B. *Axolotl.* In axolotls of about 5 to 7 cm. in length the spinal ganglia and dorsal roots were removed in the arm segments (2nd, 3rd, 4th, and 5th) on one side. Owing to motor injury, 2 out of the 10 operated animals showed partial motor paralysis. In the remaining 8 cases, however, observed from the operation up to 4 weeks afterwards, the locomotor functions of the de-afferented limbs were found to be perfectly normal and undisturbed. In walking the limbs were used in normal coordination. In a few cases there seemed to be a slight hypertonicity of the extensor and adductor muscles in the resting position, which did not, however, even in the most extreme cases, impede the normal motor activity of the limb.

7715 C

Effect of Irradiated Ergosterol and Calcium Lactate on Calcification of Trichina Cysts.*

W. W. WANTLAND. (Introduced by F. T. Jung.)

From the Department of Zoology, Northwestern University.

Workers in the field of experimental parasitology have repeatedly shown that in the normal course of infection with *Trichinella spiralis,* cysts begin to form around the coiled larvae in the muscle fibers in 4 to 6 weeks following infection. These cysts, at first extremely delicate, gradually become more conspicuous and after about 7 or 8 months there begins a deposit of calcareous material, resulting finally in calcification of the entire cyst. Complete calcification of the cyst and the contained larvae seldom occurs normally in less than one year and in many instances the calcification process is much slower.

It seems quite probable that the more general symptoms of trichiniasis, muscular pains, fever, eosinophilia, etc., are, in part at least, due to toxic products formed by the breaking down of large amounts of muscle tissue together with waste products of the larvae. Thus a continuous inoculation of the infected host with toxic proteins occurs. When cyst formation, the protective mechanism of the body for walling off the parasite, has progressed sufficiently, production of poisonous products and the effects of mechanical irritation by the larvae tend to be inhibited.

* This series of experiments was performed at Northwestern University, Department of Zoology, under the direction of Dr. Franklin D. Barker.

It is significant that the majority of deaths from trichiniasis occur 4 to 6 weeks after infection, during that period immediately preceding, or during the earlier stages of, cyst formation. It would seem then that if cyst formation and subsequent calcification could be hastened this would shorten the critical period in trichiniasis and more quickly terminate the disease.

It has been shown (Zucker and Matzner[1]) that irradiated ergosterol through the action of the active vitamin D, increases the absorption of calcium from the intestine in rats and (Harris and Moore[2]) produces a heavy calcification in heart muscle, kidneys and other organs in rabbits.

Eight white rabbits, in 2 groups of 4 each, were fed approximately 5,000 Trichinella larvae, previously digested out in artificial gastric juice.

In the first group 2 animals were given irradiated ergosterol and calcium lactate by mouth and 2 animals were kept as controls (see Table I, Series A).

An examination of Table I shows that the feeding of irradiated ergosterol and calcium lactate, in the amounts indicated, resulted in a marked acceleration of the calcification process. Marked calcification occurred within 2 months and 28 days equal in degree to that which normally requires 8 to 12 months.

The physical condition and appetite of the 2 treated rabbits were considerably better than that of the untreated controls.

In the second group, 3 rabbits were given irradiated ergosterol† and calcium lactate by mouth and one rabbit was kept as a control (see Table I, Series B). Rabbit No. 26 died approximately 4 weeks following infection. Slight calcification of cysts was noticed upon microscopic examination of the diaphragm. Rabbit No. 27 died near the end of the sixth week following infection. Marked calcification of cysts was observed in the diaphragm and various other striated muscles. Rabbit No. 25 and Rabbit No. 29, the control, were killed on this same date. Marked calcification of cysts in the diaphragm of No. 25 was evident but no calcification of cysts was visible in No. 29.

The rate of calcification varies directly with the amount of irradiated ergosterol and calcium lactate given and the length of time which the rabbits were under treatment. The differences in the

[1] Zucker, T. F., and Matzner, M. J., PROC. SOC. EXP. BIOL. AND MED., 1924, **21**, 186.

[2] Harris, L. J., and Moore, T., *Biochem. J.*, 1929, **23**, 261.

† Mead's Irradiated Viosterol in oil 10,000 International (also U. S. Pharmacopeia) units of vitamin D per gm. One Steenbock unit equals 2.7 U. S. P. units.

TABLE I.

