RESULTS OF FEEDING THYROID GLANDS OF VARIOUS TYPES OF VERTEBRATES TO TADPOLES

by

HORACE GUNTHORP

Ph. B., Hamline, 1905

A. B, Stanford, 1909

A. M., Kansas, 1912.

Submitted to the Department of Zoology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Approved by

____

Instructor in Charge.

____

Head of Department.

May 15, 1923.

CONTENTS.

RESULTS OF FEEDING THYROID GLANDS OF VARIOUS TYPES OF VERTEBRATES TO TADPOLES

Introduction.

During the past ten or more years considerable work has been done on the relationship of the thyroid gland to metamorphosis in the tadpole, and experiments have been performed in the feeding of both thyroid gland itself and pure iodine to both normal tadpoles and to specimens with the thyroids removed. The present paper records the results of experiments undertaken to determine the effect on tadpoles of the feeding of thyroids from different types of vertebrates and to determine whether or not this effect is correlated with the amount of iodine contained in the different thyroids. The experiments were started in the zoological laboratory of Washburn College, Topeka, Kansas, but owing to the removal of the writer, the later ones were carried on at the University of Washington, Seattle, Washington, where all of the measurements were made and the results tabulated.

I wish to express my thanks to Doctor Bennet M. Allen, of the Southern Branch of the University of California, under whose direction this work was carried on, for the help he has given me, and for the many suggestions he has made as the work progressed, and as this paper was in preparation.

LITERATURE .

Little has been written that can be considered in the nature of a study of the comparative effects of feeding different kinds of thyroid glands to tadpoles, most of the literature on the relationship of the thyroid to the metamorphosis of tadpoles covering other phases of the subject. However, thyroids from several different kinds of animals, and glands from the same species but of different iodine content have been fed, and some comparative work has been done.

Gudernatsch (1912 and 1914), the first person to record the fact that the feeding of thyroid hastened the metamorphosis of tadpoles, fed horse thyroid to tadpoles of different ages, and also a mixed diet of thyroid and thymus. West (1914) fed bull-frog tadpoles "daily some two grain Parke Davis & Co. sheep thyroid tablets", with positive results, and during the same year, Morse, feeding Rana pipiens, used Parke Davis & Co.'s desiccated thyroid extract and Armour and Co's. thyroid tablets as well as fresh thyroid gland obtained at a packing house, but kind not specified, all with positive results. He also found that positive results comparable to those produced by feeding thyroid could be obtained by using iodized amino-acid (3-5-Di-iodo-tyrosine) and also by feeding blood-protein iodine in the form of a commercial product called "iodalbine" manufactured by Parke Davis and Co. Positive results were also obtained when thyreoglobulin and iodothyrin, parts of the thyroid containing iodine, were used. On the other hand, he obtained negative or no results when he fed lecithin, which contains choline, extracted from the thyroid, potassium iodide, methyl iodide, iodobenzoic acid, iodoxybenzoic acid, and "metallic" iodine. Also, the feeding of sea algae containing iodine and of starch iodate caused no acceleration of metamorphosis. The same was true when he fed iodized hen's egg lecithin and iodized egg albumin. None of Morse's experiments were carried on in a comparative way.

Lenhart (1915) fed dried and powdered human thyroids (both simple and exothalmic goitres) and dog thyroid of various iodine content, but how he obtained these different iodine percentages is not stated. Also he fed ox and sheep thyroid, but did not record these results. In this case the higher the per cent of iodine the more rapid was the metamorphosis of the tadpoles. Cold and the feeding of cracker crumbs both tended to delay the change. The following year Graham fed tumorous human thyroid (adenomata) to the tadpoles of Rana pipiens, and found that the effect was closely related to the amount of iodine contained in the food, the same as in Lenhart's experiments. However, there were some exceptions to this, no explanation being given for them.

Gudernatsch (1917) later performed some experiments where thyroid and thymus glands were each split chemically into seven products and tadpoles were treated with the substances obtained. The thyroid nucleo-proteins caused the most rapid differentiation, and the precipitate from an alcoholic extract produced the greatest growth with the least differentiation. The other substances ranged between these two extremes in their action.

Rogoff and Marine (1917) found that artificially iodized blood serum caused an increase in the rate of metamorphosis when fed to tadpoles, but that its action was not as marked as that of thyroid gland.

Kendall (1917) isolated from the thyroid proteins an iodine-containing compound in pure crystalline form having a constant iodine content of 60 per cent. He later called this substance thyroxin, and its identification and properties were further discussed by Kendall and Osterberg (1919) and again by Kendall (1919). He found that this substance produced the so-called hyperthyroid symptoms, and was therefore the active element in the thyroid gland. Rogoff (1918) reduced thyroxin still further and fed both the product of this reduction and the entire thyroxin to tadpoles. Both sets of specimens metamorphosed abnormally fast, those eating the thyroxin being slightly in the lead.

McCord and Marius (1918) found that "The effect of thyroid feeding on normal tadpoles varied with the age of the individuals", and that "irradiation (subjecting them to x-rays) is without apparent effect upon normal tadpoles, but determines a slight but distinct increase in the susceptability of young tadpoles to thyroid stimulation".

Swingle (1918a) fed Armour and Co.'s powdered thyroid to tadpoles of Rana pipiens with marked acceleration of metamorphosis resulting. The same year (1918b and 1919a) he fed tadpoles iodine crystals, iodoform and potassium iodide, all with the same positive results, but those fed iodoform were slower in metamorphosing while the potassium iodide had still less effect than when iodine crystals were used. The same author later (1919b) fed different quantities of iodine to tadpoles of Rana sylvatica, and found that the larger the amount the more rapid the transformation. Bromide caused no acceleration. Still later (1919c) he reported on the comparative effect of feeding iodine to normal tadpoles and to those with the thyroid gland removed. The results were the same in both instances, but it required a larger quantity to produce an equal effect in the case of the thyroidless tadpoles. The species used was Bufo lentiginosus. Finally, Swingle (1923) gave an extended discussion of the literature covering thyroid and iodine feeding, and, following experiments on the feeding of thyroidectomized and hypophysectomized frog larvae of Rana sylvatica, reaffirmed his belief that iodine is the substance essential for anuran metamorphosis.

Emmet and Allen (1919) fed tadpoles of Rana pipiens on diets of different kinds, the fat, carbohydrate, protein and vitamine content of which were known. They were able to alter the size of the larvae and the rate of metamorphosis, but the latter process was not completed in any experiment. From experiments performed by the same authors later (1920), the food used above was evidently practically free from iodine, because when this halogen, either organic or inorganic, was added to the diet, the tadpoles completed their metamorphosis.

The effect of feeding thyroid gland to tadpoles upon which thyroidectomy had been performed was reported by Allen (1917) as successful in one case in accelerating metamorphosis, and the same author (1919a) caused similar results in both Bufo and Rana tadpoles from which both the pituitary and thyroid glands had been removed by feeding them iodine mixed with flour.

Smith and Cheny (1921) briefly report the feeding of dried and fresh anterior-lobe, as well as three kinds of commercial preparations of the same, to tadpoles. One of the commercial products which contained iodine greatly in excess of the normal amount, hastened metamorphosis, but none of the other feedings did. Iodine as KI and as thyroxin iodine were added to the dried gland in sufficient amounts to give an iodine content identical with the more active commercial product. This was fed to normal and thyroidless tadpoles, the food containing KI having no marked accelerating effect, but that containing the thyroxin iodine caused a hastened development the same as the commercial product with the high iodine content.

Uhlenhuth (1921a) gives a very good summary of the literature covering thyroid and iodine feeding experiments of the larvae of both frogs and salamanders, and later (1921b) the same writer reports the results of keeping the tadpoles of Rana sylvatica in water which from the eighteenth to the twenty-sixth day contained 0.005 gm. of Bayer's iodothyrine per 1000 c.c. of water with the result that "the fore limbs broke through the walls of the gill chamber on the thirty-third day after hatching." In similar experiments where inorganic iodine was used in place of iodothyrine, the concentration varying from one to ten drops of a 1/20 M solution of iodine per 1000 c.c. of water, the mortality was great, but "among the surviving larvae none had metamorphosed at the termination of the experiment, nor had the fore limbs broken through in a single instance; yet the limbs were considerably further differentiated than in the controls."

The physical changes that occur in the tadpoles during the process of metamorphosis and their relation to the thyroid gland are discussed by Swingle (1918a) and by Allen (1918 and 1919b).

Dickerson (1907) and Wright (1914) were used in connection with the identification and study of the life history of the tadpoles used in these experiments.

Regarding the sources of commercial thyroid preparations used in the above reports and also in the experiments carried on by the writer, under date of December 6, 1919, Armour and Company write, "Our standardized powdered thyroid substance is made from the glands of sheep only", and a letter dated December 10, 1919, from Parke Davis and Company says, "Practically all of the supplies that we have been preparing for some time past are derived from the thyroid glands of hogs rather than those of sheep."

Material and Methods.

The species used for the first three series of experiments (A, B, and C) was the common bull frog, Rana catesbiana, captured near Lawrence, Kansas. They varied somewhat in size, but so far as known at the beginning of the experiments, they were all less than a year old. The species used for the next eight series (D, E, F, G, H, K, and L) was the leopard frog, Rana pipiens. These were collected in several small ponds in and near Topeka, Kansas, by the author. For the remainder of the experiments (series M, O, P and S) the tree frog, Hyla regilla, was used. These were collected in small ponds and streams in and near Seattle, Washington, by the author. The bull frogs were kept in captivity for some time before they were used, but the experiments with the leopard frog and the tree frog were started within a short time after their capture in each case.

The series of experiments A and B on the bull frog were carried on in two rooms of different temperatures, other conditions being as nearly identical as was possible to obtain, in order to ascertain the effect, if any, of heat and cold on the rate of metamorphosis when accelerated by different kinds of thyroid feeding.

The two series K and L were also run on a comparative basis, but here the amount of water in which the separate experiments were kept was the variable quantity.

The remaining series, including A and B, were performed for the main purpose of the undertaking, namely, to ascertain the effect on tadpoles of the feeding of various kinds of thyroids, and to see whether or not the results were correlated with the iodine content of the foods.

Regularity in the time of cleaning and feeding was maintained throughout the experiments. At 10 A.M. each day the food not eaten during the preceding twenty-four hours was removed. The larger chunks were scooped from the bottom of the container with a perforated section lifter, and the finer particles were removed with a pipette. Great care had to be used in the removal of this, as it was sometimes hard to distinguish from the excreta of the tadpoles, especially in the case of the bull frogs.

At the same time the water was changed. This was accomplished by pouring the contents of the jar or dish through a clean wire sieve, and then placing the tadpoles temporarily in a second clean jar. The original jar was then thoroughly rinsed and cleaned under a running tap, and the tadpoles were returned to it. Care was taken not to contaminate one experiment with water from another, and every precaution was taken in the handling of the specimens and containers. After removing the excess food and changing the water, the fresh food was placed on the surface of the water, and the tadpoles usually began to eat it at once.

Ten tadpoles were used as the unit in most of the experiments except in 8, where the unit was twenty. These were placed in a single container and each container was considered as a single unit or experiment. In order to be sure of chance selection of animals for each experiment from the original stock, each time a series was started, the tadpoles on hand were placed in four jars, and then one specimen was taken with a dip net from each jar in turn and placed in rotation in each of the containers making up the series. After this distribution the individuals were carefully measured and recorded. In the case of the bull frogs, the measurements were made with a metric ruler, but in the case of Rana pipiens and Hyla regilla a micrometer was used. In the latter case the apparatus consisted of a Bausch & Lomb Optical Company microscope with a 7.5 micrometer eyepiece and a 40 mm. objective. The figures obtained were reduced to millimeters in order to have a single standard for all measurements.

The different series were started on the dates mentioned in each case and were closed from two to three days after the mortality had reached 50 per cent of the total number of specimens in the series. An exception to this rule occurred in series A, B and C on Rana catesbiana, which was closed while there was still about 70 per cent of the total number living.

The water used in the experiments performed at Washburn College was Topeka city water, which comes from artesian wells. The water used at the University of Washington came from Lake Washington which is fed by mountain streams. The latter contains a considerable amount of plant and animal life, but both supplies are practically free from iodine.

The method of preparing the food fed in these experiments is given below. The weighing was done on analytical balances sensitive to one-tenth milligram, but the limit of the weighing done was to the milligram. Ordinary bottles were used as containers for the food. These were carefully weighed and marked with a label corresponding to one on the jar in which were kept the tadpoles being fed the specific food. The prepared food was placed in the bottle and it was again weighed. A record was kept of all food placed in each bottle (in almost every case the supply had to be replenished more than once), and the balance remaining at the end was subtracted from the total. No weighing was done outside the bottles.

For the care of the surplus food placed in the experimental jars, but not eaten, small pieces of filter paper were weighed, and on these this excess portion was placed each day. These pieces of filter paper were kept in drawers out of the dust, and after the experiment was closed they were thoroughly dried and weighed. The difference between the weight of the filter paper alone and the paper covered with the food gave the amount fed but not eaten.

Food.

The food used in these experiments was prepared according to the directions of Doctor Allen, and after a formula worked out by him, although the proportions were modified by the writer. Ordinary wheat flour, alfalfa flour and powdered thyroid were mixed with water and rolled into flakes in the following manner: The wheat flour was run through a fine cheese cloth before using. In order to obtain alfalfa flour, alfalfa meal was purchased, and this was run through either two or three cheese cloths of varying coarseness of mesh, the finest being used last. The thyroid gland was thoroughly dried and all fat removed. It was then ground in a rough mortar and passed twice through a fine cheese cloth. The three flours were then thoroughly mixed in a dry state, and water was added, and a thin paste made. This was spread thinly on glass and allowed to dry in the open air over night. It was then placed in the oven for a short time, and was peeled from the glass as flakes. This was fed.

Two different mixtures of these three flours were used. The first one (Formula A) was fed to the tadpoles in series A, B, M, O, P and S.

The second (Formula B) was fed to the tadpoles in all the remaining series. The following are the proportions in the two formulae:

Formula A - 2 parts wheat flour
2 parts alfalfa flour
1 part thyroid.

Formula B - 2 parts wheat flour
1 part alfalfa flour
1 part thyroid

This made the proportion of thyroid (and therefore of iodine) less when Formula A was used.

Samples of the beef and the thyroids used in the following experiments were submitted to Dr. Thomas G. Thompson of the Department of Chemistry of the University of Washington, Seattle, Washington, for chemical analysis in order to ascertain the iodine content of the same. Except the last two samples, they were all dried and in powdered form, unmixed with anything else. The two exceptions, beef soaked in iodine and calf thyroid treated in the same way, were mixtures of these substances with alfalfa flour and wheat flour, prepared as described under Formula A for the preparation of food. In fact they were part of the foods left over at the end of the experiments. The results follow:

Beef (used in control)	0.0004%	iodine
Calf thyroid (raw)	.04	"
Beef thyroid (3 yrs., raw).	.01	"
Beef thyroid (mixed, cooked).	.01	"
Pig thyroid	.02	"
Dog thyroid	.20	"

U. S. P. thyroid (Parke Davis & Co.) . . .	.40%	iodine
U. S. P. thyroid (Armour & Co.)	.23	"
Human thyroid	.009	"
Buffalo thyroid	.053	"
Cat thyroid	.008	"
Bear thyroid	.025	"
Flounder thyroid.	.008	"
Beef soaked in iodine	.06	"
Calf thyroid soaked in iodine	.135	"

The method of analysis, quoting Dr. Thompson, was as follows: "One gram of the powdered thyroid was placed in a Parr Sulfur Bomb and mixed with standard sodium peroxide. Material was burned in the bomb. After cooling, the mixture was dissolved in water and filtered from small quantities of iron which came from the bomb. The solution was then treated with some freshly prepared sodium hypochloride and acidified with 1-1 phosphoric acid. The chlorine was boiled off. Upon cooling, the solution was then treated with 10 c.c. of 10% solution of potassium iodide. The iodine liberated is titrated with standard sodium thiosulphite solution. The strength of the latter is approximately two hundredth normal.

"This method is largely similar to that of the U. S. P., the only modification being the process of burning of the thyroid. The U. S. P. recommends fusion in a nickel or iron crucible with sodium carbonate and potassium nitrate. My modification of the method greatly facilitates the burning of the material."

The above figures apply only to the material used in the following experiments, as there is a wide seasonal variation in the iodine content of the thyroid gland, as shown by the work of Seidell and Fenger (1914) and of Cameron (1914 and 1915).

Tabulations.

In the tables following, which show the results of the experiments on the tadpoles and the amount of food and of iodine consumed in each experiment, the following remarks are of uniform application. The letters used for the different experiments and series are to be interpreted as follows: The first letter represents the series of experiments to be considered as a whole, and the second letter represents the unit (containing ten tadpoles usually) which was fed a certain kind of thyroid during the time the experiment was running. Therefore these second letters stand for the specific food fed in each experiment, and are to be interpreted as follows:

A, control fed ordinary beef; B, calf thyroid; C, raw beef thyroid; D, cooked beef thyroid; E, pig thyroid; F, dog thyroid; G, U. S. P. Parke Davis and Company's thyroid; H, U. S. P. Armour and Co.'s thyroid; K, human thyroid; L, buffalo (Bison bison) thyroid; M, cat thyroid; N, black or cinnamon bear (Ursus americanus) thyroid; O, flounder (Platichthys stellatus) thyroid; P, ordinary beef soaked in iodine; Q, calf thyroid soaked in iodine.

When measuring the living tadpoles before the start of the experiments, three things were taken into consideration: the total length, the body length and the length of the appendages. The fore legs were invariably absent, and the hind legs were also wanting in Rana catesbiana, but were usually present in both Rana pipiens and Hyla regilla. When it came to the examination of the specimens at the close of the different experiments, the above items were again taken into account, but also the individuals were dissected and the length of the stomach and of the intestines were recorded, and also the shape of the stomach and pancreas and the presence or absence of the pronephros and the bladder. In all the tables all measurements are recorded in millimeters and all weights in grams.

In comparing the results of the different experiments with each other it was found necessary to set up some more or less arbitrary rule by which they could be measured in order to determine the state or stage of metamorphosis reached by the specimens in the different units at the time they were closed, and from this judge the rate of development. It was soon found that no universally applicable standard could be taken, but that each species would have to be considered by somewhat different methods. A discussion of the means used in comparing the different specimens will be found under the account of the experiments on each species.

