

EFFECTS OF X-RAYS ON TRICHINÆ

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INTRODUCTION

The object of the experiments that are described in this paper was to determine whether X-rays exert deleterious influences on trichinæ (*Trichinella spiralis*), with a view to the practical application of X-ray radiation to the destruction of trichinæ in pork. These experiments were performed with the cooperation of a commercial firm that was operating X-ray machines in Florida. The experiments were planned and the details arranged by B. H. Ransom, Chief of the Zoological Division of the Bureau of Animal Industry, in consultation with the roentgenologist of the firm in question. The former supervised the tests made by the writer to determine the effects of the X-ray treatment upon the trichinæ, while the latter carried out the portions of the investigations relating to the X-ray treatment, calculations of the X-ray dosages used, etc.

The number of experiments that have been performed are insufficient to warrant any definite conclusions concerning the feasibility of applying X-ray radiation to the destruction of trichinæ in pork in routine packing-house procedure. Aside from the fact that the expense involved may render that procedure impracticable, much more experimental work than is presented in this paper would be required to demonstrate whether X-ray treatment could be depended upon to destroy trichinæ. The experimental data at hand are of interest from a general scientific viewpoint, however, and it is from that point of view that they are presented.

In a discussion of the effects of X-rays on the flour beetle (*Tribolium confusum*), Davey,¹ referring to his own work and the work of various other investigators, states:

X-rays may act upon an organism (or on a single type of cell in that organism) in one of three ways: (1) to produce a stimulation; (2) to produce a destructive effect which takes place only after a certain latent interval; (3) to produce an instant destructive effect.

That the effects of X-rays on trichinæ so far as they are injurious become evident only after the parasites are subjected to influences that stimulate them to growth and development, or, in other words, after they reach the intestine of a host in which they normally attain sexual maturity,

¹ DAVEY, Wheeler P. THE EFFECT OF X-RAYS ON THE LENGTH OF LIFE OF TRIBOLIUM CONFUSUM. *In Jour. Exp. Zool.*, v. 22, No. 3, p. 575-576. 1917.

and accordingly, that X-rays act on trichinæ in the second of the three ways mentioned above, is indicated by the results of the experiments recorded here.

METHODS OF EXPERIMENT

The trichinous meat used in these tests was obtained from hogs (series I, II, III, and V) and guinea pigs (series IV). The animals were artificially infected by feeding them trichinous pork. The infested pork to be exposed to X-rays was obtained from hogs that were killed several months after artificial infection. Trichinous guinea-pig meat was obtained from animals kept about a month after artificial infection.

Trichinous pork was packed in wooden or cardboard boxes in Washington, forwarded to Florida, where the exposure to X-rays was made, and promptly returned to Washington, where it was fed to experimental animals in order to determine the effects of the exposure. In a few cases portions of the meat that had been exposed to X-rays were digested in an acidified solution of scale pepsin, the decapsuled larvæ were examined, and the results were compared with those of the feeding experiments. Infested guinea pigs were shipped alive to Florida about 30 days after artificial infection. The animals were killed with chloroform in Florida, the skins and viscera were removed, and the carcasses were placed in boxes, exposed to X-rays, and returned to Washington.

The feeding experiments were performed in Washington. A quantity of the treated meat was ground up in a meat chopper and fed to a number of rats and, in some cases, mice. Unless they died as a result of infection with trichinæ the animals were killed at various intervals and examined for evidence of infestation with trichinæ as noted in connection with each experiment. Controls on the meat from the same lots as those exposed to X-rays showed that in all cases in which it was possible to maintain controls the untreated meat contained viable trichinæ capable of normal development. In those cases in which the entire carcasses of trichinous guinea pigs were exposed to X-ray treatment it was of course not possible to maintain controls.

EXPERIMENTS

SERIES I

X-RAY DOSAGE.—The units of dosage used in this series of experiments are described by the roentgenologist under date of January 20, 1917, on which day the exposures to X-rays were probably made,¹ as follows:

I adopted a purely arbitrary unit, 1,000 of which units are equivalent to a dosage received at a distance of 5 inches from the focal spot of a Coolidge tube with a current of 4.2 milliamperes and a pressure of 70 kilovolts across the tube terminals. Treatment continued for 42 minutes. In ordinary X-ray terms this is equivalent to 172 milliamperere minutes with a 6½-inch gap and a 5-inch distance.

