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The Effects of Thyroxine on Ribonucleic Acid Content in the Brain of Rana Pipiens Tadpoles

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M.B.Jr.

DEDICATION

This thesis is dedicated to my wife and son, Mary Lou and Maynard Brown, III for their understanding, patience, sacrifices, and encouragement during the period of investigation and preparation of this report.

M.B.Jr.

TABLE OF CONTENTS

INTRODUCTION

Studies on the effects of thyroid hormones in the Amphibia have been confined largely to the following areas of research: (a) action upon metamorphosis, (b) action upon integumentary structure, (c) action upon the nervous function, and (d) action upon oxidative metabolism. Gudernatsch (1912) fed raw glandular tissue to tadpoles and discovered that thyroid tissue contains a potent metamorphosis--stimulating factor. Since Gudernatsch's discovery, a voluminous amount of literature has been accumulated pertaining to the effects of thyroid hormone on amphibian metamorphosis.

Some of the more recent work concerned with thyroid function in amphibian larvae has been reviewed extensively by (Etkin, 1955; Kollros, 1959; and Kaye, 1961). It has been shown that virtually all organ systems of the tadpole, but especially the integumentary and nervous systems, are sensitive to the "maturational" influence of thyroid hormone. Furthermore, not all tissues are equally responsive to thyroid hormone; normal metamorphosis and development of the larvae requires a progressive increase in thyroid hormone level coordinated with the appearance of target tissue sensitivity to the hormone.

The action of thyroid hormone upon differentiation of the Amphibian central nervous system has been investigated by Kollros and his collaborators by implanting thyroxine--containing agar pellets into

specific sites in the tadpole's brain (Kollros, 1943, 1959; Kollros and Pepernick, 1952; Pesetsky and Kollros, 1956; and Kaltenbach, 1953a). Such treatment not only produces localized differentiation in the vicinity of the thyroxine pellets (mesencephalic V nucleus, Mauthner's cells), but also unilateral functional differentiation of reflexes (e.g., the blink reflex). May and Mugard (1955) have shown that thyroxine also stimulates mitosis in the differentiating brain of frog tadpoles. Weiss and Rossetti (1951) reported a marked spurt in brain growth in Rana pipiens during metamorphosis, which was accompanied by intensified mitotic activity during normal metamorphic climax. A similar increase in mitotic activity was reported by Martin (1962) in studies on development of the cerebellum in Rana pipiens, and by Reynolds (1966) in studies of the lumbo-sacral spinal cord of Rana pipiens larvae.

Hormones have a far-reaching effect on growth and differentiation. In order for a cell to survive and grow, its metabolism must function properly. This means enzymes, organic acids and other essential substances must be in continuous production. Various hormones, depending on their nature and degree of activity in the organism, exert a certain effect on the metabolism of nucleic acids. The nucleic acids have enormous biological importance, because they carry the genetic information necessary for life to continue, evoking the synthesis of specific new proteins as required. Both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) play roles in protein synthesis.

Mandel (1951) reported that after injection of thyroxine (1 mg per day) into rats, DNA content increased from 22% to 32% in the kidney. Similar increases in spleen ranged from 35% to 60% (Mandel, 1952). Others have since reported findings similar to Mandel's. Garner (1967) found that the brain in tadpoles treated with thyroxine showed a slight increase in DNA content, although the increase was not considered to be significant.

Considerable knowledge concerning RNA formation is known for developing embryos of the frog, Rana pipiens, and the clawed toad, Xenopus laevis. Esper (1962) employed the cytophotometric method of determining the quantity of RNA in pronephric, epidermal, and endodermal cells in haploid and diploid embryos of Rana pipiens (Shumway Stage 24). The cytophotometric measurement revealed that the concentration of RNA per unit area of cytoplasm is the same for haploid and diploid embryos studied. However, cell volume determination indicated that diploid cells of each tissue are twice the volume of haploid cells. The total amount of RNA in the diploid cell is therefore twice the volume of haploid cells. The study of RNA systhesis in synchronized type cells by Klevez and Stubblefield (1967a) revealed an increase in the rate of RNA synthesis after the synthesis of only a small portion of the DNA. They suggested that this phenomenon was the result of replication of functional DNA templates in the cell. Klevez and Stubblefield (1967b) conducted a similar study on synchronized mammalian cell cultures with similar findings; that most of the DNA involved in transcription was replicated in the first third of the DNA synthesis period. Therefore

the rates of RNA and protein synthesis increased because of the doubling of the active template population of each cell.

Since the literature revealed a marked increase in mitotic activity in the neural axis, which is accompanied by an increase in DNA, during metamorphic climax and following administration of exogenous thyroxine, it seemed feasible to investigate further the role of the thyroxine in stimulating nucleic acid synthesis in the brain of Rana pipiens larvae, with emphasis on the quantity of DNA and RNA produced.

METHODS AND MATERIALS

The animals used in this investigation were Rana pipiens larvae which were obtained by induced ovulation and artificial fertilization (Rugh, 1934). Eggs of Rana pipiens were reared in individual finger bowls in which food (washed spinach or mustard greens) were present at all times. The animals were maintained in tap water at room temperature (21°C) and changed every two days. All animals were staged according to the method of Taylor and Kollros (1946). Two hundred animals were selected for this study. Tadpoles chosen for this experiment were in larval stages and ranging from early stage III to late stage III. Prior works done on mitotic activity of frogs and other amphibians were performed using either stage III or stage IV as the experimental stage. Tadpoles selected were divided into two groups. One group of one hundred tadpoles was designated as the control group, and the second group of one hundred tadpoles was designated as the experimental group. These larvae were exposed to thyroxine for periods of one to ten days. The control larvae were placed in finger bowls containing tap water, and were fed spinach and changed every other day. All experimental animals were immersed in 150 milliliters of water containing 50 µg/l of dl-thyroxine. The animals were immersed in thyroxine solutions for periods of one, two, three, four, five, six, seven, eight, nine, and ten days, respectively. The solutions and food

were changed daily. After each twenty-four hour period, ten each of control and experimental tadpoles were removed from their finger bowls and frozen. At the end of 10 days the brains of the animals from each group were removed and refrozen until ready for use in second phase of the investigation. Prior to entering into the extraction technique (2nd phase) for removing DNA, the frozen brains were shaped into cubes and weighed on an analytical balance. The technique used for extraction of DNA and RNA was that of Bradshaw (1966) with some modifications which became necessary as the research progressed.

EXTRACTION OF DEOXYDRIBONUCLEIC ACID (DNA)

The initial step in DNA extraction was homogenization of frog brains in a buffer solution of 0.14 M NaCl and 0.01 M Na citrate adjusted to PH 7.3. A waring blender was used to homogenize the brain tissue. Before use, the blender was placed in the refrigerator overnight to become chilled. Fifty milliliters of DNA buffer solution were poured into the blender and the brain tissue was homogenized for a period of one minute. Homogenizing frozen tissue serves to break the cell membranes, subsequently releasing the cellular contents as a free dispersion. After homogenation, the homogenate was centrifuged in a refrigerated centrifuge (2°C) at a speed of 5000 rpm's for fifteen minutes to sediment unbroken cells, debris and deoxyribonuclear proteins (DNP). All soluble components remained in the supernatant fluid. These steps also served to separate the cellular RNA from the cellular DNA. RNA is soluble under these conditions and is found in the supernatant

fluid. The sediment was washed in DNA buffer solution and centrifuged at a speed of 5000 RPM's for fifteen minutes to obtain as much DNA as possible. The RNA extract recovered from the first centrifuging was poured into a wide mouth bottle, labeled, and frozen until ready for use in the RNA portion of this investigation. The supernatant from the second centrifuging was poured off and discarded.

During the second step twenty-five milliliters of cold 2.6 M NaCl was used to break the electrostatic bonds between protein and DNA molecules. When this dissociation takes place, protein immediately precipitates. The sediment was broken by shaking it in the salt reagent for several minutes. In order to dissolve all the DNA present, the suspension was poured into a blender and blended for one minute, then poured into a beaker and placed on a magnetic stirrer for ten minutes. The entire step takes advantage of the fact that deoxyribonucleo-- proteins are soluble in a strong salt solution. DNA is also soluble. The procedure also dissociates the proteins and histones to yield free sodium deoxyribonucleate. The DNP salt suspension was centrifuged out at a speed of 20,000 RPM's for twenty minutes at 2°C leaving the DNA dissolved in the supernatant fraction.

Once the DNA solution was separated from the proteins formerly bound to it, the DNA was precipitated by the addition of two volumes of 95% ethyl alcohol (200 ml/per 25 ml of supernatant). The resulting precipitate was a film of white fibrous material and a powder-like precipitate that was recovered by wrapping it round the stirring rod and by decanting the alcohol. The precipitated DNA was re-dissolved

in 25 ml of distilled water.

METHOD OF DETECTION AND QUANTITATION OF DNA

The nucleic acids are detectable and quantitatable by a number of different methods. The simplest method is based on the fact that DNA and RNA absorb light of 260 micro-wavelengths very strongly. Dische diphenylamine method for detection of nucleic acids conforms to this simple light absorbance, and was used for this phase of the investigation. The Dische reaction is not absolute but does give a reasonable assurance of conducting effectively assayed pure DNA samples. This method imparts a blue color to DNA. The intensity of the color is proportional to the concentration of dissolved nucleic acid over a specified range. In this case the range used was 500 micro-wavelengths.

In accordance with directions of the Dische method six test tubes were placed in a test tube rack and labeled. Five milligrams of lyophillized DNA were dissolved in 5 milliliters of distilled water. This solution was used as the standard solution of DNA (1 mg./ml). The first four test tubes are used to plot a concentration curve (each tube contained known DNA solutions) to be used as a template for measuring the amount of DNA in the unknown sample. Two milliliters of known DNA solution were pipetted into tube one, and two milliliters of distilled water were pipetted into tubes two, three, four, and five respectively. After mixing tube two well, two milliliters from this tube were transferred to tube three. The contents of tube three were mixed well, and two milliliters transferred to tube four and mixed well. Test tube five

was omitted during this dilution process. Test tube six containing no solution, received two milliliters of the extracted DNA sample. Four milliliters of Dische diphenylamine reagent were pippeted into each of the six test tubes, mixed well, and placed into a 1000 milliliter beaker containing about 500 ml of water and boiled over a bunsen burner for ten minutes. After the ten minutes heating period, all six test tubes were transferred to an ice bath for a period of five minutes. Then each was allowed to warm to room temperature. This was the final step in preparing the samples to be read on the spectrophotometer. The spectrophotometer was turned on for five minutes or more to allow for its authorized warm-up period. At the same time it was adjusted for proper reading (500 mu wave-length). After the warm-up period the machine was tested for zero by adjusting both the absorbance and transmittance scale knobs using the blank (tube #5). Tubes one through four were read and a concentration curve was prepared on graph paper. From the graph the amount of DNA in each of the unknown samples are determined.

EXTRACTION OF RIBONUCLEIC ACID

Ribonucleic acid (RNA) suspended in frozen DNA buffer solution was thawed and twenty-five ml of cold supernatant was added to twenty-five ml of trichloroacetic acid. This mixture was poured into a 250 ml flask and placed a magnetic stirrer for five minutes. Upon completion of stirring the mixture was decanted into a beaker and placed in an ice bath for five minutes. A cloudy white coloration appeared with a fine string-like precipitation. (The trichloroacetic acid aided in breaking the molecules up and provided for the precipitation of proteins containing

RNA). In order to complete the precipitation the solution was poured into two (2) centrifuge tubes and centrifuged at 2°C at 6000 RPM's for ten minutes. This procedure was repeated twice, one with cold acetone and the second with room temperature acetone. The last run was made for fifteen minutes to permit maximum precipitation of RNA and proteins. The acetone was discarded and the precipitate was poured into a 50 ml beaker under a covered hood to dry (25 minutes). The beaker was then placed into an oven to complete the drying. The next step takes advantage of the fact that RNA proteins are soluble in a in a 10% salt solution. By boiling this mixture the RNA molecules are freed. The procedure includes suspending the dried precipitate in a 10% NaCl solution in capped test tubes. Each test tube was boiled for 40 minutes. After boiling, the test tubes were placed into an ice bath for five minutes until room temperature was reached. The solute was then poured into centrifuging tubes and centrifuged at 6000 rpm's for ten minutes.

Once the RNA solution was separated from the majority of proteins formerly bound to it, the RNA was selectively precipitated by the addition of two volumes of 100% ethyl alcohol in a beaker submerged in an iced bath. One hundred and fifty (150) milliliters of ethanol were used and stirred vigorously for five minutes. A fine white powdered precipitate formed in the bottom of the beaker (RNA). The alcohol was poured off and the RNA was washed in acetone and centrifuged at 600 rpm's for ten minutes. During the last step the precipitate was dried under the hood and in the oven. This was done in capped centrifuge tubes for a twenty-four hour period.

METHOD OF DETECTION AND QUANTITATION OF RNA

The Orcinal Reaction method for detection of ribonucleic acids was used. Six test tubes were placed in a test tube rack and labeled. One milligram of commercial RNA (yeast) was dissolved in 6 millliliters of distilled water. Several drops of 0.1N NaOH were added to assist the solution. This became the stock solution of RNA. Three millliliters of distilled water were pipetted into tubes labeled #2, #3, and #4. Three millliliters of the RNA solution were pipetted into tube one, and three millliliters of the same solution were pipetted into tube #2. The contents of the tube two were mixed well, then 3 millliliters from it transferred to tube #3. The contents of tube three were mixed well, then 3 millliliters transferred to tube #4. The contents of tube four were mixed well and 3 millliliters were removed from it and discarded. Next, a blank tube (labeled #5) with 3 millliliters of distilled water was prepared along with another tube (#6) containing 0.5 milligrams of the extracted RNA dissolved in 3 millliliters of distilled water (labeled brain RNA). This last preparation was repeated for the remaining 9 control test tubes and the 10 thyroxine test tubes. Six millliliters of the acid-orcinol reagent (10%$FeCl_3 \cdot 6H_2O$ + HCl) and 0.4 millliliters of the alcohol-orcinol reagent (6 gm Orcinol + 100/95% ethanol) were added to all test tubes. The test tubes were placed into a beaker of boiling water for 20 minutes. After the 20 minutes heating period all test tubes were transferred to an ice bath for a period of five minutes to cool to room temperature. This was the final step in preparing the solution to be read on the spectrophotometer.