In regard to the tables covering the amount of food and iodine consumed, the total amount eaten was obtained by subtracting from the total amount fed the amount removed each day and afterward dried on filter paper. The total amount of iodine eaten was ascertained from the chemical analyses of the different foods used. In order to obtain the amounts eaten per day it was necessary to have some uniform time measure so as to calculate how long each experiment had run when compared to the remainder under discussion. This time measure unit was obtained by adding the number of days each individual in a single container or experiment lived, and then dividing this number by the number of individuals in the experiment, thus giving the average length of life, or the total number of days there was a full quota of specimens in each experiment. An effort was made to give the tadpoles as much as they would eat, but no more.

With some individual exceptions, development of the fore legs is generally indicative of a change in the shape of the stomach and pancreas from that of the tadpole to that of the adult. Also, a similar statement can be made regarding the presence or absence of the pronephros and the gall bladder, the former disappearing and the latter beginning to show a short time before the fore legs push out. In the tabulation of the results of the experiments these items have been omitted, but it is assumed that the above statements will be borne in mind when considering the results.

All the photographs were taken of specimens that had been prepared in formaline for some time. Where only one from each experiment is shown (series A, B and C), a typical or average sized specimen was used. In the cases where the entire experiment was photographed, they were arranged in the order of their death, unless there was no variation in this, the ones dying first being placed on the left end of the row. If a specimen was lost in any way, i.e., completely eaten by the other tadpoles before his death was discovered, a ring was placed in the series to fill the vacant place. If a specimen was dried up or damaged so that it could not be used in its proper place, a small lead slug was substituted. All the photographs are natural size.

EXPERIMENTS ON RANA CATESBIANA.

Three series of experiments, A, B and C, were performed on the tadpoles of Rana catesbiana. All of these were carried on at Washburn College, Topeka, Kansas, during the year 1920. As stated above, two objects were in view in running these experiments. The first of these, shown in the results obtained in all three of the series, was to find out whether or not the feeding of different kinds of thyroid would have identical effects on the rate of metamorphosis of the tadpoles, other conditions being equal. The second object was to ascertain what effect, if any, temperature had on the rate of metamorphosis of tadpoles when fed the different kinds of thyroid, other conditions being the same. This latter entailed a comparison of series A with series B, the former being carried on in a heated room and the latter in an unheated one.

Both of these rooms were located in a stone building with exceptionally thick walls, thus allowing for a minimum change of temperature inside in response to external changes. The unheated room (containing experiments B) was located on the third floor. It was spacious and well lighted and ventilated from the north and west. The other room, in which series A was carried on, was directly below the above mentioned one on the second floor, and was heated with steam It was generally drier and less well ventilated than the upper unheated room during the winter months. When weather conditions changed so there was practically no difference between the temperatures of the two rooms, series B was brought down to the second floor room, and from this time (June 1) on, both series were finished in this room, then the cooler of the two.

The extremes and means of temperature for the two rooms are shown in Tables I and II, the former being that for the unheated room in which series B was carried on, and the latter being for the heated room where series A was placed. All the temperatures are recorded in centigrade.

Comparing the temperatures of the two rooms, there was a constant and rather marked difference between them from the start of the record up to May 28, when they began to run practically the same. The fact is, the comparative phase of series A and B, so far as difference in temperature was concerned, was closed June 1, after the recorded temperature of the two rooms had been practically the same for four days, as it was apparent that it was no longer possible to obtain a marked difference in this respect between the two rooms without apparatus or arrangements not available.

For the month of February, the unheated room ranged from 4.0° to 13.5° cooler than the heated, with an average difference for the month of 8.8°. For March, the extremes of difference were for the unheated room from 1.5° warmer to 14.5° colder, with an average of 6.2° colder. For April it varied from 0.5° to 12.0° colder with an average 6.4°. For May the unheated room was from 1.0° warmer to 7.25° colder than the heated, with the average at 3.2°. The unheated room averaged 5.2^{0} colder than the heated room for the entire months of February, March, April and May.

The containers used in series A, B, and C were ordinary battery jars of approximately 3000 c.c. capacity. They were filled to within an inch and a half of the top, and were marked on the outside so that the amount of water placed in them each day was practically identical.

Regarding the method devised for determining the relative state of metamorphosis reached at the time the different experiments were closed, undoubtedly all of the parts measured, that is, total length, length of body, of hind legs, of fore legs, of stomach and of intestines, are of more or less importance, but it was early found there was some fluctuation in total length and in the length of appendages due to individual variations. At the same time it was seen that there was much less discrepancy between individuals from the same units as to body, stomach and intestinal lengths, but that out of these three, the more certain measurements could be taken on the body and on the intestine. If the experiments in a single series were classified according to length of intestine, placing the unit with the specimens having the shortest intestines at one end of the list as those most nearly developed into frogs, and placing the experiment with the specimens having the longest intestines at the other end, indicating that they had passed through the least development, it was found that with rare exceptions the experiments were listed in the same order as if each item of the measurements

Table I

Extremes and means of temperature in unheated room, 1920.

Month	Low		High		Average	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
February	6.0	5.5	18.0	19.0	12.6	13.5
March	3.0	6.0	21.0	22.5	15.0	13.3
April	5.0	8.0	22.0	20.0	14.5	15.8
May	16.0	17.5	24.0	27.0	19.9	21.9

Table II

Extremes and means of temperature in heated room, 1920.

Month	Low		High		Average	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
February	17.0	17.5	27.5	27.0	22.2	21.5
March	15.0	17.0	26.0	25.0	22.1	21.9
April	14.0	17.0	28.0	25.0	21.5	21.6
May	20.0	21.0	26.0	27.5	24.0	24.2
June	21.0	22.0	29.0	30.5	25.1	26.8
July	21.0	23.0	31.0	34.0	26.9	28.7

was ranked and counted. However, the length of body must be considered as of equal importance to the length of intestine, and their comparative relationship or ratio to each other was finally taken as the best criterion for judging the stage of metamorphosis reached by the several experiments. Of course, this applies only when comparing individuals or groups of the same species.

In these same comparisons, however, one other factor besides the measurements of the different parts must be taken into account. That is, the date of death of the specimens, or, in other words, the length of time the experiment ran. It is evident that if all measurements were equal at the close of two parallel experiments, the specimens reaching this state of development in the shorter time, i.e., dying the sooner, would be considered as the more advanced. Taking this fact into consideration, it was decided that the best measure by which to judge the state of metamorphosis reached was to use the number resulting from a multiplication of the ratio between the length of the intestine to that of the body by the number of days the experiment ran as the criterion. In other words, it was assumed that $\frac{a}{b} \times c = y$, where a equals length of intestine; b equals length of body; c, the number of days the experiment ran; and y, the stage of metamorphosis reached at the end of the experiment. As a short intestine and a short body indicate an advanced stage of metamorphosis, and a small number of days during which the experiment lived usually denotes a rapid rate of development, it is evident that the smaller the number y is the more frog-like the specimen concerned must have been.

The methods of care and of feeding are described above. The food for series A and B was prepared under formula A, while that for series C was mixed under formula B. The results of the three series of experiments are discussed below.

SERIES A.

Series A was started with eighty bull frogs (Rana catesbiana) on February 6, 1920. These were divided into eight groups of ten, and each group placed in a single container. The room in which these experiments were carried on was heated until June 1, when the heat was turned off for the remainder of the summer. The measurements at the beginning and at the close of the experiments are shown in Table III, and the amounts of food and iodine eaten are recorded in Table IV.

At the beginning of the experiments the specimens averaged very nearly the same in both total length and length of body, there being no hind legs visible in any individual. At the close they arranged themselves into well defined groups, the first of which comprised the first five (AA, AB, AC, AD, and AE) and the second, the remaining three units (AF, AG and AH). In the first group evidently the most robust specimens were in AB, as this experiment lead in total and body length and also in length of intestine, and was a close second in hind leg and stomach length. The order of these first five, beginning with the largest, was AB, AC, AA, AD and AE, the latter being as distinctly at the bottom of the list as AB was at the top, but the other three being hardly distinguishable from each other. The second group consisting of the last three experiments was not hard to arrange in a sequence, the order of which was AH, AG and AF. The AF specimens were the smallest and showed the most marked development of the appendages and shortening of the intestine, all three of which features point to the most advanced stage of metamorphosis. Taking the standard set for determining the stage of metamorphosis, i.e., the product of the length of intestine times the number of days the experiment was carried on, and combining the two groups, the units fall into the following order, beginning with the specimens showing the slowest metamorphosis and passing to the most rapidly developing ones: AB, AC, AA, AD, AE, AH, AG and AF.

Turning to Table IV, which shows the amount of food and iodine consumed by the tadpoles in each experiment, it is evident from the amount of uneaten food removed that probably the first five experiments (AA, AB, AC, AD and AE) were under fed if anything, as they ran for approximately 180 days in each case, and yet the maximum amount removed in any experiment was only .065 grams in AE. No record was kept of the amount of food removed in AA (control), but it was a small amount only. In the last three units (AF, AG and AH) it would seem as if an excessive amount of the food was not eaten, and therefore was removed. In fact, the first five units (AA, AB, AC, AD and AE) were fed approximately ten times as much as the remaining three (AF, AG and AH), and at the same time they ate practically all they were fed, while the last three mentioned left a

Table III

Summary of measurements in series A before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore legs Rt.	Fore legs L.	Stomach	Intestine
AA	Control	56.1	23.1	-	64.0	25.0	1.8	-	-	7.0	272.4
AB	Calf	52.8	23.2	-	68.8	26.6	1.8	-	-	8.4	344.8
AC	Beef raw	55.1	23.4	-	65.4	26.0	0.6	-	-	9.1	280.7
AD	Beef cooked	54.5	22.9	-	65.0	25.0	3.3	-	+	8.5	271.5
AE	Pig	54.3	22.7	-	59.7	24.8	0.3	-	-	7.5	224.8
AF	Dog	54.8	22.4	-	39.4	16.1	3.8	0.8	2.0	5.0	27.8
AG	P.D. and Co.	54.6	23.4	-	32.7	16.1	3.1	0.4	1.5	4.9	35.4
AH	A. and Co.	55.3	23.5	-	45.9	16.7	3.4	0.6	1.6	5.4	37.2

Table IV

Amounts of food and iodine in series A. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
AA	Control	38.661	0	38.661	.00003	179	.2160	.00000017
AB	Calf	52.418	.058	52.360	.00419	180	.2909	.00002311
AC	Beef raw	44.748	.047	44.701	.00089	180	.2483	.00000494
AD	Beef cooked	42.221	.033	42.188	.00084	180	.2377	.00000467
AE	Pig	32.412	.065	32.347	.00129	174	.1859	.00000741
AF	Dog	4.750	1.101	3.649	.00144	31	.1177	.00004645
AG	P.D. and Co.	3.509	2.243	1.266	.00101	23	.0550	.00004391
AH	A. and Co.	5.768	.624	5.144	.00237	32	.1608	.00007406

large proportion of their food untouched. A similar condition existed in all the experiments performed, not only on Rana catesbiana but also on Rana pipiens and Hyla regilla. At the outset of a series, all the tadpoles would eat readily, but after a short time those being fed food containing a relatively high per cent of iodine would seem to stop eating, growing sluggish very rapidly, and as metamorphosis advanced, would lie inert in the bottom of the container. This always resulted in a decided difference in the total amount of food eaten by units developing with abnormal rapidity and by those passing through the period of metamorphosis at a more normal rate. The total amount of food eaten was also closely associated with the number of days the experiment ran, so we would expect the first five (AA, AB, AC, AD and AE) units to have consumed much more than the last three (AF, AG and AH) because the former were fed for from six to eight times as long as the latter.

Regarding the total amount of iodine eaten in each experiment, we find this same grouping did not exist, but a new one arose. AB was far in the lead with .00419 grams, followed by AH with .00237 grams. The remainder, with the exception of the control, AA, formed a middle group with considerable variation within itself, while the amount of iodine eaten by the control was very small. This change in grouping was due partially to the total amount of food consumed, but more to the fact that there was a wide difference between the iodine content of the different foods, the last three being especially well provided with it.

When it comes to the question of the amount of iodine eaten daily, the last column indicates that the three experiments, AF, AG and AH, showed approximately ten times as much iodine eaten daily as any of the others, except AB, and they consumed between two and three times as much as that one. The amount eaten in AA, the control, was .00000017 gram which was very much less than any of the others.

In comparing Tables III and IV, that is, the condition of the specimens at the end of the experiments with the amount of food and iodine consumed, the units arranged themselves in the following order:

Development (beginning with least)	AB, AC, AA, AD, AE, AH, AG, AF
Total food (beginning with most)	AB, AC, AA, AD, AE, AH, AF, AG
Total iodine (beginning with least)	AA, AC, AD, AG, AE, AF, AH, AB
Daily food (beginning with most)	AB, AC, AD, AA, AE, AH, AF, AG
Daily iodine (beginning with least)	AA, AD, AC, AE, AB, AG, AF, AH

It is evident that in this series there was a more or less close correlation among all these items with the notable exception of experiment AB. Next to this, there was the most variation from the developmental order in the total amount of iodine eaten by each unit. With slight variations, in all the cases except these, we have the least development associated with the largest total and daily amounts of food eaten, and the least amount of iodine consumed daily.

In series A it appears that the total amount of iodine consumed had little to do with hastening metamorphosis, because the experiment showing the least developmental change (AB) consumed the greatest total amount, while AG consumed less than half as much as AH, and yet AG reached a stage of development in twenty-three days which was practically the same as that reached by AH at the end of thirty-two days. In comparing the rate of development with the amount of daily iodine consumption, however, it will be seen that there was a very close correlation, with the single exception of AB again.

Photographs of typical specimens taken from each of the experiments of series A are shown on Plate I, figs. 8-15. There was very little variation among the specimens of a single experiment.

SERIES B

As stated above, series B was run parallel to series A under identically similar conditions except that from February 6, the date of starting, to June 1 the former was conducted in an unheated room while the latter was carried on in a room supplied with heat and hence somewhat warmer and drier. The details concerning this variation in temperature are given in Tables I and II. The object in carrying on series B under different conditions was to ascertain whether or not temperature had any effect on the rate of metamorphosis of frogs fed the different kinds of thyroid. At the same time these experiments were used as a check on series A. The equipment used and the material fed were identical with those of series A.

Table V

Summary of measurements in series B before and after feeding. Length in mm.

No.	Name	Before: Total	Before: Body	Before: Hind legs	After: Total	After: Body	After: Hind legs	After: Fore legs Rt.	After: Fore legs L.	After: Stomach	After: Intestine
BA*	Control	52.5	22.9	-	61.6	24.9	1.3	-	-	3.3	236.6
BB*	Calf	52.3	22.1	-	61.9	24.5	1.3	-	-	3.0	257.9
BC	Beef raw	53.7	22.6	-	60.4	24.1	1.2	-	-	3.1	227.3
BD	Beef cooked	54.3	23.2	-	62.2	24.6	1.2	-	-	9.6	282.9
BE	Pig	52.6	22.9	-	55.6	22.6	0.2	-	-	7.2	212.3
BF	Dog	53.0	22.9	-	40.3	17.7	5.1	1.6	2.6	5.6	35.5
BG	P.D. and Co.	54.4	23.4	-	35.1	16.7	2.6	0.7	1.3	4.5	37.8
BH	A. and Co.	52.6	22.4	-	37.0	16.3	4.6	2.2	2.5	4.6	30.9

* After series B was well under way it was discovered that a single tadpole in BA and another in BB showed a remarkably rapid development of fore and hind legs, this evidently being due to these two specimens being in their second year of growth. Their measurements are not included in this table.

Table VI

Amounts of food and iodine in series B. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
BA	Control	37.680	.025	37.655	.00003	180	.2092	.00000017
BB	Calf	44.131	1.613	42.518	.00340	180	.2362	.00001889
BC	Beef raw	35.965	1.619	34.344	.00069	172	.1997	.00000401
BD	Beef cooked	37.495	1.002	36.493	.00073	180	.2027	.00000406
BE	Pig	24.352	1.636	22.716	.00091	165	.1376	.00000552
BF	Dog	7.852	.888	6.964	.00279	32	.0349	.00003402
BG	P.D. and Co.	7.178	4.590	2.588	.00207	69	.0375	.00003000
BH	A. and Co.	10.344	1.691	8.653	.00398	90	.0961	.00004422

In studying Table V, which shows the measurements in series B both before and after the experiments were performed, it will be seen that almost everything that was said concerning the similar figures in series A (as shown in Table III) can be repeated here with slight variation. The specimens were very uniform in size at the opening of these experiments, with no hind legs visible in any case, but it was soon discovered that two specimens - one in BA and another in BB - were evidently a year older than the remaining ones. These were left in the units and developed into typical young frogs, the tail being entirely absorbed. However, they were not considered in getting the average measurements of these experiments.

As occurred in series A, the experiments again group themselves into two divisions, the first five being in the one where the least metamorphosis occurred and the last three being in a group where metamorphosis was well advanced. Again taking the product of the length of intestine times the number of days as the criterion for judging the state of development, it will be seen that the order, beginning with the experiments showing the least development, was as follows: BD, BB, BA, BC, BE, BG, BF and BH.

Comparing the state of development at the close of the experiments with the amounts of food and iodine consumed as shown in Table VI, we find the following relationships:

Development (beginning with least) BD, BB, BA, BC, BE, BG, BF, BH.

Total food (beginning with most) BB, BA, BD, BC, BE, BH, BF, BG.

Total iodine (beginning with least) BA, BC, BD, BE, BG, BF, BB, BH.
Daily food (beginning with most) BB, BA, BD, BC, BE, BH, BF, BG.
Daily iodine (beginning with least) BA, BC, BD, BE, BB, BG, BF, BH.

Again we find, as in series A, a close correlation between all these items, with the exception of experiment BB, which showed a markedly large amount of iodine consumed, both total and daily, compared to the slow rate of metamorphosis of this unit. There was also considerable change of position of the units in the total amount of iodine eaten from that found in the line indicating rate of development. These same two exceptions occurred in series A. Again we can say that with some variations we had the least development associated with the largest total and daily amounts of food eaten and the least amount of iodine consumed daily.

Photographs of average specimens from each of the units in this series are shown on Plate I, figs. 16-23. The variation among the specimens in a single experiment was very slight with the exception of the two cases mentioned above.

In the summary on experiments performed on Rana catesbiana will be found some further discussion of this series, and also a comparison of it with series A as to effect of temperature.

SERIES C.

The experiments recorded under series C were on Rana catesbiana, and were started on June 15, 1920, and were closed August 4. The conditions under which these experiments were carried on were very nearly the same as those for series A, that is, they were in the same room, were placed in the same size containers, and the water used was the same. The average temperature for the total time was naturally several degrees warmer in the case of C, due to the season of the year. The specimens used were from the same lot as for both series A and B. The total number of specimens used was fifty, distributed as follows: CA, 5; CB, CC, CD, and CE, 6 each; CF, CG and CH, 7 each. The food used had a less iodine content than that in series A and B as it was prepared under formula B instead of formula A as in former cases. The purpose of the experiments was to check on the results found in the two former series. The measurements taken at the beginning and close of these experiments and the food and iodine eaten are recorded in Tables VII and VIII.

