



THE EFFECT OF INJECTED THIOURACIL ON BODY
WEIGHT AND HATCHABILITY
OF THE CHICK EMBRYO

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THE EFFECT OF INJECTED THIOURACIL ON BODY WEIGHT AND
HATCHABILITY OF THE CHICK EMBRYO

By

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AN ABSTRACT


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This study was done to determine the effect of thiouracil on early chick embryos. Most of the data published were mainly after thiourea or thiouracil were injected.

First, different dosages of thiouracil were tried to determine the toxic level of this drug. Five milligrams of thiouracil were found to be the toxic level for the chick embryos and 2.0 mg. was found to be the maximum level to be used in any physiological work.

One-tenth, 0.2, 0.5 and 2.0 mg. of thiouracil was injected into the small end of fertile eggs that had been incubated for 48 hours.

Two groups of controls were kept. One group was injected with Ringer's solution and one group received no injection. The eggs were then incubated in forced draft incubator at 37.5° C and a relative humidity of 66%. The eggs were turned every two hours and were candled at 48 hour intervals after the treatment.

Between the 7th and the 21st day of incubation, twelve eggs from each of the treatments were randomly selected and sacrificed so embryos' body weights could be determined.

The average body weights of all thiouracil injected embryos autopsied on the 7th through the 21st day of incubation were lower than those of controls. Significant weight differences between individuals receiving different levels of thiouracil were noted after the 11th day of incubation.

In each group, 12 fertile eggs were left in the incubator after the 21st day of incubation until they hatched or died. The experiment lasted for 6 more days and on the 27th day all the remaining eggs were sacrificed. Embryos receiving 0.1, 0.2, 0.5 and 1.0 mg. hatched by the 25th day of incubation. On the 27th day, some live embryos were found in the eggs which had received an injection of 2.0 mg. of thiouracil.

Both groups of controls hatched on the 20th and 21st day of incubation. All of the control embryos showed retraction of the yolk sac at hatching time; whereas in many of the treated embryos, yolk sacs were still outside the body cavity at hatching time.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

History. Certain drugs such as sulfocyanide, thiourea and thiouracil, inhibit the formation and secretion of thyroid hormone and thus are a powerful goitrogenic substance in many different animals and man (MacKenzie, MacKenzie and McCollum, 1941; Ritcher and Clisby, 1941; Kennedy, 1942; Astwood, Sullivan, Bissell, and Tyslowitz, 1943; Mackenzie and Mackenzie, 1943). These workers further reported that the characteristic morphological changes produced in the thyroid gland by these drugs are a loss of colloid, hyperemia, and enlargement (hypertrophy) and multiplication of cells (hyperplasia) leading to an increased size and weight of the thyroid.

Adams and Bull (1949) injected thiourea and thiouracil (Deracil) into yolk sac of developing chick embryos of White Plymouth Rocks. Dosages used were 0.5 ml. of a solution of 0.4% thiourea and 0.1% to 0.2% thiouracil on the 8th, 14th, and 18th day of incubation. They reported that the main effects were retardation of hatching in those eggs incubated to the 21st through 24th day and lack of retraction of the yolk, decreased body and limb growth, enlargement of the thyroid gland with typical hyperemia, hypertrophy and hyperplasia, a marked increase in height of follicular epithelium and colloid depletion.

Grossowitez (1946) found that chick embryos whose yolk sacs were injected with thiourea between the 7th and 17th days of incubation were delayed in hatching and their yolk sacs were not retracted. McCreight (1950) reported similar reaction in another strain of chicks. Adams and Buss (1952) working with White Plymouth Rock chick embryos which were given a single injection of thiourea and methyl thiouracil found that cell proliferation occurred in the thyroid gland.

Vidal (1952) reported that thiourea injected at the 7th day and 15th day of incubation caused generalized retardation of growth with no specific abnormality but high mortality.

Romanoff and Lauffer (1956) reported the effect of injected thiourea on the development of some organs of the chick embryo. They confirmed some of the above findings and showed that the administration of thiourea resulted in prolongation of embryonic development and high mortality. In all cases the thyroid was markedly hypertrophied, and increased in weight. They also reported an increase in the weight of the adrenal and testes and a considerable decrease in liver and ovarian weights.

Many investigators have reported hypertrophy and hyperplasia of the thyroid gland in fish, amphibians, reptiles, birds and mammals from treatment with anti-thyroid drugs; with emphasis on the increased size of the follicular

cells and weight of the thyroid (Charipper and Gordan, 1947; Adams and Bull, 1949; Adams and Craig, 1949, 1950, 1951; D'Angelo, Gordon and Charipper, 1947; Domm and Beivaiss, 1948; Goldsmith, 1949; Lynn and Wachowski, 1941; McCreight, 1950).

Mitotic activity shortly after injection of anti-thyroid drugs or goitrogenic compounds have been reported (Paschkis, Cantarow, Rakoff, and Rothenberg, 1945; Adams and Bull, 1949). Paschkis et al, 1945, reported that thiouracil given daily to rats in drinking water produced a hyperplasia as soon as 24 hours after treatment. Adams and Buss, 1952, determined the mitotic activity in chick embryos given one injection of an anti-thyroid drug on the 14th day of incubation and killed after 24 additional hours of incubation. The 14th day was chosen for the single injection because on the 13th day of incubation the follicles of the thyroid have become distinct and contain colloid (Bradway, 1929). The gland is completely developed by the 15th day of incubation (Sem, 1932; Martindale, 1941; Bull, 1948). The results from Adams and Buss (1952) using White Plymouth Rock chick embryos showed that counts of mitosis in comparable areas with cell proliferation began as early as the cellular hypertrophy and colloid loss in the thyroids of the treated embryos. Twenty-four and forty-eight hours after injection mitosis in the injected embryos exceeded that in the controls. Thus they confirmed that the weight of the thyroid of chick

embryos treated with anti-thyroid drugs was not only due to hypertrophy of follicular cell but also due to a marked increase in the number of cells by mitosis.

Working with day-old chicks, Astwood, Bissell and Hughes (1944) found that when 0.1% thiouracil was given in the ration for 10 days the thyroid was enlarged 5 - 7 times more than that in the controls. Turner and Schultze (1945) reported that the action of thiouracil on body weight is relatively greater in young, growing birds. Glazener and Jull (1946) reported that thiouracil decreased the growth and feed consumption of broilers.

Moreng and Shaffner (1949); Shaffner (1951); indicated that thiouracil depressed weight gains in chickens except when fed at low levels (0.012 to 0.05 per cent).

Thiouracil fed to hens at the level of 0.1 per cent for six months does not affect egg production or fertility and hatchability in chickens but the higher level (0.3 per cent) reduced these traits (McCartney and Shaffner, 1949).

Chicks hatched from thiouracil treated hens had enlarged thyroids and hypoactive thyroids (Wheeler and Hoffman, 1948, 1949, 1950; McCartney and Shaffer, 1949; Booker and Sturkie, 1949). These latter workers injected thyroxine into these chicks hatched from the hypothyroid hens and reported a reduction in the size of the thyroid.

Mellen (1957) working with metabolic rate (M.R.) of chicks found that thiouracil depressed the M. R. as long as the drug was given in feed, but lasted for only 18 hours when the drug was withheld after which the M.R. averaged the same as in the controls.

Mode of Action. The exact mode of action of anti-thyroid compounds is not known, but evidence supports the theory that the thiocarbamides among them prevent the iodination of tyrosine. Pitt-Rivers (1950); Larson, Keating, Peacock, and Rawson (1945) found that thiouracil does not decrease the uptake of inorganic iodine, but prevents the gland from binding to protein or decreases the organic bond. Dempsy (1944) by histological technique, found that the enzyme peroxidase is present in thyroid cells and disappears after thiouracil administration. The hypertrophy and hyperplasia of the thyroid gland following treatment with anti-thyroid drugs evidently results from excessive secretion of thyrotrophic hormone (T.S.H.) by the anterior pituitary gland. Stimulation causing this excessive T.S.H. results from the decreased amount of thyroid hormone in the circulating blood.

CHAPTER II

OBJECTIVE

Thyroid inhibiting drugs have been used in an attempt to measure the relative requirement for the thyroid hormone in varying physiological conditions.

The objective of these experiments were:

1. To determine the dosage level of thiouracil for further physiological and embryological experiments and to determine the level of toxicity of this drug.
2. To determine the effect on subsequent livability and growth after injections of thiouracil into 48 hour embryos. In most reports in the literature, injections have been made after the 7th day of incubation; therefore after the thyroid has started forming. No previous workers have determined the effects of injections of thiouracil into 48 hour embryos.
3. To determine the effect on hatchability, after injections of thiouracil into 48 hour chick embryos.

CHAPTER III

METHODS AND PROCEDURE

Experiment No. 1. - To Determine the Toxicity Level of Thiouracil on the Chick Embryo.

Twelve dozen fertile eggs of White Leghorn hens (*Gallus gallus d.*) were obtained from the Michigan State University Poultry Research Farm. The eggs were selected for good shell texture and uniformity of size and weight. The eggs were incubated in a force-draft incubator at 37.5°C and a relative humidity of 60%. The maintenance of temperature and humidity at this level is recommended by the manufacturer of the incubator and also by Romanoff (1956) who states that at this level the maximum hatchability is obtained. The eggs were turned every two hours and were candled at 48 hour intervals after treatment started.

After 48 hours of incubation the eggs were divided into 7 groups, each group containing 12 eggs. The groups were injected as follows: 0.1 mg. of thiouracil, 0.5 mg. of thiouracil, 1.0 mg. of thiouracil, 2.0 mg. of thiouracil and 5.0 mg. of thiouracil; and two controls--one with the injection of Ringer's solution and one without any injection at all.

The mode of injection was via the Yushok methods (1950). This constitutes an injection being made into

the albumin at the small end of the egg so as not to strike the major allantoic blood vessel area.

The thiouracil was difuted in Ringer's solution (sodium chloride, 9 gm., potassium chloride, 0.42 gm., calcium chloride, 0.29 gm. and distilled water, 1000 ml.). The solutions were sterilized by autoclaving for 30 minutes.

The small ends of the eggs were washed with 70% ethyl alcohol and dried. The operator's hands were also scrubbed and washed with ethyl alcohol. The egg shell was pierced by a needle which was flamed and inserted at the small end. Then 0.5 ml. of sterile thiouracil was injected for each of the different treatments and 0.5 ml. of Ringer's solution was injected to one of the controls. Then the hole made by the needle was sealed with liquid paraffin. All eggs were then incubated. As a spot check to test for contamination and whether or not the treatments had killed the started embryos, eggs were candled 24 hours after injections were made.

The eggs were left in the incubator up to the 21st day and then opened. Results of the above experiment at 21st day of incubation are shown in Table I.

The embryos in Group 5 which contained 5 mg. thiouracil, started to die 48 hours after injection and all the embryos were dead on the 9th day of incubation.

TABLE I

Effects of different dosages of thiouracil on chick embryos injected at 48 hours of incubation.

<u>Group</u>	<u>Dosages</u>	<u>No. of Dead Embryos</u>	<u>No. of Eggs Set</u>	<u>No. of Live Embryos</u>
1	0.1 mg. of thiouracil	3	12	9
2	0.5 mg. of thiouracil	2	12	10
3	1.0 mg. of thiouracil	4	12	8
4	2.0 mg. of thiouracil	7	12	5
5	5.0 mg. of thiouracil	12	12	0
6	Control with 0.5 ml. of Ringer's solution	1	12	11
7	Control without injection	2	12	10

This demonstrated that 5.0 mg. of thiouracil was toxic thus inhibited embryonic development and completely suppressed body metabolism and the death of the embryos resulted.

Experiment No. 2. - Effect of Thiouracil on Body Weight and Hatchability.

As the 5.0 mg. was toxic to the embryos the dosage level used in this experiment was: 0.1 mg. of thiouracil, 0.2 mg. of thiouracil, 0.5 mg. of thiouracil, 1.0 mg. of thiouracil and 2.0 mg. thiouracil. Controls consisted of uninjected eggs and eggs injected with Ringer's solution.