¹ The meat was received in Washington on January 22, and feeding experiments were begun on January 23.

EXPERIMENT 1.—Strength of dosage, 2,899 units.

Twelve rats and two mice were fed in this experiment.

Rats 1, 2, and 3 were fed on January 23. Rats 1 and 2 were chloroformed on January 26. No trichinae were found in the intestines. Rat 3 died on February 23; diaphragm negative.

Rats 4 to 9, inclusive, were fed on January 25. Rat 4 was killed on January 26. Trichinae were found in the intestine. The parasites were about ready to molt. They were somewhat paler than normal. Rat 5 was killed on January 27. No trichinae were found in the intestine. No. 6 was killed on February 26; diaphragm negative. No. 7 was killed on March 12; diaphragm negative. No. 8 and 9 were killed on March 15; diaphragms negative.

Rats 10 to 12, inclusive, were fed on January 30. Rats 10 and 11 were killed on January 31. A few trichinae were found in the intestines of each animal. The parasites showed evidences of growth. Most of them were dead, however, having undergone granular degeneration.

Rat 12 was killed on February 1. A few trichinae were found attached to the mucosa of the intestine. These showed evidence of growth.

Two mice were fed some of the treated meat on January 29. Mouse 1 was killed on January 30. No trichinae were found in the intestines. Mouse 2 was killed on the same date. A few trichinae were found in the intestines. The parasites were dead but showed evidence of growth. No details of structure were made out because the parasites had undergone granular degeneration.

EXPERIMENT 2.—Strength of dosage, 966 units.

Nine rats were fed in this experiment. Rats 1 to 3 were fed on January 23. Rat 1 was killed on January 26. No trichinae were found in the intestines. Rat 2 was killed on February 2. A few trichinae, apparently fully grown, were found in the intestines. The parasites showed rather striking malformations, which were especially pronounced in the reproductive organs. The gonads were shrunken. The uterus of female specimens contained eggs, but the latter were full of minute granules. The receptaculum seminis, which in normal females is crowded with spermatozoa, was empty.

Rat 3 was killed on February 6. No trichinae were found in the intestines.

Rats 4 to 9 were fed on January 25. Rat 4 was killed on January 27; intestines negative. Rat 5 was killed on February 1; a few trichinae were found in the intestines. The parasites showed marked evidence of degeneration. The cuticle was wrinkled; internally numerous vacuoles were seen; the sex cells appeared undeveloped; the worms showed very feeble movements. No. 6 was killed on February 26; diaphragm negative. No. 8 and 9 were killed on March 15; diaphragms negative.

EXPERIMENT 3.—Strength of dosage, 191 units.

Six rats were fed in this experiment. Three rats were fed on January 25. Rat 1 was killed on January 26. Trichinæ were found in the intestine. The parasites appeared normal as to size and structure. Rat 2 was killed on January 29; trichinæ in intestines normal; uterus of females packed with embryos. No. 3 was killed on February 2. Numerous trichinæ were found in the intestines; apparently normal.

Three rats were fed on January 26. One rat died on February 6. Numerous larvæ were found in the fluid expressed from the diaphragm. Intestines showed numerous trichinæ. The second rat was killed on February 12. Numerous unencysted larvæ were found in the diaphragm. The third rat died on February 24. Numerous encysted trichinæ were found in the diaphragm.

EXPERIMENT 4.—Strength of dosage, 81 units.

Five rats were fed in this experiment. Rats were fed on January 23. Rat 1 was killed on January 26; numerous live trichinæ were found in the intestines. Rat 2 was killed on January 29; intestines negative. Rat 3 was killed on February 3; numerous live trichinæ in intestines. Rats 4 and 5 were killed on March 15; diaphragms heavily infested with trichinæ.

EXPERIMENT 5.—Strength of dosage, 35 units.

Five rats were fed in this experiment. Rats 1 and 2 were fed on January 23. Rat 1 was killed on January 26; numerous live trichinæ in intestines. Rat 2 was killed on January 27; results as in No. 1. Rats 3 to 5 were fed on January 29. Rat 3 died on February 5; numerous live trichinæ in intestines. Rat 4 died on February 24; diaphragm heavily infested with trichinæ. Rat 5 died March 2; results as in No. 4.