The spectrophotometer was turned on and adjusted for reading purposes at 660 microwave length. After a period of about five minutes (time alloted for the spectrophotometer to warm up) tubes one through four were read and a concentration curve was prepared on graph paper. From the graph the amount of RNA in each of the unknown samples was determined.

RESULTS

An investigation was made on the effects of thyroxine on the DNA and RNA content in the frogs' brain. Two hundred stage III larvae of Rana pipiens, whose average body length was 32 mm, were used in this investigation. One hundred animals, designated as the experimental group, were immersed in 50 µg/l of dl-thyroxine, while the control larvae were immersed in 150 ml of tap water.

GENERAL METAMORPHIC EFFECTS

Moderate acceleration of general metamorphosis occurred which was presumably caused by small quantities of thyroxine which entered the body fluids. Change in the symmetry of the head outline from a broad round larval type to a narrow more pointed adult type was the result of exposure to thyroxine. Changes noticed on the fifth day were: slight narrowing of the body, development of additional blood vessels around the limb buds, resorption of the tail, widening of the mouth, and a change in pigmentation (color from dark to light brown). Also, the blood vessels in the gills were very much pronounced. The changes became progressively more pronounced throughout the ten day experimental period. Only one animal exposed to thyroxine was lost. This mortality was not necessarily attributed to over exposure to thyroxine. Many of the above cited metamorphic changes normally do not occur until metamorphic climax at larval stage XX (Taylor and Kollros, 1946). Since the concentration of the thyroxine solution used was small, metamorphosis,

although accelerated, was rather sequential. Animals exposed to strong thyroxine solutions usually do not live beyond five or six days. The control larvae advanced from stage III to larval stage V or VI. The experimental larvae advanced up to stage VI or VII during the ten day period.

SPECTROPHOTOMETRIC MEASUREMENT OF DEOXYDRIBONUCLEIC ACID

The amount of DNA extracted from the brain of each group of Rana pipiens tadpoles, as determined by the Dische diphemnylamine method of detection, was assayed on a spectronic 20 spectrophotometer at a 100% transmittance and zero absorbance. The results are summarized in Table I and Figures I, and III, through XIV.

Figures III through XIV, show a comparison of DNA content extracted from the brains of the control and experimental tadpoles with respect to the percent transmittance and absorbance for each experimental period through ten days. The control animals showed an average reading of 96.6% transmittance and 0.0148 or 0.015 absorbance. The average readings obtained for each of the ten experimental groups were 95.8% transmittance and 0.019 absorbance. The average percentage difference is 1.1% increase in DNA with an average absorbance difference of 0.004. The extraction procedure used (Bradshaw, 1966) is abbreviated and somewhat crude. Undoubtedly, a certain amount of DNA was lost in this procedure.

SPECTROPHOTOMETRIC MEASUREMENT OF RIBONUCLEIC ACID

The amount of RNA extracted from the brain of each group of

Rana pipiens tadpoles was determined by the Orcinol Reaction method of detection on a spectrophotometer at 100% transmittance and zero absorbance. The results are summarized in Table II.

Figures XV through XXII show a comparison of RNA content extracted from the brains of the control and experimental tadpoles with respect to the percent transmittance and absorbance.

The control animals showed an average reading of 96.1% transmittance and 0.023 absorbance. The experimental animals showed an average reading of 89.9% transmittance and 0.048 absorbance. The individual readings obtained from each experimental group from one through ten days are presented in Table II, Figures II and XV through XXII.

A comparison of the percentage RNA increase reveals an increase from the 1st day through the 10th day in each group. The control group showed a 4.0% increase compared to a 7.5% increase within the experimental group. (See Table II and Figures II and XV through XXII). The average readings of each group are also reflected in Table II.

TABLE I

A COMPARISON OF DNA CONTENT EXTRACTED FROM THE BRAIN OF THYROXINE-TREATED AND CONTROL TADPOLES OF *RANA PIPIENS*

Day Periods	Control Group % Transmittance	Experimental Group % Transmittance	% Difference From Control	Control Absorbance	Experimental Absorbance	Absorbance Difference
1st Day	99	98.8	+0.2	0.006	0.009	+0.003
2nd Day	97.5	97.5	0.0	0.01	0.01	0.000
3rd Day	97	96.0	+1	0.015	0.019	+0.004
4th Day	96	97.0	-1	0.019	0.015	-0.004
5th Day	97	95.0	+2	0.015	0.02	+0.005
6th Day	97.5	95.5	+2	0.01	0.02	+0.01
7th Day	97	95.1	+1.9	0.015	0.02	+0.005
8th Day	96.5	94.5	+2	0.0195	0.024	+0.0045
9th Day	96	94.0	+2	0.019	0.025	+0.006
10th Day	95.8	94.1	+1.7	0.0198	0.025	+0.052
Over-all Averages	96.93%	95.75%	1.18%	0.015	0.019	0.0094

TABLE II

A COMPARISON OF RNA CONTENT EXTRACTED FROM THE BRAIN OF THYROXINE-TREATED AND CONTROL TADPOLES OF RANA PIPIENS

Day Periods	Control Group % Transmittance	Experimental Group % Transmittance	% Difference From Control	Control Absorbance	Experimental Absorbance	Absorbance Difference
1st Day	97	92.5	+4.5	.012	.035	+.023
2nd Day	96.5	91.5	+5.0	.015	.040	+.025
3rd Day	96.5	96.0	+0.5	.015	.020	+.005
4th Day	96.5	91.5	+5.0	.015	.040	+.025
5th Day	99.0	92.5	+5.5	.050	.035	+.015
6th Day	96.5	90.0	+6.5	.015	.045	+.030
7th Day	97.5	87.0	+10.5	.010	.060	+.050
8th Day	96.0	86.0	+10.0	.020	.065	+.045
9th Day	92.5	86.5	+6	.035	.063	+.028
10th Day	93.0	85.0	+8	.030	.070	+.040
Over-all Averages	96.1%	89.85%	6.15%	0.022	0.048	0.033

DISCUSSION

The results of studies on RNA content in the brain of Rana pipiens larval following treatment have been presented.

The present analysis shows that, with the technique used, there is a distinguishable increase in RNA content of experimental animals over control animals for the same time period. The question is whether the exposure of thyroxine had marked effect on the brain to cause an increase in DNA and subsequently, RNA. Another question of interest could be whether or not there was a discrepancy to species variation or to artefacts during the preparations. In an attempt to answer the latter question, the procedure used was (Bradshaw, 1966). Although this method was followed closely to preclude human error, it was found to be elementary in nature, and did induce a partial loss during the macromolecule isolation. However, this loss was described to be neglible and the procedure did permit findings sufficient to make a quantitative analysis for statistical study. The procedure was modified somewhat as proceedings progressed. It was noted in each phase of the method, (RNA extraction and RNA extraction) that the frogs' brains had to be centrifuged three times as long to permit sediment of desired macromolecules. The weight of the brain promoted this observation. In separating the DNA by use of alcohol, 95% ethanol, instead of using twice the volume of the nucleoprotein extract to cause RNA to precipitate out, 200 ml of alcohol were used proportional to about 40 ml of extract. For RNA extraction 150 ml of 100% of alcohol were used, in lieu of two

volumes of RNA extract. During the RNA extraction, the first step was modified; in order to allow adequate precipitation of macromolecules in the presence of trichloracetic acid, 25 ml of each solution were placed on a magnetic stirrer for five minutes and subsequently into an ice bath for five minutes. The last modified step was also in the RNA extraction phase. In place of centrifuging precipitated RNA in alcohol solution for 10 minutes, it was allowed to settle for 5 minutes and washed with acetone as called for in the proceedings, centrifuged for 10 minutes, and dried in an oven for twenty-four hours. These modifications did not affect the end product. Trial runs were made to ensure no errors. Findings and procedures by Garner, 1967, confirmed these actions.

Garner, (1967) reported a slight increase in DNA content of the brain of Rana clamitans tadpoles after five days exposure to 200 µg/l of dl-thyroxine in 500 milliliters of water. His findings revealed an average of 92% transmittance and 0.04 absorbance in the control group, and an increase 06.5% transmittance and 0.034 absorbance in the experimental group.

Previous work done on the nervous system of Rana pipiens dealing with mitotic activity under the influence of thyroid hormone, revealed that there was an initial increase in mitotic rate followed by a decline after prolonged treatment (Weiss and Rossetti, 1951; Martin, 1962; and Reynolds, 1966). These investigators suggested that thyroxine brings about a precocious maturation of cells resulting from mitosis, thus, there are more interphase cells present in animals treated for ten days than those subjected to thyroxine for fewer days or probably

those not exposed to thyroxine. Mandel (1951) reported that after the injection of thyroxine (1mg.per day) into rats, DNA content increased from 22% to 32% in the kidney. Similar increases in spleen ranged from 35 to 60% (Mandel, 1952).

The results of this investigation support the findings of Mandel (1951, 1952) on rats. Rana pipiens stage III larva showed a slight increase in DNA when immersed in thyroxine for a ten day period, although the increase was not statistically significant. The present findings revealed an increase in the RNA content of the brain of Rana pipiens after being immersed in thyroxine solution for a ten day period. The control groups showed an average increase 4.0% compared to 7.5% increase within the experimental groups. On the basis of previous works done on this phase of this investigation, these data are in good agreement with the fact that the present results show a gradual quantitative difference between DNA and RNA.

Miyagi, Kohl and Flickinger, (1967) reported that RNA preparations from livers and kidneys of adult chickens were qualitatively distinguished by DNA-RNA hybridizations involving double saturation experiments. This technique was used to study the temporal and regional synthesis of liver-like RNA in the developing chicken embryo. It was found that the amount of liver-like RNA in the embryo increases from two and one half to three and one half days of development. Furthermore, this liver-like RNA was found in greater amount in the areas of three and one half days embryos containing the differentiating liver rudiment.

It has been shown by Martin (1962) that the brain of thyroxine

treated tadpoles becomes better differentiated than untreated tadpoles. The increase in RNA content obtained in this investigation can possibly be attributed to the differentiating brain tissue, which is accelerated under the influence of thyroid hormone. These results concur with the temporal studies of Miyagi et al (1967) on liver RNA from differentiating liver rudiments in the chick embryo.

Klevez and Stubblefield (1967a) found an increase in the rate of RNA synthesis after the synthesis of only a small portion of the DNA. They suggested that this phenomenon was the result of replication of functional DNA templates in the cell. Klevez and Stubblefield (1967b) again reported similar findings in mammalian cell cultures: that most of the DNA involved in transcription was replicated in the first third of the DNA synthesis period. Therefore the rates of RNA and protein synthesis increased because of the doubling of the active template population of each cell.

The treatment of tissue with various chemicals and hormones has been found to have varying effect on RNA, DNA content. Rao, (1968) reported that exposure of HeLa cells to nitrous oxide had no significant effect on the synthesis of DNA, RNA, or proteins. The progress of the cells through mitotic cycle was also unaffected. However, a high degree of mitotic synchrony was obtained in suspension cultures of HeLa cells treated with thymidine during exponential growth, resuspended fresh medium, and then exposed to nitrous oxide. Michels, Cason and Sokoloff, (1963) reported that treatment of rats with L-thyroxine increases the incorporation in vivo of radioactive amino acids into protein of liver,

kidney, and heart, but not of spleen, testis, or brain. The distribution of the effects among the organs is the same as that observed in thyroxine stimulation of oxidative metabolism. Thus, stimulation of protein synthesis seems to be a physiological action of the thyroid hormone. This finding also suggests that the effects of thyroxine on oxidative metabolism are secondary to its effects on protein synthesis. Subsequent studies by Sokoloff and his associates (1963) further characterized the thyroxine stimulation of protein biosynthesis, and localized it to the step in protein synthesis involving the transfer of soluble RNA-bound amino acid to microsomal protein. The effect of thyroxine treatment in vivo has been confirmed in cell-free rat liver preparations from thyroidectomized rats by Stein and Gross (1962) and most recently by Tata and his associates, (1962).

The present findings support the conclusions of those investigators that thyroxine stimulates an increase in RNA content. However, the work of Michels et al (1963) indicated that rats treated with L-thyroxine did not show an increase in the incorporation of radioactive amino acids into protein in the brain. Since adult rats were used and brain tissue becomes differentiated early these factors may account for their results. In the present study young tadpoles were used in which the brain tissue was still undergoing differentiation. Tissue specificity as well as species specificity are probably important factors which may account for differences in quantity of RNA synthesis in various animals.

It is suggested that further investigations using different hormones be made using the same animals, to compare effects on the

tadpole's brain with those revealed in this report and that of Garner (1967). It is also suggested that a concomitant study be made on the RNA content of the tadpole brain in a series of larval stages I through XXV, using normal and thyroxine-treated tadpoles.

SUMMARY

1. Rana pipiens tadpoles from select stages were used in order to determine the effects of thyroxine on RNA content of the tadpole brain.
2. It was found that the brain in tadpoles treated with thyroxine showed an increase in DNA and RNA content.
3. The increase in RNA content of the tadpole brain in experimental animals doubled that of the control group and assayed DNA content of the same experimental group.
4. It was concluded that thyroxine stimulated in RNA synthesis in the developing brain of Rana pipiens tadpoles.