One hundred and twelve dozen fertile eggs were obtained from Michigan State University Poultry Research Farm. All the eggs were from White Leghorn hens (*Gallus gallus d.*). They were selected for uniformity of size and shape.

Random selection during the setting of the eggs in the incubator trays was used. Each group was set with 16 dozen eggs in the forced draft incubator under the previously stated condition of temperature and humidity. Eggs were again turned every 2 hours.

Following the technique of Yushok (1950) as previously described, injections were made after 24 hours of incubation. After 24 hours of incubation the eggs were candled by an ordinary egg candler to check fertility.

On each day from the 7th through the 21st day of incubation, twelve eggs from each treatment were randomly selected and sacrificed at which time the embryo body weights were taken. In addition to body weight, the number of live embryos was recorded so as to determine fertility. After the 21st day one dozen of the eggs in each group was allowed to remain in the incubator until they hatched or died. This procedure was used to determine the effect of an anti-thyroid drug (thiouracil) on the delay of hatching as reported for thiourea (Grossowicz, 1946; Adams, 1949; Adams and Buss, 1952; Vidal, 1952).

CHAPTER IV

RESULTS AND DISCUSSION

A. The Influence of Different Dosages of Thiouracil:-

Before starting the final experiment, the toxic level of thiouracil was determined as discussed in Chapter III.

The experiment showed that 5.0 mg. of the thiouracil when injected into chick embryos resulted in 100% mortality after 9th day of incubation. 2.0 mg. was less toxic to the embryos, thus it was included in the experiments. (Table I)

Romanoff and Lauffer (1956) injected 2, 3, 5 and 10 mg. of thiourea and obtained 100% mortality in the 5 and 10 mg. of treated embryos. Similar results were obtained by Vidal (1952) with 5.0 mg. of thiourea.

There are no data available regarding the toxic level of thiouracil.

B. Effect of Thiouracil on Body Weight:-

From an examination of Table II it is evident that in general the average body weight of chicks injected with thiouracil was less than that of the controls injected with Ringer's solution or left untreated. The embryo weights of the eggs treated with thiouracil were less than

the controls, until the 11th day of incubation at which time the thyroid had become functional (Hansbrough and Khan, 1951). Before the 11th day there is no significant difference between the thiouracil treatments (except the 0.1 mg. level--the low level) but all the treated embryos with thiouracil are significantly different than the controls. Thus it indicates that the earlier injection, i.e., injection of different doses of thiouracil, does inhibit the body weight before the thyroid is completely formed. This suggests that thiouracil inhibits body development even though the thyroid per Se is not functional and indicates that colloid, which is present at the seventh day, may be involved in development.

There are no published data to show the effect on embryos of injections of thiouracil before the development of the thyroid except Vidal (1952) who injected high levels of thiourea to determine any abnormality in development and mortality. Vidal (1952) mentioned that injecting thiourea at 0 or 48 hours of incubation caused no abnormality in development although it did cause high mortality: however, he did not study the effect on body weight and hatchability. His primary object was to determine the effect of the injections on subsequent livability of embryos. With the exception of the controls, most of the embryos died before the 11th day of incubation.

As shown in Table II there is a change in significant differences with individual treatments after the 11th day of incubation, the period when the thyroid is completely formed (Khan, 1951). Reduction of body weight caused by 2.0 mg. of thiouracil was significantly greater than the reduction caused by 1.0 mg. of thiouracil. But there is no significant difference between treatments of 0.1, 0.2 and 0.5 mg. of thiouracil when injected into chick embryos. The greatest difference is between the controls and treated embryos.

The average body weights of all embryos autopsied on the 7th through the 21st days of incubation were lower than those of controls. From the 17th day of incubation through the 21st, those of the embryos injected with thiouracil were consistently lower than those of the controls. The difference between the higher concentration injected (2.0 mg. of thiouracil) and controls are approximately 13 gms. on the 21st day, 10 gms. on the 20th, 8 gms. on 19th, 6 gms. on the 18th and 5 gms. on the 17th day of incubation. There was a pronounced difference in weight between individuals receiving different treatments of thiouracil. There is an approximate difference of 6 to 7 gms. between 2.0 mg. and 0.1 mg. of thiouracil injected chick embryos on the 21st day of incubation. There is a difference in body weight of approximately 3 - 5 gms. between the 2.0 mg. and 0.1 mg. treatments from the 17th to 21st day.

TABLE II

Average body weight in gms. of chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

Age of Embryos	Weight of Chick Embryos with Different Doses of Thiouracil					
	2.0 mg.	1.0 mg.	0.5 mg.	0.2 mg.	0.1 mg.	Control <u>1</u> Control <u>2</u>
7 days old	<u>0.450</u>	<u>0.566</u>	<u>0.633</u>	<u>0.601</u>	<u>0.682</u>	<u>0.867</u> <u>0.993</u>
8 days old	<u>1.093</u>	<u>1.142</u>	<u>1.143</u>	<u>1.165</u>	<u>1.293</u>	<u>2.047</u> <u>1.974</u>
9 days old	<u>1.236</u>	<u>1.265</u>	<u>1.442</u>	<u>1.471</u>	<u>1.655</u>	<u>3.119</u> <u>3.212</u>
10 days old	<u>1.830</u>	<u>1.971</u>	<u>2.229</u>	<u>2.321</u>	<u>2.305</u>	<u>4.243</u> <u>4.258</u>
11 days old	<u>2.362</u>	<u>2.772</u>	<u>2.964</u>	<u>3.010</u>	<u>3.213</u>	<u>5.203</u> <u>5.609</u>
12 days old	<u>2.799</u>	<u>3.045</u>	<u>3.566</u>	<u>3.883</u>	<u>4.156</u>	<u>7.019</u> <u>7.259</u>
13 days old	<u>4.36</u>	<u>5.05</u>	<u>5.50</u>	<u>6.05</u>	<u>6.68</u>	<u>10.86</u> <u>10.72</u>
14 days old	<u>6.06</u>	<u>6.74</u>	<u>7.50</u>	<u>7.95</u>	<u>8.56</u>	<u>12.95</u> <u>12.94</u>
15 days old	<u>11.01</u>	<u>11.98</u>	<u>13.20</u>	<u>13.82</u>	<u>14.37</u>	<u>16.36</u> <u>15.97</u>
16 days old	<u>12.90</u>	<u>13.63</u>	<u>13.77</u>	<u>14.55</u>	<u>16.22</u>	<u>18.15</u> <u>18.45</u>
17 days old	<u>15.19</u>	<u>15.74</u>	<u>16.20</u>	<u>16.60</u>	<u>17.87</u>	<u>19.98</u> <u>20.41</u>
18 days old	<u>18.44</u>	<u>18.98</u>	<u>19.94</u>	<u>20.61</u>	<u>21.40</u>	<u>24.74</u> <u>24.81</u>

Continued next page

TABLE II (Continued)
Weight of Chick Embryos with Different Doses of Thiouracil

<u>Age of Embryo</u>	<u>2.0 mg.</u>	<u>1.0 mg.</u>	<u>0.5 mg.</u>	<u>0.2 mg.</u>	<u>0.1 mg.</u>	<u>Control</u> <u>1</u>	<u>Control</u> <u>2</u>
19 days old	<u>19.44</u>	<u>20.44</u>	<u>21.49</u>	<u>22.86</u>	<u>24.01</u>	<u>27.68</u>	<u>27.66</u>
20 days old	<u>19.64</u>	<u>20.41</u>	<u>21.72</u>	<u>23.28</u>	<u>24.55</u>	<u>28.65</u>	<u>29.25</u>
21 days old	<u>18.83</u>	<u>19.83</u>	<u>21.16</u>	<u>23.66</u>	<u>25.50</u>	<u>30.66</u>	<u>31.33</u>

Control 1 - Untreated

Control 2 - Injected .05 ml. of Ringer's solution

—— - Solid line means non-significant at 5% level

The difference is approximately the same in all the treatments from 13th to the 21st day of incubation.

The retardation of growth with exposure of chick embryos to anti-thyroid compound, was first studied by Andrews and Schnetzler (1945) who raised chicks from eggs laid by hens given thiouracil. They demonstrated the presence of the drug in the yolk of such eggs, but they did not describe any modification in development of the embryos. The second paper, that of Grossowicz (1946) already mentioned, emphasized retardation of hatching and lack of retraction of yolk sac. Adams and Bull (1949) injected 0.4% of thiourea on the 14th day of incubation and embryos killed daily thereafter showed a statistically significant difference. They also found that when a series of chick embryos were injected with thiourea on the 8th, or 8th and 14th, or 8th, 14th and 18th days of incubation, the body weights at autopsy on the 21st day were consistently less than those of the controls. Decreased body weight in New Hampshire chicks after doses of thiourea and thiouracil have been reported (Macright, 1950). These chicks were injected on the 6th, 12th and 18th days of incubation and killed at daily intervals thereafter. Some papers, however, include no data on body weights or growth of chicks injected with thiourea or hatched from eggs laid by hens fed thiouracil.

Romanoff and Lauffer (1956) injected a single dose of 5.0 mg. of thiourea into different groups on successive

days from 0 to 18 days of incubation. All the embryos died except for eight, which hatched on the twenty-first day of incubation from eggs injected on the 18th day. The body weights of dead embryos were less than controls. This indicated that 5.0 mg. of thiourea was toxic to chick embryos. The above results are in agreement with data presented in Table II.

There are several reports on the administration of goitrogenic drugs to very young chicks from one day to a week old, that have a bearing on the possible influence of such compounds on early growth. In these experiments, the anti-thyroid drug, thiourea, or thiouracil was included in the food or drinking water for varying periods of time and in varying quantities (Astwood, Bissell and Hughes, 1944; Mixner, Reineke and Turner, 1944; Schultze and Turner, 1949; Briggs and Lillie, 1946; D'Angelo, Gordon and Charipper, 1947). One day old chicks of several breeds usually responded to two weeks of age on 0.1% thiouracil in their food by slight losses in body weights (Mixner, et. al., 1944). Thiouracil (0.1% to 0.8%) included in food or White Leghorn chicks for two weeks from the day of hatching lowered the body weights approximately 10 gms. compared with the controls (Sultze and Turner, 1945). Weak solutions of thiourea (0.05% or 0.07%) caused only negligible reduction in the weight of White Rock chicks that were fed the drug for 3

weeks from hatching. However, thiourea of 0.1% strength reduced the weight of female chicks 48.8 gms. (37.2%) and of males 34.8 gms. (28.2%) below those of their controls (Schultze and Turner, (1945)). There was a reduction of 27.2% in chicks of a New Hampshire cross given 0.5% thiouracil in their food for 5 weeks (Briggs and Lillie, 1946). Body weights lower than those of their controls were also reported in chicks of a Barred Rock X New Hampshire cross fed 0.1% thiourea in the ration for 20 weeks (D'Angelo, Gordon and Charipper, 1947).

In these experiments, the general effects upon the body weight of newly hatched chicks of treatment with moderately strong solution of anti-thyroid drugs were: (1) long treatment of 3 to 20 weeks usually reduced it. (2) short ones sometimes reduced it and sometimes did not; breeds differed in their responses; and thiourea was more effective than thiouracil.

In mammals many instances of retarded growth after administration of anti-thyroid drugs have been reported (Astwood, et. al., 1943; Christensen, 1945; Dempsy and Astwood, 1943; Fitzhugh and Nelson, 1947; Goldsmith, Gordon and Charipper, 1945; Higgins, 1945; Hughes, 1944; William Weinlass, Bissell and Peter, 1944). Only dogs and monkeys seem to be refractory to the treatment with these drugs (Aranow, Engle and Sperry, 1946; Donowski, Man and Winkler, 1946; Mayer, 1947). These drugs inhibit metamorphosis in

anuran and urodele tadpole (Gordon, Goldsmith and Charipper, 1943), but in some instances body growth in the anurans has continued although limb growth has not (Adams and Craig, 1949a).

C. Effect of Thiouracil on Hatching and Yolk Sac Retraction

In each group, 12 fertile eggs were left in the incubator after the 21st day of incubation until they hatched or died.