EXPERIMENT 6.—Dosage, 19 units.

Three rats were fed on January 23 with the meat treated in this experiment. Rat 1 was killed on January 26; intestines contained many live trichinæ. Rat 2 died on February 12; diaphragm not infested. Rat 3 died February 26; diaphragm heavily infested.

ARTIFICIAL DIGESTION TESTS IN EXPERIMENTS 1 TO 6.—Some of the meat used in each experiment was digested in an artificial gastric juice January 23. The trichinæ thus freed from their capsules were examined microscopically. They showed no visible evidence of injury, being active under heat stimulation and remaining tightly coiled at room temperature and thus behaving like normal trichinæ.

RESULTS OF EXPERIMENTS OF SERIES I.—These experiments indicate that trichinæ are seriously injured by sufficiently high dosages of X-rays. Although the trichinæ in all six experiments when freed from their cysts by artificial digestion showed no apparent evidence of having been affected by the X-ray treatment, the parasites in the meat that had been exposed to the heaviest dosage (experiments 1 and 2) failed to complete their development when fed to experimental animals. Instead of growing and developing in a normal manner, after the molt

that regularly occurs soon after the parasites reach the intestines, they underwent degenerative changes, and even in those cases in which the parasites developed to sexual maturity the reproductive processes were seriously disturbed. That the reproductive organs are especially susceptible to X-ray injury is clearly shown by the results of experiment 2. In this experiment the larvæ succeeded in attaining maturity, but the sex cells evidently failed to function.

It is also interesting to note that despite the fact that several rats in experiment 3 were not fed until 6 days after the meat had been exposed to X-rays, the animals developed an infection. Thus, in this experiment there was evident neither an immediate nor a delayed effect of the X-ray treatment upon the encysted parasites.

SERIES II

Three experiments are included in this series. The units of dosage used in this series have the same relative values as those in series I. Under date of February 5, 1917, the roentgenologist writes as follows:

The package marked "A" (experiment 7) was given 600 units, the package marked "B" (experiment 8) 300 units, and the package marked "C" (experiment 9) 350 units. The 300 units given to package "B" were given with low density and extra long time. The packages marked "A" and "C" were given the 600 and 350 units, respectively, at high tension—that is, close to the tube and with short time.

EXPERIMENT 7.—Strength of dosage, 600 units. The meat was exposed 19 minutes.

Three rats were fed on February 8. Rat 1 died on March 1; diaphragm negative. Rat 2 died on March 2; diaphragm showed a slight infestation with trichinæ. Rat 3 died on March 6; diaphragm slightly infested with trichinæ.

EXPERIMENT 8.—Strength of dosage, 300 units. The meat was exposed 46 minutes.

Three rats were fed on February 8. Rats 1 and 2 died February 12; live trichinæ were found in the intestines. Rat 3 died on February 26; numerous larvæ were found in the fluid expressed from the diaphragm.

EXPERIMENT 9.—Strength of dosage, 350 units. The meat was exposed 10½ minutes.

Four rats were fed on February 8. Rat 1 died on February 21; numerous live trichinæ in intestines. Rat 2 died on February 28; diaphragm infested with encysted trichinæ. Rat 3 died on March 1; results as in No. 2. Rat 4 died on March 2; diaphragm heavily infested with trichinæ.

RESULTS OF EXPERIMENTS OF SERIES II.—The parasites in the meat used in experiment 7 were evidently affected by the exposure. That some of them, however, escaped the injurious influences of the exposure to X-rays may be concluded from the results of the feeding experiments which resulted in rather slight infections.

SERIES III

In this series, which includes 12 experiments, the dosages used had the same relative values as those of the preceding series. The time of exposure and distance from the focal spot in the X-ray treatment of the various samples of meat in this series of experiments were not given in concrete terms, but in experiments designated by the letter A the meat was placed at twice the distance from the focal spot and held four times as long as in experiments designated by the letter B.

Two rats were used in each feeding experiment. The rats were fed on May 14.

EXPERIMENT 10A.—Dosage, 674 units.

Both rats were killed on June 15; diaphragms heavily infested with trichinae.

EXPERIMENT 10B.—Dosage, 674 units.

Rat 1 died on May 29; intestines negative; diaphragm negative. Rat 2 died on June 12; diaphragm negative.