At the beginning, the specimens were somewhat larger and more mature than was the case at the start of either A or B, but at the close their development had reached very nearly the same stage as the corresponding previous ones. The grouping at the close again showed the two classes, the more mature including the three last groups, and the least developed, the first five. Judged by the same standard as used in previous cases, their arrangement in the order of the state of their development, beginning with the more tad-pole-like, follows: CE, CD, CB, CC, CA, CH, CF and CG.

The correlations between the state of development at the close of the experiments and the amount of food and iodine consumed (Table VIII) follow:

Development (beginning with least) CE, CD, CB, CC, CA, CH, CF, CG.

Total food (beginning with most) CD, CA, CC, CE, CB, CF, CH, CG.

Total iodine (beginning with least) CA, CC, CD, CE, CH,CF, CG, CB.

Daily food (beginning with most) CA, CC, CD, CE, CB, CF, CH, CG.

Daily iodine (beginning with least) CA, CD, CC, CE, CB, CH, CF, CG.

As in former series, we had a wide variation from the expected in the case of CB regarding both the total amount of iodine consumed and also in the daily consumption of the same. CA and CE also showed rather wide variation in the same respect. In fact, there appeared to be here less uniformity between the stage of development and the total iodine consumption than occurred in either of the previous series.

In examining Table VIII, it will be seen that much smaller amounts were fed in the experiments of this series and at the same time very little uneaten food was removed, the latter fact indicating, at least in the first five units (CA, CB, CC, CD and CE), the probability of underfeeding having occurred. On the other hand, the short length of time this series ran accounts for the smallness of the total consumption of food. The actual maximum period covered was fifty days, but this is reduced to thirty in the table because the number of days there recorded presupposes ten specimens in each unit, and there were less in this series from the beginning.

One other factor not shown in the tables probably enters into these results, especially in the very rapid metamorphosis of the specimens in units CF, CG and CH. This factor is temperature.

Table VII

Summary of measurements in series C before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore legs Rt.	Fore legs L.	Stomach	Intestine
CA	Control	57.1	24.5	-	62.2	25.6	2.3	-	-	7.4	218.1
CB	Calf	54.9	23.9	-	58.9	23.7	1.2	-	-	3.2	255.0
CC	Beef raw	50.8	21.8	-	59.5	22.8	1.8	-	-	3.2	227.0
CD	Beef cooked	55.8	23.7	-	62.6	24.1	0.8	-	-	7.1	256.4
CE	Pig	55.1	23.4	-	58.8	23.1	0.9	-	-	3.3	265.6
GF	Dog	57.1	25.0	-	34.4	17.1	2.9	-	1.1	6.0	39.6
CG	P.D. and Co.	55.6	23.9	-	29.7	16.9	2.3	-	0.8	5.6	36.9
CH	A. and Co.	50.9	22.0	-	40.3	16.6	1.7	-	0.4	5.0	50.6

Table VIII

Amounts of food and iodine in series C. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten Food	Total eaten Iodine	Days	Eaten daily Food	Eaten daily Iodine
CA	Control	8.433	.022	8.411	.00001	16	.5257	.00000053
CB	Calf	7.560	0	7.560	.00076	30	.2520	.00002533
CC	Beef raw	8.218	0	8.218	.00021	24	.3424	.00000875
CD	Beef cooked	9.042	.016	9.026	.00022	30	.3009	.00000733
CE	Pig	7.613	0	7.613	.00038	30	.2538	.00001207
CF	Dog	1.065	.040	1.025	.00051	6	.1708	.00008500
CG	P. D. and Co.	0.753	.096	0.657	.00066	5	.1314	.00013200
CH	A. and Co.	0.962	.132	0.830	.00048	6	.1383	.00008000

This series was carried on during the summer months, and the average heat was considerably higher than occurred in either series A or B. The average morning temperature for the last half of June was 25.1° and for July, 26.9°, making the average morning temperature 26.3° for the entire series. The afternoons averaged slightly warmer, being 26.5° for the last half of June and 28.7° for July, making the total average for the afternoons 28.0°. From Tables I and II, it will be seen that the averages for series A were 23.6° for the forenoons and 24.1° for the afternoons, while for series B they were 19.0° and 20.0° respectively.

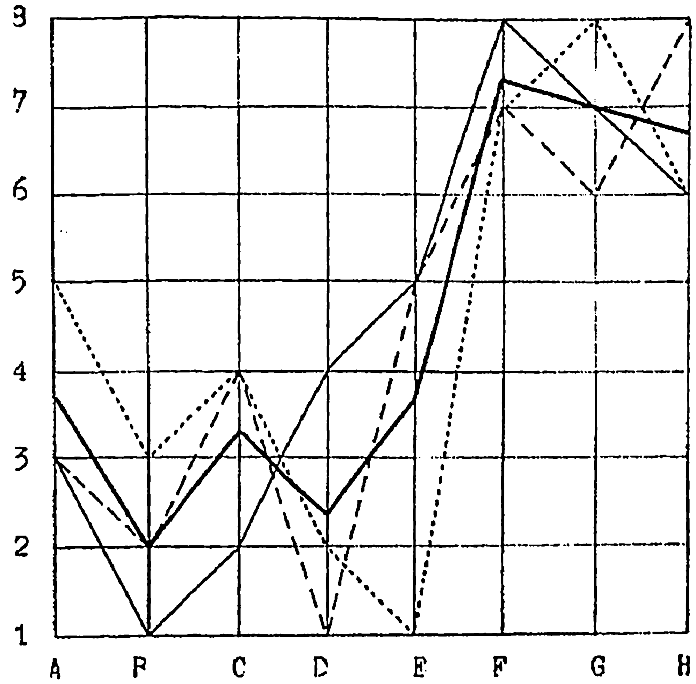
The figures (24 to 31) on Plate II show average specimens taken from the eight experiments in series C. There was practically no variation among the individuals in a single unit.

Further discussion of this series will be found below.

SUMMARY OF EXPERIMENTS ON RANA CATESBIANA

In considering the stage of development reached by the specimens in the different experiments, it is necessary to compare the results as shown in the separate series. These are shown in Fig. 1 where the letters represent the parallel experiments in which the same food was fed, and the numbers stand for the relative rate of metamorphosis of the different experiments, the highest being the most fully developed, and therefore having had the most rapid rate. The average for the three series is also shown. There was some variation between all three of the series, this being the greatest in the case of those fed on pig thyroid (E), followed by those fed on cooked beef thyroid (D), while the dog thyroid experiments (F) showed the least difference.

While many of the conditions under which these three series were carried on were identical, there were some noteworthy differences. One of these was the matter of the nature of the food, series A and B being fed food prepared under formula A, while the food used in series C was prepared under formula B. That meant that the first two series had a food of stronger thyroid and therefore of iodine content, but as this was uniform for all the experiments in a series, it would have no effect on the comparison of series C with the other two. However, it would tend to make the differences between the rates of metamorphosis in the



Series A ——— Series B – – – – Series C ·······
Average ━━━━

Fig. 1. Experiments on Rana catesbiana. Graph showing the comparative stages of development reached in the experiments comprising series A, B and C; also the average for the three series.

same series more marked. A second factor that was quite different in the three series was that of temperature, and in addition the specimens in series C were five months older at the time of starting than those in either series A or B. Another factor entering into at least parts of all three series was that parallel experiments in different series were carried on for different lengths of time, due usually to the different rates of development. All of the experiments in series C were of shorter duration than the corresponding ones in series A and B. Besides these there was some variation in the way the different specimens reacted to the feeding of the same material, and in some units chance apparently threw together groups of somewhat physiologically similar individuals with the result that the total of their reactions differed in measurable quantity from units fed the same material but in other series.

The stages of development of the three series A, B, and C, taken as a whole, arrange themselves in the following order, beginning with the one that shows the least change: B, fed calf thyroid; D, fed cooked beef thyroid; C, fed raw beef thyroid; A, control and E, fed pig thyroid (tied); H, fed Armour and Co.'s thyroid; G, fed Parke Davis and Co.'s thyroid; F, fed dog thyroid.

As stated before, series A and B were carried on under different temperature conditions during the first four months for the purpose of determining whether or not that factor would cause any marked variation in the effect of feeding the different kinds of thyroid. During this period experiments F, G and H in both series died, and so a comparison of them introduces no new factors. In the case of the other five, A, B, C, D and E, however, while the first four months were carried on under quite different temperature conditions, the last two were spent in the same room under identical circumstances. This would naturally be very likely to cause some obliteration of any differences that might have developed between the two series during the first part of the experiments.

First, comparing experiments AF, AG and AH with BF, BG and BH respectively, it will be seen from Tables III and V that the specimens in each instance averaged very nearly the same in total size and size of various parts both at the beginning of the work and at its close. But it will be noted by Tables IV and VI that the units BF, BG and BH lived for approximately three times as long as did their companion experiments in series A. In other words, it took them three times as long to reach the same stage of development. From these same tables, we find that these three units of series B ate approximately twice as much total food and total iodine as AF, AG and AH, but due to the longer time they ran, their daily consumption was only about two-thirds that of the latter.

Turning now to the temperature of the two series as shown in Tables I and II, during February and March, the only months during which experiments F, G and H of both series were running parallel, the difference between the rooms in which series A and series B were being run was during February, 9.6° for the morning and 8.0° for the afternoon, and during March, 7.1° for the morning and 8.6° for the afternoon. In other words, series B was kept under a temperature of slightly less than 8.5° colder than series A during the time those units fed F, G and H were running parallel.

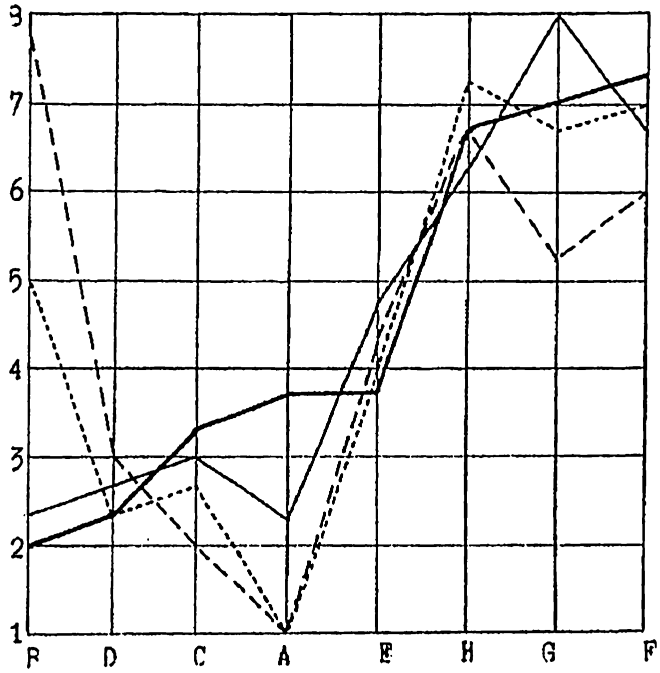
Comparing experiments F, G and H of these two series, then, all conditions except temperature being the same, in view of the fact that the same stage of development was reached in the corresponding units of each series, it would seem that a lowering of the temperature about 8.5° caused a slowing up of the metabolic processes to approximately one-third of what they were when the tadpoles were kept in the warmer place.

Very similar results to the above are shown in the three last units (F, G and H) of series C when they are compared to the same three units of series A and B. This series was carried on in the same room as was series A, but was not started until three months after experiments AF, AG and AH had been closed, consequently the temperature was quite different. The average for February and March, the time these three of the A series were living, was 22.2° for the morning and 21.7° for the afternoon, while for June, when the corresponding units of the C series were being carried on, it was 25.1° for both morning and afternoon making a difference of 3.6° in favor of the C series. It will be seen by comparing Tables III and VII that the tadpoles in these two corresponding groups of experiments were very nearly the same size at the start. At the close, in both series they were by far the most advanced, but in series A the tail and body were somewhat shorter and the legs were better developed. Also, the stomach and intestine were shorter in series A. All of these things indicate AF, AG and AH had advanced to a somewhat later stage of development than had the corresponding units of series C when they died.

Two other factors, both mentioned above, besides temperature were different in series C to what they were in series A, both of which would favor a more rapid development. The first of these was that these specimens were six months older than those in series A when it was started, and the second was that formula B was used in the preparation of the food for series C, while that of series A was prepared under formula A, hence the C experiments received food somewhat richer in thyroid. Also in comparing Tables IV and VIII, it appears that CF and CG ate more food daily than AF and AG respectively, and that all three of the C experiments consumed more iodine daily, the latter condition being the least marked in the last pair of experiments, H. Admitting that these two factors hastened in a measurable degree the development of the last three units in series C, it yet seems apparent that the warmer temperature had considerable to do with the shortening of the period of similar development to a third of the time taken by series A.

Turning to the first five experiments in the three series, A, B, C, D and E, in order to determine what effect the different temperature conditions had on them, a study of Tables III, V and VII, and Fig. 1 fails to show any uniform differences applicable to all three series. It is possible, in fact probable, that if these experiments had been carried on for a sufficient length of time under different temperature conditions, they would also have shown that a lower temperature caused delay in metamorphosis when these foods were fed. Or it may be possible that differences between series A and B were obliterated by their being under identical (warm) conditions during the last two months they were carried on.

From the above facts, it seems safe to conclude that if Rana catesbiana are fed dog thyroid (E), Parke Davis and Co.'s thryroid (G) and Armour and Co.'s thyroid (H), all other conditions being similar, a lower temperature will delay the time of metamorphosis. A difference of approximately 8.5^{0} caused metamorphosis to be delayed about three



Stage of development ———— Total food ————

Total iodine – – – – Daily iodine ··········

Fig. 2. Experiments on Rana catesbiana. Graph showing the relationship between the average stage of development reached and the average amounts of food and iodine consumed in the different esperiments.

times as long as when the tadpoles were fed the same substances under warmer conditions. On the other hand, no difference in rate of metamorphosis was discernible when control (A), calf thyroid (B), raw beef thyroid (C), cooked beef thyroid (D) or pig thyroid (E) were fed under similar variations of temperature.

Turning to the question as to what, if any, relationship there was between the stage of development attained and the food and iodine consumed, we have this graphically shown in Fig. 2 which is based on the information contained in Tables IV, VI and VIII. The line representing the stage of development reached is that of the average for the three series A, B and C. Both the total and daily iodine lines are drawn so that the higher the point is the more there was eaten, while the food line is just the reverse, the higher it is, the less there was consumed. The experiments are rearranged in order to make a regular curve for the stage of development line. The total amount eaten is omitted from the graph because it so closely follows the line for daily food, being one-third of a space lower for experiments C and D, but otherwise exactly the same.

There was a close relationship between the amount of food, both total and daily, consumed and the state of development reached, as these two lines parallel each other quite closely through all the experiments. The total amount of food consumed would naturally be closely associated with the length of time the experiment was carried on, but this does not apply to the daily consumption. The experiments fed A, control, B, calf thyroid, C, raw beef thyroid, and D, cooked beef thyroid, consumed distinctly larger quantities of food than the other experiments, those fed E, pig thyroid, lagging only a short distance behind, ~~while the last~~

while the last three, F, dog thyroid, G, Parke Davis and Company's thyroid, and H, Armour and Company's thyroid, were much behind in both daily and total consumption. As stated before, this lack of food consumption was due to the tadpoles in these three last experiments refusing to eat anything after the first few days of the experiment. They ate as well and probably as much for the first fourth of the time they lived, but after that were seldom seen to eat at all; and yet they continued to metamorphose rapidly, becoming perceptably smaller and more frog-like each day. Food consumption in the tadpole is probably dependent upon the condition of the alimentary canal, and this in turn serves as a measure of development. Just as the alimentary tract shortens so does the ability to eat diminish. This is correlated with the natural fact that must take place as the animal undergoes changes from adaptation to one kind of food to adaptation to another. In the experiments mentioned above (F, G and H) this change in the physiological condition of the alimentary canal was evidently due to the excessive amount of iodine contained in the food.

When it comes to the question of whether or not there was any correlation between the stage of development reached and the amount of iodine consumed, a first glance at Fig. 2 might lead one to say there was none, but a closer study disproves this statement.

First, taking up the total amount of iodine consumed; this naturally will be related to the total amount of food eaten and to the per cent of iodine in the particular food. This accounts for the decidedly large amount of iodine eaten by experiments B, fed calf thyroid, and for the very small amount in experiments A, control. With these two exceptions, the total iodine line follows more or less closely the developmental line, but not quite as closely as does the food line.

Turning to the daily amount of iodine consumed in each case, here we find that experiments A, control, still had the least consumption, but experiments B had dropped from the highest to the fifth place. However, both A and B show the most marked variation from the developmental line here as in the case of total iodine consumed. But on the whole, the daily iodine line is considerably closer to the developmental line throughout its length than the total iodine line is, and with the exceptions of experiments A and B, its points approximate the developmental line closer than the food line does.

Why experiments A showed a high degree of development correlated with a large amount of food and a low amount of iodine eaten while experiments B showed a low state of development correlated with a large amount of food and a high amount of iodine consumed is not clear. These were both exceptions to what seems to be the general plan of more development being associated with small amounts of food and large amounts of iodine eaten. Regarding the B experiments, it is possible that the age of the thyroid fed was responsible, as all the remainder were fed thyroids taken from mature animals while that used in the units B was taken from calves. This would mean that either the iodine content of the calf thyroid was in combination with other things which made it less available for use by the tadpoles, or some element usually associated with the iodine was less abundant, or some repressing (so far as the iodine or iodine-containing element is concerned) substance was in the young thyroid, but not present in the old.

EXPERIMENTS ON RANA PIPIENS

The experiments on Rana pipiens were carried on during the summer of 1920, the first being started June 20 and the last one closed July 28. The first five series, D, E, F, G and H, were run for the purpose of determining the effect of feeding different kinds of thyroids to tadpoles of this species and recording the variations, if any existed. Series K and L were performed in order to determine whether or not the amount of water in which the specimens were kept during the experiment had any effect on the results. They were all conducted in the room in which the previously described series A and C were performed, all the conditions being identical with the exception of the food formula and the size of the containers. The food was prepared under formula B. As the tadpoles of Rana pipiens are much smaller than those of Rana catesbiana, smaller dishes were used. Those for series D, E, F, G, H, and K were about five inches in diameter and half as deep, each being filled with approximately 200 c.c. of water. For series L, larger shallow dishes eight inches in diameter were used, in which 1000 c.c. of water was placed.