Chick embryos receiving the different dosages of thiouracil were delayed in hatching if the eggs containing them were incubated to hatching dates or beyond. However, no chicks injected with thiouracil hatched on or before the 21st day of incubation with the exception of 3 chicks out of 12 fertile eggs of Group 1 receiving 0.1 mg. of thiouracil and 4 chicks in Group 2 receiving 0.2 mg. of thiouracil and 2 chicks from Group 3 receiving .5 mg. of thiouracil.

The results of this phase of the experiments are shown in Table III. The results show that chicks receiving 2.0 mg. of thiouracil had not hatched at the 27th day of incubation. At this time they were sacrificed and embryos were removed. Four embryos were alive and eight were dead.

In Groups I to IV all the chicks hatched by the 25th day of incubation.

The controls receiving Ringer's solution and without any treatment hatched on the 20th and 21st days of incubation;

and the yolk sacs were entirely within the body.

This effect was similar to that reported by Grossowicz (1946) but less striking since the eggs were only allowed to go six days beyond normal hatching, compared with ten days in his experiment. He reported a correlation between the time of injection (the 7th to the 17th day) and dosages administered (0.3 to 3.0 mg. of thiourea) and the degree of retardation. Adams and Bull (1949) used 2.0, 4.0 and 6.0 mg. of thiourea and found no differences between treatments. Beginning treatment on the 8th or 14th day made no apparent difference. In further studies by Adams and Buss (1952), they found no chicks injected with thiourea or propyl thiouracil hatched on or before the 21st day of incubation.

Romanoff and Lauffer (1956) compared the effect of different doses when eggs were injected with 2, 3, 5 and 10 mg. of thiourea on the eleventh day of incubation (a state of incubation at which the thyroid is known to be functional). Observation showed that the administration at this time resulted in prolongation of embryonic development by 3 to 10 days beyond the normal period of 21 days and increased pre-natal mortality up to 100% in some cases, as compared to 25% mortality among the controls.

Similar results were obtained in the present experiment when the chicks received thiouracil before the formation of thyroid. There is no information in the literature on the action of anti-thyroid drugs administered before thyroid

formation, i.e. before the 11th day of incubation.

The present experiment shows that thiouracil, when injected into 48 hour embryos will prolong the incubation period and will delay yolk sac retraction.

TABLE III

Effect of Thiouracil on Length of Incubation
Period

<u>Days</u>	<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u>2.0 mg.</u>	<u>Control</u>
21st	3	4	2	-	-	10
22nd	1	0	1	-	-	
23rd	4	6	5	6	-	
25th	3	1	2	4	-	
27th	-	-	-	-	12 (sacrificed)	

CHAPTER V

SUMMARY

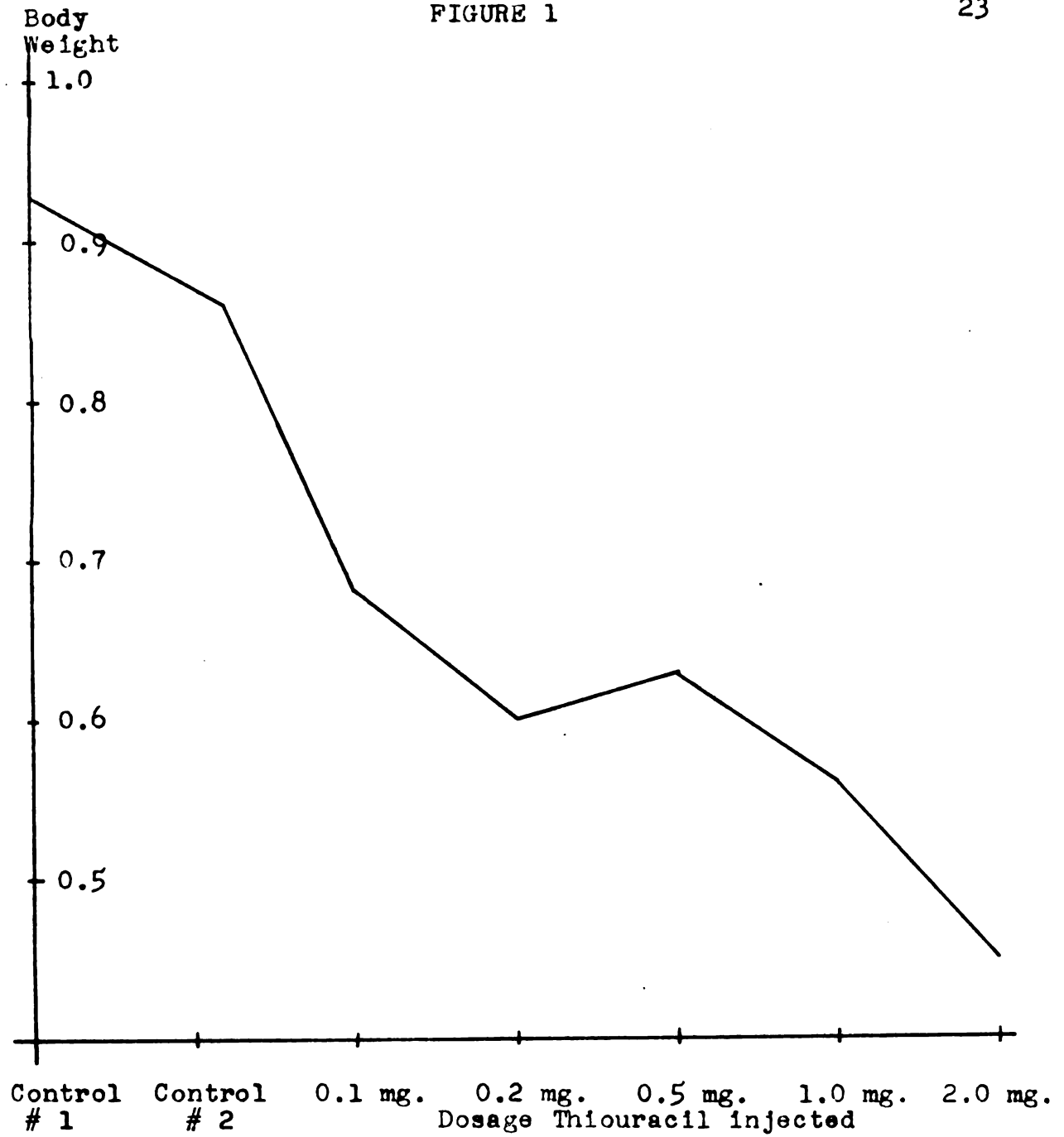
Thiouracil, in amounts varying from 0.1 to 2.0 mg., was injected into the albumen of chicken (*Gallus gallus d.*) eggs after 24 hours of incubation. The embryos were sacrificed or were permitted to develop until they hatched or died. The controls received Ringer's solution or were not injected.

Observation showed that administration of 5.0 mg. of thiouracil after 48 hours of incubation caused 100% mortality by the ninth day of incubation.

There was a highly significant loss of body weight of all the treated chick embryos compared to controls. Significant differences between individual treatments were noted after the 11th day of incubation.

Experiments showed that the administration of thiouracil resulted in prolongation of embryonic development beyond the normal period of 21 days and lack of retraction of the yolk sac.

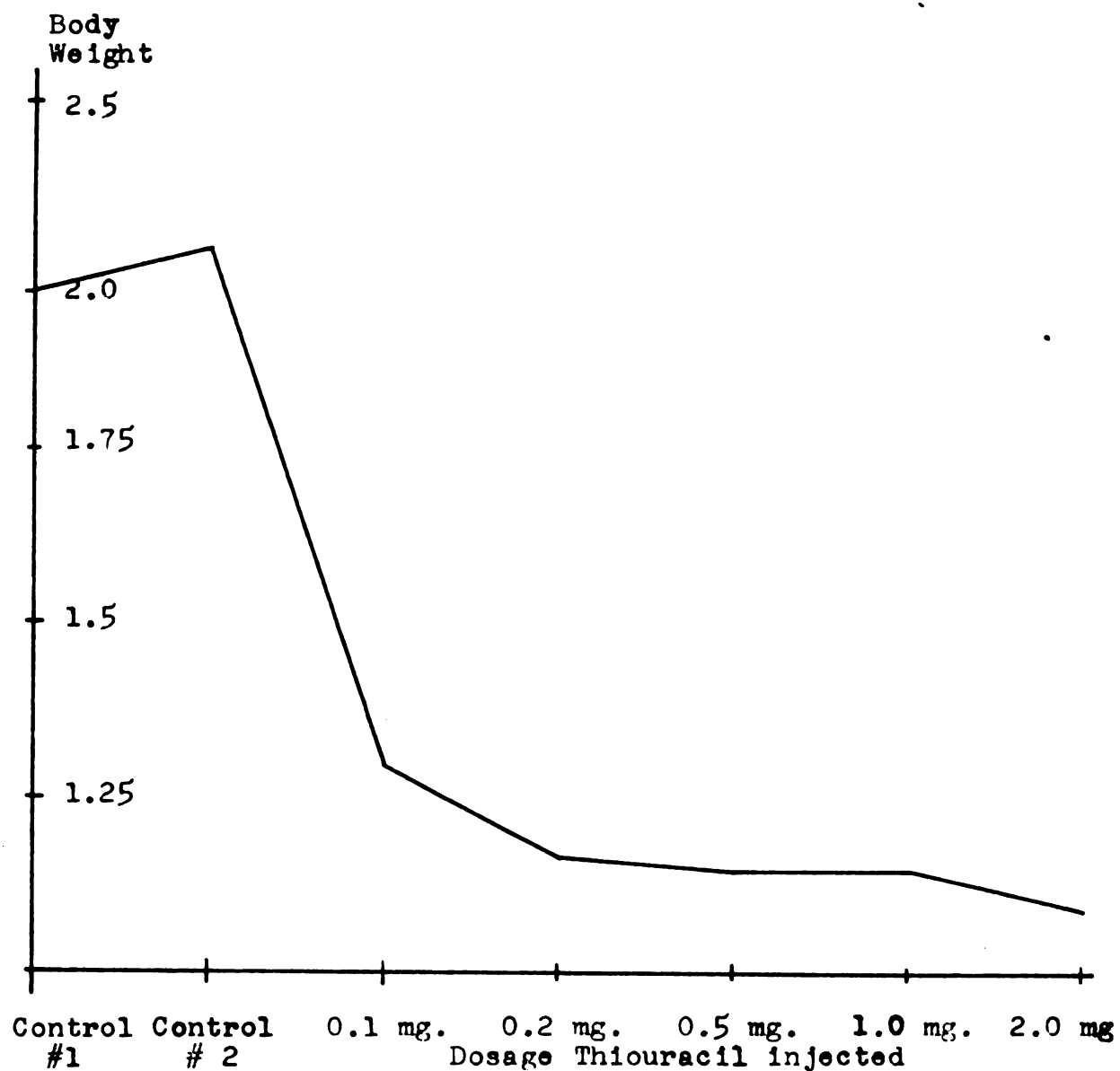
FIGURE 1



Average body weight in gms. of 7 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