	Series A				Series B			
	Controls		Treated		Control	Treated		
	R8	R9	R11	R12	R29	R25	R26	R27
Approximate No. larvae fed	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000	4000-5000
Date of infection	12/5/33	12/5/33	12/5/33	12/5/33	3/17/34	3/17/34	3/17/34	3/17/34
Treatment begun			2/5/34	2/5/34		3/20/34	3/20/34	3/20/34
Dosage per day ergosterol and calcium lactate	None	None	16 drops 0.5-1 gm.	20 drops 0.5-1 gm.	None	80 drops 1 gm.	100 drops 1.5 gm.	150 drops 2 gm.
Autopsy	3/4/34 (Killed)	3/3/34 (Killed)	3/3/34 (Killed)	3/3/34 (Killed)	4/27/34 (Killed)	4/27/34 (Killed)	4/16/34 (Died)	4/27/34 (Died)
Calcification of cysts	None	None	Marked	Marked	None	Marked	Slight	Marked

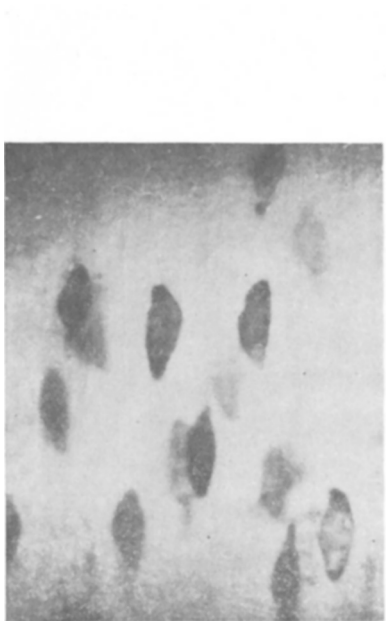

FIG. 1.

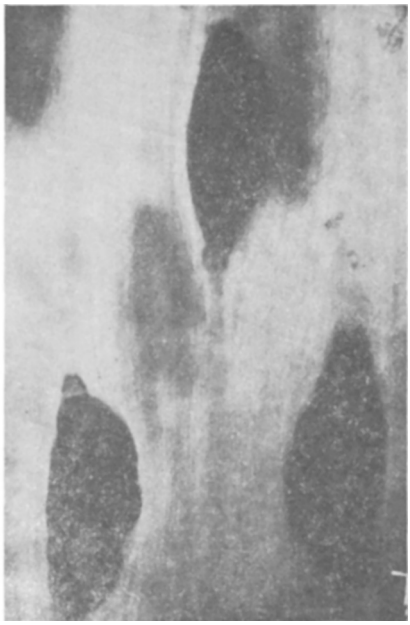

FIG. 2.

FIG. 1. Photomicrograph of fresh diaphragm of Rabbit No. 27. 32.5 ×. Animal received 150 drops of irradiated ergosterol plus 2.0 gm. calcium lactate every day.

FIG. 2. Another region, Rabbit No. 27. 85 ×.

degree of calcification are shown in the photomicrographs of the fresh diaphragms.

That too large an amount of ergosterol may be given is indicated by the death of Rabbit No. 27. This animal had presumably passed through the more dangerous period of the disease but suddenly near the end of the sixth week, exhibited extreme muscular weakness, dyspnea and paroxysmal tachycardia. It is probable that large doses of activated ergosterol lead to a hypercalcemia and subsequent calcium rigor.

A test was made on another rabbit (No. 24) infected on the same date as the above group in Series B. This animal had been receiving 60 drops of ergosterol plus 0.5 gm. of calcium lactate every other day. On April 27 the dosage was increased to 180 drops of ergosterol plus 2.0 gm. of calcium lactate per day. On May 1 the animal refused most of its food and on May 2 exhibited extreme weakness, increased heart rate and dyspnea. The animal was killed and marked calcification of cysts was noted.

Five other white rabbits were infected on the same date and with approximately the same number of larvae as the animals in Series

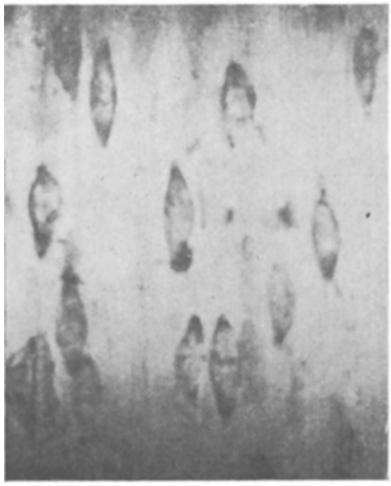

FIG. 3.

FIG. 4.

FIG. 3. Photomicrograph of fresh diaphragm of Rabbit No. 25. 32.5×. Animal received 80 drops of irradiated ergosterol plus 1.0 gm. calcium lactate every day.

FIG. 4. Another region, Rabbit No. 25. 85×.

B. Two of these animals (R-22 and R-23) received respectively 30 drops of ergosterol plus 0.5 gm. of calcium lactate and 40 drops of ergosterol plus 0.5 gm. of calcium lactate every other day since the third day following infection. The remaining 3 were control animals.