EXPERIMENT 11A.—Dosage, 924 units.

Rat 1 died May 28; intestine negative; diaphragm negative. Rat 2 died on June 12; diaphragm negative.

EXPERIMENT 11B.—Dosage, 924 units.

Both rats killed on June 15; diaphragms heavily infested.

EXPERIMENT 12A.—Dosage, 1,363 units.

The rats were killed on June 15; diaphragms negative.

EXPERIMENT 12B.—Dosage, 1,363 units.

The rats died on June 17; diaphragms negative.

EXPERIMENT 13A.—Dosage, 2,162 units.

The rats were killed on June 15; diaphragms negative.

EXPERIMENT 13B.—Dosage, 2,162 units.

The rats were killed June 15; diaphragms negative.

EXPERIMENT 14A.—Dosage, 1,081 units.

Rat 1 dead June 5; one unencysted larva found in diaphragm. Rat 2 killed June 15; diaphragm heavily infested.

EXPERIMENT 14B.—Dosage, 1,081 units.

Rat 1 dead June 12; diaphragm heavily infested. Rat 2 killed June 15; results as in No. 1.

EXPERIMENT 15A.—Dosage, 3,094 units.

Rats killed June 15; diaphragms negative.

EXPERIMENT 15B.—Dosage, 3,094 units.

Rats killed June 15; diaphragms negative.

RESULTS OF EXPERIMENTS OF SERIES III.—In this series of experiments trichinous meat subjected to dosages up to 1,081 units proved to be infective, whereas in experiment 2 (series I) a dosage of 966 units impaired the vitality of the reproductive cells of the parasites. Whether this can be accounted for on the basis of variation of trichinae to the effects of X-rays or whether other factors were involved can not be stated.

SERIES IV

Under date of June 28, the roentgenologist states that the meat used in this series of experiments was—

exposed to the direct action of the rays at a distance of very nearly 25 cm. from the focal spot of a Coolidge-type tube. The pressure across the tube terminals was 73 kilovolts, measured by standard sphere gap, and also by ratios. The current through the tube varied during the time of treatment, which extended over a period of about 3 hours. The lowest reading was 4.2 milliamperes, the highest 4.9. This high reading, however, was for only a short time after the tube was started. The current gradually dropped during 10 minutes to 4.3 milliamperes, and during the rest of the treatment fluctuated between 4.2 and 4.3 milliamperes.

The boxes were so placed that the rays from other tubes in the machine had very little influence on the contents. By calculation it shows as negligible.

Box A was given an exposure of 42 minutes; box B an exposure of 84 minutes; box C an exposure of 126 minutes; and box D an exposure of 168 minutes. Following the system of measurement used by Davey,¹ which has the merit of being a complete expression of X-ray quantity, these dosages would read:

$$\text{Box A } 180 \frac{\text{MAM}}{25^2} \text{ at } 73 \text{ KV.}$$

$$\text{Box B } 361 \frac{\text{MAM}}{25^2} \text{ at } 73 \text{ KV.}$$

$$\text{Box C } 542 \frac{\text{MAM}}{25^2} \text{ at } 73 \text{ KV.}$$

$$\text{Box D } 722 \frac{\text{MAM}}{25^2} \text{ at } 73 \text{ KV.}$$

The rats used in this series of experiments were fed on July 31 and August 3. Five rats were fed in each experiment.

EXPERIMENT 16 (BOX A), 42 MINUTES.—Rats 1 and 2 died August 4. A few trichinæ were found in the intestines. The parasites showed evidence of growth. The sex cells were strikingly disorganized. Other organs also showed evidence of injury. Rat 3 was killed on August 20; diaphragm moderately infested. Rat 4 died on August 29; diaphragm moderately infested. Rat 5 died on September 16; diaphragm moderately infested.

EXPERIMENT 17 (BOX B), 84 MINUTES.—Rat 1 died on August 6; intestines negative. Rat 2 died on August 17; intestines and diaphragm negative. Rat 3 died on August 18; results same as in rat 2. Rat 4 died on August 20; results same as in rat 2. Rat 5 was killed on September 10; diaphragm negative.

EXPERIMENT 18 (BOX C), 126 MINUTES.—Rats 1 and 2 were killed on August 20; diaphragms negative. Rats 3 and 4 were killed on September 10; diaphragms negative. Rat 5 was killed on September 10; diaphragm slightly infested.