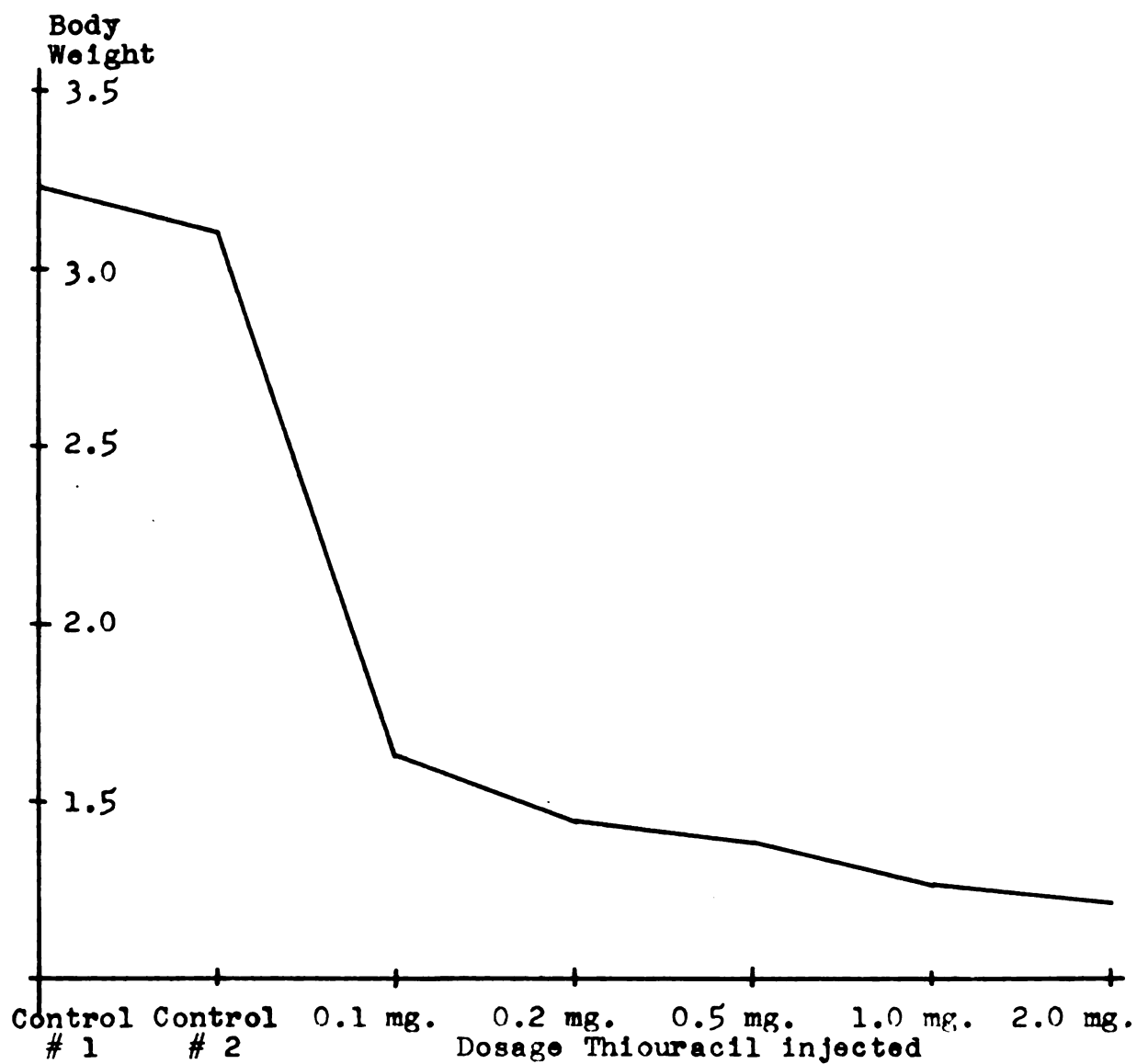
FIGURE 2

24



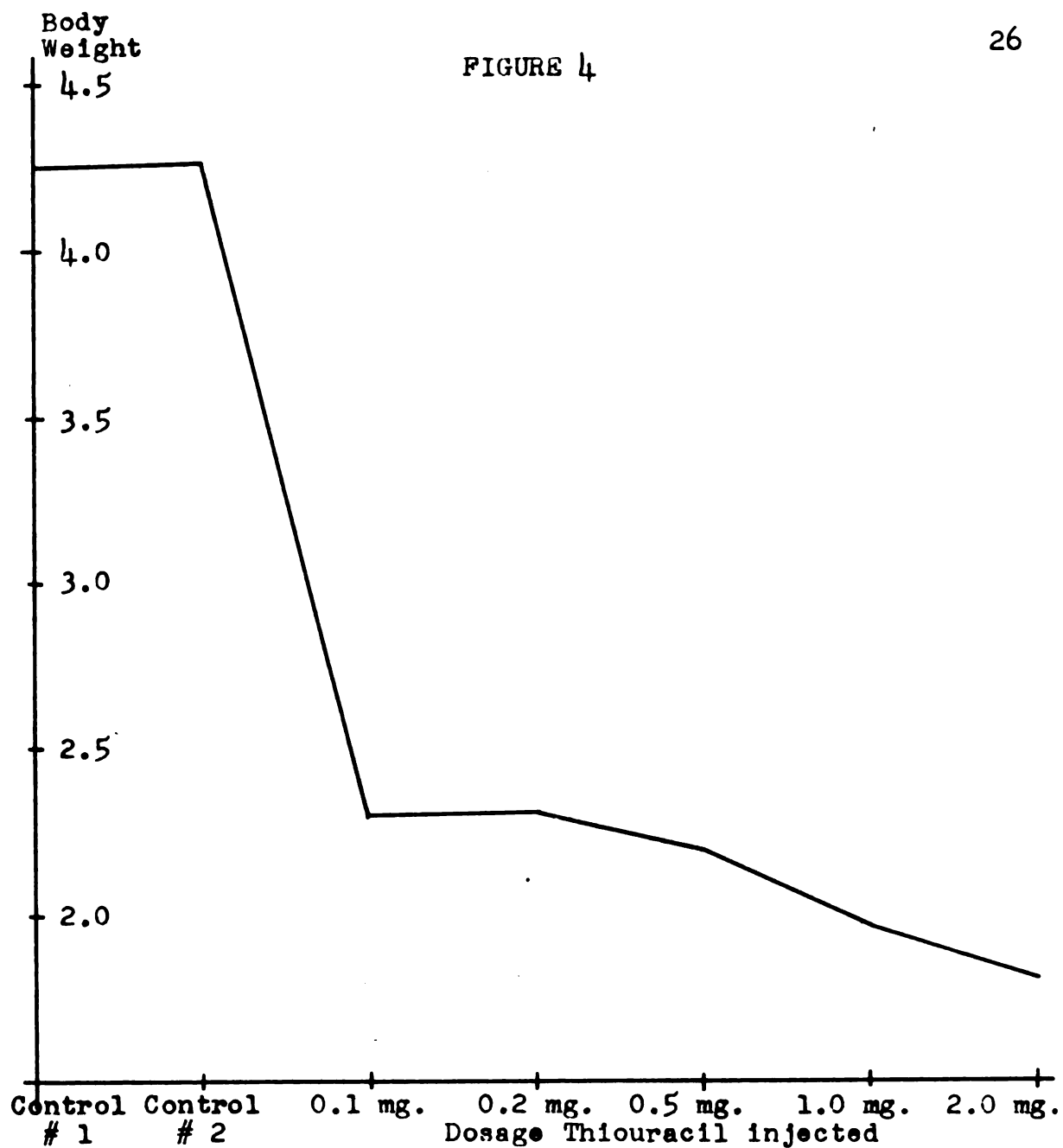
Average body weight in gms. of 8 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

FIGURE 3



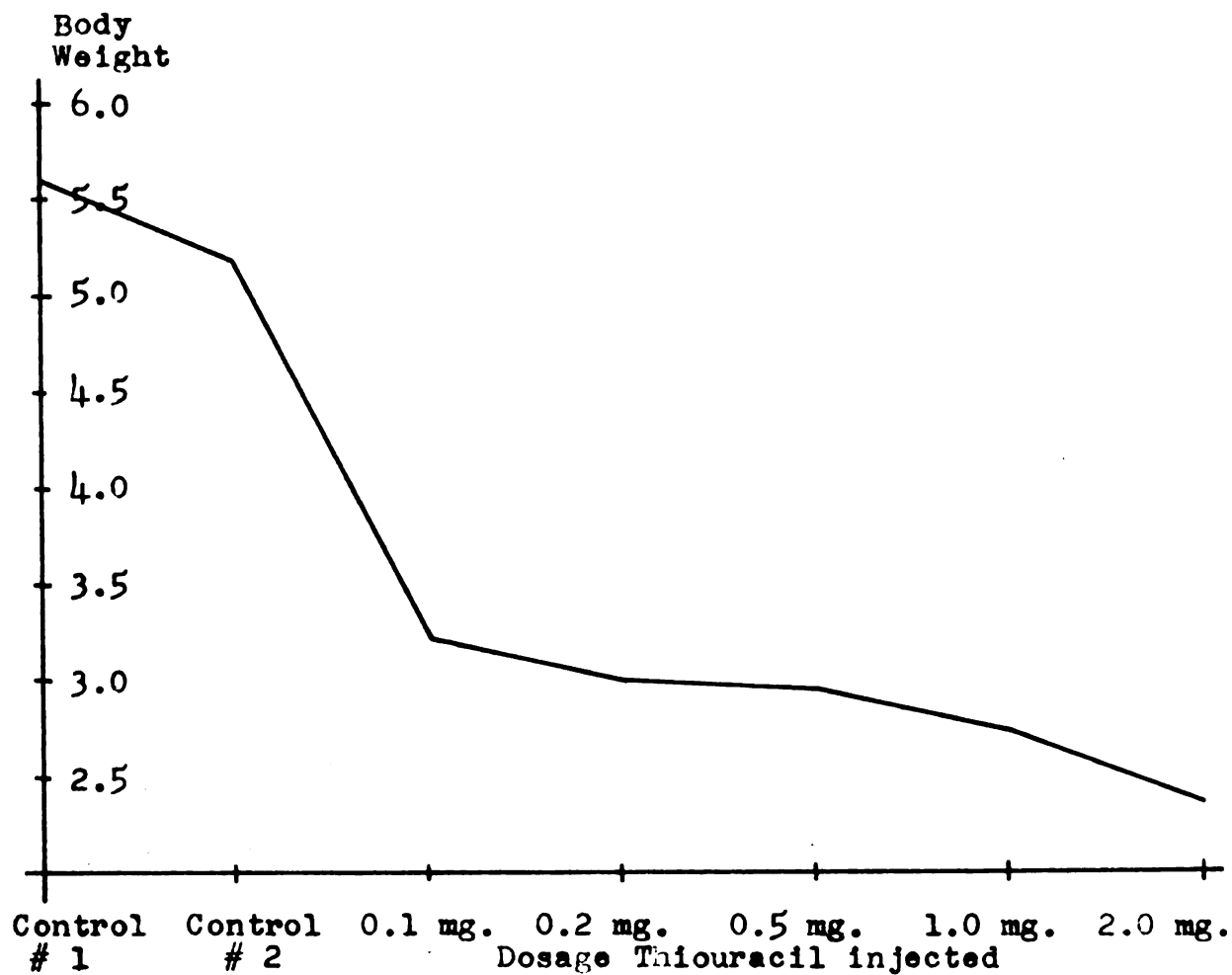
Average body weight in gms. of 9 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