The tadpoles of Rana pipiens develop into frogs during the one season. Consequently a shorter time was necessary to complete a series of experiments. It will be noted that most of the specimens used had rudimentary hind legs present when the experiments started, a condition not true in Rana catesbiana.

In determining the stage of development reached in the different experiments, it was found that the method used in the experiments on the bull frog was not satisfactory in the case of Rana pipiens. Consequently a new method was devised which based the determination largely on the ratio between the body length and leg length, but also took into consideration the length of time the experiment ran. That is, $\frac{a}{b} \times c = y$, where a equals body length; b equals hind leg length; c, number of days the experiment ran; and y, the stage of development reached. The smaller y is, the more advanced is the stage of metamorphosis. Of the several leg measurements, those of the hind legs were chosen because after they start to develop they grow continuously during the life of the tadpole and are always in plain sight and easily measured. In some few instances other measurements were also used, especially the intestinal length and that of the fore legs, but these were only considered when the results from the above formula were close. Each experiment, excepting those in series F, consisted of ten specimens, making a total of eighty tadpoles used in each series. The results are discussed below.

SERIES D.

Series D, on Rana pipiens, was started June 20, 1920, and was closed July 20. The results of these experiments are shown in Tables IX and X.

At the close, the experiments fell into the following order when classified as to stage of development reached, the one showing the slowest rate coming first: DC, DA, DD, DB, DE, DH, DF and DG. These arranged themselves into two distinct groups similar to those found in the previous series, the first five being distinctly less developed than the last three. Taking up the items enumerated in Table X, their relation to the order of development is as follows:

Table IX

Summary of measurements in series D before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore legs Rt.	Fore legs L.	Stom-ach	Intes-tine
DA	Control	18.31	7.90	1.34	17.95	8.24	4.58	1.38	1.41	2.75	31.91
DB	Calf	17.81	7.82	1.14	21.35	9.02	5.56	0.88	0.80	3.21	44.78
DC	Beef raw	17.80	7.49	1.10	17.72	8.32	3.41	0.91	0.91	2.67	30.67
DD	Beef cooked	16.65	7.24	1.01	16.20	7.61	4.08	1.35	1.35	3.34	34.26
DE	Pig	16.61	7.23	1.01	17.89	8.69	5.17	1.81	1.86	2.73	31.92
DF	Dog	18.35	7.86	1.15	10.23	6.15	3.34	1.07	1.82	1.87	9.58
DG	P.D. and Co.	17.35	7.53	1.02	8.56	5.39	2.45	0.20	1.21	1.59	8.94
DH	A. and Co.	17.50	7.35	1.07	10.44	5.90	3.66	1.01	1.70	1.68	7.39

Table X

Amounts of food and iodine in series D. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
DA	Control	.727	.045	.682	.000001	25	.02728	.000000027
DB	Calf	.756	.020	.736	.000074	23	.03200	.000003217
DC	Beef raw	.557	.035	.522	.000013	27	.01933	.000000481
DD	Beef cooked	.631	.034	.597	.000015	27	.02211	.000000556
DE	Pig	.713	.010	.703	.000035	26	.02704	.000001346
DF	Dog	.138	.051	.087	.000044	8	.01088	.000005500
DG	P. D. and Co.	.093	.057	.036	.000036	7	.00514	.000005143
DH	A. and Co.	.099	.050	.049	.000028	11	.00446	.000002561

Development (beginning with least)	DC, DA, DD, DB, DE, DH, DF, DG
Total food (beginning with most)	DB, DE, DA, DD, DC, DF, DH, DG
Total iodine (beginning with least)	DA, DC, DD, DH, DE, DG, DF, DB
Daily food (beginning with most)	DB, DA, DE, DD, DC, DF, DG, DH
Daily iodine (beginning with least)	DA, DC, DD, DE, DH, DB, DG, DF

At the start of the experiments, the specimens of the different units averaged very nearly the same size. Those in DA were slightly larger than the rest, while those in DD and DE were somewhat smaller, but these differences were hardly great enough to effect the results as shown by the fact that the unit DE forged ahead into fifth place by the end of the experiments in spite of this initial handicap.

Regarding the total amount of food consumed, as shown in Table X, there was not a great deal of difference among the first five experiments, DA, DB, DC, DD and DE, and the same can be said of the daily consumption. The unit DC ate the least in both instances, while DB used the most. Even if the difference is not great between these two, yet it must be taken into consideration when the rate of metamorphosis is considered, as the former developed to a less degree than the latter, as seen above. The last three units, DF, DG, and DH, consumed much less food than the first five. This is associated with the short time they lived--eight to eleven days--as compared to the much longer period of life of the first five--twenty-three to twenty-seven days. Also, the units DF, DG and DH acted very much like the corresponding experiments on Rana catesbiana did. They ceased feeding shortly after the experiments were started, and lay inert in the bottom of their containers.

The same grouping that was seen for food consumption does not hold when the total amount of iodine is considered. The unit DB lead with

DF, DG, DE and DH following in the order named, while the remaining three, DA, DC and DD, were a considerable distance behind. This condition is correlated to the iodine content of the food and to the amount eaten. When the daily iodine consumption is considered, the number of days the experiments ran comes into the consideration. Here we find that DF and DG were away in the lead, with DB and DH following with approximately half as much, while the rest had a considerably lower amount.

At the close of the experiments, there was a general inverse correlation between the amount of food consumed and the stage of development reached, but it was not universal, as DB, fed calf thyroid, DC, fed raw beef thyroid, and DE, fed pig thyroid, were all exceptions. But with the exception of DB, there was a marked tendency for those which received the most iodine, both as to total and daily amounts, to develop the fastest. This same tendency for the experiment that was fed calf thyroid (DB) to show less development in the presence of high iodine consumption was noticeable in the experiments on Rana catesbiana as well as here.

The specimens used in series D as they appeared at the close of the experiments are shown on Plate III, figs. 32 to 39 inclusive. There was a great deal more individual variation apparent in this species than in the bull-frog, as some of the specimens reached the frog stage and died while others still resembled half grown tadpoles in all respects. This was probably due to individual differences regarding the rates of metabolism and of development. In some cases there was probably a difference in the ages of the different specimens at the beginning of the series.

SERIES E.

Series E on Rana pipiens was run as a parallel to series D, all the conditions being the same. Experiment DA was used as the control for this series also, but is here listed again as EA. The results are shown in Tables XI and XII.

At the commencement of the experiments, the average size of the specimens in the different units was quite uniform. On the whole, they averaged slightly larger than those used in series D, the largest being in units EE and EG, and the smallest in the control, EA.

At the end of the experiments, the units took on the following order, putting the experiment showing the least development first; EA, EC, ED, EB, EE, EF, EH and EG. From a study of the tables and of Plate IV, figs. 40 to 47 inclusive, which show photographs of this series, it will be seen that the units group themselves into two well marked divisions again, as in all previous series, the last three having developed much faster than the first five.

Taking up the items covered in Table XII, their relation to the order of development is as follows:

Development (beginning with least) EA, EC, ED, EB, EE, EF, EH, EG.
Total food (beginning with most) EA, EB, ED, EE, EC, EH, EG, EF.
Total iodine (beginning with least) EA, EC, ED, EE, EF, EB, EH, EG.
Daily food (beginning with most) EB, ED, EA; EE, EC, EH, EG, EF.
Daily iodine (beginning with least) EA, EC, ED, EE, EB, EF, EH, EG.

Here in series E the total food consumed in the different experiments runs almost parallel to the stage of development, the most being eaten by those showing the least advance in metamorphosis. The correlation between daily consumption and rate of development is not quite so close. Regarding the iodine eaten, both as to daily and total

Table XI

Summary of measurements in series E before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore legs Rt.	Fore legs L.	Stomach	Intestine
EA	Control	18.31	7.90	1.34	17.95	8.24	4.58	1.38	1.41	2.75	31.91
EB	Calf	18.37	8.01	1.17	13.17	9.23	7.85	3.82	3.83	2.58	15.69
EC	Beef raw	18.72	8.01	1.18	19.10	9.37	5.13	1.97	1.62	2.15	36.86
ED	Beef cooked	18.65	8.19	1.23	15.96	9.54	6.89	2.82	2.83	2.78	26.60
EE	Pig	20.37	8.47	1.52	11.73	6.90	7.00	1.46	1.87	2.06	10.50
EF	Dog	19.89	8.43	1.39	15.40	9.37	3.71	3.03	3.05	2.59	22.51
EG	P.D. and Co.	19.72	8.47	1.41	11.78	6.80	3.49	0.26	1.66	2.02	11.26
EH	A. and Co.	19.06	8.01	1.18	11.07	6.52	3.43	1.74	1.82	1.91	10.01

Table XII

Amounts of food and iodine in series E. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
EA	Control	.727	.045	.682	.000001	25	.02728	.000000027
EB	Calf	.725	.062	.663	.000056	21	.03157	.000002667
EC	Beef raw	.597	.002	.595	.000015	24	.02479	.000000625
ED	Beef cooked	.679	.021	.658	.000016	22	.02991	.000000727
EE	Pig	.635	.014	.621	.000031	23	.02700	.000001348
EF	Dog	.096	.030	.066	.000033	7	.00943	.000004714
EG	P. D. and Co.	.133	.056	.077	.000077	7	.01100	.000011000
EH	A. and Co.	.171	.067	.104	.000060	9	.01156	.000006667

amounts, there is a rather close correlation in position, the former being exactly the same except for a slight shifting among the last three units, while in the latter case, this shifting includes the last four experiments.

Turning again to Table XII, in regard to the total amount of food consumed: there is no great difference among the first five experiments, EA, EB, EC, ED and EE, but all of the last three units, EF, EG, and EH, fall below these to a marked extent. As to the daily amount, the same is true, but we find a somewhat larger variation in the first group, and the amount that the last three consumed is not nearly as low in proportion as when the total is considered. Both these facts are due to the difference in the number of days the experiments were carried on. In the first group, the time extended from twenty-five days in the control, EA, to twenty-one days in EB, fed calf thyroid, while in the second group, the units were fed for about a third as long.

The unit EG consumed the largest total amount of iodine, followed closely by EH and EB. Next came EF and EE with approximately one-half as large an amount, followed by ED, EC and EA in that order. But this order was not retained when it came to the daily consumption, again due to the variation in the number of days the different units were carried on. Here we found EG far in the lead, followed by EH and EF. Then after a considerable gap came the remaining five in the same order as when the total amount was considered.

As in series D, there was considerable variation in the rate of metamorphosis in each experiment. This is shown on Plate IV, figs. 40 to 47 inclusive.

SERIES F.

Series F was on Rana Pipiens, and was started on June 20,1920, and closed July 20. This series was the same as series D and E in every way except that the unit used was seven in each experiment instead of ten. DA was used as the control for this series as well as for the two preceeding, but was here called FA.

Due to the fact that bacterial infection started in experiments FB, FC, FD and FE and killed twenty-one of the total twenty-eight specimens in these units six days after they were started, the results obtained so far as these particular ones were concerned were of no value. The remaining three, FF, FG, and FH, were not affected by the bacteria. They developed at approximately the same rate as the corresponding units in series D and E, and their arrangement at the close of the experiments was in the following order, beginning with the one showing the least rapidity of development:FG, FF, FH.

The relationship between rate of development and the amount of food and iodine consumed is shown below:-

Development (beginning with least)	FG, FF, FH
Total food (beginning with most)	FF, FH, FG
Total iodine (beginning with least)	FG, FH, FF
Daily food (beginning with most)	FF, FH, FG
Daily iodine (beginning with least)	FH, FF, FG

The results of the experiments of this series are omitted from the general discussion of the work on Rana pipiens, and the tables are not included because of their unsatisfactory nature.

SERIES G.

The experiments in series G were on Rana pipiens, and were started July 7, 1920, and were closed July 28. They were carried on under the same conditions as the former series D, E and F. They consisted of ten specimens in each unit, and were fed under formula B. Tables XIII and XIV give the results in tabular form, and Plate V, figs. 48 to 55 inclusive show the specimens at the close of the experiments.

These were all smaller tadpoles than had been used in the previous experiments, and probably due to this their metamorphosis was somewhat more rapid. Also the mortality was greater than in previous sets, except in the case of series F where bacterial trouble set in. This high death rate was probably due to the small size of the specimens, and also to the high temperature during July. The control, GA, fared worse than any other single experiment, and it was due to this fact that the lot were closed a week before it was originally intended to do so. For these reasons the results are not as trustworthy as those shown in series D and E. The bottle containing the specimens from GB was broken in transportation and the specimens dried up. It was impossible to examine the internal organs in this instance, but the external measurements were taken and they are used in estimating the rate of development. The same method for determining the rate of development was used in this series and the following one as in series D and E, but here the shortness of the time the experiments were allowed to run and the immaturity of the specimens at the start made it apparent that the results obtained from the use of the formula $\frac{a}{b} \times c = y$ were not as accurate as in the cases of the previous series. Taking this into consideration a study of the

Table XIII

Summary of measurements in series G before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore Rt.	legs L.	Stom-ach	Intes-tine
GA	Control	11.71	4.55	0.09	10.30	4.67	0.15	-	-	1.68	15.01
GB	Calf	10.66	4.73	0.06	12.30	5.77	0.10	-	-	?	?
GC	Beef raw	9.74	4.31	0.02	13.50	5.77	0.25	-	-	1.88	32.58
GD	Beef cooked	10.63	4.75	0.08	14.35	5.97	0.21	-	-	1.96	31.39
GE	Pig	10.46	4.61	0.04	14.58	6.18	0.26	-	-	2.31	34.21
GF	Dog	10.21	4.52	0.02	6.30	3.47	0.80	0.10	0.57	1.35	4.70
GG	P.D. and Co.	10.56	4.63	0.07	6.18	3.47	0.65	-	0.57	1.48	5.00
GH	A. and Co.	11.04	4.82	0.10	6.59	3.34	0.74	0.01	0.35	1.34	5.82

Table XIV

Amounts of food and iodine in series G. Unit used, gram.

No.	Name	Amt. fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
GA	Control	.292	.112	.180	.000000+	14	.01286	.000000013
GB	Calf	.486	.027	.459	.000046	21	.02185	.000002190
GC	Beef raw	.211	.027	.184	.000005	19	.00968	.000000263
GD	Beef cooked	.187	.058	.129	.000003	16	.00806	.000000188
GE	Pig	.275	.034	.241	.000012	21	.01148	.000000571
GF	Dog	.078	.028	.050	.000025	7	.00714	.000003571
GG	P. D. and Co.	.099	.033	.066	.000066	6	.01100	.000011000
GH	A. and Co.	.097	.037	.060	.000035	8	.00750	.000004375

dimensions leads one to conclude the order of development at the close of the experiments was as follows, beginning with the one showing the least metamorphosis: GA, GB, GD, GC, GE, GH, GG, GF.

Comparing this order with the food and iodine eaten as shown in Table XVII, we get the following:

Development (beginning with least)	GA, GB, GD, GC, GE, GH, GG, GF
Total food (beginning with most)	GB, GE, GC, GA, GD, GG, GH, GF
Total iodine (beginning with least)	GA, GD, GC, GE, GF, GH, GB, GG
Daily food (beginning with most)	GB, GA, GE, GG, GC, GD, GH, GF
Daily iodine (beginning with least)	GA, GD, GC, GE, GB, GF, GH, GG

Again the experiments fell into two groups, the one showing the least development consisting of GA, GB, GC, GD and GE, while the one showing the more advanced stage of metamorphosis contained the last three, GF, GG and GH. This is in line with what occurred in all previous series. Also the single experiment, GB, consumed an exceptionally large amount of iodine, both total and daily, a condition shown in all previous units where calf thyroid (B) was fed. The amount of food eaten, both total and daily, appears to have had little correlation with the rate of development, as it varied in one experiment in one direction and in the next in the opposite way. But in regard to the amount of iodine consumed; with the single exception of GB as just stated, the relationship between both the total and the daily amounts and the rate of development was exceptionally close in all the units.

Going into the question of food consumption more in detail it will be seen from Table XIV that the experiment GB ate nearly twice as much food both as to total and daily amounts as any other single unit.

Just why this group ate so much is not clear. Except for this one case, there is no decided difference among the group comprising the first five experiments, GA, GB, GC, GD and GE, in either total or daily amounts. The second group, comprising the last three experiments, GF, GG and GH, ate a much smaller total amount of food, but due to the fewer days they ate, the daily amount is little less (none in the case of GG) than the lowest ones of the preceeding group.

In regard to the total amount of iodine eaten by the different units, GG was well in the lead, followed by GB, GH and GF in this order, the remaining four experiments being far behind these. In daily consumption of iodine, the same order prevailed except that GB fell below both GH and GF, due to its having been fed approximately three times as long as the other two. Here again, as in the case of the total consumption of iodine, the remaining four units fell a long ways behind the others.

A study of Plate V, figs. 48 to 55, shows there was little variation among the individuals of the single experiments in this series, this being in marked contrast to the condition prevailing in the two previous ones. In this case, the reason for more uniformity is probably due to the fact the specimens were much more nearly the same in sige at the commencement of the experiments than they were in either series D or E. Also, series G ran for a fourth less length of time than did the two preceeding ones; hence, they had less time to develop individual variations due to different metabolic rates.

SERIES H.

The experiments in series H were parallels in every way with those in series G, and experiment GA was used as the control for series H also, it being designated as HA in this discussion. As stated before under series G, the control used was not very satisfactory, as the mortality was abnormally high, causing the closing of this and the previous series a week before the time intended. This together with the small size of the specimens used made the results obtained less accurate than in series D and E which covered this same species. The results are shown in Tables XV and XVI and the specimens on Plate VI.

The same methods of estimating the stage of maturity were used as in series G. Beginning with the one showing the least development, the following was the order at the close of the experiments: HA, HC, HB, HE, HD, HH, HF and HG.

At the beginning of the series, the different units averaged nearly the same size. At the close comparing the rate of development with the food and iodine eaten as shown in Table XVI, we had:

Development (beginning with least)	HA, HC, HB, HE, HD, HH, HF, HG.
Total food (beginning with most)	HC, HB, HA, HE, HD, HH, HF, HG.
Total iodine (beginning with least)	HA, HD, HE, HC, HB, HF, HG, HH.
Daily food (beginning with most)	HC, HA, HB, HE, HH, HF, HG, HD.
Daily iodine (beginning with least)	HA, HD, HE, HC, HB, HF, HG, HH.