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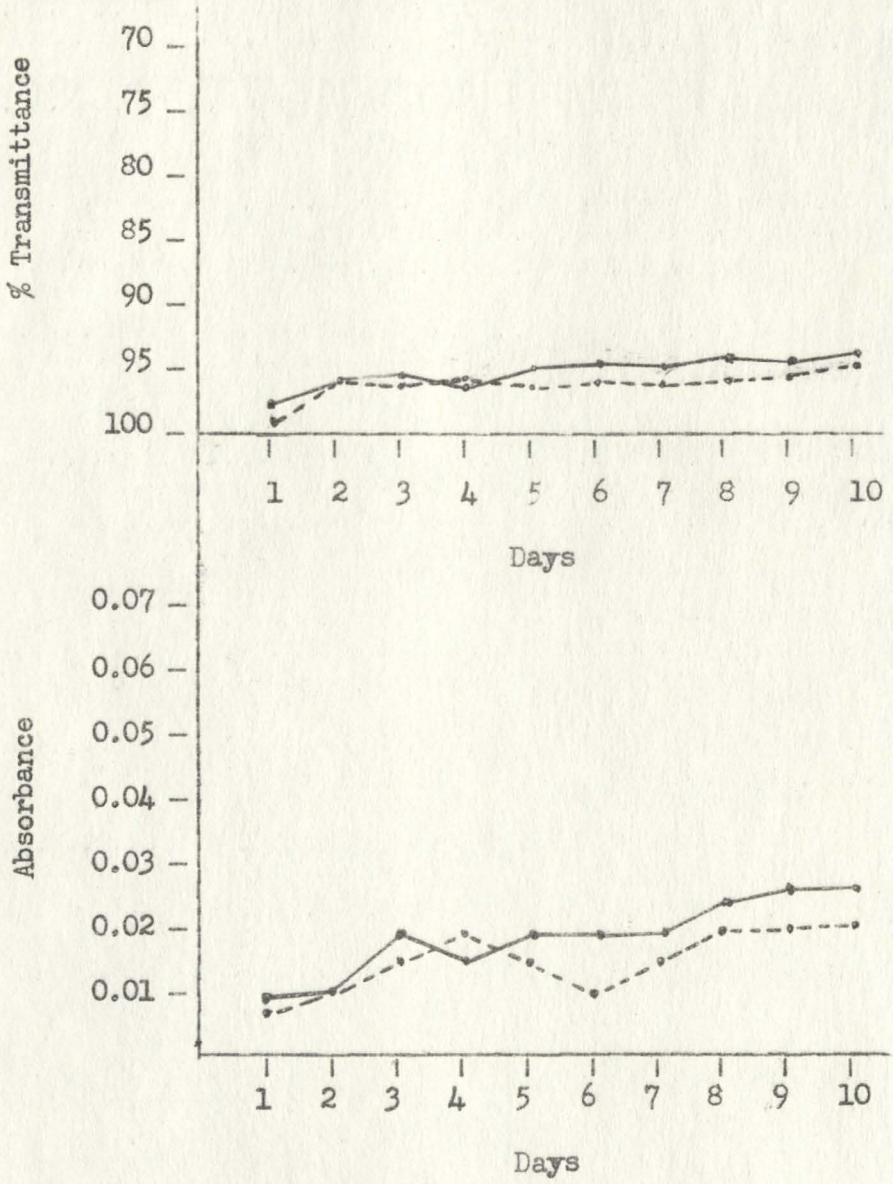
APPENDIX

Figure I

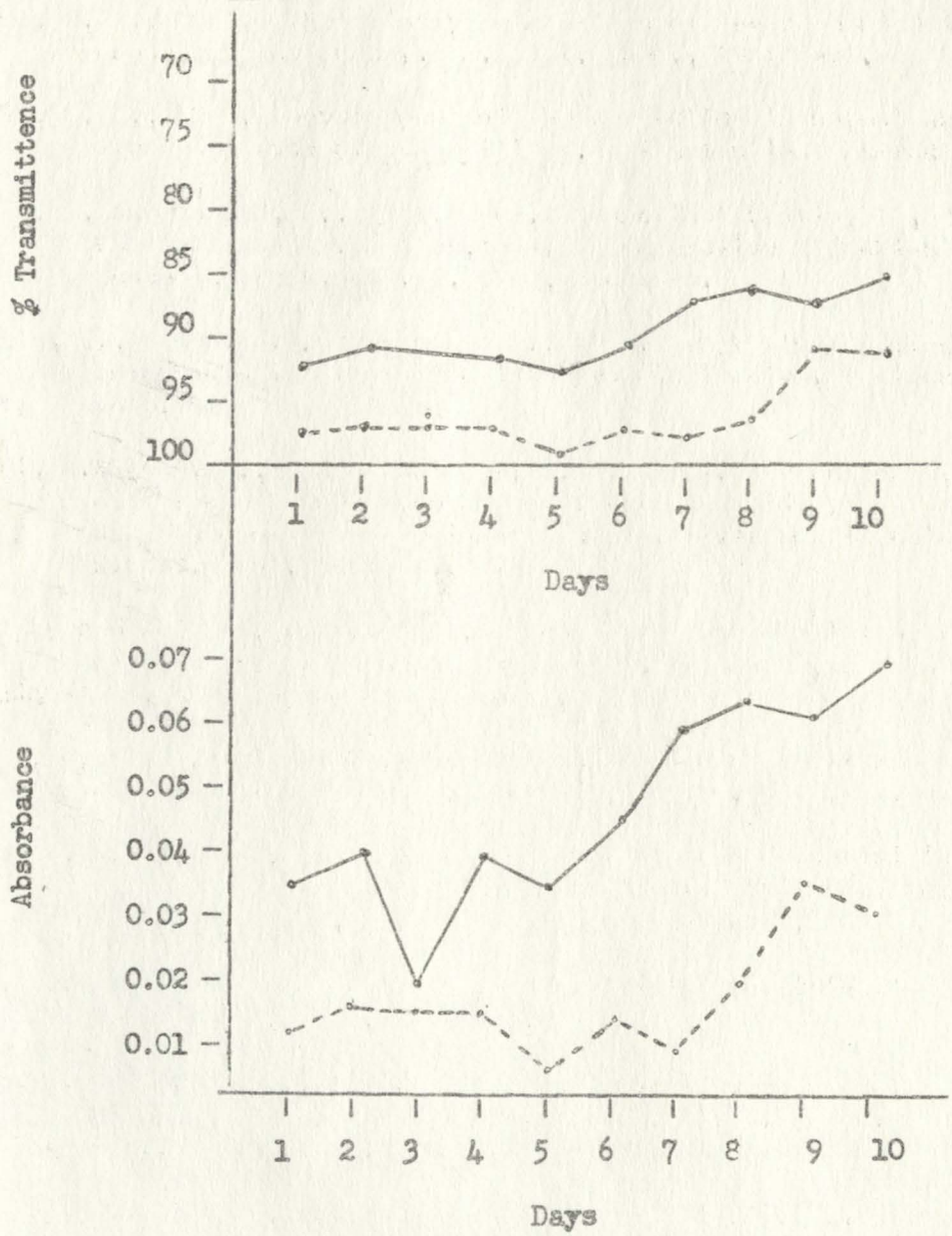


A comparison of DNA content increase in proportion to Days Exposed To Thyroxine.

_____ Experimental

_ _ _ _ Control

Figure II

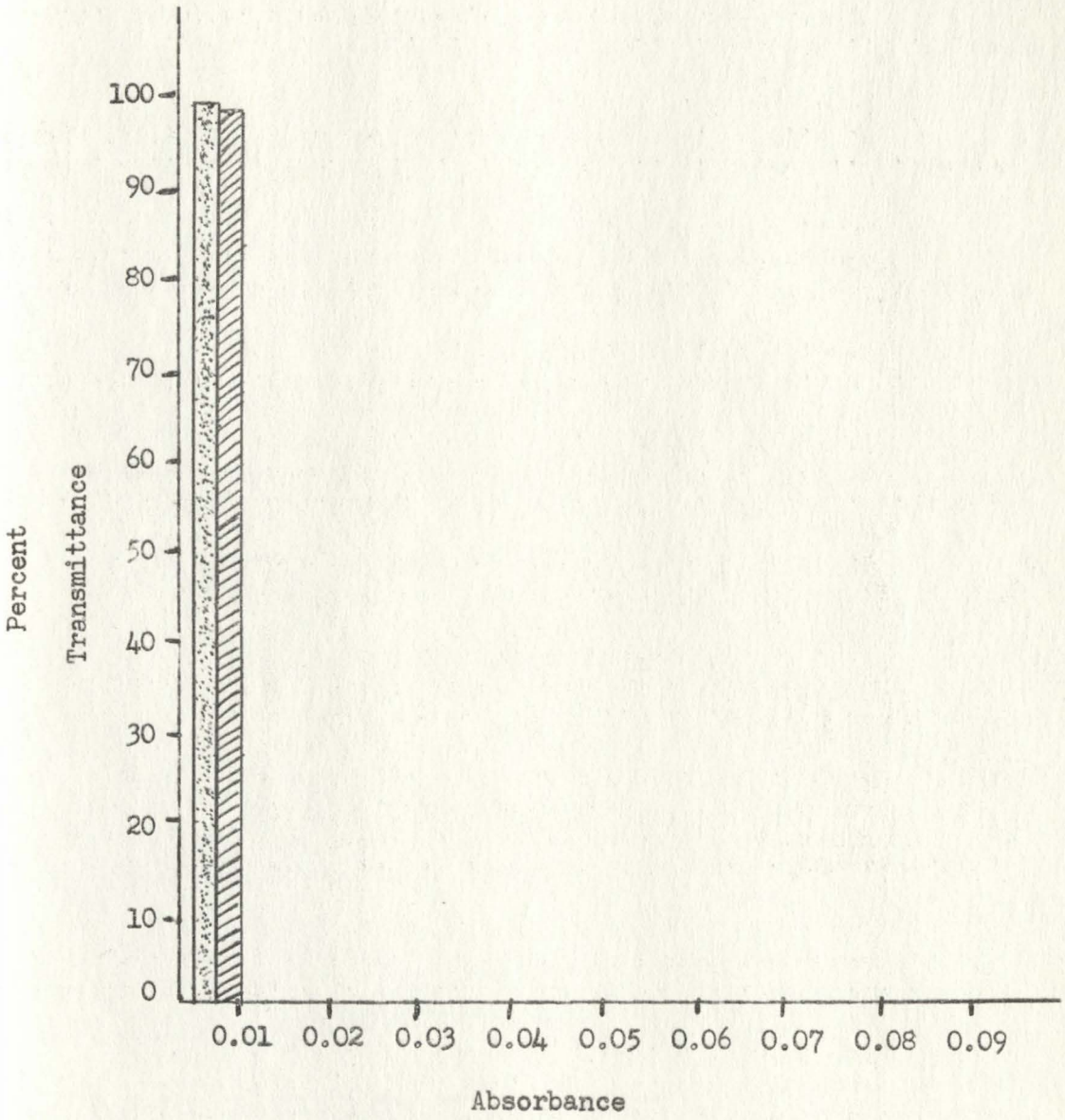


A comparison of RNA content increase in proportion to Days Exposed To Thyroxine.

_____ Experimental

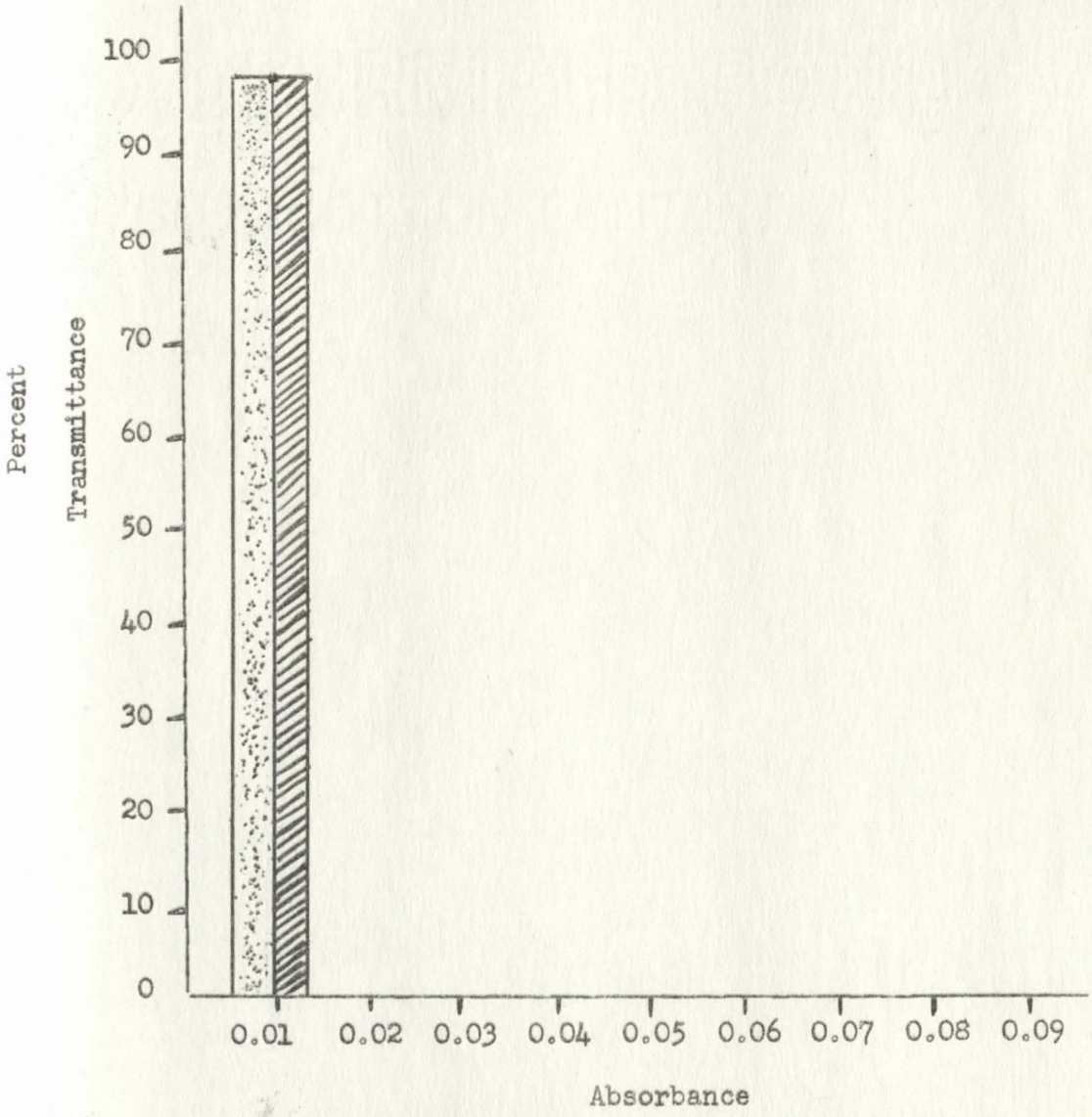
_ _ _ _ _ Control

Figure III



A comparison of DNA between the control and the Thyroxine treated tadpoles for one day