FIGURE 4



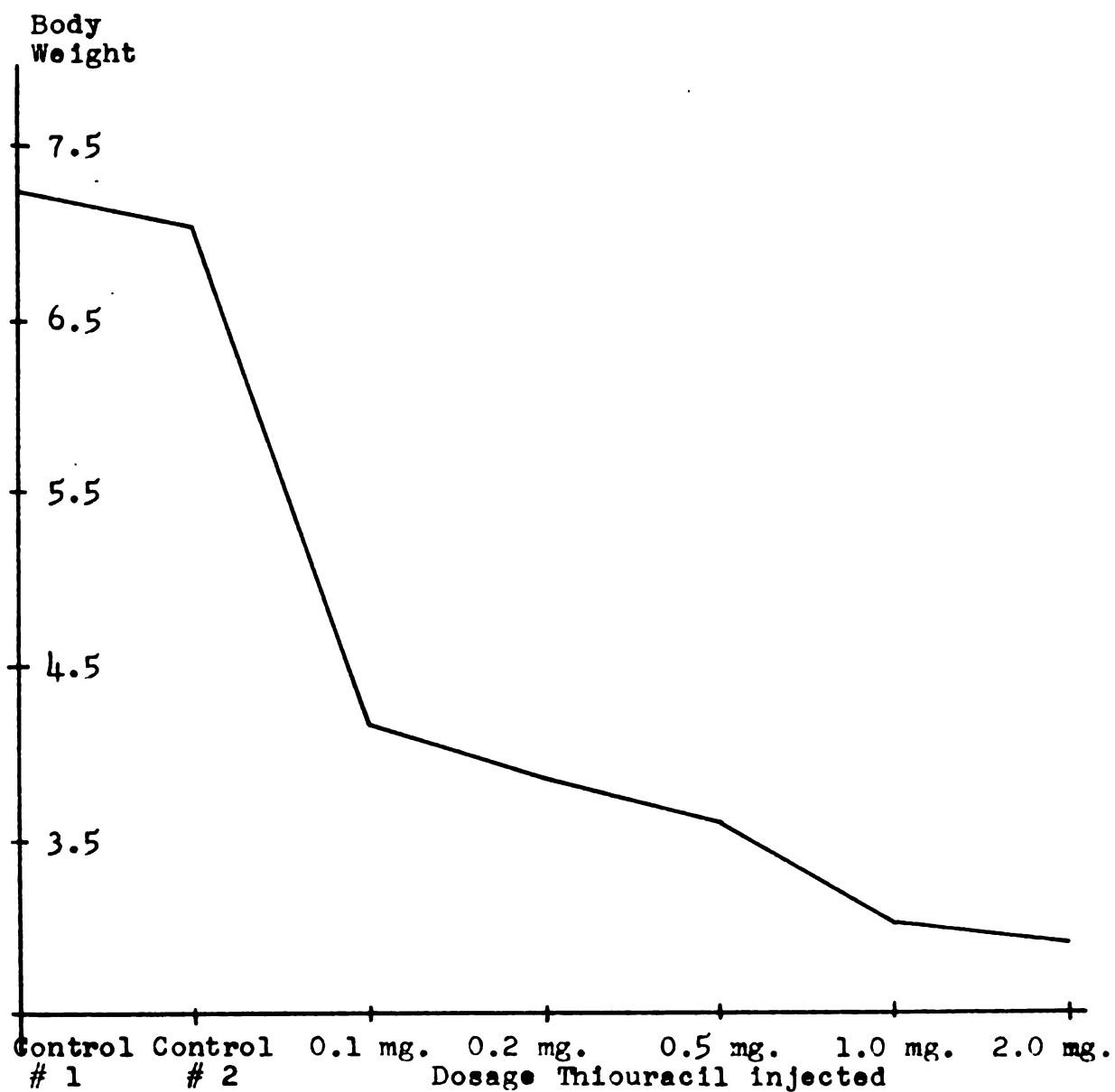
Average body weight in gms. of 10 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

FIGURE 5



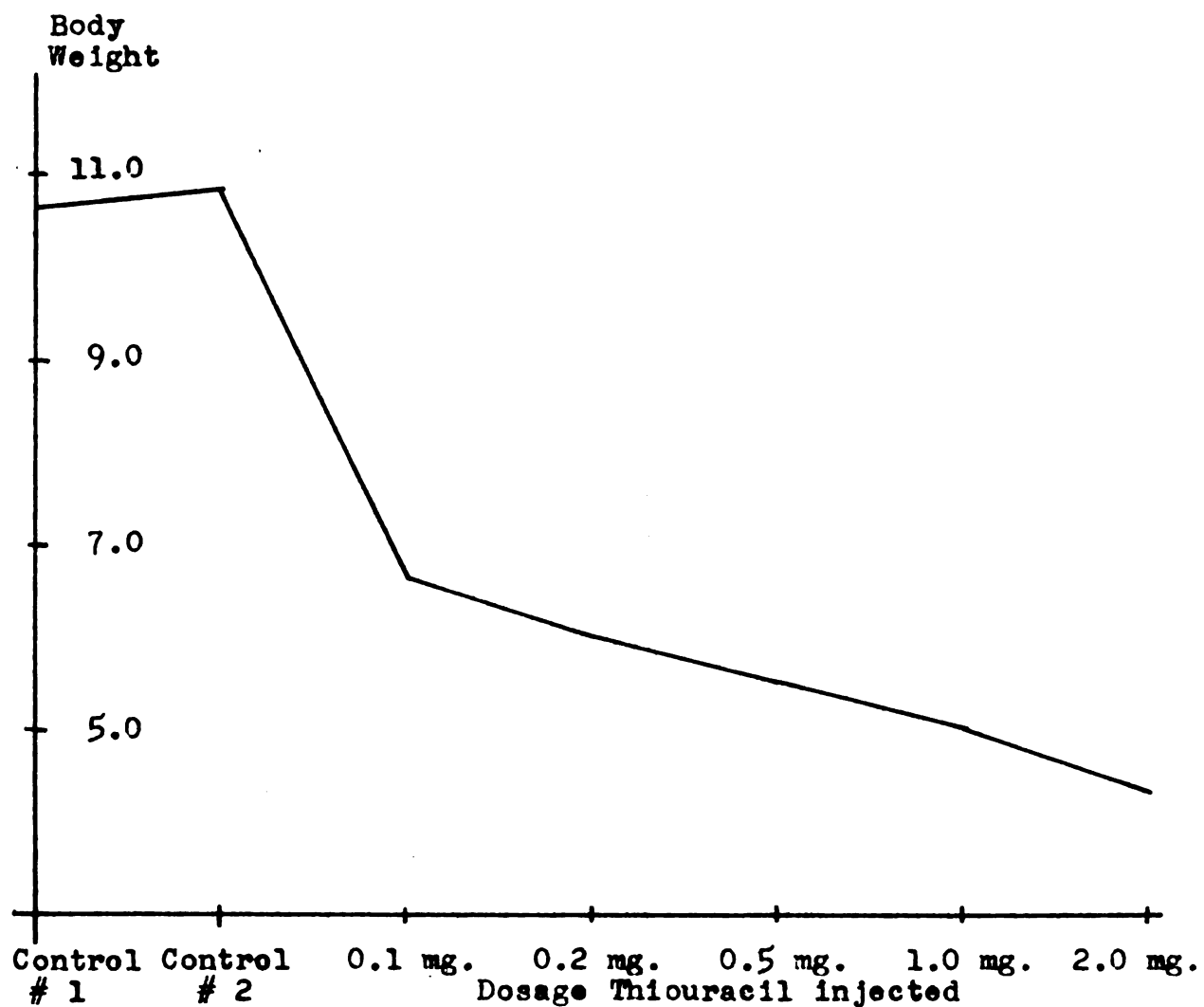
Average body weight in gms. of 11 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

FIGURE 6



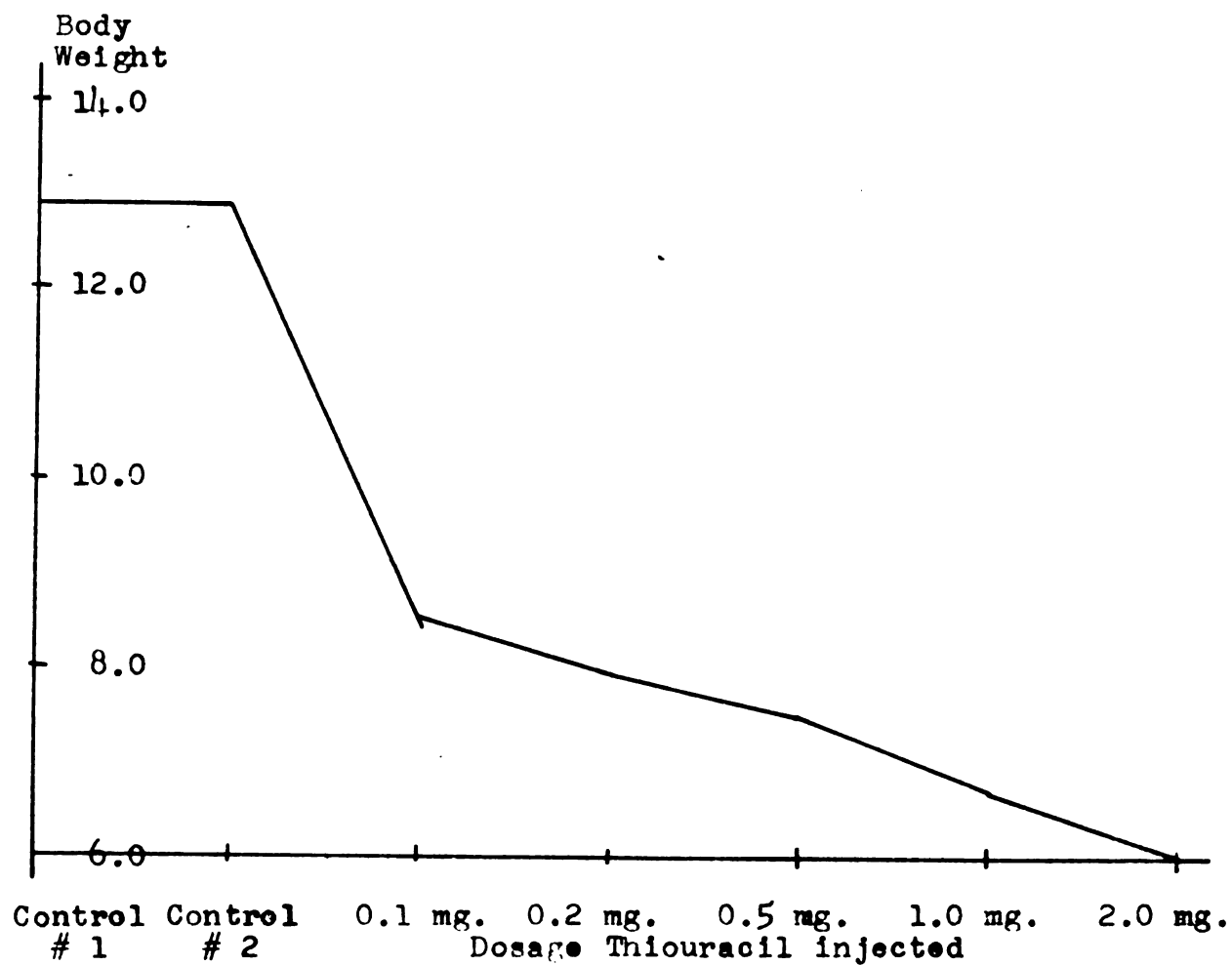
Average body weight in gms. of 12 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

FIGURE 7



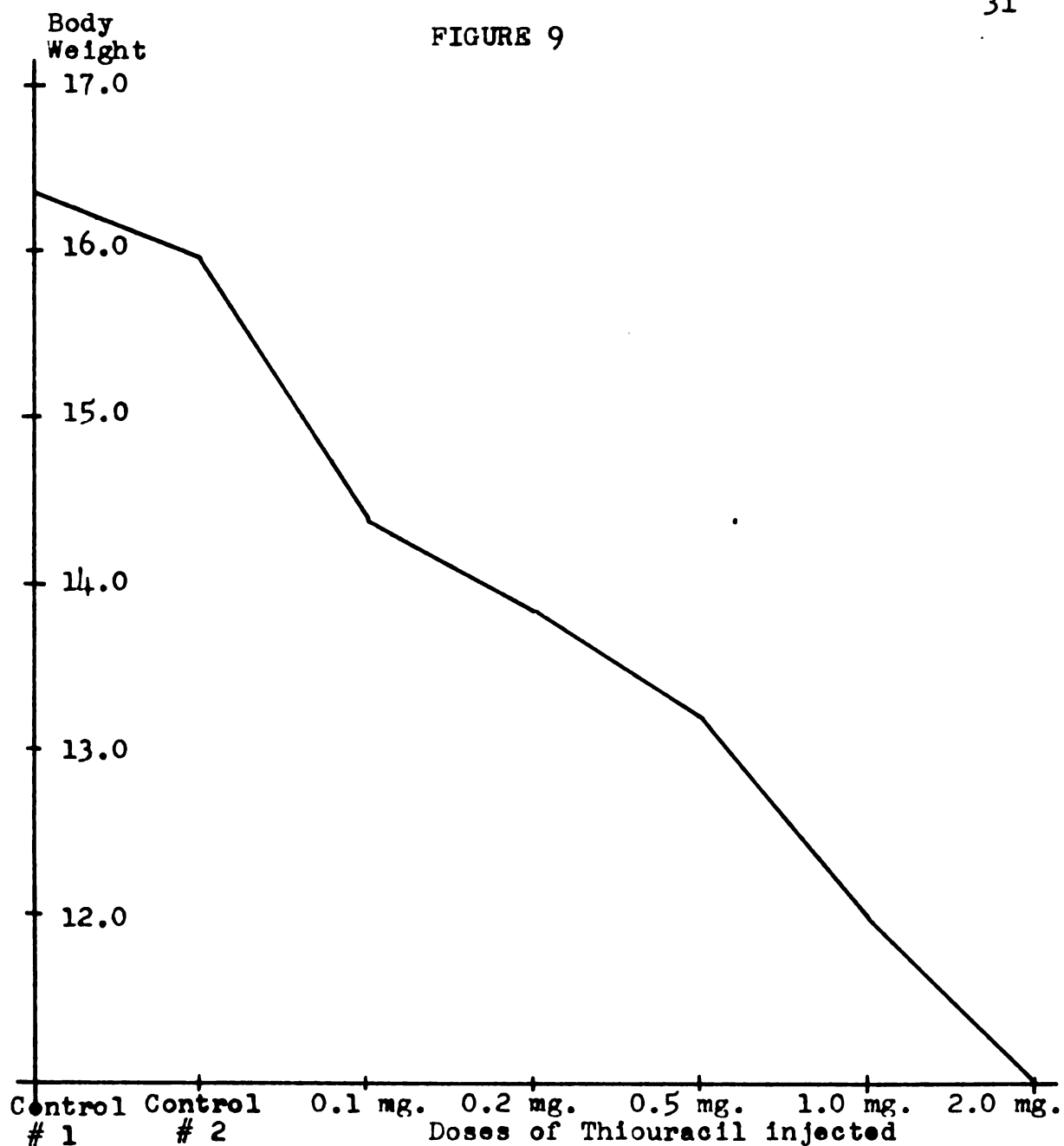
Average body weight in gms. of 13 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 8