In studying this comparison, it will be seen that so far as both total and daily consumption of food were concerned, there was a close correlation between these and the rate of development except in the case of daily food in experiment HD. Both the total and daily amounts of iodine eaten showed considerable variation from the rate of development. The experiment fed B, calf thyroid, did not act in its usual manner. This food generally seemed to cause a slow

Table XV

Summary of measurements in series H before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore legs Rt.	Fore legs L.	Stomach	Intestine
HA	Control	11.71	4.55	0.09	10.30	4.67	0.15	-	-	1.68	15.01
HB	Calf	10.87	4.85	0.06	13.62	5.87	0.29	-	-	1.87	23.90
HC	Beef raw	10.40	4.56	0.05	13.43	5.56	0.21	-	-	1.97	30.95
HD	Beef cooked	10.37	4.53	0.05	11.34	4.77	0.26	-	-	1.97	23.09
HE	Pig	10.74	4.74	0.07	12.53	5.13	0.24	-	-	1.50	27.06
HF	Dog	10.27	4.23	0.03	5.39	3.35	0.75	0.19	0.40	1.48	4.62
HG	P.D. and Co.	10.30	4.62	0.03	5.00*	3.17	0.69	0.12	0.57	1.19	4.29
HH	A. and Co.	10.47	4.47	0.04	6.10	3.33	0.70	-	0.41	1.34	4.47

Table XVI

Amounts of food and iodine in series H. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
HA	Control	.292	.112	.180	.000000+	14	.01286	.000000013
HB	Calf	.236	.016	.220	.000022	19	.01158	.000001158
HC	Beef raw	.489	.049	.440	.000011	21	.02095	.000000524
HD	Beef cooked	.155	.083	.072	.000002	19	.00379	.000000105
HE	Pig	.163	.019	.144	.000007	18	.00800	.000000389
HF	Dog	.077	.034	.043	.000022	7	.00614	.000003071
HG	P.D. and Co.	.082	.047	.035	.000035	8	.00438	.000004375
HH	A. and Co.	.103	.044	.059	.000035	8	.00738	.000004375

rate of metamorphosis in previous series, but the large amount of it eaten resulted in an abnormally high iodine consumption, both as to total and daily amounts, when these were compared to the stage of development reached. In this series the unit HB ate more than any other experiment except HC, and the total amount of iodine consumed was surpassed only by HG and HH. However, in the amount of iodine eaten daily, experiment HF was also above it.

With minor changes within the divisions, the same major grouping of experiments as to rate of development and as to the amounts of food and of iodine consumed was found here as in the previous series G. Also, as in this previous series, there was very little variation among the individuals of the experiments probably for the same reasons. Photographs of the specimens in series H taken at the close of the work are shown on Plate VI, figs. 56 to 63 inclusive.

SERIES K AND L

Series K and L were run as parallel sets of experiments in order to ascertain whether or not the volume of water used had any effect on the results. As it had been discovered that the effects of feeding dog thyroid and Parke, Davis and Co.'s and Armour and Co.'s preparations were the most rapid in causing premature metamorphosis, these three were chosen as the foods for this comparative study. The conditions under which these were carried on were identical with those of series D, E, F, G and H except for the volume of water used. They were fed under formula B. The experiments in series K were kept in shallow dishes containing 200 c.c. of water, and those in series L were in dishes containing 1000 c.c. of water. The latter containers were larger than the former, so the depth of water in both was practically the same. The water was carefully measured each day as changed. They were started July 3, 1920, and finally closed July 14. However, each pair, except the controls, were closed on the day when the last specimen in one of them died. That is, as soon as the last specimen in an experiment died, the remaining specimens in the corresponding experiment in the other series were killed. The dates of closing follow:

KA and LA on July 14, ten KA and nine LA killed,

KF and LF on July 10, ten LF killed,

KG and LG on July 11, ten LG killed, and

KH and LH on July 14, nine LH killed.

The results of these experiments are shown in Tables XVII to XX inclusive.

In comparing Tables XVII and XIX, it will be seen that the specimens in the experiments were practically the same size at the start, and that the controls, KA and LA, were the same at the end, unless LA could be considered as somewhat more developed than KA. On the other hand, with the possible exception of length of intestine, KF, KG and KH were markedly more mature in all respects than the corresponding LF, LG and LH. Also, the fact that the specimens in the experiments in series K, with the exception of the control, reached their maximum stage of development possible under the experimental conditions and died before the corresponding ones in series L did, would indicate the same. Still further, an examination of Plate VII shows the same condition without question. From these facts, it is evident that series

Table XVII

Summary of measurements in series K before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore Rt.	legs L.	Stom-ach	Intes-tine
KA	Control	14.41	6.26	0.39	15.83	7.15	0.49	-	-	2.80	45.78
KF	Dog	14.55	6.23	0.35	7.08	4.56	1.75	0.93	1.24	1.81	7.76
KG	P.D. and Co.	14.07	6.10	0.30	7.02	4.34	1.57	0.31	0.95	1.65	5.26
KH	A. and Co.	14.59	6.26	0.39	8.88	4.62	1.75	0.15	0.91	1.79	5.97

Table XVIII

Amounts of food and iodine in series K. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
KA	Control	1.322	.006	1.316	.000001	11	.11964	.000000120
KF	Dog	.208	.064	.144	.000072	7	.02057	.000010286
KG	P.D. and Co.	.286	.109	.177	.000177	7	.02529	.000025286
KH	A. and Co.	.201	.121	.080	.000046	10	.00800	.000004600

Table XIX

Summary of measurements in series L before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore Rt.	legs L.	Stom-ach	Intes-tine
LA	Control	14.58	6.36	0.37	13.26	6.58	0.52	-	-	2.42	38.30
LF	Dog	14.25	6.10	0.35	11.80	4.90	1.73	-	0.10	1.83	6.23
LG	P.D. and Co.	14.56	6.34	0.49	10.37	5.05	1.66	0.02	0.98	1.75	5.33
LH	A. and Co.	14.43	6.31	0.37	10.95	4.84	1.65	0.29	0.52	1.75	5.33

Table XX

Amounts of food and iodine in series L. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
LA	Control	.354	.123	.231	.000000+	10	.02310	.000000023
LF	Dog	.185	.056	.129	.000064	7	.01843	.000009143
LG	P.D. and Co.	.180	.092	.098	.000098	8	.01225	.000012250
LH	A. and Co.	.230	.132	.098	.000056	11	.00891	.000005091

K, with the exception of KA, the control, developed more rapidly in 200 c.c. of water than did series L in 1000 c.c.

Turning to the amount of food and iodine consumed in the corresponding experiments as shown in Tables XVIII and XX, it is seen that the K series led the L series in amount eaten with the exception of KH and LH, where the reverse was true. In the case of KF and LF, however, this lead was so slight that it probably had little to do with the results, but the same cannot be said of KA and LA, nor of KG and LG. In both these cases the K experiments consumed from two to four times as much as the corresponding L ones, certainly enough to account for any more rapid development the former may have shown. As stated before, KG developed more rapidly than LG, but it was hard to distinguish much difference between the specimens in KA and LA, the two controls, at the close of the experiments. So it is evident that at least in the cases where dog thyroid (F) and Armour and Co.'s thyroid (H) were fed, it was not the greater amount of food consumed that caused the more rapid development, but it must have been the only other variable quantity, that is, the amount of water in which the specimens were kept.

Turning to a more detailed study of events in connection with these two series, after the first two days the experiments were carried on, with the exception of the controls, KA, and LA, little food was consumed in any of the units. That is, almost all of the food actually eaten by the tadpoles was consumed during the first two days. After that, the specimens stayed on the bottom of the containers, motionless practically all the time except when disturbed. The flakes of food were placed on the surface of the water directly after it had been changed every morning at ten o'clock. They floated there until they became waterlogged when the sunk to the bottom of the dish, and remained there until they were removed the following morning.

The question arises as to just why the specimens in the smaller amount of water developed so much faster than the ones in the larger amount. It seems to the writer that the only answer to this is to assume that some active substance which causes acceleration of metamorphosis in the tadpoles had dissolved out of the food, and that this was naturally five times as concentrated in the experiments of series K as in those of series L because it was in one-fifth as much water. It acted through the body wall and membranes of the tadpoles, its action being the same, with the possible exception of speed, as when taken into the alimentary canal. The specimens, series K, in the more concentrated solution would naturally react more rapidly than those, series L, in the weaker one. That the larvae of frogs and toads do not swallow water but rather absorb it through the integument is the contention of Boulenger (1914), which is in line with the above conclusion. Also, Swingle (1919c) performed some experiments on the larvae of Bufo lentiginosus in which he did not vary the amount of water in which they were kept, but did place them in solutions consisting of different concentrations of iodine with the result that those which obtained the most iodine metamorphosed the most rapidly. This would be in line with the present results, except that here it is not clear just what the active substance is that dissolved out of the food and caused the speeding up of metamorphosis.

However, it is quite clear that this active substance was either iodine or some compound of which iodine was a part, as a chemical analysis was made of the Parke Davis and Company's thyroid preparation (used in the preparation of food G) after it had been soaked for twelve hours in water, then thoroughly dried and ground to a powder. It was found that the iodine content was only 0.112 per cent where it was 0.40 per cent before it was soaked. At the end of half the period the food was in the water, almost three-fourths of the iodine content had dissolved out. The same method of making the chemical analysis was used here as in previous cases.

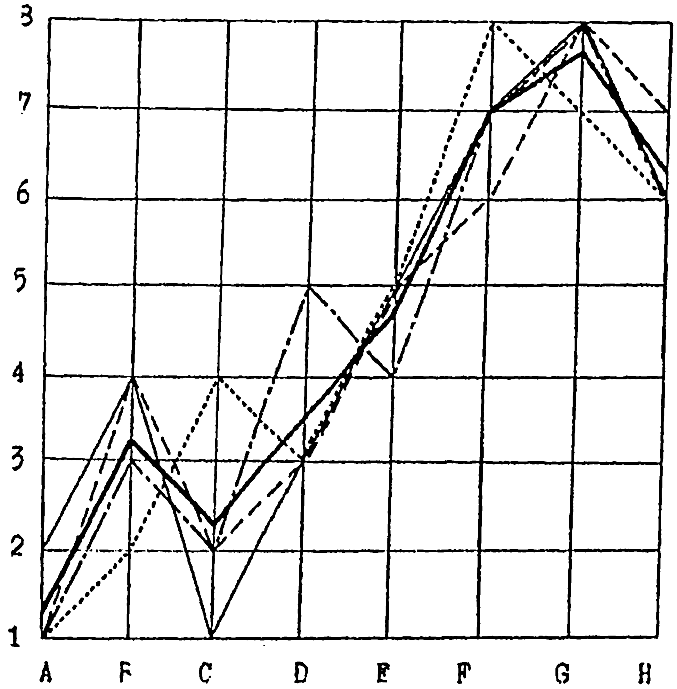
SUMMARY OF EXPERIMENTS ON RANA PIPIENS

A summary of the results of the experiments on Rana pipiens entails a comparison of the series D, E, G and H. This is graphically shown in fig. 3 where the letters represent the kind of food and the numerals the rank of the experiment in relation to the other units. The lower the number is the slower the rate of development was. As stated before, the results of series F are not included in either the graphs or the discussion following because, with the possible exceptions of the FF, FG and FH experiments, they were questionable owing to bacterial action.

In these series all of the conditions under which they were carried on were identical with the possible exception of temperature. Even in this respect there was less than half a degree variation between the average for series D and E on the one hand and series G and H on the other.

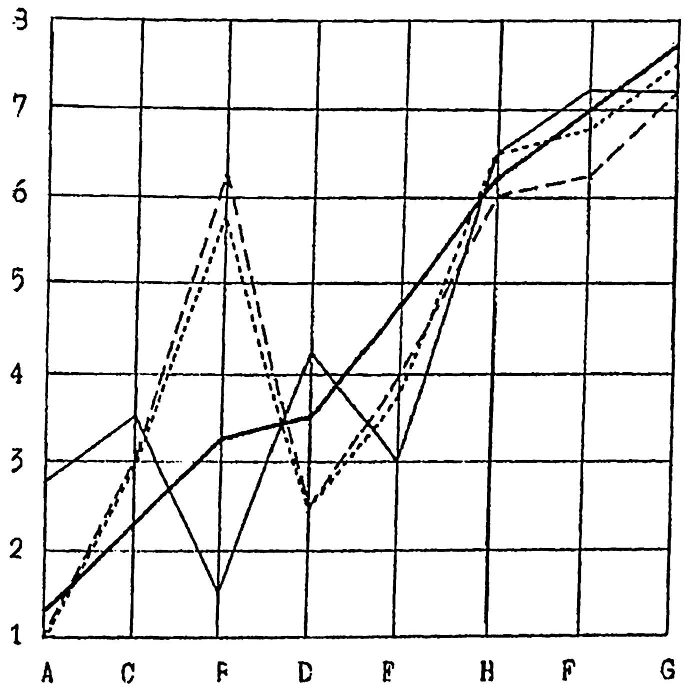
There was much less variation between the different series in the case of Rana pipiens than was seen in the experiments on Rana catesbiana. The largest difference was in the case of those fed C, raw beef thyroid. On the whole, with this one exception, the rate of development among similar units in the different series is remarkably uniform. The average rate of development for all the four series is as follows, beginning with the one showing the slowest rate: A, control; C, raw beef thyroid; B, calf thyroid; D, cooked beef thyroid; E, pig thyroid; H, Armour and Co.'s thyroid; F, dog thyroid; G, Parke Davis and Co.'s thyroid.

In regard to the relationship of the rate of development to the



Series D ——— Series E – – – – Series G ....
Series H —.-— - Average ———

Fig. 3. Experiments on Rana pipiens. Graph showing the comparative stages of development reached in the experiments comprising series D, E, G and H; also the average for the four series.



Stage of development ———— Total food ————
Total iodine - - - - - Daily iodine ---------

Fig. 4. Experiments on Rana pipiens. Graph showing the relationship between the average stage of development reached and the amounts of food and iodine consumed in the different experiments.

material eaten, we turn for information to Tables IX to XVI inclusive. The averages of the total food and the total and daily iodine consumed for the four series was taken, and the graph shown in fig. 4 was made from these. The average rate of development for the four series is also shown, the experiments being arranged so that this line will form a regular curve. The curve for the daily food was omitted because it follows so closely the total food line that the one may be considered as standing for both items for all practical purposes. The lower numerals on the graph stand for slower rate of development, and a less amount of iodine consumed. Regrading the food, the reverse is true, the lower number standing for the larger amount eaten.

Regarding the relationship between the amount of food eaten and the rate of development, with the exceptions of experiments B, fed calf thyroid, and possibly E, fed pig thyroid, it appears that the more rapid the rate of metamorphosis the less the amount of food consumed. The exceptions are so few that it is very probable the statement holds true for all feeding experiments on tadpoles, especially when it is remembered that there was even more uniformity between these two items in the experiments on Rana catesbiana than there is in the series under discussion here.

There is a very close correlation between the rate of development and the amount of iodine, both total and daily consumed with the one exception of experiments B, fed calf thyroid. That is, the more iodine consumed the more rapid the rate of metamorphosis. In the case of experiments B, they consumed an equal or larger total of iodine than the units E, fed pig thyroid, or F, fed dog thyroid, or H, fed Armour and Company's thyroid, and yet each of these developed much more rapidly. On the other hand, the only experiment of these three that B surpassed in daily consumption of iodine was E. This correlation between the amount of iodine eaten and the rate of development is much clearer here than was the case in the experiments on Rana catesbiana.

This exceptional behavior on the part of the specimens in experiments B seems to always be associated with a very large total consumption of iodine, but the amount of this substance eaten daily is not relatively so high due to the fact that these units invariably were long lived. This would indicate that it was not the total amount of iodine consumed that caused the acceleration of metamorphosis so much as it was a large amount used daily. In all of the series of experiments on Rana pipiens the last three units, F, fed dog thyroid, G, fed Parke Davis and Company's thyroid, and H, fed Armour and Company's thyroid, developed with the greatest rapidity. However, in every one of the four series, the units fed B, calf thyroid, consumed as much or more total iodine that at least one of these three, and in the case of series D, almost twice as much as any of them. Again in this case no explanation can be given for the exceptional conduct of the specimens in experiments B, unless it was that the age of the thyroid had something to do with it.

A full discussion of series K and L, carried on to determine the effect of the amount of water in which the experiments were kept on the results of thyroid feeding is given above under the treatment of those series. The results there obtained probably indicate that the active substance of the thyroid glands dissolves out into the water in which the specimens are kept, and this fact probably accounts for the rapid rate of metamorphosis of the last three experiments in each series, F, fed dog thyroid, G, fed Parke Davis and Company's thyroid, and H, fed Armour and Company's thyroid, a speed associated with small food consumption and a practically inert condition after the first two or three days.

EXPERIMENTS ON HYLA REGILLA

The experiments on Hyla regilla, the tree frog, were carried on during the summer of 1922 at the University of Washington, Seattle, Washington. Their purpose was to ascertain further the effects of feeding the different kinds of thyroid previously used, to add to these material from other species, and to study the reactions of a new genus to thyroid feeding. The conditions under which the experiments were performed were as nearly identical with those for the ones that were done in Kansas as it was possible to make them. They were placed in shallow white enamel-ware dishes of a capacity of 1250 c.c. which were filled about two-thirds full. These dishes were kept on tables in a small room with two large windows opening towards the southeast. The summer was exceptionally smoky, and the illumination on the experiments was probably about the same as in the case of all previous ones. The methods of caring for the specimens, feeding, etc., were the same as used in all the preceeding series. All the food was prepared under formula A. The temperature, recorded in centigrade, will be found in Table XXI.

Table XXI.

Extremes and means of temperature, 1922.

Month	Low		High		Average	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
June	18.0	19.5	24.0	26.5	20.7	21.6
July	18.5	20.0	24.5	28.0	21.4	23.0
August	18.0	19.0	25.0	26.0	19.6	21.9

Several new kinds of food were used in these series, and all of the previous varieties were tried at least once. The new kinds, with their letter symbols, follow: K, human thyroid, L, buffalo thyroid; M, oat thyroid; N, bear thyroid; O, flounder thyroid; P, beef soaked in iodine; Q, calf thyroid soaked in iodine.

The same method of determining stage of development reached at the time of closing the different experiments was followed as in the previous series on Rana pipiens. The number of specimens in each experiment in series M, O and P was ten, while in series S it was twenty.

SERIES M

Series M consisted of experiments on Hyla regilla which were started June 6, 1922, and were closed June 27. The following kinds of foods were fed: A, control; C, raw beef thyroid; G, Parke Davis and Co.'s thyroid; H, Armour and Co.'s thyroid; K, human thyroid, L, buffalo thyroid; M, oat thyroid; N, bear thyroid; O, flounder thyroid. The results of these experiments are shown in Tables XXII and XXIII.