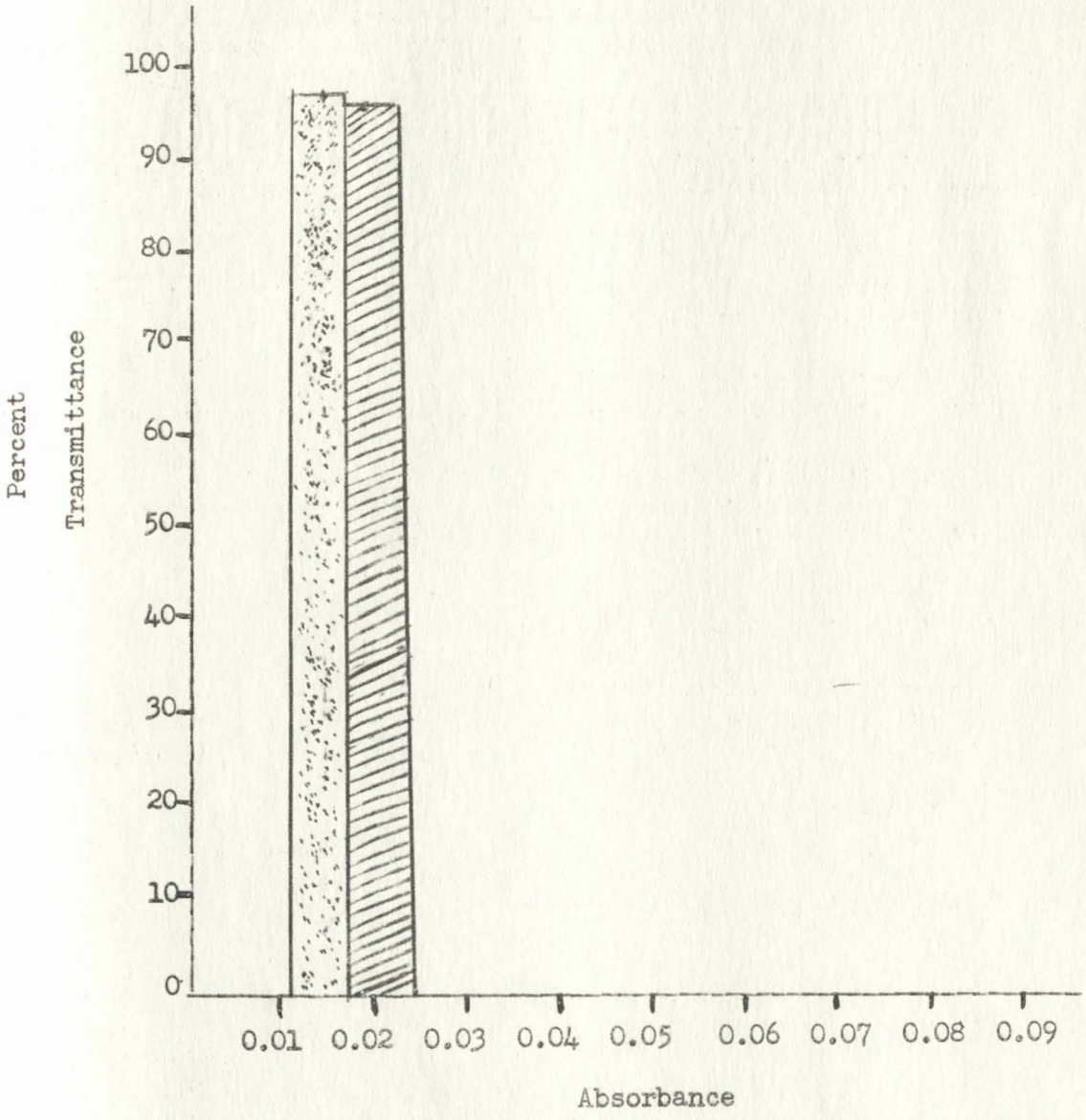
Figure IV



A comparison of DNA between the control and the Thyroxine treated tadpoles for two days

Experimental

Control

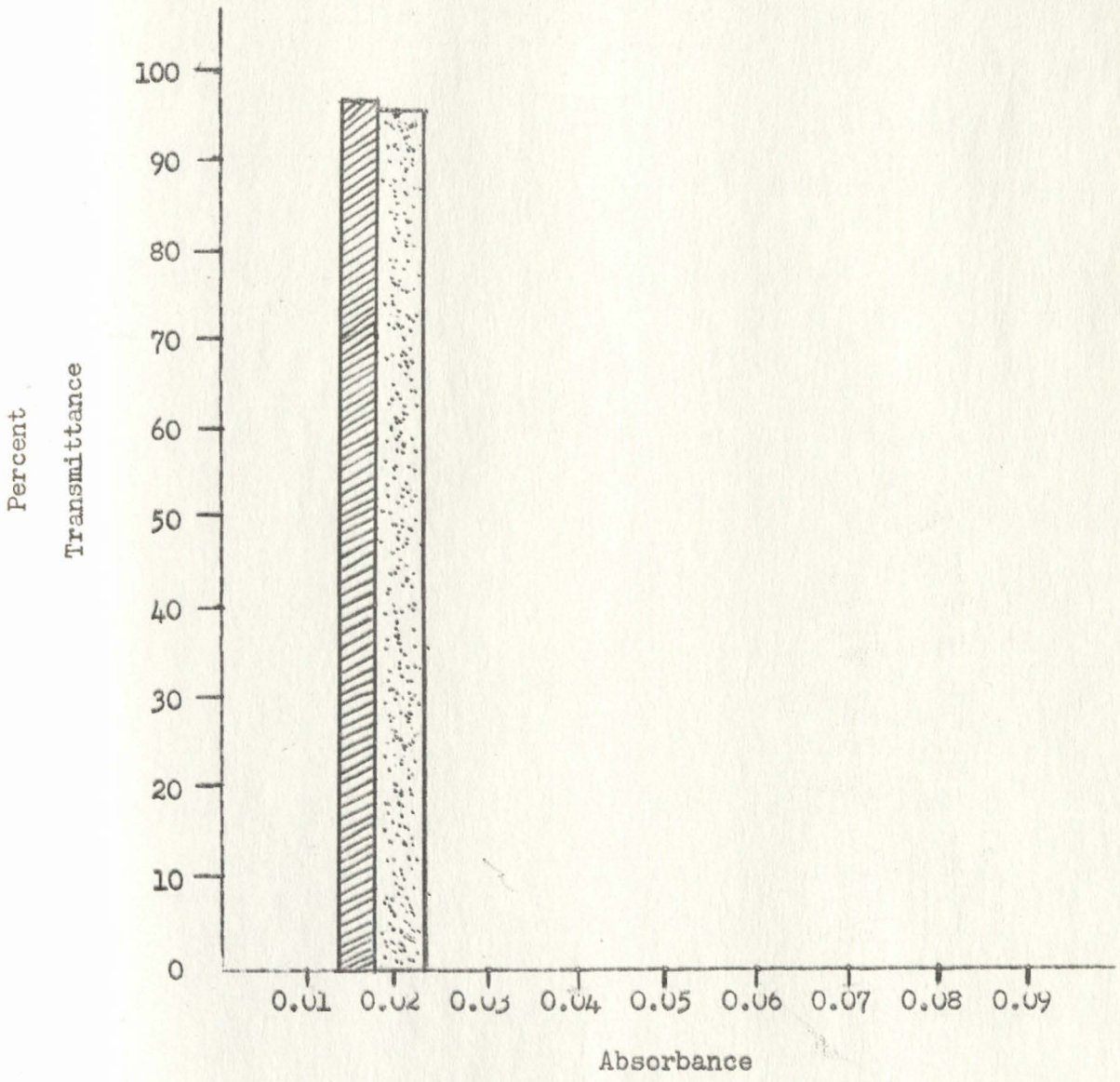


A comparison of DNA between the control and the Thyroxine treated tadpoles for three days

Experimental

Control

Figure VI

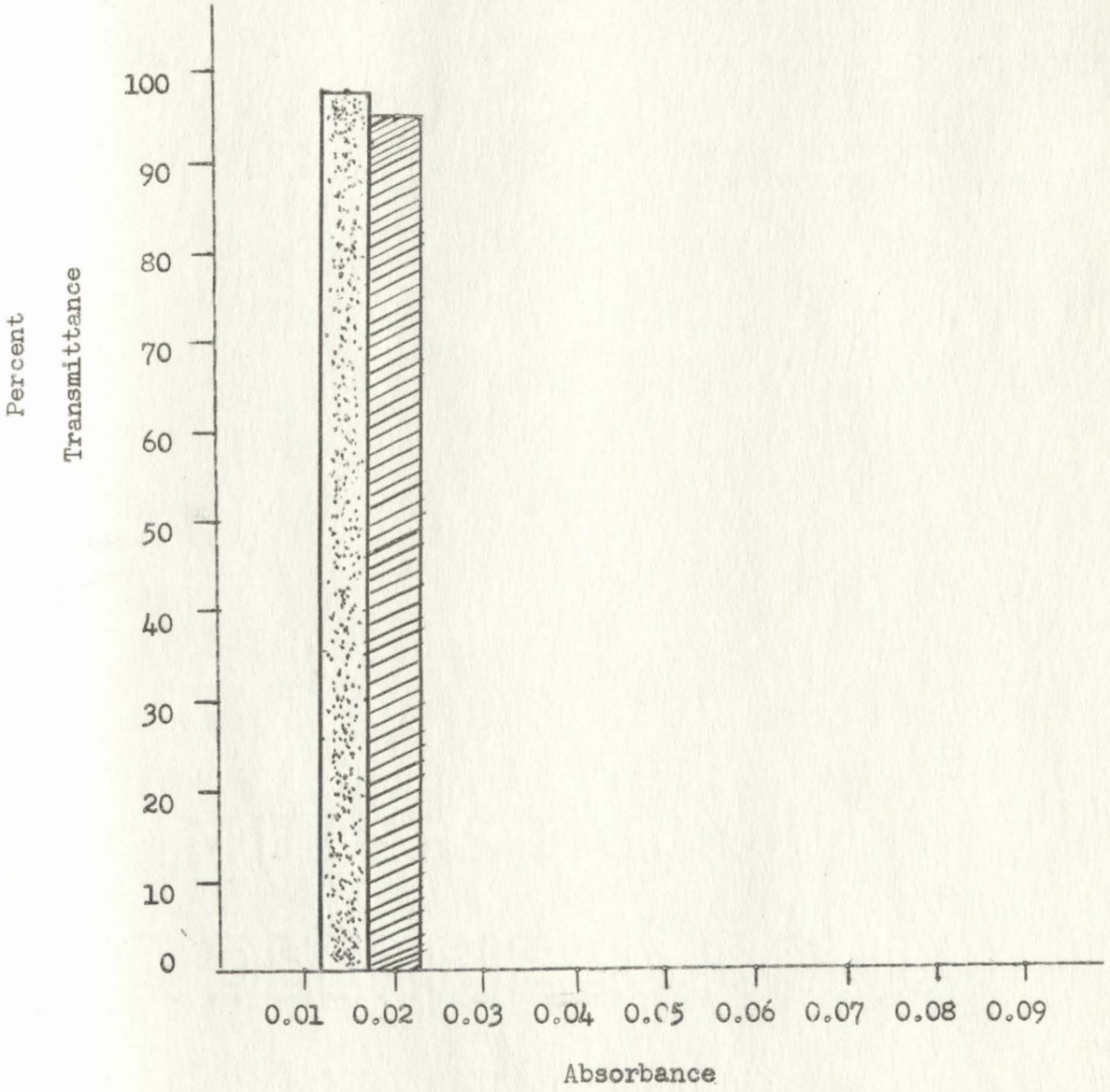


A comparison of DNA between the control and the Thyroxine treated tadpoles for four days

Experimental

Control

Figure VII

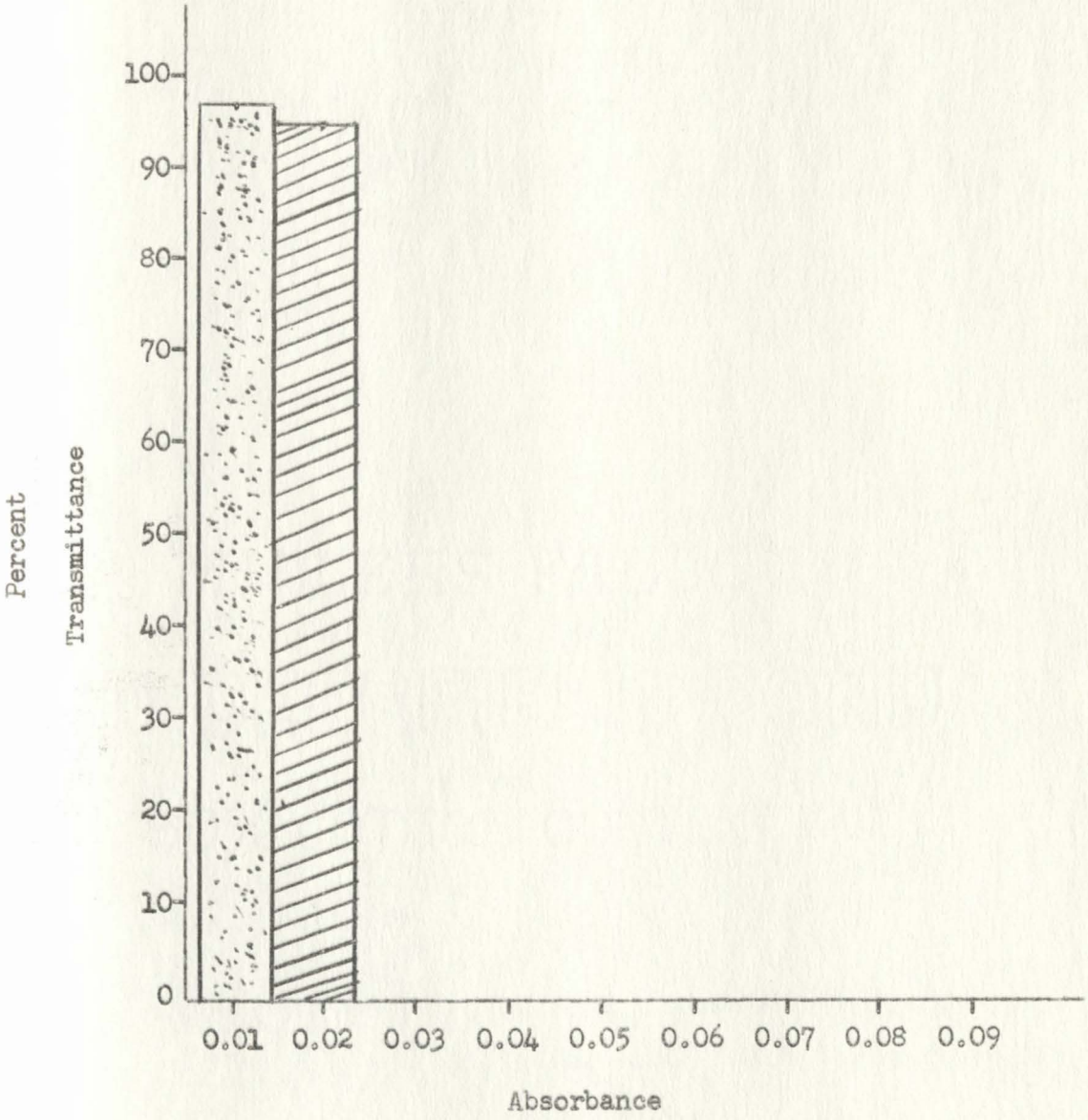


A comparison of DNA between the control and the Thyroxine treated tadpoles for five days