¹ Davey (OP. CIT., p. 586) states: "The voltage and distance are given directly and the product of the current and time is given, thus, '100 milliamperes-minutes at 25 cm. distance at 50 kilovolts.' This is usually contracted to read $100 \frac{\text{MAM}}{25^2}$ at 50 kv. It will be noticed that distance is expressed in terms of its square. This is because the intensity of X-rays varies inversely as the square of the distance."

EXPERIMENT 19 (BOX D), 168 MINUTES.—Rat 1 was killed on August 7; intestine negative. Rat 2 was killed on August 20; intestine negative and diaphragm negative. Rat 3 died on September 5; diaphragm negative. Rats 4 and 5 were killed on September 10; diaphragms negative.

RESULTS OF EXPERIMENTS OF SERIES IV.—The X-ray dosages used in these experiments were clearly injurious to the trichinæ. The smallest dosage used (experiment 16) had some effect, though it did not destroy the reproductive functions of all the parasites. In the three other experiments in which considerably larger dosages were used only 1 infection occurred among the 15 experimental animals on which the infectiousness of the meat was tested, and that infection was slight.

SERIES V

This series included six experiments. The dosages used in these experiments were not indicated, except that two samples were given similar dosages and that the remaining samples received graded dosages. Furthermore, the samples were mixed so that it is not known which samples received the larger or the smaller dosages. The samples were treated on March 24. Experimental rats were fed in Washington on March 27.

EXPERIMENT 20.—Rat 1 died on April 5; no trichinæ were found in the intestines. Rat 2 was killed on April 9; intestines contained live trichinæ; female trichinæ contained many embryos; diaphragm negative. Rat 3 was killed April 16; intestines positive; diaphragm positive. Rat 4 was killed on April 24; diaphragm heavily infested.

EXPERIMENT 21.—Rat 1 was killed on April 9; intestines contained live trichinæ, normal in appearance; female trichinæ contained eggs and embryos. Rat 2 was killed on April 16; intestines contained many live trichinæ. Rat 3 died on April 24; diaphragm heavily infested.

EXPERIMENT 22.—Rat 1 was killed on April 8; intestines negative; diaphragm negative. Rat 2 was killed on April 16; diaphragm negative. Rat 3 died on April 17; diaphragm negative. Rat 4 died on April 24; diaphragm heavily infested.

EXPERIMENT 23.—Rat 1 was killed on April 9; live trichinæ were found in the intestines; sex cells were atrophied; no larvæ were found in the diaphragm. Rat 2 was killed on April 16; no trichinæ were found in the intestines; diaphragm negative. Rat 3 was killed on April 23; diaphragm negative. Rat 4 was killed on April 23; one encysted larva was found in the diaphragm.

EXPERIMENT 24.—Rat 1 was killed on April 2; intestines contained numerous live and apparently normal trichinæ. Rat 2 was killed on April 8; live trichinæ were found in the intestines; diaphragm negative. Rat 3 was killed on April 16; intestines contained trichinæ, apparently dead; diaphragm negative. Rat 4 was killed on April 24; diaphragm heavily infested. Rat 5 was killed on April 24; diaphragm negative.

EXPERIMENT 25.—Rat 1 was killed on April 9; intestines contained live trichinæ; sex cells of trichinæ atrophied; diaphragm negative. Rat 2 was killed on April 16; diaphragm negative. Rat 3 died on April 19; diaphragm negative. Rats 4 and 5 were killed on April 24; diaphragm negative.

RESULTS OF EXPERIMENTS OF SERIES V.—The results of these experiments are in harmony with the results of the experiments recorded in the preceding pages. Trichinæ that showed sex-cell injuries (experiments 23 and 25) failed to produce a new generation. That a few larvæ in experiment 23 escaped injury is evident from the results of the feeding experiment with rat 4. It is interesting to note, however, that despite the fact that the parasites showed evidence of injury they were still alive on the fourteenth day after artificial infection. This indicates that X-rays exert a selective action on the sex cells of trichinæ and that injuries to the sex cells do not necessarily affect the other vital functions of the parasites.

