	Control	27	31-39	None	++++
10	Control	27	29-36	C.L.O. 4 gtt. for last 5 days	++++ Healing ++
11	Control	27	28-38	C.L.O. 4 gtt. for last 5 days	++++ Healing +

Consistent results were obtained in all the experiments. The experimental data can best be presented in Tables I to VI, which summarize the essential findings.

*Result of Experiment Shown in Table I.*—Complete prevention of rickets in parathyroidectomized Rat 3. Rickets in untreated parathyroidectomized Rat 7; and in unoperated Controls 9, 10 and 11. Positive curative effect of 4 drops of C.L.O. after 5 days in parathyroidectomized Rat 8 and in Controls 10 and 11.

*Result of Experiment Shown in Table II.*—Complete prevention was obtained in parathyroidectomized Rats 5 and 6 with 4 drops of C.L.O. daily. Marked healing occurred in parathyroidectomized Rats 2 and 4, and in control unoperated Rats 8 and 9 after 5 doses of 2 drops of C.L.O. Marked rickets without spontaneous healing in control unoperated Rat 7.

Serial sections of the neck organs of B-2, B-4, B-5 and B-6 showed no remains of parathyroid tissue at the site of extirpation, and absence of

TABLE II  
*Litter B*

Rat No.	Parathyroidectomy	Days on diet	Weight	Treatment	Rickets
2	I/24/30. Complete	34	37-78	C.L.O. 2 gtt. 5 days	++++ Healing +++
4	I/24/30. Complete	34	52-62	C.L.O. 2 gtt. 5 days	++++ Healing +++
5	I/24/30. Complete	26	33-53	C.L.O. 4 gtt. daily	0
6	I/24/30. Complete	26	33-50	C.L.O. 4 gtt. daily	0
7	Control	22	30-36	None	++++
8	Control	34	28-46	C.L.O. 2 gtt. 5 days	++++ Healing +++
9	Control	34	32-50	C.L.O. 2 gtt. 5 days	++++ Healing +++

any accessory glands. The entire thymus was included in the series of B-2, B-4 and B-6, and particular search was made for intra-thymic rests of parathyroid tissue. None were found.

*Result of Experiment Shown in Table III.*—Incomplete prevention with 2 drops of C.L.O. daily in Rat 1; complete protection in Rat 2. Marked healing with 2 to 4 drops of C.L.O. after 5 doses in parathyroidectomized Rats 3 and 4, and in unoperated Controls 5 and 6. No spontaneous healing in unoperated rachitic control Rat 7.

The experiments in Table IV show that similar results were obtained with irradiated ergosterol.

*Result of Experiment Shown in Table IV.*—Complete prevention was obtained in parathyroidectomized Rats 1, 2 and 3 with a daily dose of 1 gtt. of Acterol\* diluted to equivalent strength of 1 gtt. of C.L.O. Marked healing was obtained after 5 doses of 1 gtt. of the same preparation in parathyroidectomized Rats 4 and 6, and in control unoperated Rats 8 and 9. It was noted that a less severe rickets developed in the operated than in the control rats, probably due to the more rapid gain in weight in the latter group. Complete serial sections

TABLE III

*Litter C*

Rat No.	Parathyroidectomy	Days on diet	Weight	Treatment	Rickets
1	II/3/30. Complete	28	24-30	C.L.O. 2 gtt. daily	+
2	II/3/30. Complete	28	30-40	C.L.O. 2 gtt. daily	0
5	II/3/30. Complete	39	38-62	C.L.O. 2 gtt. for 5 days	++ Healing ++
4	II/3/30. Only one Ek in sections; series incomplete	39	30-59	C.L.O. 4 gtt. for 5 days	++ Healing +++
5	Control	39	22-39	C.L.O. 2 gtt. for 5 days	+++ Healing +++
6	Control	39	28-43	C.L.O. 4 gtt. for 5 days	++++ Healing +++
7	Control	28	28-42	None	+++

of the neck organs of Rat D-6, including thymus gland, showed no remains of parathyroid tissue or accessory glands.

The experiments shown in Tables I to IV may be briefly summarized. Complete prevention of rickets in young rats on a Steenbock low phosphorus diet was obtained with daily dosage of 2 to 4 drops of cod

\* Acterol was the trade name of the Mead Johnson irradiated ergosterol preparation, until supplanted by the official U.S.P. designation of Viosterol.

liver oil in spite of complete extirpation of the parathyroids. In one rat (C-1) complete protection was not afforded by a daily dose of 2 drops. Six parathyroidectomized rats given curative doses of 2 to 4 drops of cod liver oil for 5 days, all showed distinct healing.