Experimental

Control

Figure VIII

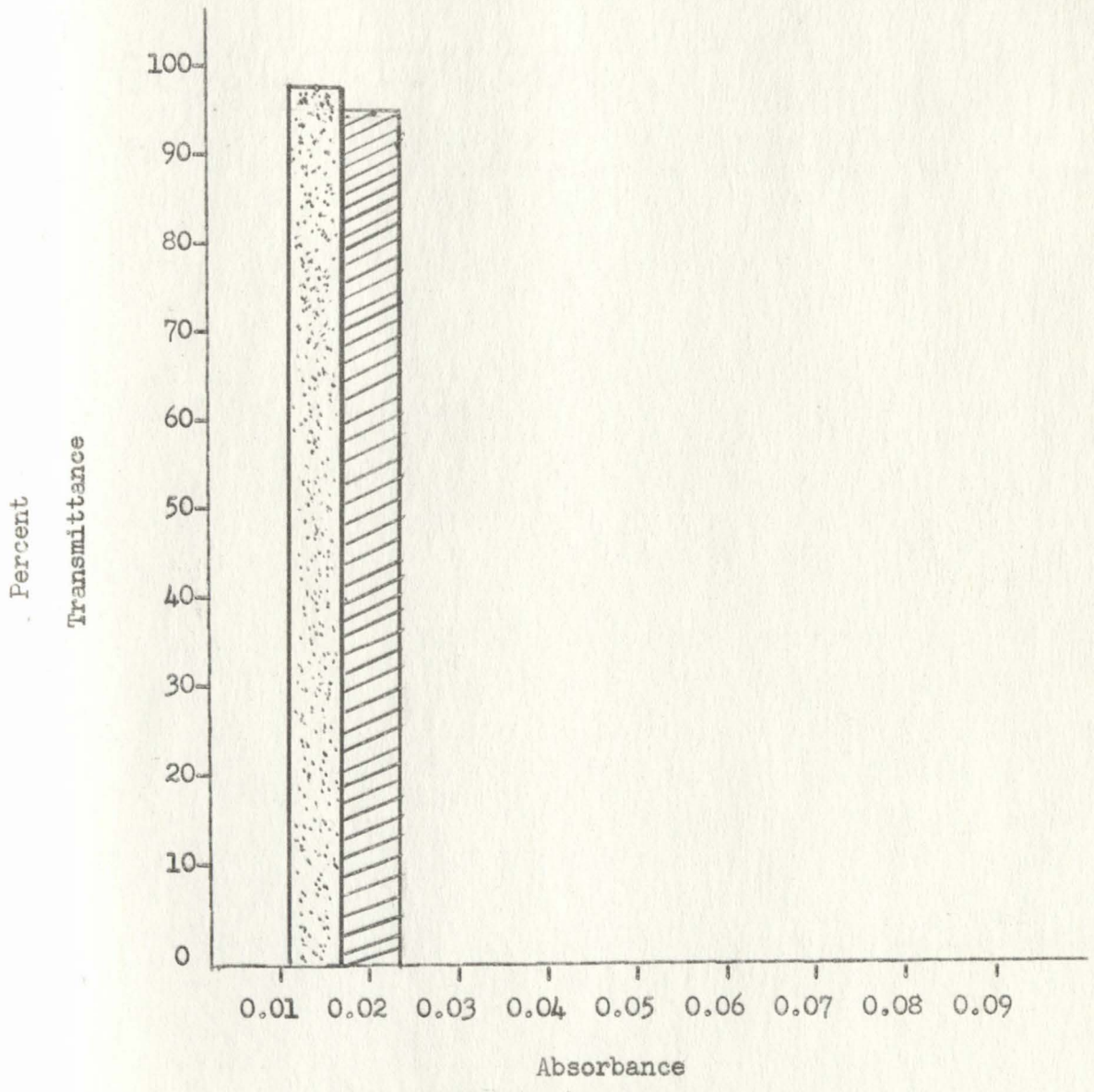


A comparison of DNA between the control and the Thyroxine treated tadpoles for six days

Experimental

Control

Figure IX

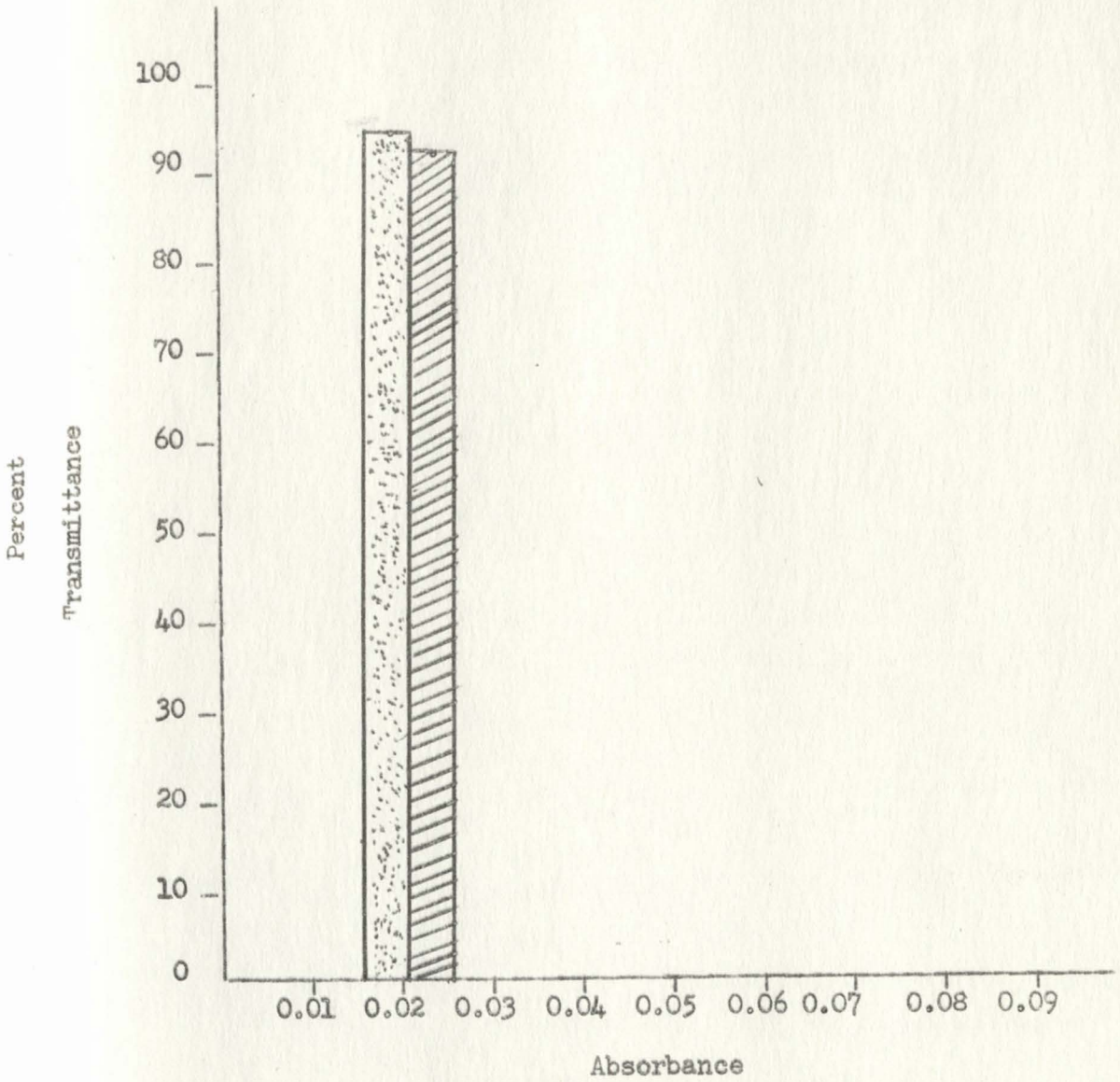


A comparison of DNA between the control and the Thyroxine treated tadpoles for seven days

Experimental

Control

Figure X

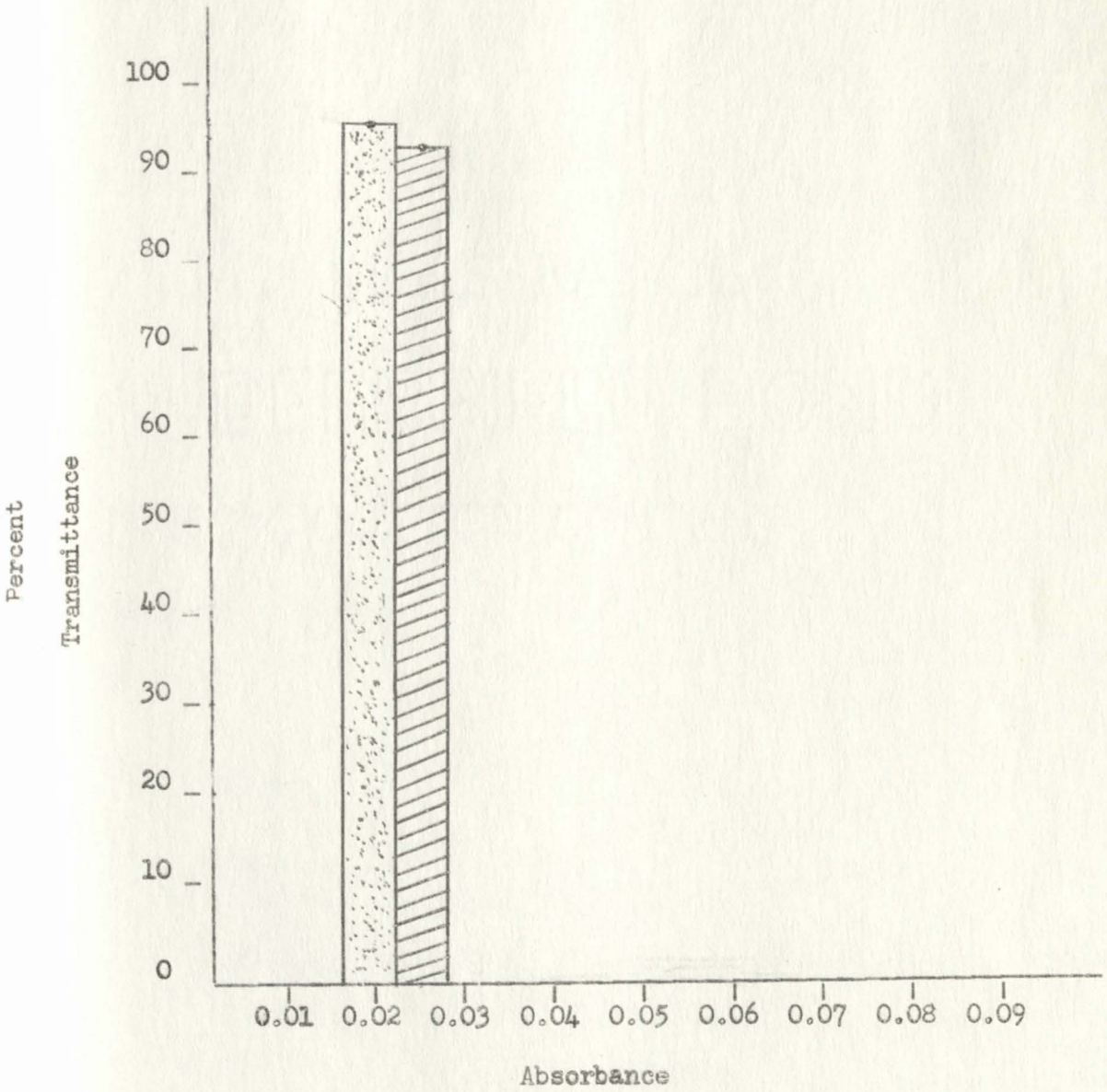


A comparison of DNA between the control and the Thyroxine treated tadpoles for eight days