DISCUSSION

The results of the experiments described in the foregoing pages show that trichinæ may be seriously injured by X-ray radiation. It is interesting to note that in experiments 1 to 6 inclusive (series I), larvæ isolated from the treated meat by artificial digestion appeared to be unaffected. These larvæ were normal as to color and general appearance, as viewed through the microscope and as indicated by their reactions to heat stimulation. The examination was made three days after treatment. The larvæ from the meat treated in experiments 1 and 2 (series I) were incapable, however, of attaining full sexual maturity in the intestines of rats or mice. Those in experiment 1 and some of those in experiment 2 underwent granular degeneration, while others in the latter experiment succeeded in attaining maturity without being capable of functioning sexually. The fact that no spermatozoa were found in the receptaculum seminis of the female indicates that successful copulation had not taken place.

It is also of interest to observe that a considerable degree of variation in resistance to X-rays is exhibited by trichinæ, since certain dosages proved to be destructive in some cases and not in others. This is possibly due, however, to other factors. It may be noted in this connection that trichinæ exhibit considerable variation in their resistance to cold¹ and in their resistance to heat.²

Assuming that a reliable and practically possible method of destroying the vitality of the sex cells in trichinæ by means of X-ray treatment of infested meat can be perfected, which is quite uncertain, it is still questionable whether such a method would be acceptable as a prophylactic

¹ RANSOM, B. H. EFFECTS OF REFRIGERATION UPON THE LARVÆ OF TRICHINELLA SPIRALIS. *In Jour. Agr. Research*, v. 5, no. 18, p. 819-854. 1916. Literature cited, p. 853-854.

² ——— and SCHWARTZ, Benjamin. EFFECTS OF HEAT ON TRICHINÆ. *In Jour. Agr. Research*, v. 17, no. 5, p. 201-221. 1919. Literature cited, p. 220-221.

measure, inasmuch as trichinæ are not inoffensive as intestinal parasites apart from the damage done by their migrating larvæ. Rats, for example, commonly die from intestinal trichinosis prior to the migration of the larvæ, and human beings also often suffer seriously from the effects of the intestinal stage of the parasites during the first few days after infection before the migrating larvæ have been produced. Consequently, unless the X-ray treatment has the effect of diminishing the injurious action of the intestinal stage of trichinæ upon the host as well as of destroying their powers of reproduction, it can scarcely be considered a satisfactory prophylactic measure. It is of interest to note in this connection that Tyzzer and Honeij¹ found that encysted trichinæ that had been subjected to radium radiation failed to develop in mice. These investigators also determined that whereas radium radiation failed to destroy sexually mature trichinæ in live rats, trichinæ in rats which were radiated beginning with the second day after ingestion of trichinous meat showed retardation in development. Radiation of the larvæ in rats before they have begun to develop proved fatal to them.

SUMMARY

(1) Encysted trichinæ are injured by relatively heavy dosages of X-rays. So far as has been determined the injuries are not visible in the encysted or artificially decapsuled larvæ as structural or functional disturbances but become apparent only when the larvæ reach a suitable host animal in whose intestine they are normally capable of continuing their development.

(2) Trichinæ from meat that has been exposed to strong dosages of X-rays undergo rapid granular degeneration in the intestines of suitable hosts before they attain maturity.

(3) Encysted larvæ that have been exposed to lower but still injurious dosages of X-rays are able to continue development in the intestines of suitable hosts. Such larvæ, however, do not attain structural and functional sex maturity. The sex cells appear to be atrophied, and no evidence of successful copulation can be found. X-rays, therefore, appear to exert a more or less selective action on the gonads of trichinæ.

(4) Trichinæ appear to exhibit considerable variation in their susceptibility to X-rays, since certain dosages injured some parasites and failed to injure others. Whether the apparent variation in susceptibility of trichinæ to X-rays is an expression of an actual physiological variation or may be accounted for by other factors has not been determined.

(5) The experiments described in this paper do not warrant any definite conclusions as to the feasibility of using X-ray radiation as a practical means of destroying trichinæ in pork.

¹ TYZZER, E. E., and HONEIJ, James A. THE EFFECTS OF RADIATION ON THE DEVELOPMENT OF TRICHINELLA SPIRALIS WITH RESPECT TO ITS APPLICATION TO THE TREATMENT OF OTHER PARASITIC DISEASES. *In* Jour. Par., v. 3, no. 2, p. 43-56, 1 pl. 1916.