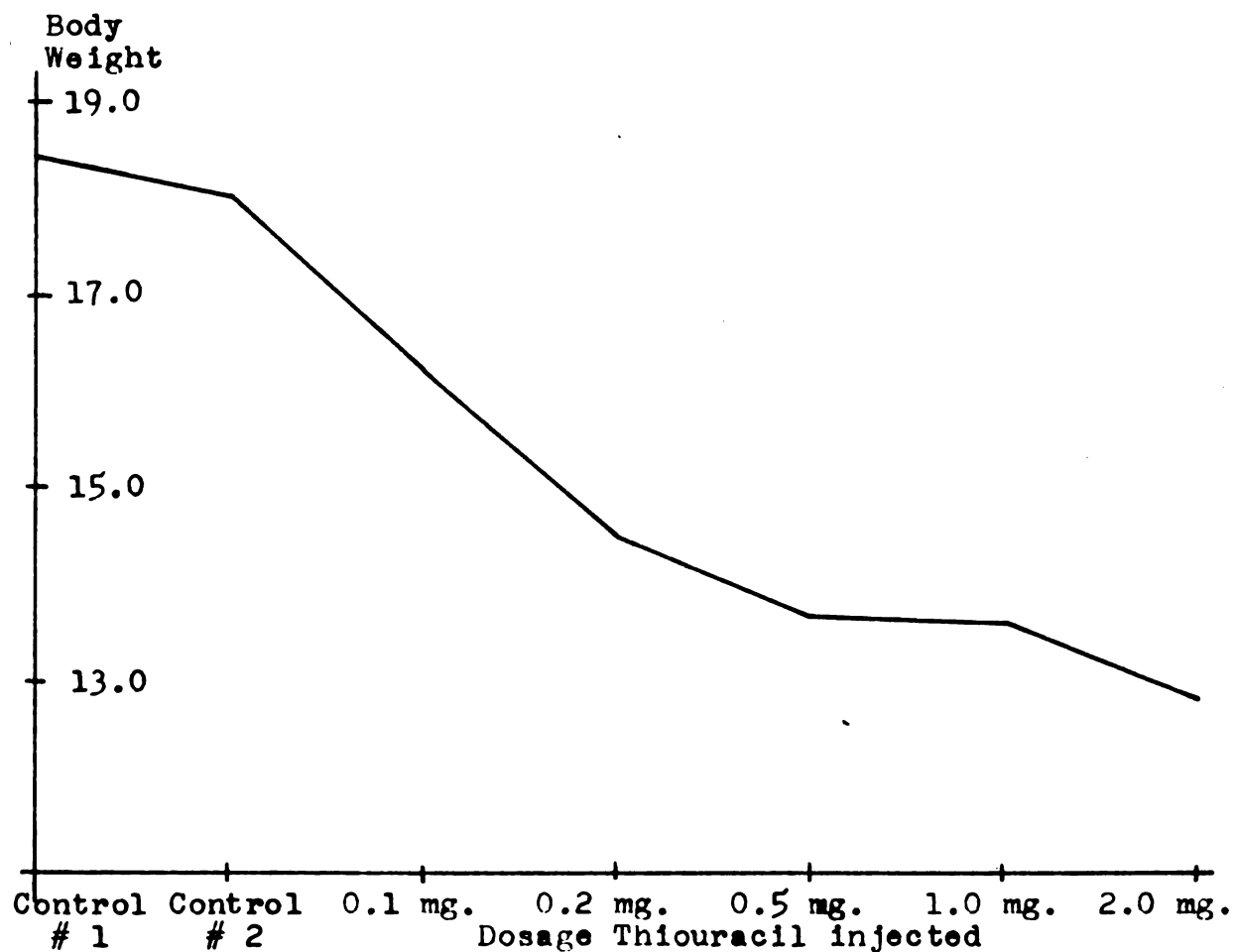
Average body weight in gms. of 11 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 9



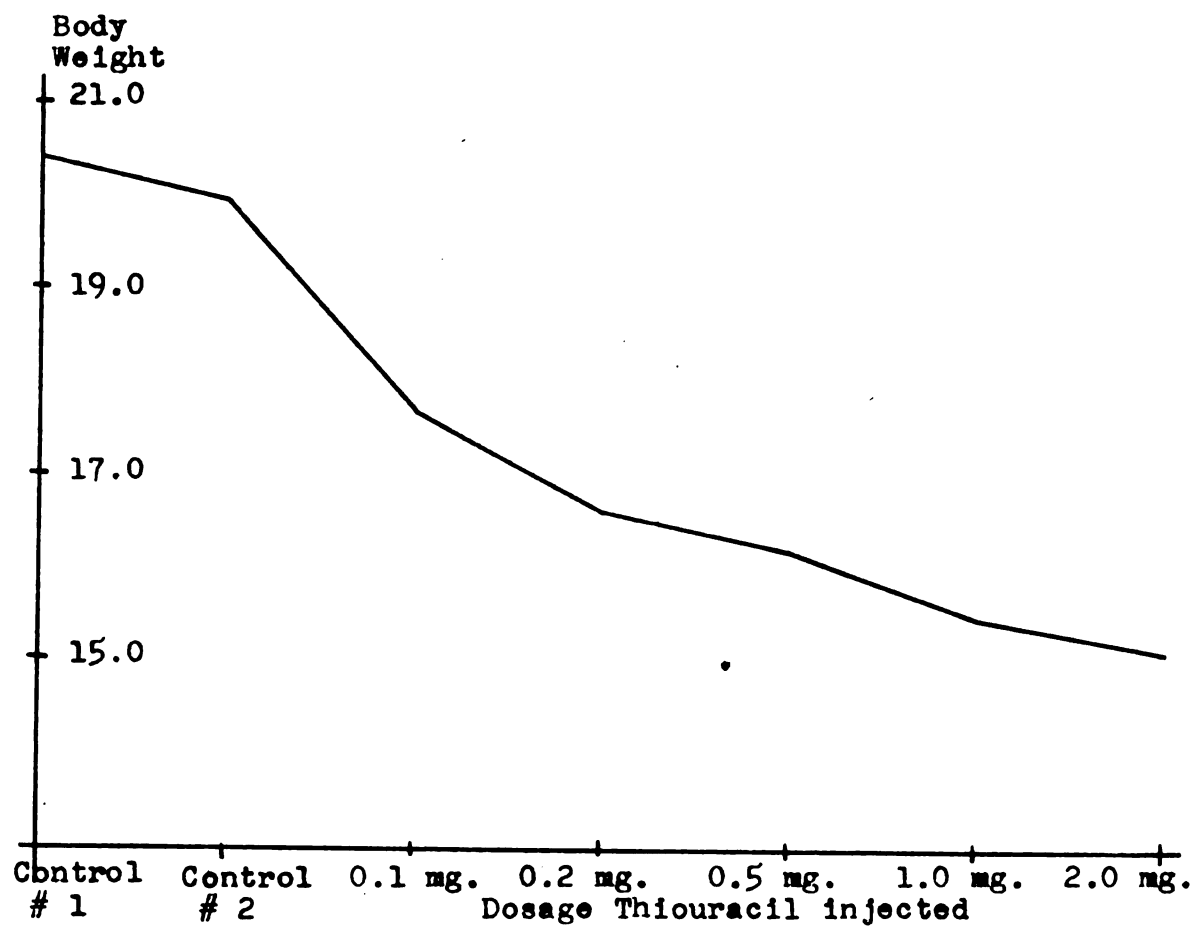
Average body weight in gms. of 15 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 10



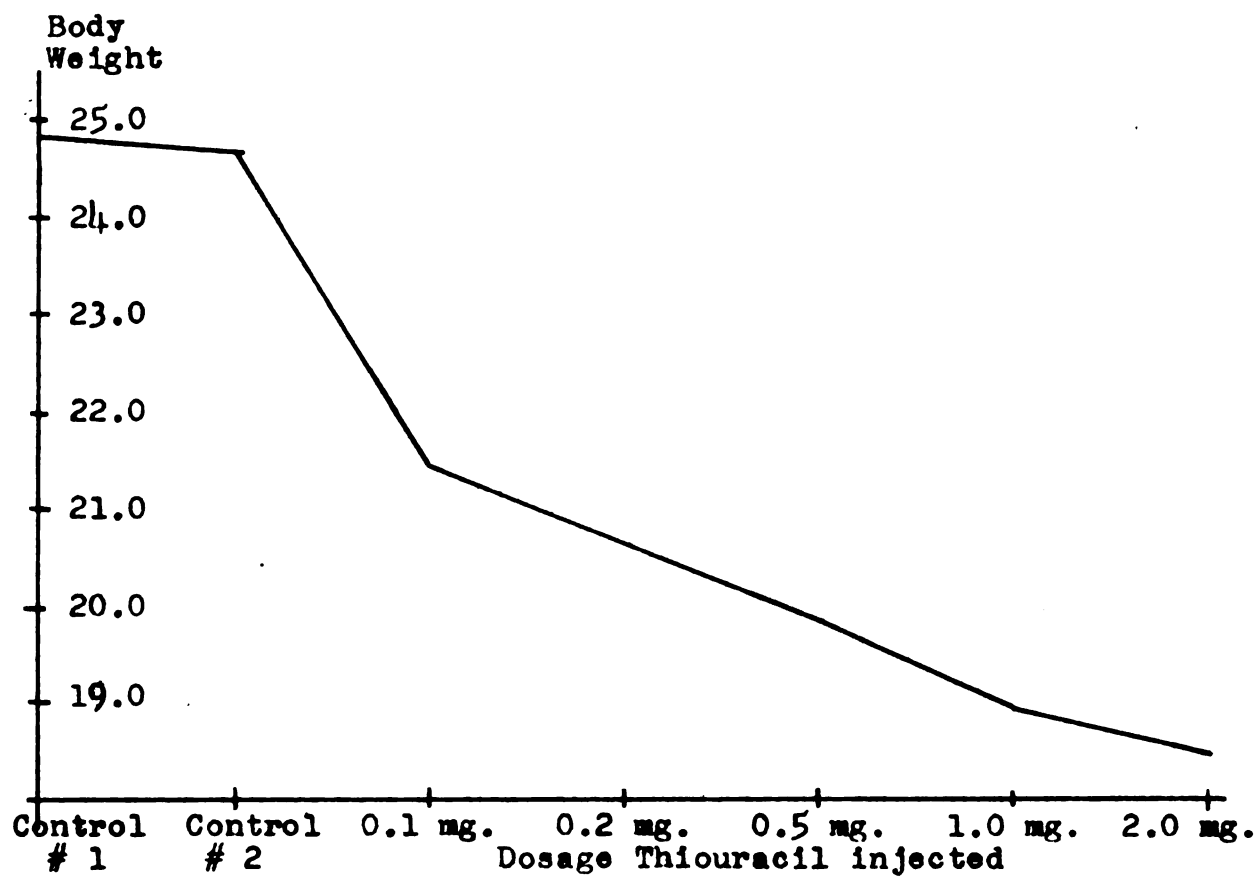
Average body weight in gms. of 16 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 11