Experimental

Control

Figure XI

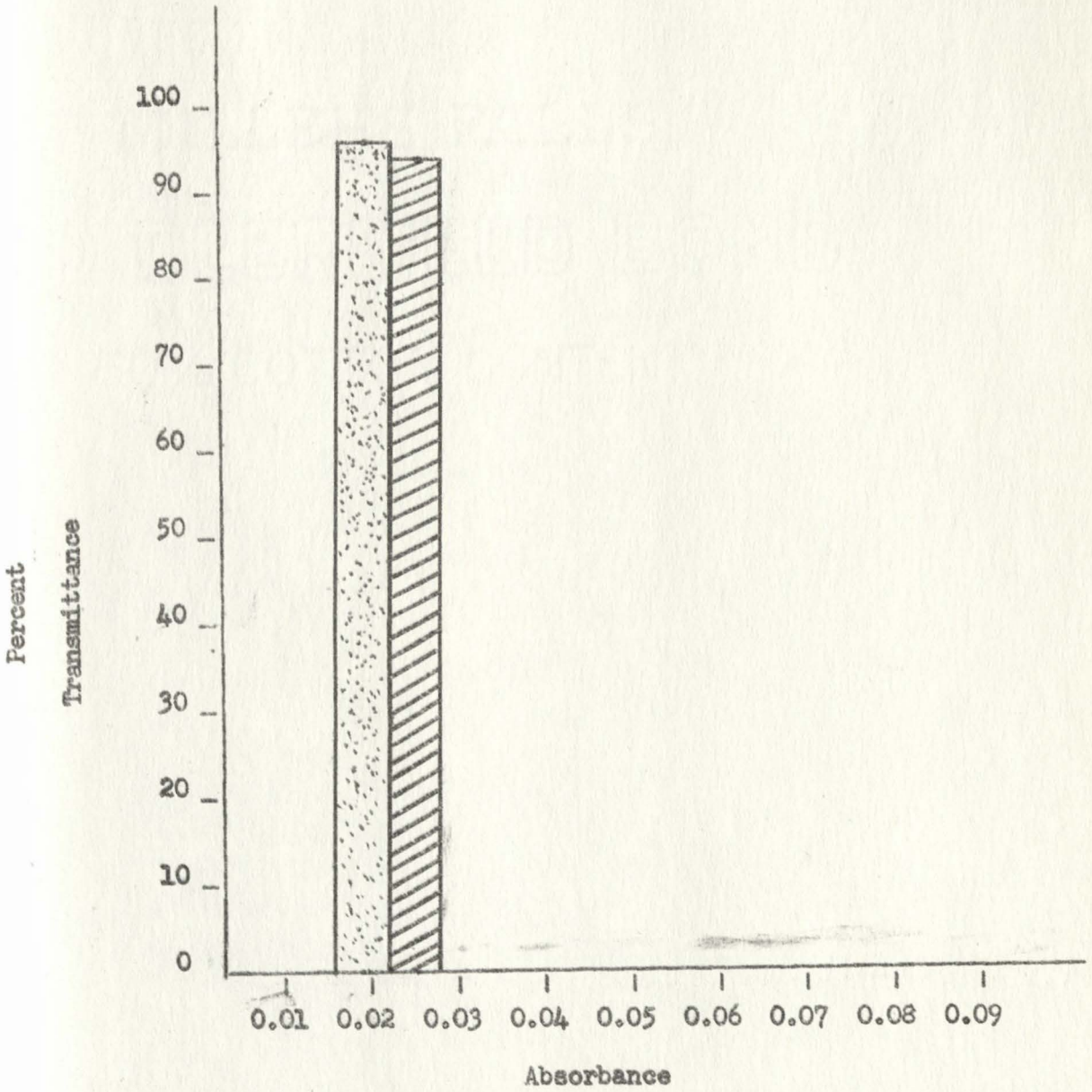


A comparison of DNA between the control and the Thyroxine treated tadpoles for nine days

Experimental

Control

Figure XII

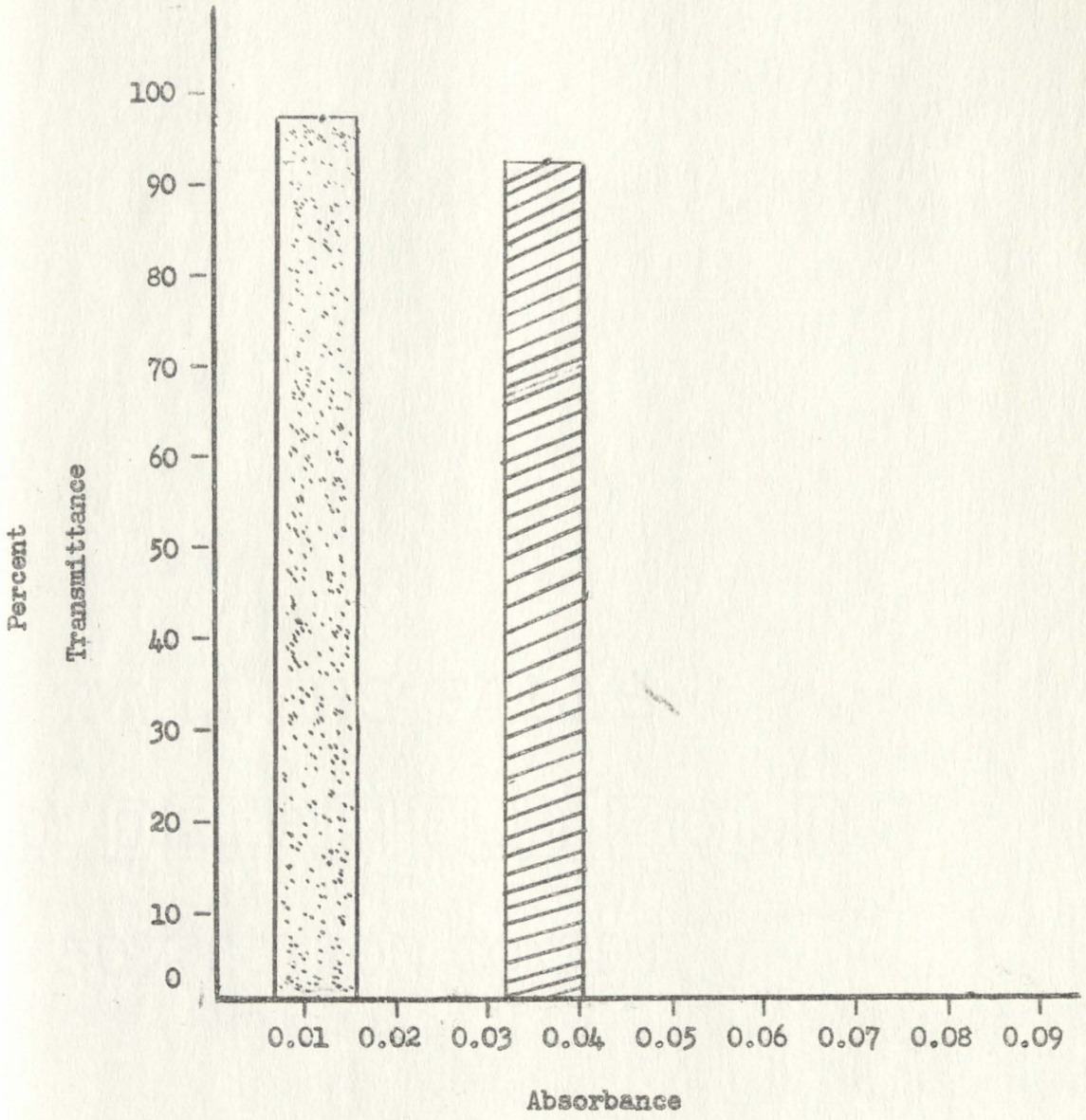


A comparison of DNA between the control and the Thyroxine treated tadpoles for ten days

Experimental

Control

Figure XIII

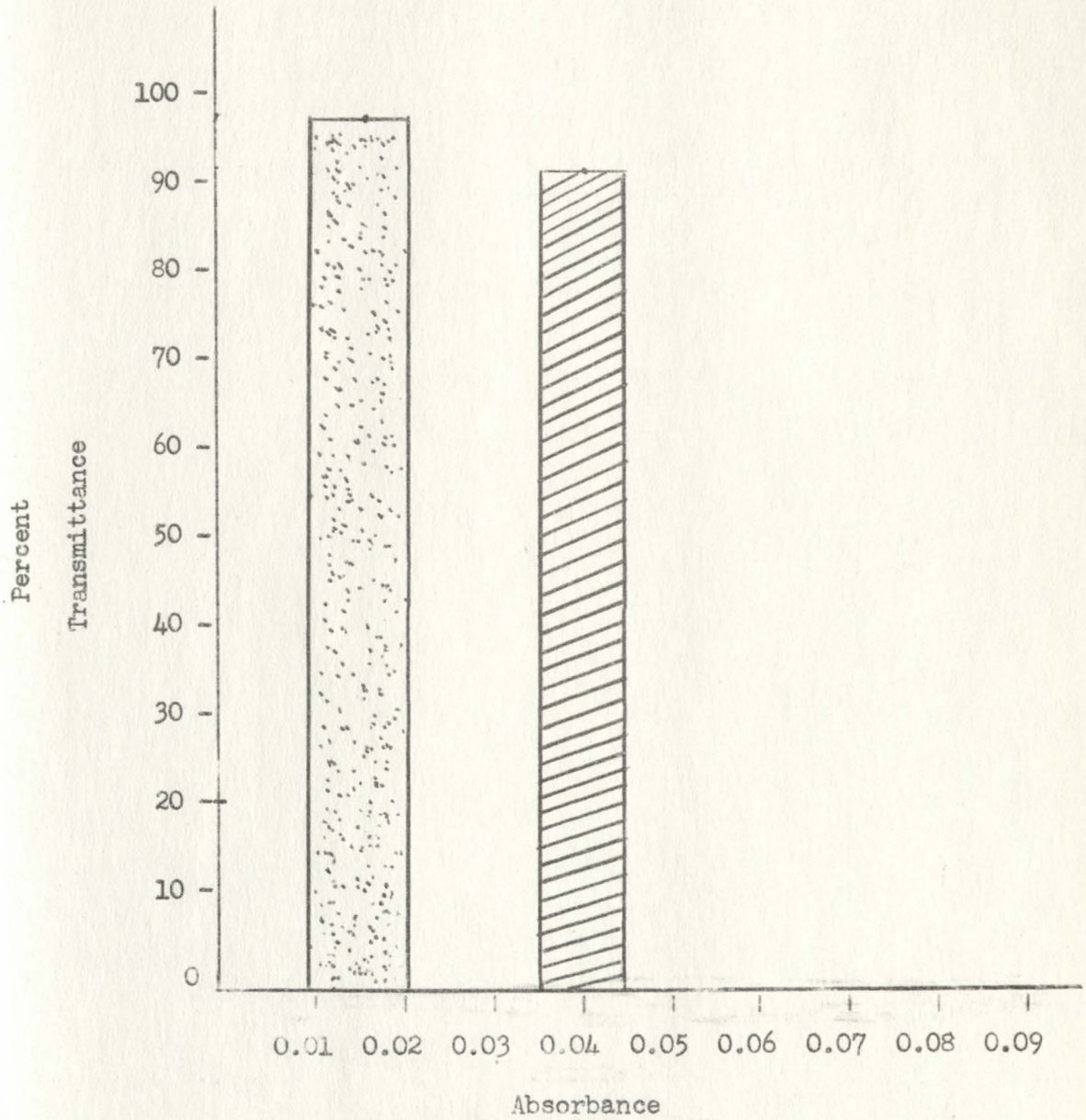


A comparison of RNA between the control and the Thyroxine treated tadpoles for one day

Experimental

Control

Figure XlV

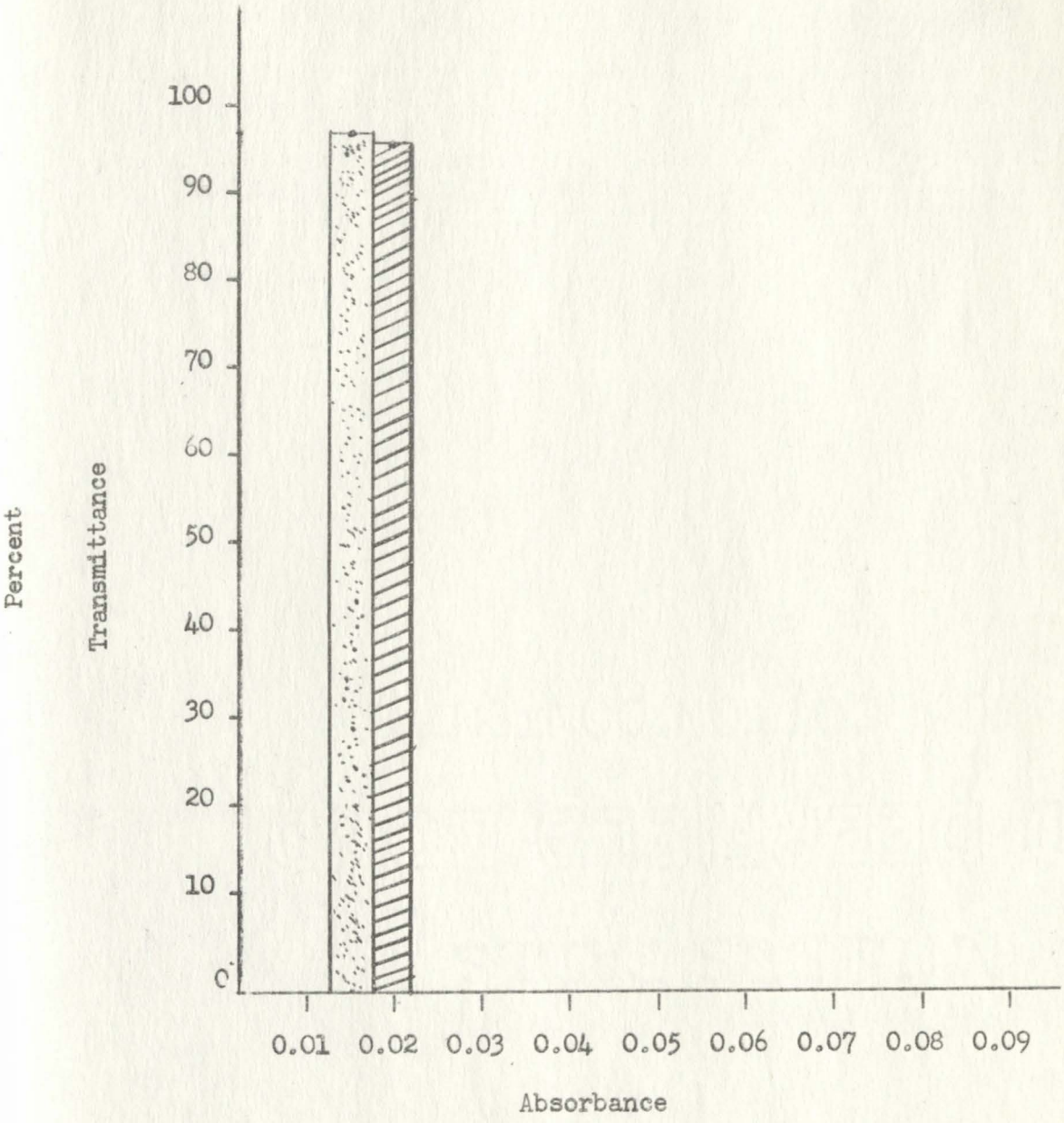


A comparison of RNA between the control and the Thyroxine treated tadpoles for two days