In the beginning, the specimens in the different units averaged about the same size except that those in MG and MO were slightly smaller than the rest. At the close, in spite of this handicap, MG had made the most rapid advancement. From a study of Table XXII, taking into consideration the length of time the different experiments ran, one is led to the conclusion that the following was the arrangement of the units at the close, so far as state of development was concerned, beginning with the one showing the slowest progress: MA, MO, MC, MM, ML, MN, MK, MH and MG.

Table XXII

Summary of measurements in series M before and after feeding. Length in mm.

No.	Name	Before Total	Before Body	Before Hind legs	After Total	After Body	After Hind legs	After Fore Rt.	After legs L.	After Stom-ach	After Intes-tine
MA	Control	17.36	7.62	-	19.07	8.09	0.49	-	-	2.19	42.14
MC	Beef raw	16.58	7.58	-	17.07	7.54	0.54	-	-	2.09	33.25
MG	P.D. and Co.	15.84	6.96	-	9.70	4.80	0.75	-	0.22	1.44	9.61
MH	A. and Co.	17.67	7.90	-	10.04	5.09	0.86	-	-	1.41	7.35
MK	Human	17.74	8.11	-	11.86	5.62	1.01	-	-	1.57	9.70
ML	Buffalo	17.65	8.09	-	12.60	5.92	1.18	-	-	1.69	13.40
MM	Cat	17.10	7.61	-	16.44	6.57	1.96	-	-	2.03	17.78
MN	Bear	17.46	7.63	-	12.25	5.48	1.41	-	-	1.52	7.15
MO	Flounder	16.24	7.04	-	19.49	6.78	0.46	-	-	2.39	34.11

Table XXIII

Amounts of food and iodine in series M. Unit used, gram.

				Total eaten			Eaten daily	
No.	Name	Amt.fed	Removed	Food	Iodine	Days	Food	Iodine
MA	Control	.309	.123	.186	.000000+	14	.01329	.000000011
MC	Beef raw	.256	.111	.145	.000003	13	.01115	.000000223
MG	P.D. and Co.	.207	.194	.013	.000010	9	.00144	.000001111
MH	A. and Co.	.202	.183	.019	.000009	11	.00173	.000000818
MK	Human	.098	.063	.035	.000001	11	.00318	.000000057
ML	Buffalo	.175	.125	.050	.000005	10	.00500	.000000530
MM	Cat	.124	.030	.094	.000002	15	.00627	.000000100
MN	Bear	.164	.114	.050	.000003	17	.00294	.000000147
MO	Flounder	.274	.078	.196	.000003	14	.01400	.000000224

Comparing this with Table XXIII, where the amount of food and iodine consumed is shown, we find the following relationships;

Development (beginning with least)	MA, MO, MC, MM, ML, MN, MK, MH, MG
Total food (beginning with most)	MO, MA, MO, MM, ML, MN, MK, MH, MG
Total iodine (beginning with least)	MA, MK, MM, MO, MO, MN, ML, MH, MG
Daily food (beginning with most)	MO, MA, MC, MM, ML, MK, MN, MH, MG
Daily iodine (beginning with least)	MA, MK, MM, MN, MC, MO, ML, MH, MG

From the above it will appear that there was a very close inverse correlation between the amount of food eaten and the rate of development. This applied to both the daily and total amounts. In regard to the iodine, however, with the exception of the first four experiments, fed A, control; C, raw beef thyroid; G, Park Davis and Co.'s thyroid; and H, Armour and Co.'s thyroid, there seemed to be little or no relationship. The experiment fed on K, human thyroid, showed an exceptionally low iodine consumption in relation to the rate of development. On the other hand, in the case of MO, fed flounder thyroid, the reverse was true, but not in so marked a degree.

The consumption of food in this series was exceptionally small. The reason for this was not clear, but it was not because the specimens did not have an opportunity to eat, as in every instance a considerable portion of the material fed was removed. Of course there was a correspondingly small quantity of iodine eaten also. In spite of these facts, there was considerable difference in the length of time the experiments ran, and a marked difference between the various units at the close of the work, both things indicating a distinct difference in the rates of development.

The units composing this series arranged themselves into four well marked groups when the rate of development or stage of metamorphosis reached, was considered. Beginning with the group showing the least change, they were as followw: (1) MA, MC and MO; (2) ML, MM and MN; (3) MK; (4) MG and MH. The grouping was as distinct and exactly the same when the total amount of food consumed was considered, and the same was true of the daily food with the single exception of experiment MK which ate sufficient to throw it into the second group. However, when considering the total amount of iodine eaten, this grouping entirely disappeared with the single exception of the fourth one, the two units of which had by far the most iodine. This was also true when the daily iodine consumption was considered.

SERIES O.

The experiments on Hyla regilla listed under series O were started June 15, 1922, and were closed July 8 following. They were carried on under identical conditions to those under which the experiments in series M were run, but in this case all of the different kinds of food so far tested were used. The results are shown in Tables XXIV and XXV.

From the former table, taking into consideration the number of days each experiment ran, after they were all through, the following seems to be their arrangement, beginning with the one showing the slowest rate of development: OA, OB, OD, OE, OC, OO, OM, ON, OL, OK, OH, OG and OF.

The relationship between the rate of development and the amount of food and iodine consumed was as follows:

Table XXIV

Summary of measurements of series O before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore Rt.	legs L.	Stom-ach	Intes-tine
OA	Control	13.51	5.64	-	19.30	7.31	0.20	-	-	2.37	39.40
OB	Calf	13.22	5.32	-	19.81	7.71	0.35	-	-	2.35	44.50
OC	Beef raw	12.71	5.46	-	18.69	7.70	0.46	-	-	2.68	48.90
OD	Beef cooked	12.44	5.22	-	17.20	6.88	0.29	-	-	2.16	36.05
OE	Pig	12.69	5.09	-	19.55	7.73	0.43	-	-	2.42	44.40
OF	Dog	11.73	5.01	-	5.38	3.21	0.55	0.16	0.15	1.18	4.70
OG	P.D. and Co.	11.81	5.08	-	6.73	3.47	0.34	-	0.12	1.37	4.60
OH	A. and Co.	11.91	5.29	-	7.86	3.48	0.38	-	-	1.21	5.25
OK	Human	12.66	5.34	-	9.77	4.31	1.66	0.18	0.18	1.30	5.25
OL	Buffalo	12.03	5.07	-	9.47	3.87	0.79	-	0.08	1.22	5.55
OM	Cat	12.07	5.17	-	15.27	5.48	2.65	-	-	1.35	14.25
ON	Bear	12.29	5.12	-	8.92	3.84	0.92	-	0.05	1.25	6.35
OO	Flounder	12.35	5.04	-	15.60	6.28	0.56	-	-	1.84	29.44

Table XXV

Amounts of food and iodine in series O.   Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten Food	Total eaten Iodine	Days	Eaten daily Food	Eaten daily Iodine
OA	Control	.565	.057	.508	.000000+	23	.02209	.000000018
OB	Calf	.311	.075	.236	.000019	23	.01026	.000000826
OC	Beef raw	.513	.093	.420	.000008	20	.02100	.000000420
OD	Beef cooked	.296	.105	.191	.000004	23	.00830	.000000174
OE	Pig	.196	.081	.115	.000005	23	.00500	.000000274
OF	Dog	.164	.082	.082	.000033	12	.00683	.000002750
OG	P.D. and Co.	.232	.115	.117	.000094	11	.01064	.000008545
OH	A. and Co.	.214	.150	.064	.000029	14	.00457	.000002071
OK	Human	.345	.054	.291	.000005	20	.01455	.000000262
OL	Buffalo	.272	.122	.150	.000016	17	.00882	.000000935
OM	Cat	.313	.024	.289	.000005	22	.01314	.000000210
ON	Bear	.386	.116	.270	.000014	18	.01500	.000000750
OO	Flounder	.384	.071	.313	.000005	20	.01565	.000000250

Development (beginning with least) OA, OB, OD, OE, OC, OO, OM, ON, OL, OK, OH, OG, OF.

Total food (beginning with most) OA, OC, OO, OK, OM, ON, OB, OD, OL, OG, OE, OF, OH.

Total iodine (beginning with least) OA, OD, OE, OO, OM, OK, OC, ON, OL, OB, OH, OF, OG.

Daily food (beginning with most) OA, OC, OO, ON, OK, OM, OG, OB, OL, OD, OF, OE, OH.

Daily iodine (beginning with least) OA, OD, OM, OO, OK, OE, OC, ON, OB, OL, OH, OF, OG.

It can again be said that those units showing the least development consumed the most food as a rule, both as to daily and total amounts. The experiment which was fed K, human thyroid, was the most marked exception to this, as it showed a rapid rate of development associated with a comparatively large amount of food eaten. Exceptions in the other direction, i.e., a slow rate of metamorphosis associated with a comparatively small food consumption, were found in the experiment fed E, pig thyroid, and to a less extent in the units fed B, calf thyroid, and D, cooked beef thyroid.

Regarding the iodine consumption, a large amount was generally associated with a rapid rate of development. There were two exceptions to this--one in the case of the experiment fed K, human thyroid, where a rapid rate of metamorphosis went with a comparatively small amount of iodine eaten, especially regarding the daily amounts, and a second in the case of the unit OB, fed calf thyroid, where the development was slow but the amount of iodine consumed was high.

At the start the specimens used in this series were considerably smaller than those used in series M, but were quite uniform in size.

At the close, the units could be divided into three large groups; those which had undergone a considerable change, those which had developed to only a slight degree, and those which occupied a middle position in relation to the other two groups. OF, OG, OH, OK, OL and ON showed the most rapid development; OA, OB, OC, OD, OE and OO underwent the least, while the middle group consisted of the single experiment OM. The last three of the first group, OK, OL and ON, approach this middle group, due to the fact that it took them a considerably longer time to develop than it did the first three.

SERIES P.

Series P, on Hyla regilla, was started July 4, 1922, and was closed July 18. The specimens used in this series were considerably smaller at the start than those used in the two preceeding series. This fact probably accounted for a high rate of mortality which caused the series to be closed at the end of two weeks whereas the preceeding ones had been carried on for three. For this reason, the results of these experiments were not satisfactory and are not included in the summary of experiments on Hyla regilla following. The following foods were used: PA, control; PE, pig thyroid; PF, dog thyroid; PG, Parke Davis and Co.'s thyroid; PH, Armour and Co.'s thyroid; PK, human thyroid; PL, buffalo thyroid; PM, cat thyroid; PN, boar thyroid; PO, flounder thyroid.

It was very hard in a series that had run for as short a time as this one had and hence showed little differentiation between some of the units to place them in order so as to show relative rapidity of development, because the more slowly developing experiments had not had sufficient time to differentiate. The following arrangement is as accurate as could be made under these conditions. Beginning with those showing the slowest rate of development, they fell into approximately the following order: PM, PA, PO, PE, PN, PK, PL, PH, PF and PG.

SERIES S

The last experiments on Hyla regilla, series S, were started on August 10, 1922, and were closed August 31. In this series each unit had twenty specimens in it, otherwise the conditions under which they were performed were the same as for the three preceeding series. The results are shown in Tables XXVI and XXVII. In the latter table the number of days the experiments ran is figured on the basis of there being only ten specimens in each unit, thus making these numbers on a uniform basis with all the other series.

In addition to the foods used in the previous experiments on Hyla regilla, two new ones were added here. They were P, beef soaked in iodine, and Q, calf thyroid soaked in iodine. The former was prepared by soaking some of the cooked beef from the same lot as that used in the control (A) for forty-eight hours in a strong solution of iodine which had been made by dissolving iodine crystals in 95% alcohol. This was then thoroughly dried and made into the regular food under formula A. For food Q, the same thing was done with calf thyroid taken from the same lot as that used in making food B.

As to the rate of development, taking into consideration the length of time the experiments ran, the data in Table XXVI shows the following to be the order, beginning with the slowest: SA, SQ, SP, SO,

Table XXVI

Summary of measurements in series S before and after feeding. Length in mm.

No.	Name	Before			After						
		Total	Body	Hind legs	Total	Body	Hind legs	Fore Rt.	legs L.	Stom-ach	Intes-tine
SA	Control	18.58	7.78	-	18.44	7.93	-	-	-	3.18	37.04
SK	Human	17.51	7.52	-	11.76	5.48	0.73	-	0.04	2.00	14.20
SL	Buffalo	19.35	8.16	-	10.20	5.42	0.99	-	0.03	1.90	8.81
SM	Cat	18.65	7.89	-	14.88	5.18	1.28	-	-	1.99	10.41
SN	Bear	17.89	7.82	-	9.57	5.49	1.02	0.23	0.39	1.82	6.37
SO	Flounder	18.11	7.69	-	17.44	7.14	0.33	-	-	2.06	31.70
SP	Control + I	17.88	7.50	-	18.05	7.13	0.05	-	-	2.06	27.11
SQ	Calf + I	17.17	7.03	-	21.13	7.95	0.11	-	-	2.52	40.13

Table XXVII

Amounts of food and iodine in series S. Unit used, gram.

No.	Name	Amt.fed	Removed	Total eaten		Days	Eaten daily	
				Food	Iodine		Food	Iodine
SA	Control	.805	.130	.775	.000001	33	.02348	.000000019
SK	Human	.221	.089	.132	.000002	21	.00629	.000000113
SL	Buffalo	.247	.132	.115	.000012	26	.00442	.000000469
SM	Cat	.199	.054	.145	.000002	30	.00483	.000000077
SN	Bear	.323	.155	.168	.000003	24	.00700	.000000350
SO	Flounder	.363	.111	.252	.000004	24	.01050	.000000168
SP	Control + I	.509	.150	.359	.000215	18	.01994	.000011967
SQ	Calf + I	.776	.056	.720	.000972	26	.02769	.000037386

SM, SK, SL and SN. As in most previous series, there was a distinct grouping of the units into a division which developed comparatively rapidly, and another one which developed much slower. In the former group of rapid developers we can place SK, SL and SN, the latter showing this in the most marked degree. At the close of the series, the specimens in these units all had the typical dwarfed appearance characteristic of precocious development due to thyroid feeding. The group showing little development, consisting of experiments SA, SQ, SO, SP and SM, did not show this abnormal appearance. There was a heavy mortality in experiment SP, the cause of which is discussed below.

The relationship between the rate of development and the amount of food and iodine consumed as shown in Table XXVII was as follows:

Development (beginning with least)	SA, SQ, SP, SO, SM, SK, SL, SN
Total food (beginning with most)	SA, SQ, SP, SO, SN, SM, SK, SL
Total iodine (beginning with least)	SA, SM, SK, SO, SN, SL, SP, SQ
Daily food (beginning with most)	SQ, SA, SP, SO, SN, SK, SM, SL
Daily iodine (beginning with least)	SA, SM, SK, SO, SN, SL, SP, SQ

Regarding the total amount of food, the control, SA, and the unit fed calf thyroid soaked in iodine, SQ, consumed over twice as much as any other unit, and yet these same two showed the lowest rate of development. Experiment SN, fed bear thyroid, was the only marked exception to a close relationship between rapid development and small amount of food consumed.

Turning to the question of iodine and its relation to the rate of metamorphosis; both SK, fed human thyroid, and SM, fed cat thyroid, showed a relatively low iodine consumption, both total and daily, combined with a rather rapid rate of development. Exactly the reverse was true in the case of the units SP, fed ordinary beef soaked in iodine and SQ, fed calf thyroid soaked in iodine. These two experiments are discussed further below. In the remaining four units a large consumption of iodine was associated with a rapid development, or the reverse. These were similar conditions to those shown in series M and O, except that foods P and Q were fed for the first time in the present series.

In the two experiments that were fed on food soaked in iodine, SP and SQ, we find a very slow rate of development associated with a large consumption of food, as was usually the case in all the experiments. At the same time, these units apparently consumed the most iodine, both as to total and daily amounts, of any of the experiments in this series. In fact, according to Table XXVII, the specimens in SP ate about eighteen times as much total iodine and over twenty-five times as much each day as those in SL, the next highest, while those in SQ consumed in all eighty times as much as the specimens in SL, and their relative daily consumption was about the same. At first sight it would appear that these results were at marked variance with the general tendency as shown in practically all the other experiments here performed, but a closer examination of the experiments throws considerable doubt upon such a supposition. The specimens in these two units were very active, and those that survived continued so until they were killed. The mortality in SP was high just after the series was started, as it was also in SQ, but in the former case this high death rate kept up for a longer time than in the latter, as is shown in Table XXVIII which gives the dates of the death of the specimens in these two experiments. This high mortality was probably due to the excessive amount of free iodine present,

Table XXVIII

Dates of death of specimens in experiments

P and Q in series S.

	Date		SP	SQ
	Aug.	11	5	2
	"	12	0	0
	"	13	3	2
	"	14	0	4
	"	15	0	0
	"	16	1	0
	"	17	0	0
	"	18	3	1
	"	19	1	0
	"	20	0	0
	"	21	0	0
	"	22	1	0
	"	23	2	1
	"	24-30	0	0
Killed	"	31	4	10
Total			20	20

as it is toxic in too large quantities. Individual variation among the specimens would account for the survival of some and the death of others. From a casual examination of the food removed, it seemed evident that a considerable per cent of the added iodine in both these foods was dissolved out of the food by the water in which the tadpoles lived. In order to determine whether or not this was the case, a chemical analysis was made of the food Q, calf thyroid soaked in iodine, after it had been in water for two hours. It was thoroughly dried, and the analysis was made by the same method used in all previous determinations. The analysis of this food at the beginning of the experiment showed a 0.135 per cent iodine content, while after it had been in the water for two hours, it had only 0.061 per cent iodine in it. From the method of preparing foods P and Q, i.e., soaking the beef and calf thyroid in iodine, then drying, and mixing this preparation with wheat flour and alfalfa flour to make the paste which was fed, it is probable that there was very little of the iodine that entered into combination with the other substances, as was evidently the case when Allen (1919a) fed iodine mixed with flour. This would account for the large per cent which was so readily separated from this food by soaking for a short time. It is evident that this would tend to reduce the amount of iodine the specimens actually consumed to a large extent and this reduction would be more marked the longer the food remained in the water uneaten. But the food first consumed must have been high in iodine content, and also, as mentioned before, according to Boulenger (1914) and Swingle (1919c), tadpoles absorb water and hence in all probability iodine dissolved in it, through the integument. Nevertheless, the amount of iodine which was eaten and absorbed by the specimens in experiments SP and SQ was probably small.