Muscle tissue from rabbit No. 23 showed marked calcification of cysts 2 months and 13 days from the date of infection. Calcification of cysts was observed in Rabbit No. 22, but in a lesser degree, 3 months and 10 days from the date of infection. These 2 treated animals did not show any symptoms of trichiniasis. They remained active, increased in weight and were in excellent condition at the time of killing.

The 3 control animals on the other hand exhibited symptoms in varying degrees. Dyspnea appeared, extreme muscular weakness and loss of appetite. In all 3 cases, however, the symptoms subsided (except for general weakened condition) during the sixth week of infection and the animals lived until they were killed 3 months and 10 days from the date of infection. No calcification of cysts had taken place in these control animals.

Six rabbits infected with from 4,000 to 5,000 larvae and treated

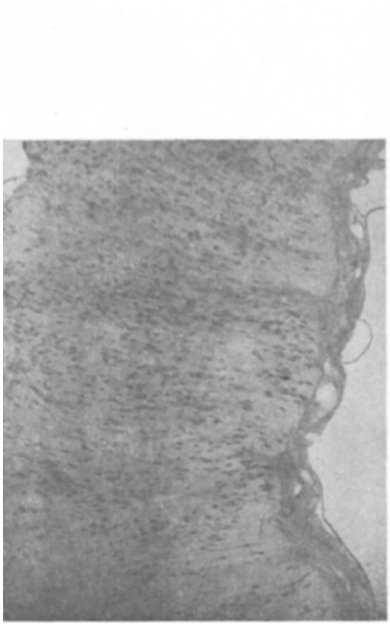

FIG. 5. FIG. 6.

FIG. 5. Camera view of region of diaphragm, Rabbit No. 27. 1.5 ×. Small dark bodies are cysts.

FIG. 6. Photomicrograph of fresh diaphragm of Rabbit No. 29 (control animal). 85 ×. Animal infected same date as Nos. 25 and 27. No treatment.

with from 16 to 20 drops of irradiated ergosterol plus from 0.5 gm. to 1.0 gm. of calcium lactate every other day showed no calcification of cysts at autopsy (4 weeks after infection). In 2 other infected animals, however, that had received respectively 50 drops of ergosterol plus 1.5 gm. calcium lactate and 60 drops of ergosterol plus 2.0 gm. calcium lactate every other day, slight calcification was observed during the fourth week of infection. At the end of the fourth week the poles of the cysts show medium to marked calcification in animals that have received between 30 and 60 drops of irradiated ergosterol plus from 0.5 gm. to 2.0 gm. of calcium lactate every other day. The onset of the calcification process in treated animals evidently takes place during the early part of the fourth week following infection.

The optimum dosage of activated ergosterol as evidenced from the above experiments is from 30 to 60 drops every other day. This amount apparently has no deleterious effects yet markedly accelerates the calcification of trichina cysts in white rabbits. The statement that in the normal course of infection cysts begin to show

signs of calcification only after 7 or 8 months from the date of infection has been set forth by various observers in the field of parasitology and is in agreement with the experiments performed in this laboratory. The results of the experiments here reported are in striking contrast to the normal course of trichiniasis as shown by the marked calcification obtained in less than 6 weeks in treated animals by the administration of irradiated ergosterol and calcium lactate.

The treatment of trichinized rabbits with irradiated ergosterol apparently has a definite therapeutic value. It still remains to be tested in human cases of trichiniasis.

7716 P

Fibrinolytic Streptococci from Lower Animals.*

R. R. MADISON. (Introduced by W. H. Manwaring.)

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In addition to hemolytic streptococci specifically lytic for human fibrin there are at least 2 apparently distinct fibrinolytic strains of *S. hemolyticus* (or strains intermediary between *S. hemolyticus* and

TABLE I.

Lysis of Lower Animal Fibrins by *S. hemolyticus*.

24-hour veal-infusion broth cultures of *S. hemolyticus* tested against veterinary fibrins by the serum-free fibrin-clot technic of Tillett and Garner.[1]

++++ represents complete liquefaction of the fibrinogen-thrombin complex within 10 minutes; +++, 30 minutes; ++, 1 hour; and +, 2 to 3 hours.

+++ and ++++ fibrinolytic strains are + and ++ thrombolytic, by the plasma-clot technic.

Origin of strain	Fibrin				
	Horse	Hog	Cow	Rabbit	Man
Horse, "Strangles" (10 strains)	+	0	0	0	0
" "	++	0	0	0	0
Colt, "Navel ill"	+++	0	0	0	0
Rabbit, "Pneumonia"	+	0	0	0	0
Man, "Prostate abscess"	+	0	0	0	+
" "Knee "	+	0	0	0	++++
Hog, "Septicemia" (2 strains)	0	++++	0	0	+
" "Mixed infection"	0	+	0	0	0
Control (Autolytic test)	0	0	0	0	0

* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.