Experimental

Control

Figure XV

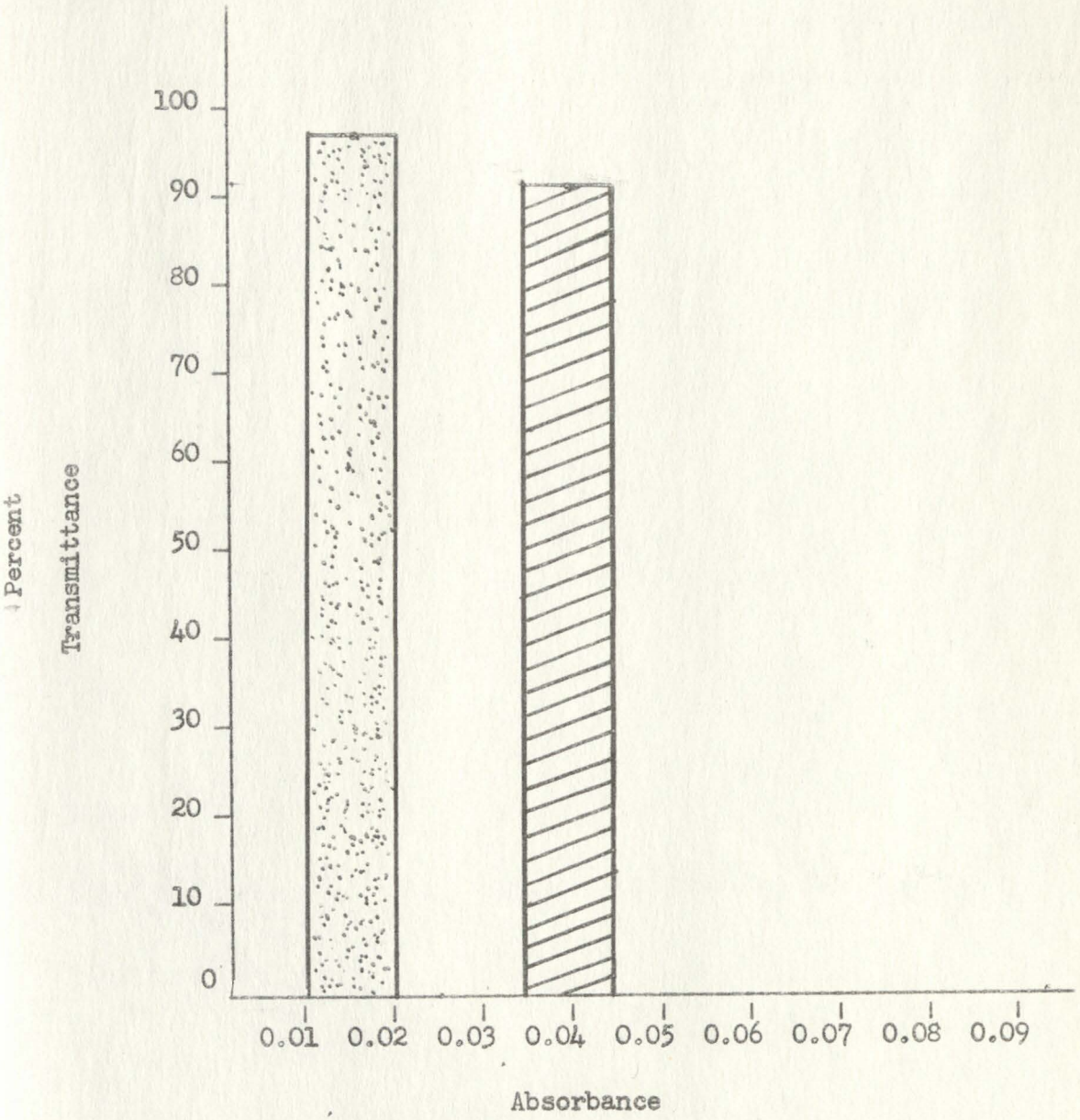


A comparison of RNA between the control and the Thyroxine treated tadpoles for three days

Experimental

Control

Figure XVI

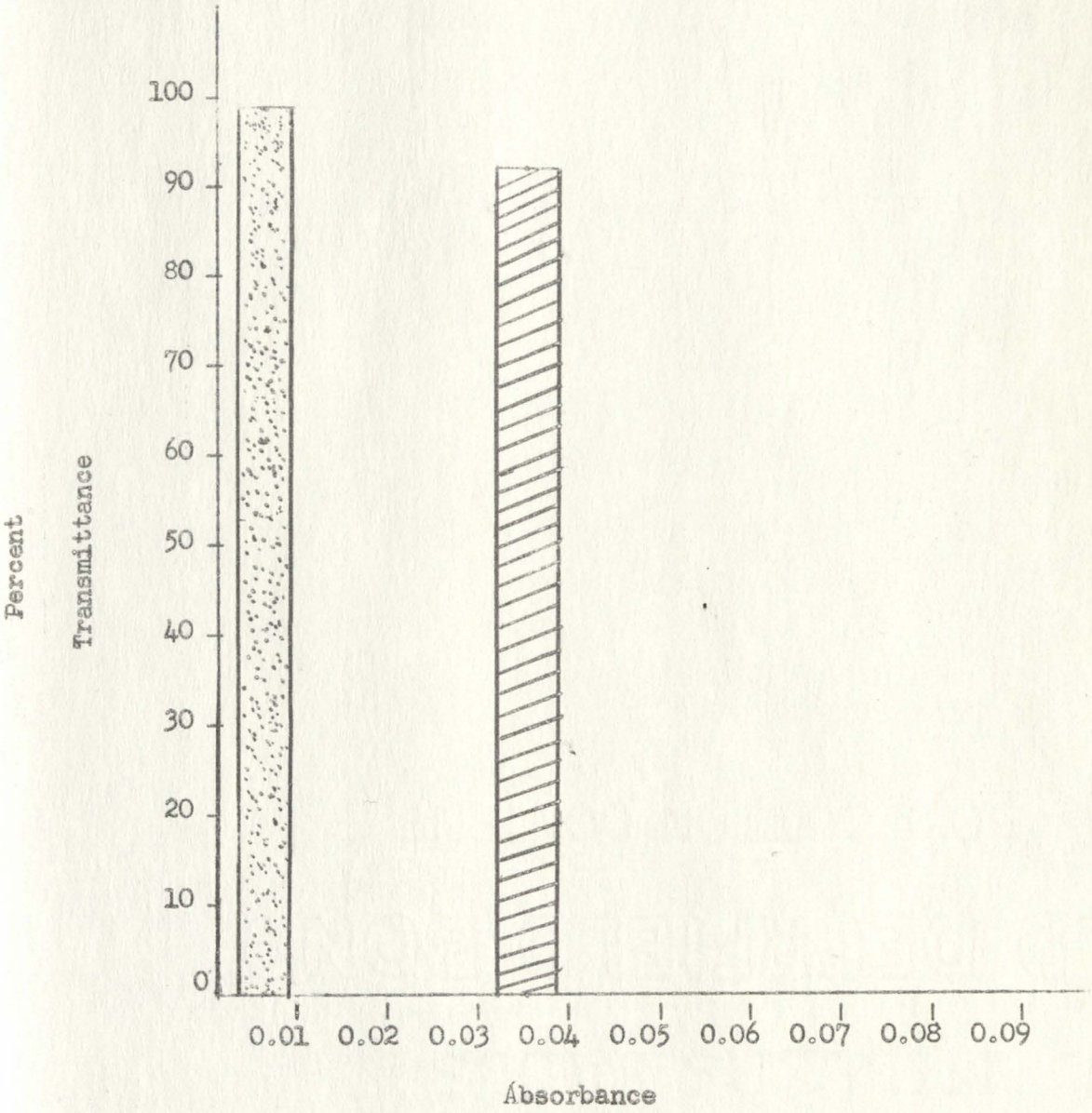


A comparison of RNA between the control and the Thyroxine treated tadpoles for four days

Experimental

Control

Figure XVII

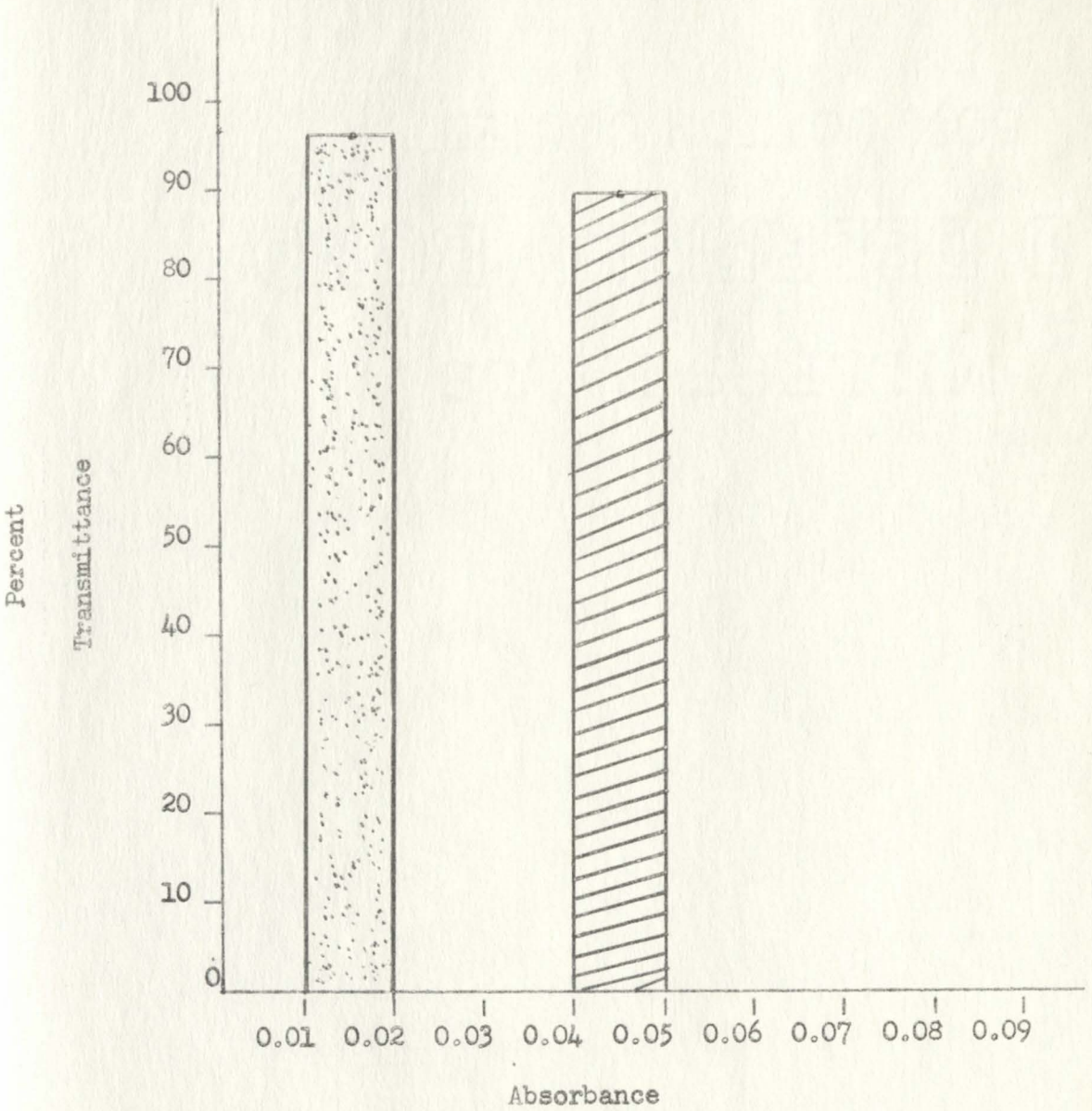


A comparison of RNA between the control and the Thyroxine treated tadpoles for five days