In experiment SP, fed ordinary beef soaked in iodine, there was no trace of thyroid material, but in the unit SQ, fed calf thyroid soaked in iodine, one-fifth of the food was thyroid. The presence of this large amount of thyroid, and the attendant iodine which was part of it, apparently had little effect because what slight difference there was noticeable between the rates of development in these two experiments was in favor of SP, the one without any thyroid. However, it must be remembered that of all the different kinds of thyroids used in the preceeding experiments, the one which had the least effect in hastening metamorphosis was this same calf thyroid, even in spite of the fact that the specimens eating it always consumed a proportionately larger amount of iodine than other units developing more rapidly.

The generally accepted idea is that the feeding of iodine to tadpoles of the tailless Amphibia will cause an acceleration of metamorphosis. This is largely based on the work of Swingle (1918a, 1918b, 1919b, 1919c and 1923) and of Allen (1919a). In regard to the effect of feeding iodine to the larvae of salamanders, on the other hand, Uhlenhuth (1921a, 1922a and 1922b) found it had no effect in hastening metamorphosis. In the present experiments (SP and SQ), it would appear that the feeding of an iodine-soaked food to the tadpoles of Hyla regilla had no effect in hastening metamorphosis, but it is certain that the specimens actually obtained a very much smaller amount of iodine than the figures in Table XXVII would indicate, and it is reasonable to believe that further experimentation will show that this form is no exception to the other anurans. Also, the high rate of mortality in these two experiments (Table XXVIII) reduced the number of specimens so greatly that it is obviously not possible to draw definite conclusions from the small number remaining.

SUMMARY OF EXPERIMENTS ON HYLA REGILLA.

The work on Hyla regilla consisted of four series of experiments, M, O, P and S. In this summary and comparison of the different experiments, those of series P are omitted for reasons stated before. A comparison of the different rates of development of corresponding experiments in the three series and the average of the same are shown in fig. 5 where the numerals represent the comparative rate of metamorphosis, the lowest being the slowest, and the letters the experiments and the food consumed by them. In computing the average rate, series S was given twice as much weight as either of the others because it had twice as many individuals in each experiment.

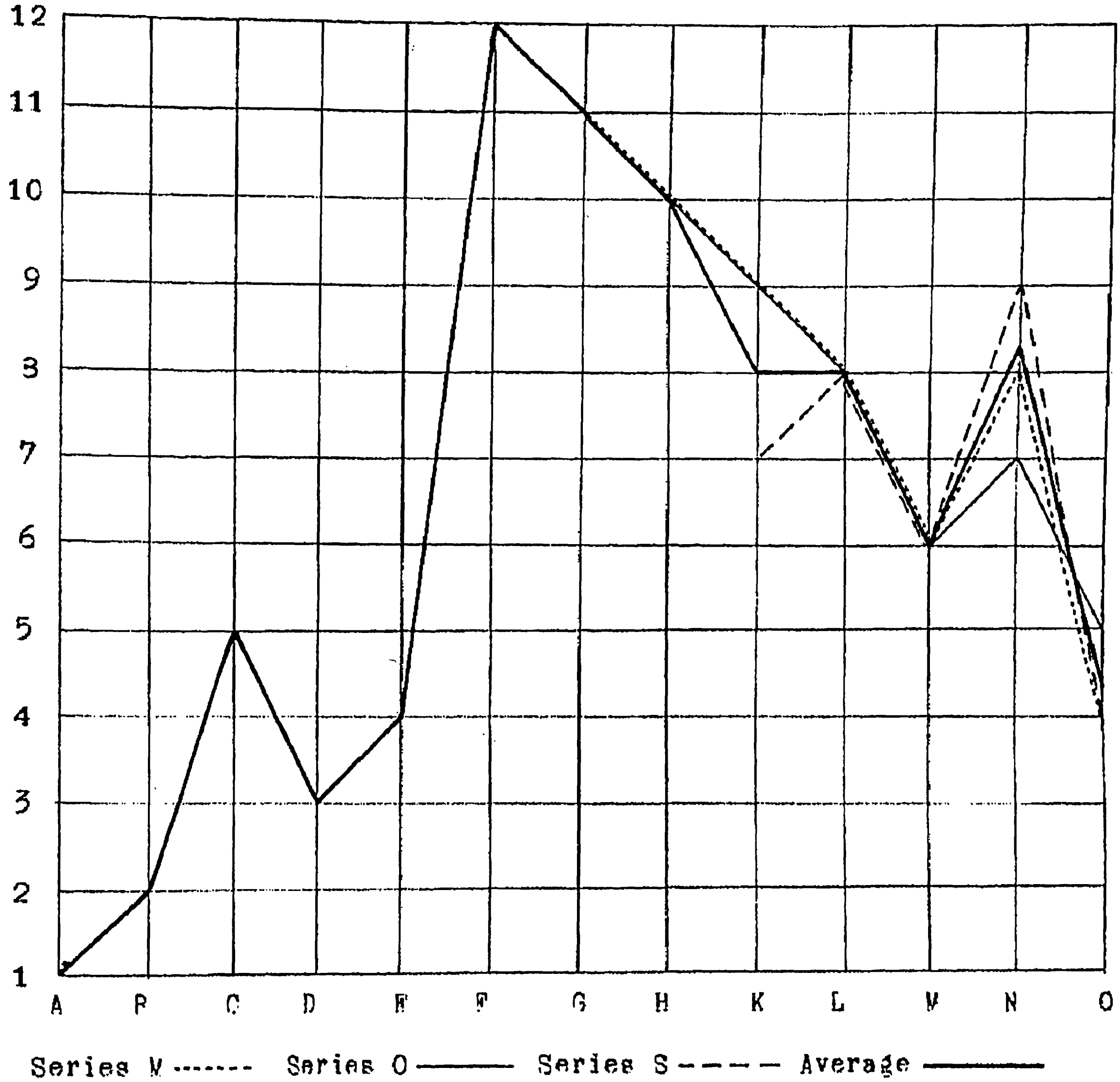
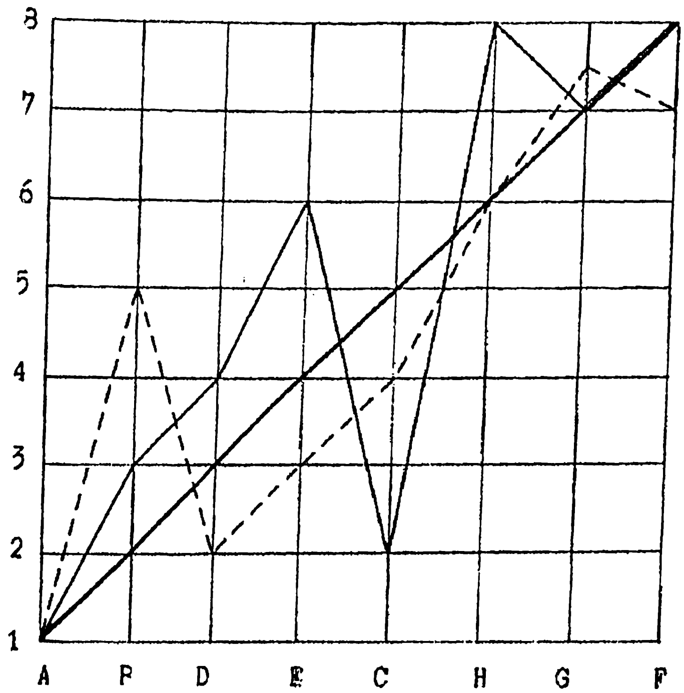


Fig. 5. Experiments on Hyla regilla. Graph showing the comparative stages of development reached in the experiments comprising series M, O and S; also the average for the three series. Series O and the average are both represented by the average line between A and H, and L and M as they are identical between these points.

The conditions under which these several series were carried on were identical, with the possible exception of temperature. But in this respect the variation was only slight, as shown in Table XXI.

The following is the order in which the experiments fell in regard to their average rate of development, beginning with the slowest: A, control; B, calf thyroid; D, cooked beef thyroid; E, pig thyroid; O, flounder thyroid; C, raw beef thyroid; M, cat thyroid; K, human thyroid, and L, buffalo thyroid (tied); N, bear thyroid; H, Armour and Co.'s thyroid; G, Parke Davis and Co.'s thyroid; F, dog thyroid. Those fed B, D, E and F were only in one series, while C, G, and H were omitted from the last series. There was a remarkably slight difference in the rate of development in the corresponding experiments in the different series. Units K, fed human thyroid, and N, fed bear thyroid, showed the least uniformity.

In studying the relationship between the average rate of development and the amount of food and iodine consumed it was found necessary to divide the experiments on Hyla regilla into two groups owing to the fact that all of the series did not cover the complete list of foods used, but at the same time the groups can be compared due to the presence in both of them of three of the units. The first of these groups consisted of those fed foods A, B, C, D, E, F, G and H, the same ones used in the experiments on the tadpoles of Rana catesbiana and Rana pipiens. The relationships of this group of experiments are shown in figure 6. The second group consisted of the units fed A, G, H, K, L, M, N and O, and their relationships are shown in figure 7. The data from which these graphs are made is contained in Tables XXII to



Stage of development ———— Total food ————

Total and daily iodine (identical) - - - - - -

Fig. 6. Experiments on Hyla regilla. Graph showing the relationship between the average stage of development reached and the amounts of food and iodine consumed in the experiments fed A, B, C, D, E, F, G and H.

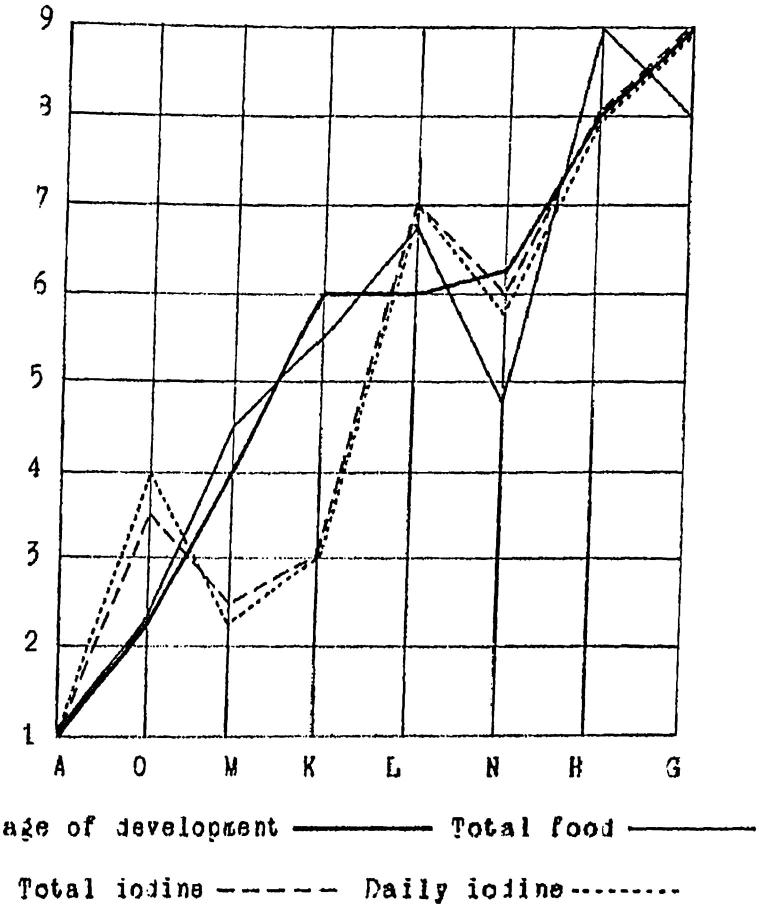


Fig. 7. Experiments on Hyla regilla. Graph showing the relationship between the average stage of development reached and the amounts of food and iodine consumed in experiments fed A, G, H, K, L, M, N and O.

XXVII inclusive. The same arrangement of the experiments so as to give a gradual advance in rate of development from the slowest to the fastest was made as in the summaries of previous series. The higher the number on the left of the graph, the more rapid was the development of the specimens in the experiment. The higher numbers also stand for a larger consumption of iodine, but the reverse is true of the food, the lower the number the more food there was eaten.

In regard to the relationship between the amount of food eaten and the rate of development; it seems that the less food consumed the more rapid the development, with some exceptions. This would be the expected relationship, as the units showing the more rapid metamorphosis were naturally the shortest lived, and consequently ate for the shorter period. In the first group mentioned above, as shown in Fig.6, the relationship of the amount of food consumed to the rate of development varies to a considerable extent, but this is at least partially due to the fact that the graph represents the results obtained from only a single experiment in most of the cases. The lines parallel each other much more closely when the relationships of the second group, shown in Fig.7, are considered.

The relationship between the average rate of metamorphosis and both total and daily iodine consumption can be considered as one because of very little variation between the total and the daily iodine lines. In the case of experiment O, fed flounder thyroid, the amount eaten daily is proportionately somewhat higher than the total amount, but otherwise the two lines are almost parallel. In a general way, large iodine consumption went with fast development. But experiment B, fed calf thyroid, was a marked exception to this as it showed a proportiona ately high iodine consumption in connection with a slow rate of development, a condition apparently usual for experiments fed with this preparation. Also, the units D, fed cooked beef thyroid, K, fed human thyroid, and M, fed cat thyroid, were just the reverse to B, showing an exceptionally low iodine consumption associated with a more rapid rate of metamorphosis. As stated before, it is possible that the age of the thyroid in food B had something to do with the fact that it appeared less active in proportion to the amount of iodine contained in it than did the other thyroids. But this does not account for the other exceptions. It seems probable that the active agent of the thyroid gland varies in intensity in different animals, and that the per cent of iodine contained in the gland is in a general way a gauge by which its action may be estimated, but that there are more or less marked exceptions to this, and the exceptions may extend in either direction from what might be called the normal.

Experiments SP, fed beef soaked with iodine, and SQ, fed calf thyroid soaked with iodine, are not discussed in this summary because they were treated in detail under the general discussion above.

GENERAL SUMMARY.

In comparing the results of the different series we find that the first eight experiments in each series uniformly arranged themselves in two groups, the first showing a slower rate of development and the second a more rapid one. Invariably the units fed A, control, B, calf thyroid, C, raw beef thyroid, D, cooked beef thyroid, and E, pig thyroid, were found in the first group, and those fed F, dog thyroid, G, Parke Davis and Co.'s thyroid and H, Armour and Co.'s thyroid, were in the second. There was some variation in the arrangement of the experiments in the different series as shown by the following comparison where the units are arranged in order, the one which showed the slowest rate of development being placed first:

Rana catesbiana	B, D, C, A, E, H, G, F.
Rana pipiens	A, C, B, D, E, H, F, G.
Hyla regilla	A, B, D, E, C, H, G, F.

The variation within the two groups mentioned above was considerable and seemed to follow no rule. In series 5 where additional kinds of food were used, we find that the units fed O, flounder thyroid, showed the slower rate of development characteristic of the first group above, while those fed K, human thyroid, L, buffalo thyroid, and N, bear thyroid, had a distinctly rapid metamorphosis, thus placing them in the second group, although they all had a slower rate than those fed either F, G or H. The experiments fed M, cat thyroid, were midway between these groups so far as the rate of development went.

Regarding the relationship between the rate of development and the amounts, both total and daily, of food eaten, it may be said that with few exceptions, the greater amount of food consumed was associated with the slower rate of development. As stated before, this is correlated with the length of time the experiments ran, as those living longer would naturally eat a greater amount, and the longer lived experiments were the ones that developed the slowest.

As to the correlation between the amount of iodine used and the rate of development, it may be said that with some exceptions the greater amount of iodine consumed was associated with the more rapid rate of development. One marked exception to this was seen in all the experiments fed on B, calf thyroid. In these there was invariably a very high relative amount of iodine eaten compared to a much slower relative rate of development. Also, in the experiments on Hyla regilla, those fed K, human thyroid, M, cat thyroid, and D, cooked beef thyroid, showed the opposite condition, namely, a low iodine consumption together with a relatively rapid development and in the experiments on Rana catesbiana, those fed A, control, showed the same condition.

CONCLUSIONS.

The following are the conclusions reached from a study of the foregoing experiments:

1. Those tadpoles that develop the most rapidly eat the least, this being due to the fact that the longer lived individuals have more opportunity for food consumption.

2. A lower temperature tends to slow up the process of metamorphosis. However, there was little evidence that it effected the rate of development of the specimens fed one kind of thyroid any more than it did those fed other kinds.

3. In experiments where thyroid is fed to tadpoles, while the rate of metamorphosis is usually related to the amount of iodine consumed by the specimens, those obtaining the larger amounts, both total and daily, developing the more rapidly, yet there are distinct exceptions to this.

4. These exceptions to the above seem to be associated with some specific kinds of thyroid. For instance, calf thyroid may be fed in large enough quantities so that a relatively large amount of iodine is consumed, and yet no correspondingly rapid metamorphosis ensues. On the other hand, human and cat thyroid seem to produce an abnormally rapid development when the small amount of iodine consumed is considered.

5. Food soaked in iodine, and hence having a much higher iodine content than the same food not so treated, does not always appear

to have a greater effect in accelerating the rate of metamorphosis than untreated food.

6. From the above (4 and 5) it would appear probable that the iodine alone is not the active agent in the thyroid gland, but that it is combined with or part of the active agent, and that this latter varies in composition in the glands of different animals.

7. The larger the volume of water in which the specimens were kept, the slower was the rate of development. This indicates that the active substance of the thyroid not only acts through the alimentary canal, but also dissolves out of the food and effects the tadpoles through their integuments.

BIBLIOGRAPHY

Allen, Bennet M., 1917. Extirpation of the hypophysis and thyroid glands in Rana pipiens. Anat. Rec., 11:486-

Allen, Bennet M., 1918. The relation of normal thyroid gland development to bodily growth and differentiation in Rana, Bufo and Amblystoma. Anat. Rec., 14:26-

Allen, Bennet M., 1919a. The relation of the pituitary and thyroid glands of Bufo and Rana to iodine and metamorphosis. Biol. Bulletin 36: 405-417.

Allen, Bennet M., 1919b. The development of the thyroid glands of Bufo and their normal relation to metamorphosis. Jl. Morph., 32:489-504.

Boulenger, E. G., 1914. Reptiles and Batrachians. London and New York.

Cameron, A. T., 1914. Contributions to the biochemistry of iodine. Part I. The distributions of iodine in plant and animal tissue. Jl. Biol. Chem., 18:335-380.

Cameron, A. T., 1915. Contributions to the biochemistry of iodine. Part II. The distributions of iodine in plant and animal tissues. Jl. Biol. Chem., 23:1-39.

Dickerson, Mary C., 1907. The frog book. New York.

Emmet, A. D., and Allen, F. P., 1919. Nutritional studies on the growth of frog larvae (Rana pipiens). Jl. Biol. Chem., 38: 325-343.