TABLE IV  
*Litter D*  
*Steenbock Diet*

Rat No.	Parathyroidectomy	Days on diet	Weight	Treatment	Rickets
1	II/11/30. Complete	22	26-44	Acterol (conc.) 1-10,000; 1 gtt.	0
2	II/11/30. Complete	22	23-36	Acterol (conc.) 1-10,000; 1 gtt.	0
3	II/11/30. Complete	22	27-48	Acterol (conc.) 1-10,000; 1 gtt.	0
4	II/11/30. Complete	31	27-45	Acterol (conc.) 1-10,000; 1 gtt. III/5-III/9	++ Healing ++++
6	II/11/30. Complete	31	27-45	Acterol (conc.) 1-10,000; 1 gtt. III/5-III/9	+++ Healing ++++
7	II/11/30. Only one parathyroid in sections	22	25-41	None	++
8	II/11/30. Control	31	26-66	Acterol (conc.) 1 gtt. III/5-III/9	++++ Healing ++++
9	II/11/30. Control	31	26-62	Acterol (conc.) 1 gtt. III/5-III/9	++++ Healing ++++

Irradiated ergosterol (1 drop of a 1-10,000 dilution of Mead Johnson's concentrated Viosterol, stated to have an anti-rachitic potency 10,000 times that of cod liver oil) gave complete protection in 3 parathyroidectomized rats, and brought about healing after 5 doses in 2 rats.

It seems proved by these experiments that the antirachitic action of cod liver oil and of Viosterol is effective in the absence of the two principal parathyroid glands.

The possibility of accessory functional parathyroid tissue deserves consideration. Whereas Erdheim (9), in serial sections of the neck organs, found in every instance, minute collections of parathyroid cells, especially abundantly near the apex of the thymus or within its substance, Farner and Klinger (10), on the other hand, were able to demonstrate accessory glands in only 6 out of 35 serially sectioned animals, and in another series, in but 2 out of 64 rats. Bayer and Form (11) found them completely absent. In 6 of our experimental animals, complete serial sections were made of the neck organs, and in no instance could accessory parathyroid tissue be discovered. It would appear then that in the hooded strain used for these experiments accessory parathyroid tissue occurs exceptionally, if at all, and this possible source of error in the interpretation of the results may be definitely excluded.

In spite of the completeness of the removal of all parathyroid tissue, frank tetany did not develop in any of the animals, and the mortality, aside from deaths during the operation, has been low. Hammett (12) has shown that different strains of rats react differently as regards development of post-operative tetany and mortality. It is possible that the high calcium content of the Steenbock rickets-producing diet may have exerted a protective influence.

## *II. The Effect of Thymectomy*

The literature bearing upon the relation of the thymus gland to the skeletal system is voluminous, but conflicting. In most of the older work indicating an influence of thymectomy upon the calcification of the bones, neither dietary nor hygienic factors were taken into consideration. Pappenheimer (13) was unable to confirm the alleged production of rickets in rats following thymus extirpation.

In spite of the lack of convincing evidence that the thymus has a definite relation to bone development or calcification, the recent report of Nitschke (14), that injection of thymus extracts produces a marked fall in blood calcium and inorganic phosphate, brings up the possibility that antirachitic agents may be modified in their effect in the absence of this tissue.

In the experiment shown in Table V, which is illustrative of several others, it is shown that removal of the thymus does not interfere with the protective or curative action of cod liver oil in experimental rickets.

*Result of Experiment Shown in Table V.*—Complete protection of thymectomized Rats 1, 5 and 8 with 2 gtt. daily of C.L.O., the control unoperated Rat 4 also being protected. Five doses of 2 gtt. of C.L.O.

TABLE V  
*Litter F*  
*Steenbock Diet*

Rat No.	Thymectomy	Days on diet	Weight	Treatment	Rickets
1	II/14/30. Complete	18	43-54	C.L.O. 2 gtt. daily	0
5	II/14/30. Complete	27	41-74	C.L.O. 2 gtt. II/20-III/12	0
8	II/13/30. Complete	27	44-71	C.L.O. 2 gtt. II/19-III/11	0
4	II/14/30. Control unoperated	19	41-42	C.L.O. 2 gtt. II/20-III/5	0
10	II/14/30. Complete	38	34-72	C.L.O. 2 gtt. III/13-III/17	+++ Healing ++
6	II/14/30. Control unoperated	38	41-107	C.L.O. 2 gtt. II/13-III/17	+++ Healing +++++
7	II/14/30. Control unoperated	38	32-64	C.L.O. 2 gtt. III/13-III/17	+++ Healing ++

brought about marked healing in thymectomized Rat 10, as well as in the unoperated controls, Rats 6 and 7.

### *III. The Effect of Combined Parathyroidectomy and Thymectomy*

It seemed of interest to ascertain whether irradiated ergosterol would retain its antirachitic activity in the absence of both parathyroids and thymus. The experiment reported in Table VI shows that such is the case.

Serial sections were made of the excised parathyroids and of the entire neck tissues removed at autopsy. The completeness of the extirpation of both parathyroids and thymus was proven.

One may deduce from the experiment that Acterol is antirachitic in the absence of both thymus and parathyroids.

TABLE VI  
*Litter I*  
*Steenbock Diet*

Rat No.	Thymectomy	Parathyroid-ectomy	Days on diet	Weight	Treatment	Rickets
4	III/25/30	III/31/30	27	66-108	Acterol (1-10,000) 1 gtt. daily IV/1-IV/19	0
5	Control— unoperated	—	27	57-83	None	+++
6	Control— unoperated	—	27	71-98	None	+++

#### CONCLUSIONS

Cod liver oil and Viosterol in therapeutic doses are antirachitic in the absence of the parathyroid gland, or of the thymus, or of both.

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