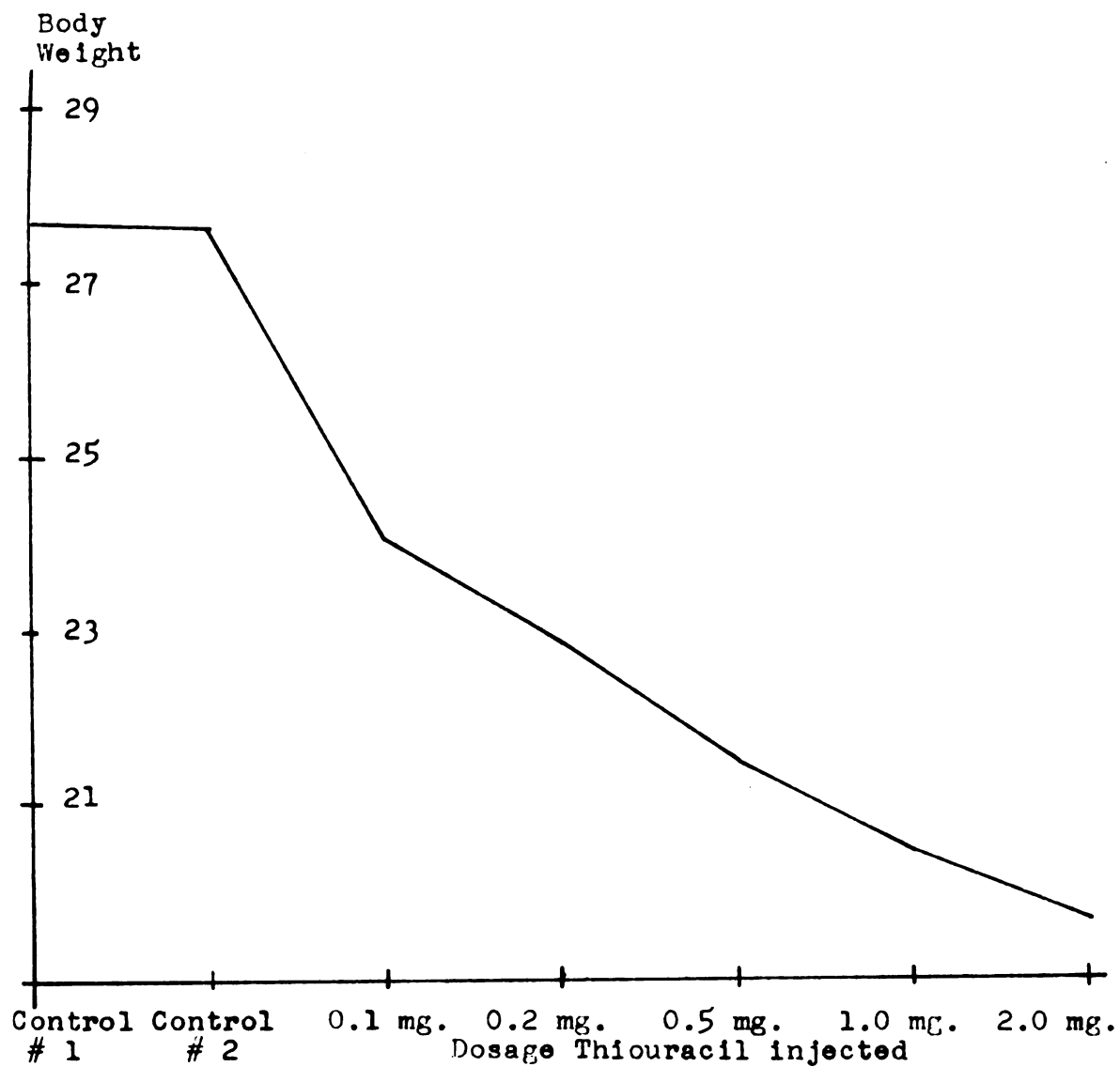
Average body weight in gms. of 17 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 12



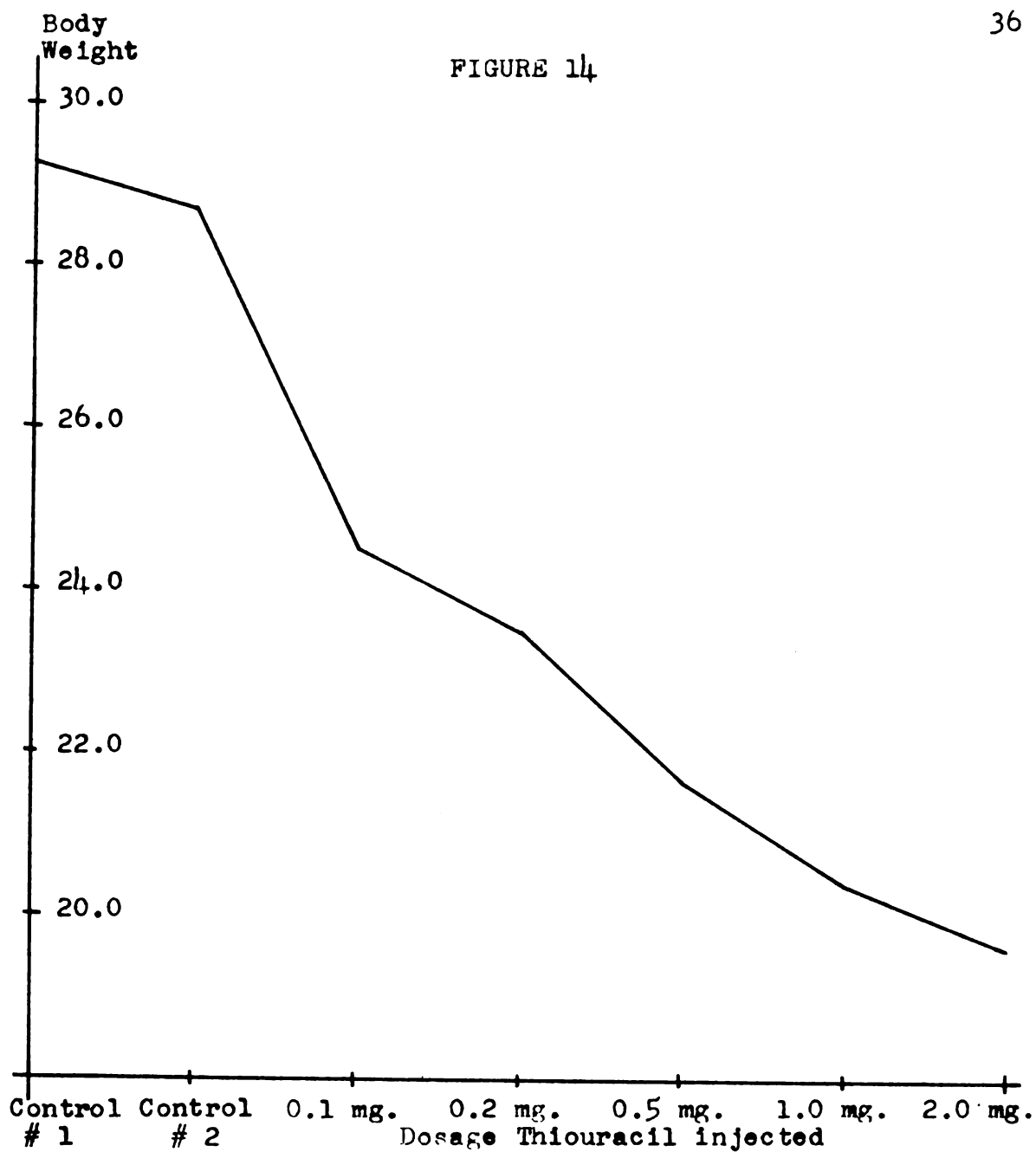
Average body weight in gms. of 18 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 13



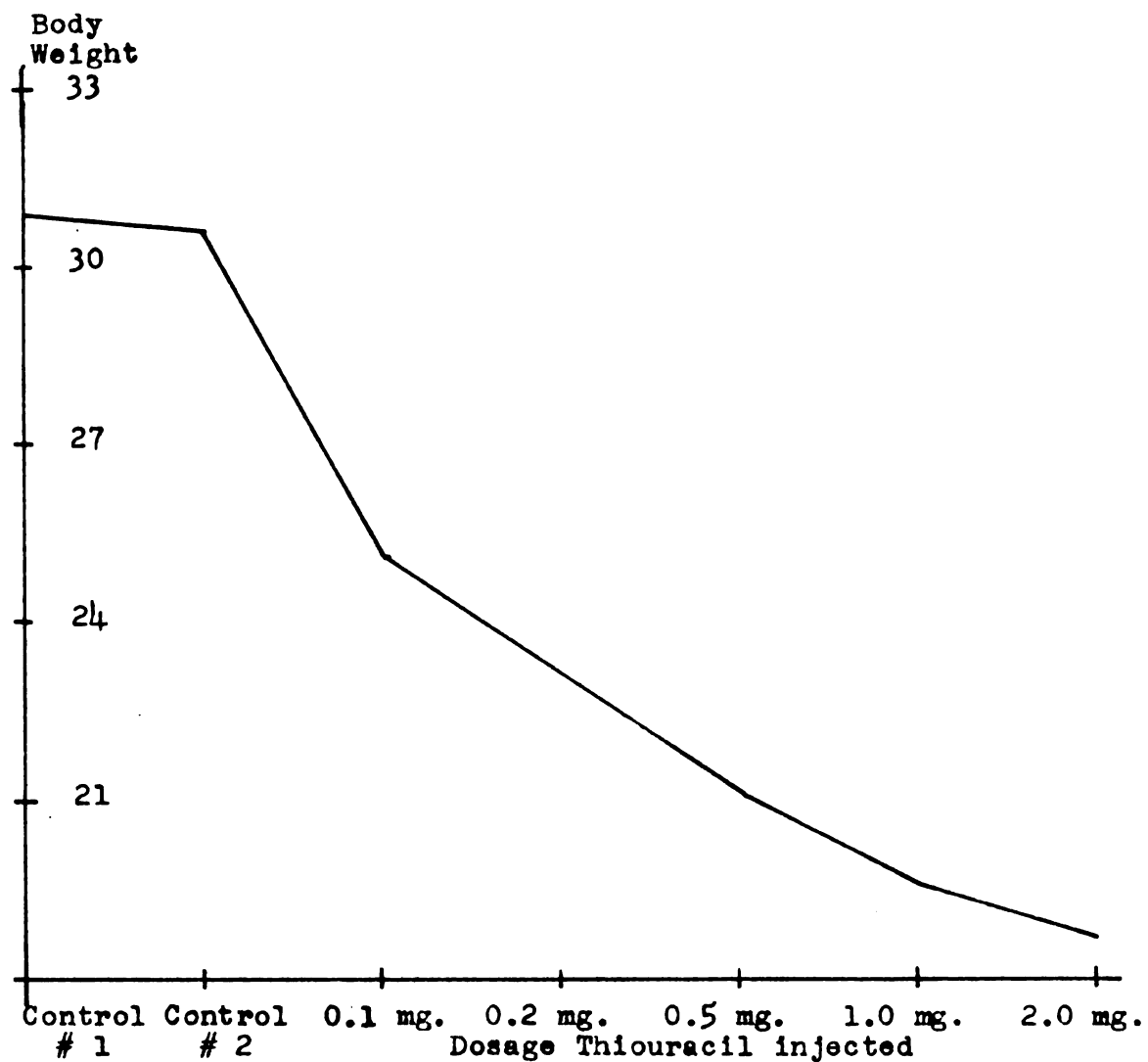
Average body weight in gms. of 19 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 14



Average body weight in gms. of 20 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

FIGURE 15



Average body weight in gms. of 21 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