Experimental

Control

Figure XVIII

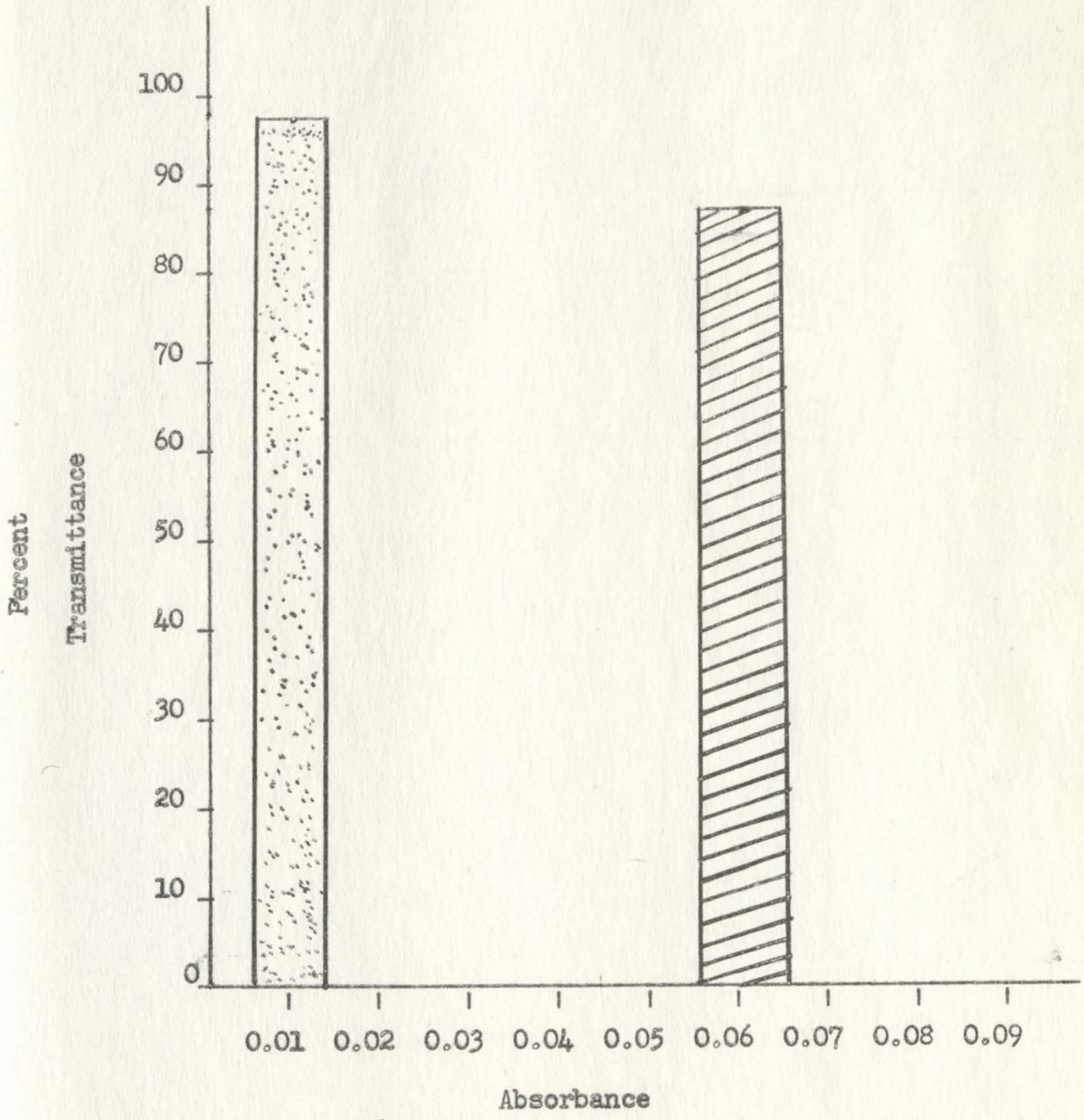


A comparison of RNA between the control and the Thyroxine treated tadpoles for six days

Experimental

Control

Figure XIX

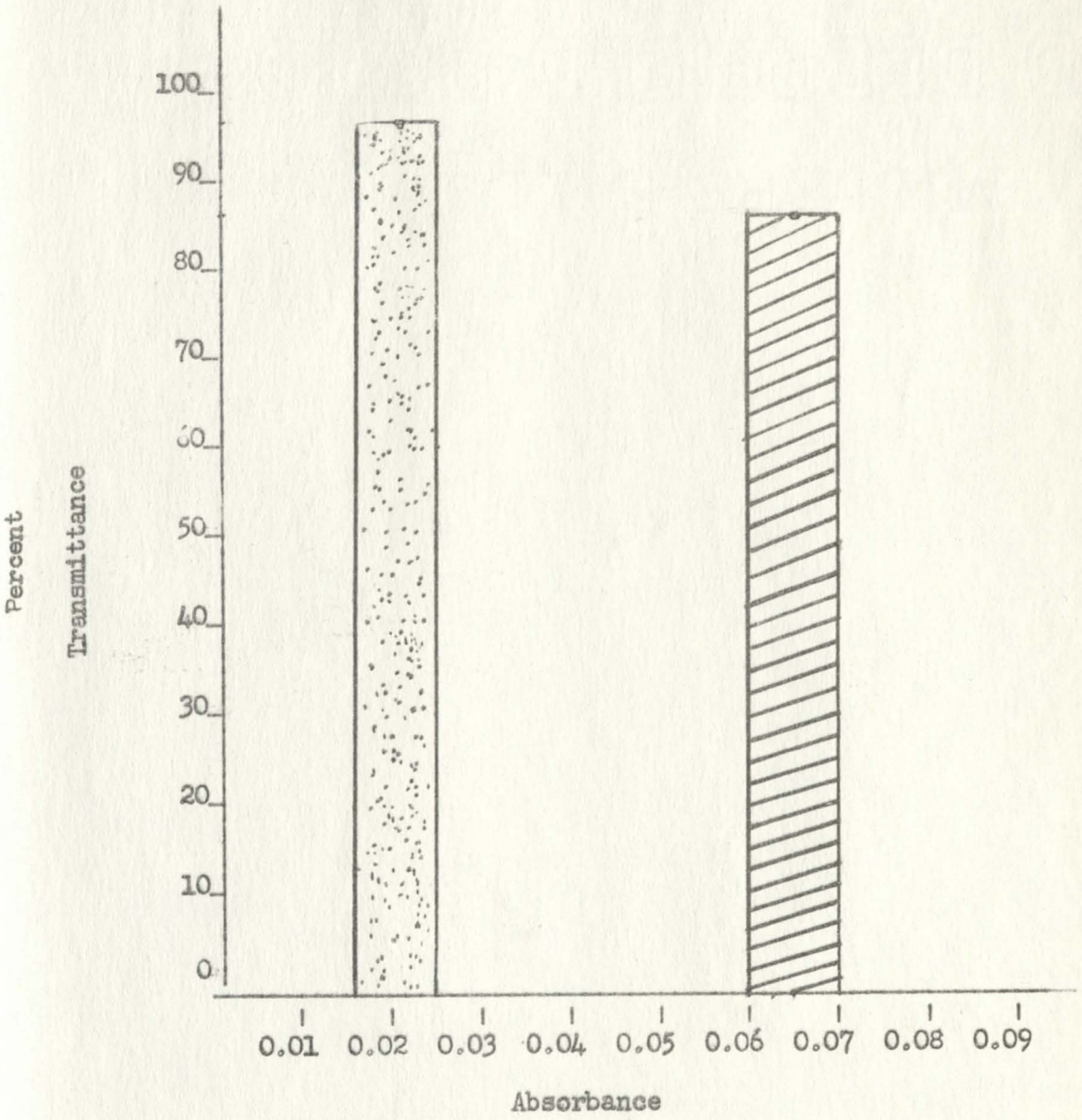


A comparison of RNA between the control and the Thyroxine treated tadpoles for seven days

Experimental

Control

Figure XX

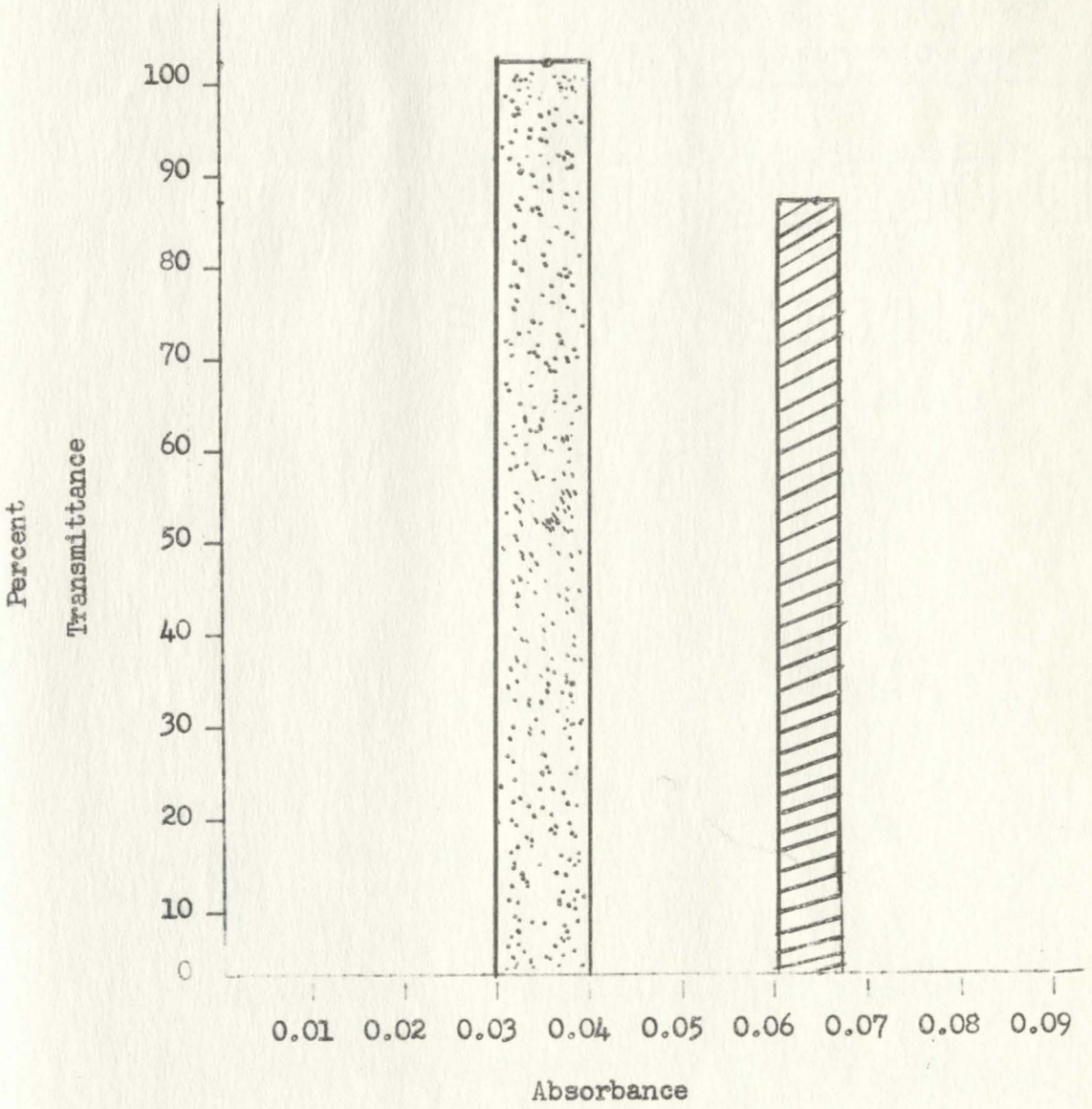


A comparison of RNA between the control and the Thyroxine treated tadpoles for eight days

Experimental

Control

Figure XXI

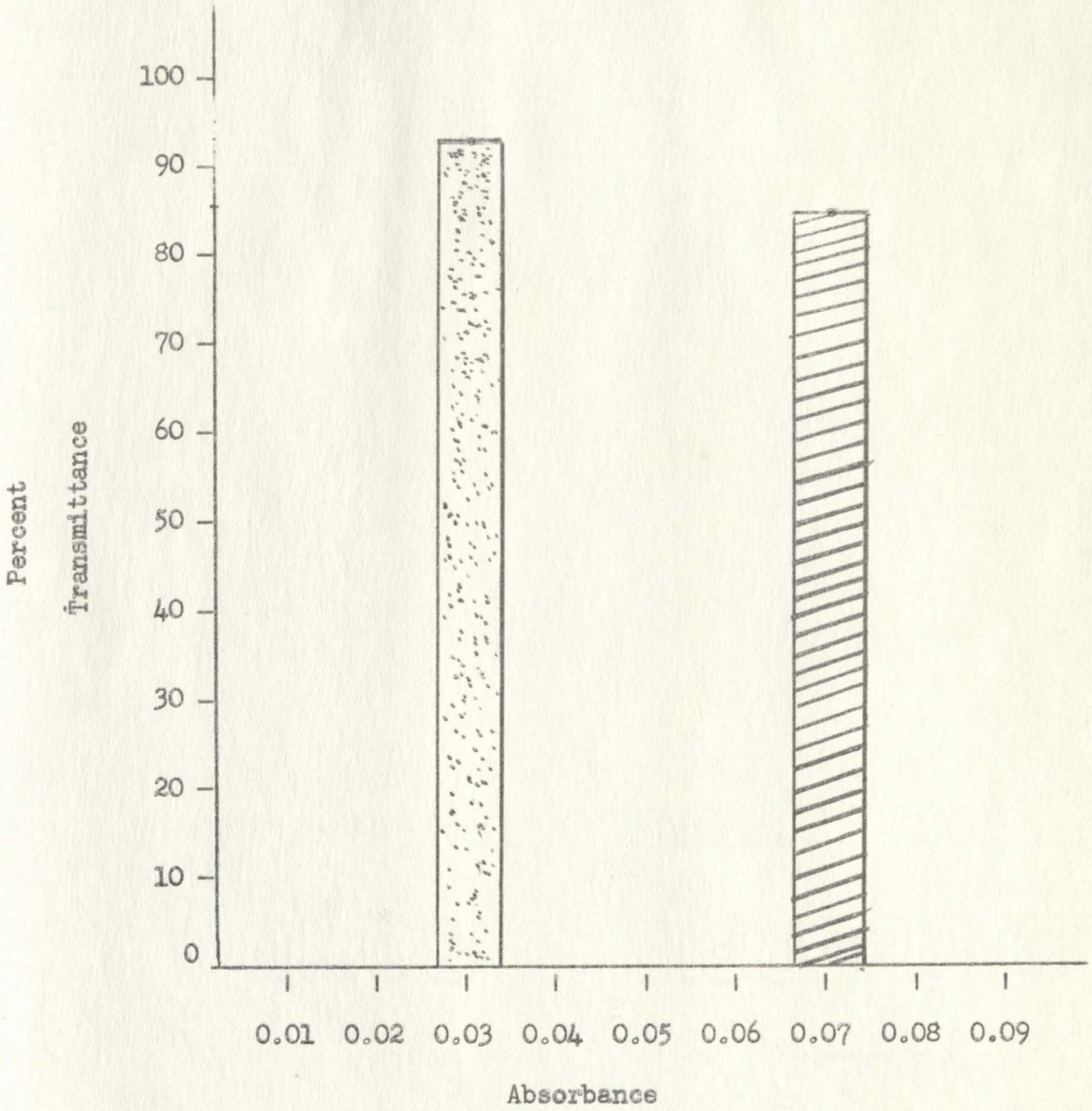


A comparison of RNA between the control and the Thyroxine treated tadpoles for nine days

Experimental

Control

Figure XXII



A comparison of RNA between the control and the Thyroxine treated tadpoles for ten days

Experimental

Control