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APPENDIX

TABLE IV

Body weight in gms. of 7 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>Dosage Thiouracil</u> <u>0.5 mg.</u>	<u>Injected</u> <u>1.0 mg.</u>	<u>2.0 mg.</u>	<u>Control</u> <u># 1</u>	<u>Control</u> <u># 2</u>
0.6	0.5	0.7	0.5	0.4	0.9	0.8
0.8	0.5	0.6	0.6	0.5	1.1	0.9
0.8	0.6	0.6	0.7	0.6	0.8	1.2
0.7	0.6	0.8	0.5	0.5	0.9	1.0
0.6	0.8	0.6	0.5	0.4	0.8	0.9
0.6	0.6	0.5	0.6	0.3	0.7	0.8

<u>Source of Variance</u>	<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance	41	1.5		
Subclass	6	1	.16666	11.66 ^{††}
Error	35	.5	.0142857	Significant at 1% level

TABLE V

Body weight in gms. of 8 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>Dosage Thiouracil Injected</u>		<u>Control</u>	<u>Control</u>
		<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u># 1</u>	<u># 2</u>
1.210	1.170	1.036	1.092	1.960	1.990
1.370	1.230	1.185	1.112	1.980	2.065
1.214	1.080	1.105	1.190	1.954	1.850
1.248	1.201	1.136	1.056	2.450	1.995
1.440	1.158	1.172	1.240	1.950	1.982
1.280	1.155	1.226	1.166	1.990	1.965
<u>Source of Variance</u>		<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance		41	7.1		
Subclass		6	6.2	1.033	40.1 ^{††}
Error		35	0.9	.02571	Significant at 1% level

TABLE VI

Body weight in gms. of 9 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>Dosage Thiouracil Injected</u> <u>0.5 mg.</u>	<u>1.0 mg.</u>	<u>2.0 mg.</u>	<u>Control</u> <u># 1</u>	<u>Control</u> <u># 2</u>
1.552	1.610	1.375	1.330	1.255	3.240	3.507
1.750	1.340	1.472	1.170	1.356	3.060	2.951
1.564	1.435	1.295	1.210	1.230	3.359	2.931
1.650	1.645	1.384	1.362	1.156	3.445	3.545
1.594	1.452	1.765	1.325	1.155	2.950	3.421
1.824	1.348	1.365	1.195	1.265	2.661	2.922
Source of Variance		<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>	
Total Variance		41	177.534			
Subclass		6	152.750	25.458	7.1591 ^{††}	
Error		35	124.487	3.556	Significant at 1% level	

TABLE VII

Body weight in gms. of 10 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

0.1 mg.	0.2 mg.	Dosage Thiouracil Injected		Control # 1	Control # 2
		0.5 mg.	2.0 mg.		
2.150	2.235	2.156	1.956	4.460	3.915
2.345	2.266	2.205	2.057	3.940	4.135
2.405	2.450	2.356	2.155	4.650	4.810
2.312	2.259	2.150	1.865	4.300	4.352
2.205	2.265	2.156	1.752	3.660	3.515
2.415	2.456	2.354	2.046	4.750	4.825
Source of Variance		d.f.	S.S.	M.S.	
Total Variance		41	43.168		
Subclass		6	40.561	89.9 ^{††}	
Error		35	2.607	.0744	
				Significant at 1% level	

TABLE VIII

Body weight in gms. of 11 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

0.1 mg.	Dosage Thiouracil Injected			Control # 1	Control # 2
	0.2 mg.	0.5 mg.	1.0 mg.	2.0 mg.	
3.0685	3.4413	3.2576	2.4595	2.4363	4.7784
2.8756	2.7765	3.3773	2.5226	2.4242	6.0894
3.5365	3.4652	3.0433	3.0557	2.3214	5.1793
2.6076	2.5522	2.8164	2.7827	2.5703	5.6935
3.6666	3.0724	2.6544	2.8773	2.1443	4.6547
3.5262	2.7566	2.6388	2.9418	2.2822	4.8272
Source of Variance	d.f.	S.S.	M.S.	f.	
Total Variance	41	66.027			
Subclass	6	48.346	8.057	15.95 ^{††}	
Error	35	17.681	0.5051	Significant at 1% level.	

TABLE IX

Body weight in gms. of 12 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution and untreated.

0.1 mg.	Dosage Thiouracil Injected			Control	
	0.2 mg.	0.5 mg.	1.0 mg.	# 1	# 2
3.8053	3.9000	3.5298	3.0835	7.2322	7.7543
4.3793	3.5665	3.4931	3.4394	6.4545	6.3123
4.0424	4.5042	3.6841	3.1063	6.3535	5.9549
4.3465	3.7715	3.2496	2.7814	7.1654	8.1955
3.7276	3.6581	3.6544	2.7514	8.0458	7.8001
4.6470	3.9013	3.7517	3.1112	6.8656	7.5400
Source of Variance		d.f.	S.S.	M.S.	f.
Total Variance		41	130.12		
Subclass		6	122.14	20.35	92.50 ^{††}
Error		35	7.98	0.22	Significant at 1% level.

TABLE X

Body weight in gms. of 13 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>Dosage Thiouracil Injected</u>		<u>Control</u>	<u>Control</u>
		<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u># 1</u>	<u># 2</u>
5.9822	5.2247	4.6127	4.3276	10.0764	11.3795
6.3296	5.5015	5.3855	4.8853	11.7533	10.1435
6.9563	6.8487	5.3905	4.8383	9.8814	10.3084
6.8085	6.0490	5.6310	5.3938	11.4038	11.7043
7.0473	6.3484	6.1174	5.3996	11.7632	9.9655
7.0394	6.1005	5.8908	5.5475	10.3093	10.9005
Source of Variance		<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance		41	268.06		
Subclass		6	256.53	42.75	129.78††
Error		35	11.53	0.3294	Significant at 1% level.

TABLE XI

Body weight in gms. of 14 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>Dosage Thiouracil Injected</u>		<u>Control # 1</u>	<u>Control # 2</u>
		<u>0.5 mg.</u>	<u>1.0 mg.</u>		
9.3704	8.2085	8.0045	7.6964	10.9855	11.6244
7.5777	7.4437	6.9737	6.0455	11.5305	12.8175
8.4536	8.1035	7.7316	6.8525	13.4751	12.5795
8.7006	7.8185	7.0145	6.5827	14.1413	13.1473
8.5319	8.0956	7.3056	6.1047	14.8976	14.7281
8.7474	8.0955	8.0335	7.2044	12.7457	12.8101

<u>Source of Variance</u>	<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance	41	312.87		
Subclass	6	290.68	48.444	76.40 ^{††}
Error	35	22.19	0.634	Significant at 1% level

TABLE XII

Body weight in gms. of 15 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

	Dosage Thiouracil Injected			Control	
	<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u>2.0 mg.</u>
				<u># 1</u>	<u># 2</u>
13.5495	13.5214	12.9781	10.5425	9.1176	15.4265
14.2407	13.6715	13.4125	12.2655	11.0051	17.4568
14.0865	14.2304	13.2655	13.4345	10.1251	15.1561
14.8005	14.2204	13.7855	11.3752	11.8491	16.0575
15.2763	13.3104	13.3724	12.4271	11.4562	15.8543
14.3441	13.9897	12.4161	11.8751	12.3125	15.9505
Source of Variance	<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>	
Total Variance	41	184.84			
Subclass	6	135.48	22.58	16.01 ^{††}	
Error	35	49.36	1.41	Significant at 1% level	

TABLE XIII

Body weight in gms. of 16 day old chick embryos injected with Thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

	Dosage Thiouracil Injected			Control	
	<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u>2.0 mg.</u>
				<u># 1</u>	<u># 2</u>
15.6859		14.3573	13.2758	12.9212	13.2771
16.7585		15.2517	14.1851	13.5425	13.1035
17.7385		15.9826	14.5054	14.1655	13.2123
14.9804		14.3321	13.1569	12.5435	12.0025
15.2346		13.3872	13.7885	13.8557	13.0165
16.9768		14.0145	13.7545	14.8136	12.8496
				17.6465	18.3296
				18.2169	18.5635
				19.0255	20.0291
				17.7774	16.8134
				18.0545	18.1441
				18.2407	19.1415
Source of Variance	<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>	
Total Variance	41	206.28			
Subclass	6	183.28	30.54	46.98 ^{††}	
Error	35	23.00	0.65	Significant at 1% level	

TABLE IXV

Body weight in gms. of 17 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>Dosage Thiouracil Injected</u>		<u>Control</u>	
		<u>0.5 mg.</u>	<u>2.0 mg.</u>	<u># 1</u>	<u># 2</u>
16.4004	16.7426	15.3255	14.9459	79.1845	20.9624
18.9314	16.0225	15.9083	14.7357	20.0697	21.2742
17.4075	16.6883	16.3276	16.0005	19.1506	19.2736
18.5115	16.0916	16.3586	15.5829	21.3447	20.7335
19.7357	18.5696	16.6005	17.5525	20.7448	20.0155
16.2525	15.5116	16.7291	15.6665	19.4105	20.2738
Source of Variance		<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance		41	186.08		
Subclass		6	153.91	25.65	28.18††
Error		35	32.17	0.91	Significant at 1% level

TABLE XV

Body weight in gms. of 18 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

	Dosage Thiouracil Injected			Control <u># 1</u>	Control <u># 2</u>
	<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>0.5 mg.</u>		
	21.7895	20.9494	19.6966	23.5478	23.8356
	20.3686	19.7335	18.9876	24.6105	24.0186
	23.2875	22.5076	20.2024	24.9884	24.3236
	19.1314	20.5076	20.0815	25.6105	25.3511
	20.9815	19.8774	19.8784	24.4005	26.9057
	22.9214	20.1625	20.3547	25.3547	26.4511
Source of Variance	d.f.	S.S.	M.S.	f.	
Total Variance	41	282.67			
Subclass	6	240.27	40.04	33.09††	
Error	35	42.40	1.21	Significant at 1% level	

TABLE XVI

Body weight in gms. of 19 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

<u>0.1 mg.</u>	<u>Dosage Thiouracil Injected</u>		<u>Control</u>	
	<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u># 1</u>	<u># 2</u>
23.8276	22.9343	21.3245	27.7656	26.0745
24.1786	23.9945	22.1911	28.2334	27.1437
24.6937	20.4855	19.6452	28.2606	28.3036
24.0153	21.3905	20.6591	26.9615	28.5284
24.0072	19.4908	18.9006	28.3832	27.7896
23.9642	20.6897	19.9464	26.5294	28.1633
<u>Source of Variance</u>		<u>d.f.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance		41		
Subclass		6	65.04	112.13††
Error		35	0.58	Significant at 1% level

TABLE XVII

Body weight in gms. of 20 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

<u>0.1 mg.</u>	<u>Dosage Thiouracil Injected</u>		<u>Control</u>	
	<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u># 1</u>	<u># 2</u>
	<u>0.2 mg.</u>	<u>2.0 mg.</u>		
24.2516	22.7656	20.8753	28.1265	27.4325
24.2725	23.8754	19.8743	29.8724	28.8745
25.7642	22.7412	20.5012	29.7120	29.9125
24.2154	23.7546	19.8756	27.3711	30.8761
24.7416	22.1576	20.9453	29.9741	28.8776
24.1235	24.4251	20.4214	27.0576	29.5976
Source of Variance	<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance	41	558.35		
Subclass	6	524.13	87.35	90.05††
Error	35	34.22	0.97	Significant at 1% level

TABLE XVIII

Body weight in gms. of 21 day old chick embryos injected with thiouracil at 48 hours of incubation compared with controls injected with Ringer's solution or untreated.

<u>0.1 mg.</u>	<u>0.2 mg.</u>	<u>Dosage Thiouracil Injected</u>		<u>Control</u>	<u>Control</u>
		<u>0.5 mg.</u>	<u>1.0 mg.</u>	<u># 1</u>	<u># 2</u>
26	24	22	20	29	32
24	23	20	19	30	30
25	24	21	21	32	32
26	23	20	18	30	31
27	24	22	20	31	32
25	24	22	21	32	31
<u>Source of Variance</u>		<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>f.</u>
Total Variance		41	944.29		
Subclass		6	908.29	151.38	148.4††
Error		35	36.00	1.02	Significant at 1% level

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