Emmet, A. D., Allen, F. P., and Sturtevant, M., 1920. Relation of vitamines and iodine to the size and development of the tadpole. Jl. Biol. Chem., 41:31-71.

Graham, A., 1916. A study of the physiological activity of adenomata of the thyroid gland in relation to their iodine content, as evidenced by feeding experiments on tadpoles. Jl. Exp. Medi., 24: 345-359.

Gudernatsch, J. F., 1912. Feeding experiments on tadpoles. I. The influence of specific organs given as food on growth and differentiation. Arch. fur. Entwick. d. Organ., 35:457-

Gudernatsch, J. F., 1914. Feeding experiments on tadpoles. A further contribution to the knowledge of organs with internal secretions. Amer. Jl. Anat., 15:431-478.

Gudernatsch, J.F., 1917. Studies in internal secretion. IV. Treatment of tadpoles with thyroid and thymus extracts. Anat. Rec., 11:357-

Kendall, Edward C., 1917. On the crystalline compound containing iodine which occurs in the thyroid. Endoc., 1:153-169.

Kendall, E. C., 1919. The chemical identification of thyroxin. Jl. Biol. Chem., 40:125-147.

Kendall, E. C., and Osterberg, A. E., 1919. Isolation of the iodine compound which occurs in the thyroid. Jl. Biol. Chem., 39: 265-334.

Lenhart, C. H., 1915. The influence upon tadpoles of feeding desiccated thyroid gland in variable amounts and of variable iodine contents. Jl. Exp. Medicine, 22:739-746.

McCord, Carey P., and Marinus, Carlton J., 1918. The effect of the x-ray upon the response of tadpoles to thyroid stimulation. Endoc., 2:189-300.

Morse, Max, 1914. The effective principal in thyroid accelerating involution in frog larvae. Jl. Biol. Chem., 19:421-429.

Rogoff, J. M., 1917. A method for the standardization of thyroid preparations. Jl. Pharm. and Exp. Therap., 10:199-208.

Rogoff, J. M., 1918. Note on the preparation of a soluable concentrated product of the thyroid gland. Jl. Pharm. and Exp. Therap., 12:207-209.

Rogoff, J. M., and Marine, D., 1917. Attempts to produce a substance with thyroid-like activity by the artificial iodization of proteins. Jl. Pharm. and Exp. Therap., 10:321-325.

Seidell, Atherton, and Fenger, Frederic, 1914. Seasonal variation in the composition of the thyroid gland. Bull. No. 9, Hygienic Lab., U. S. Public Health Service, pp. 67-82.

Smith, Philip E., and Cheny, Garnett, 1921. Does the administration of anterior lobe to tadpole produce an effect similar to that obtained from thyroid feeding? Abstracts Am. Assoc. Anat., Anat. Rec., 21:84.

Swingle, W. W., 1918a. The acceleration of metamorphosis in frog larvae by thyroid feeding, and the effects upon the alimentary canal and sex glands. Jl. Exp. Zool.,24:521-543.

Swingle, W. W., 1918b. Iodine as the active principal of the thyroid gland. Endoc., 2:283-288.

Swingle, W. W., 1919a. Studies on the relation of iodine to the thyroid. I. The effects of feeding iodine to normal and thyroidectomized tadpoles. Jl. Exp.Zool., 27:397-416.

Swingle, W. W., 1919b. Iodine and the thyroid. III. The specific action of iodine in accelerating Amphibian metamorphosis. Jl. Gen. Phys., 1:593-606.

Swingle, W. W., 1919 c. Iodine and the thyroid. IV. Quantátive experiments on iodine feeding and metamorphosis. Jl. Gen. Phys., 2:161-171.

Swingle, W. W., 1923. Thyroid transplantation and anuran metamorphosis. Jl. Exp. Zool., 37:219-257.

Uhlenhuth, Eduard, 1921a. The internal secretions in growth and development of Amphibians. Amer. Nat., 55:193-221.

Uhlenhuth, Eduard, 1921b. The effect of iodine and iodothyrine on the larvae of salamanders. II. The relation between metamorphosis and limb development in salamander larvae. Biol. Bulletin, 41:307-317.

Uhlenhuth, Eduard, 1922a. The effect of iodine and iodothyrine on the larvae of salamanders. I. The effect of iodothyrine and iodine on the metamorphosis of Amblystoma maculatum. Endoc., 6:102-116.

Uhlenhuth, Eduard, 1922b. The effect of iodine and iodothyrine on the larvae of salamanders. III. The role of the iodine in the specific action of the thyroid hormone as tested in the metamorphosis of the Axolotl larvae. Biol. Bulletin, 42:143-152.

West, Paul Ashley, 1914. Notes on a sheep thyroid experiment with frog tadpoles. Sci., 39:918-979, 1 fig.

Wright, A. H., 1914. North American Anura. Carnegie Pub. No.197. Washington, D. C.

INDEX.

PLATE I

Rana catesbiana

Fig. 8. From experiment AA, control, fed beef.

Fig. 9. From experiment AB, fed calf thyroid.

Fig. 10. From experiment AC, fed raw beef thyroid.

Fig. 11. From experiment AD, fed cooked beef thyroid.

Fig. 12. From experiment AE, fed pig thyroid.

Fig. 13. From experiment AF, fed dog thyroid.

Fig. 14. From experiment AG, fed Parke Davis and Co.'s thyroid.

Fig. 15. From experiment AH, fed Armour and Co.'s thyroid.

Fig. 16. From experiment BA, control, fed beef.

Fig. 17. From experiment BB, fed calf thyroid.

Fig. 18. From experiment BC, fed raw beef thyroid.

Fig. 19. From experiment BD, fed cooked beef thyroid.

Fig. 20. From experiment BE, fed pig thyroid.

Fig. 21. From experiment BF, fed dog thyroid.

Fig. 22. From experiment BG, fed Parke Davis and Co.'s thyroid.

Fig. 23. From experiment BH, fed Armour and Co.'s thyroid.

The specimens shown in Figs. 9, 10, 11, 12, 16 and 20 were dissected before the photographs were taken.

The specimens photographed were individuals as near the average for the experiment they represent as it was possible to obtain.

Plate I

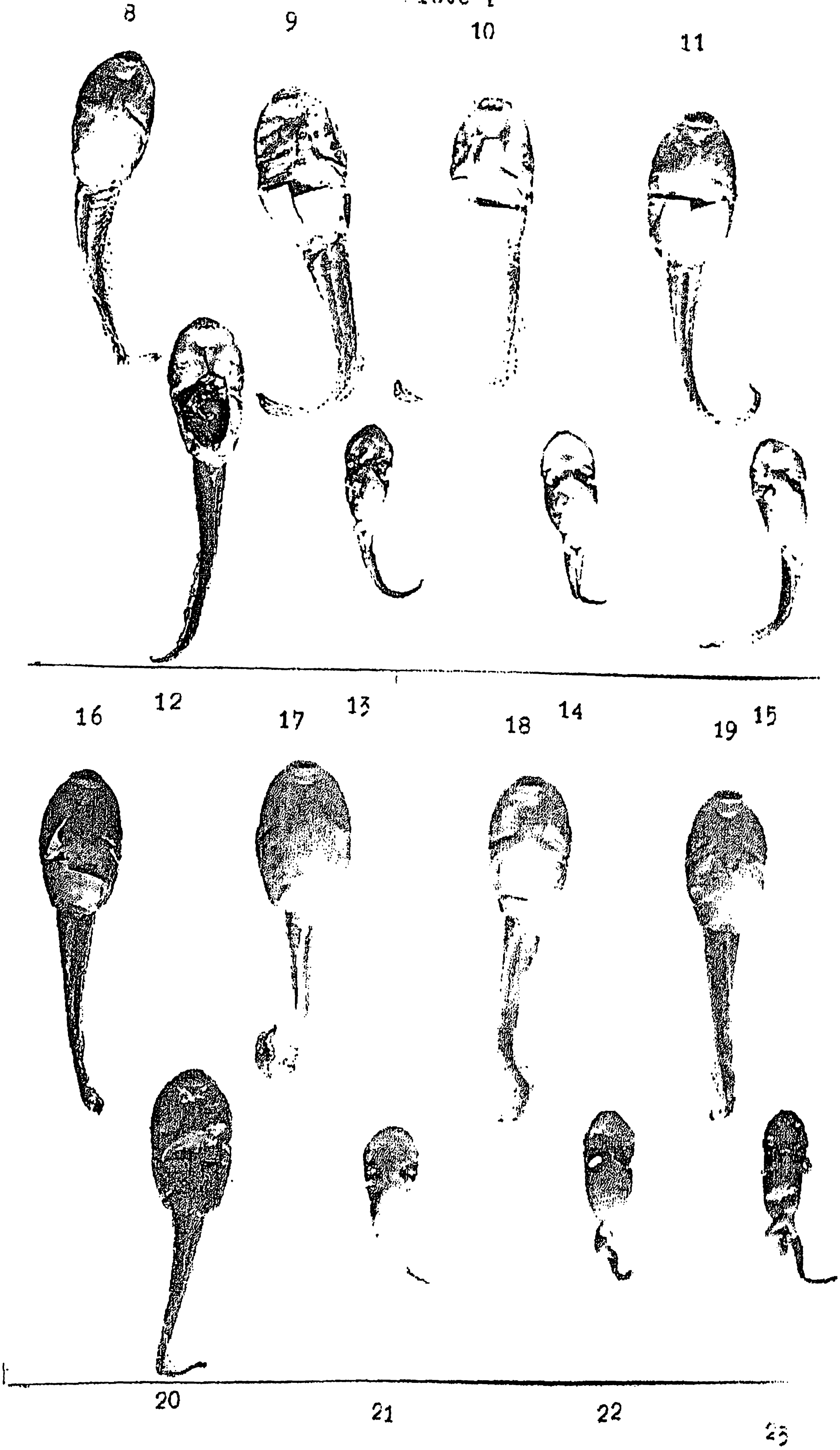


PLATE II

Rana catesbiana

Fig. 24. From experiment CA, control, fed beef.

Fig. 25. From experiment CB, fed calf thyroid.

Fig. 26. From experiment CC, fed raw beef thyroid.

Fig. 27. From experiment CD, fed cooked beef thyroid.

Fig. 28. From experiment CE, fed pig thyroid.

Fig. 29. From experiment CF, fed dog thyroid.

Fig. 30. From experiment CG, fed Parke Davis and Co.'s thyroid.

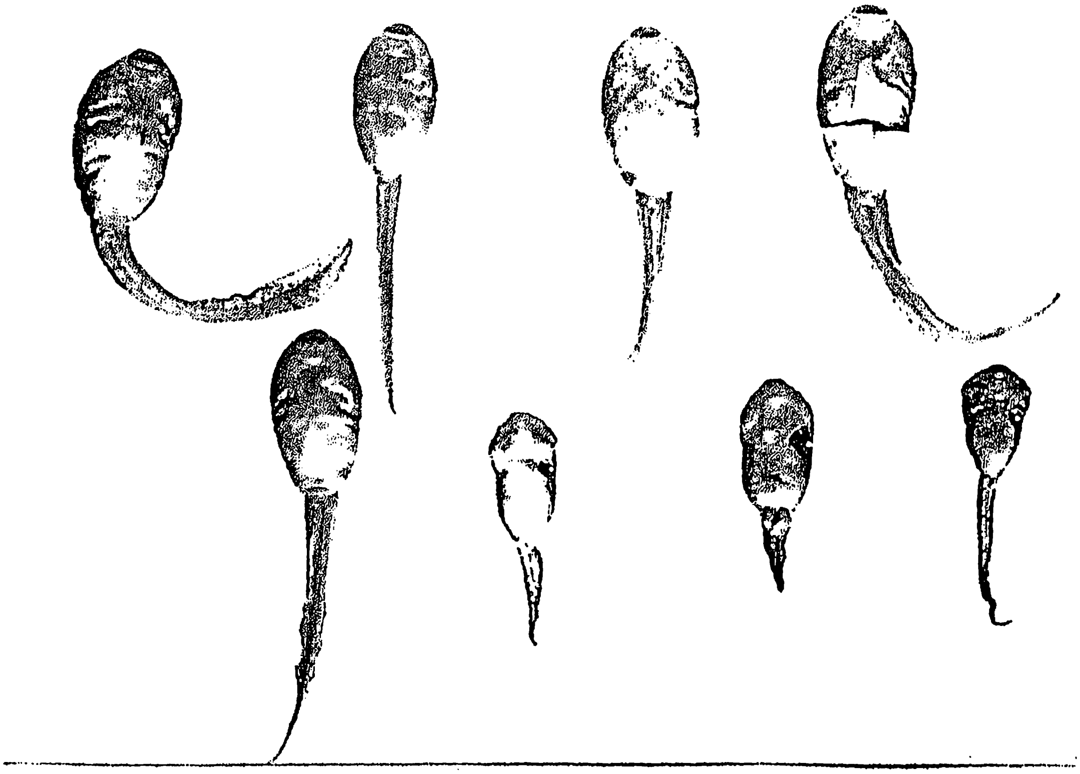
Fig. 31. From experiment CH, fed Armour and Co.'s thyroid.

The specimen shown in Fig. 27 was dissected before the photograph was taken.

The specimems photographed were individuals as near the average for the experiment they represent as it was possible to obtain.

Plate II

24 25 26 27



28 29 30 31

PLATE III

Rana pipiens

Fig. 32. Specimens in experiment DA, control, fed beef.

Fig. 33. Specimens in experiment DB, fed calf thyroid.

Fig. 34. Specimens in experiment DC, fed raw beef thyroid.

Fig. 35. Specimens in experiment DD, fed cooked beef thyroid.

Fig. 36. Specimens in experiment DE, fed pig thyroid.

Fig. 37. Specimens in experiment DF, fed dog thyroid.

Fig. 38. Specimens in experiment DG, fed Parke Davis and Company's thyroid.

Fig. 39. Specimens in experiment DH, fed Armour and Company's thyroid.

In Fig. 35, the first two specimens from the left were dried up.

Plate III

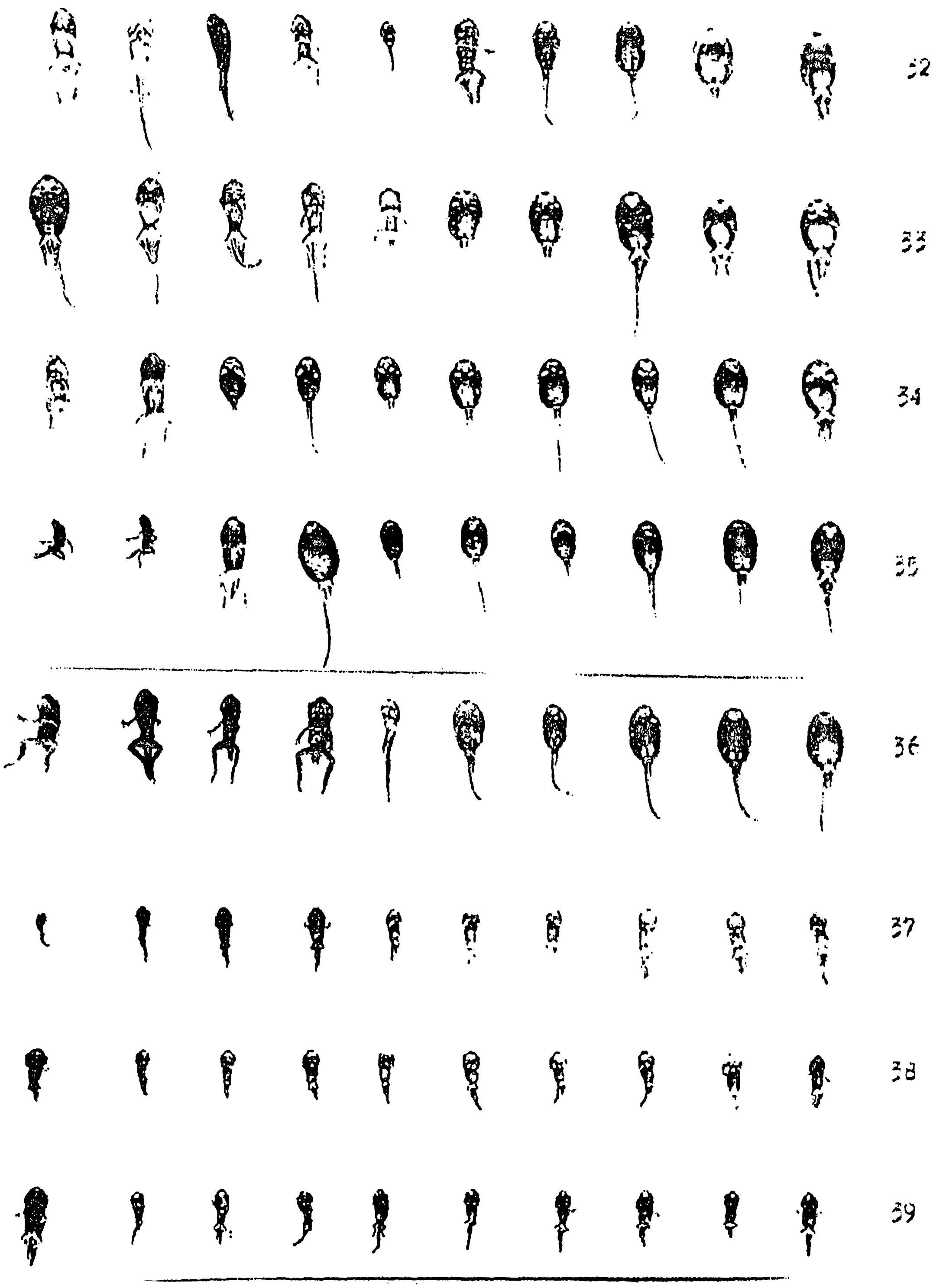


PLATE IV

Rana pipiens

Fig. 40. Specimens in experiment EA, control, fed beef. This experiment was also the control for series D, and is, therefore, a duplicate of Fig. 32.

Fig. 41. Specimens in experiment EB, fed calf thyroid.

Fig. 42. Specimens in experiment EC, fed raw beef thyroid.

Fig. 43. Specimens in experiment ED, fed cooked beef thyroid.

Fig. 44. Specimens in experiment EE, fed pig thyroid.

Fig. 45. Specimens in experiment EF, fed dog thyroid.

Fig. 46. Specimens in experiment EG, fed Parke Davis and Company's thyroid.

Fig. 47. Specimens in experiment EH, fed Armour and Company's thyroid.

In Fig. 42, the first four specimens from the left were partially eaten before being removed from the experiment. The lead slug in Fig. 46 represents a specimen that accidently dried up in transit and could not be photographed.

Plate IV

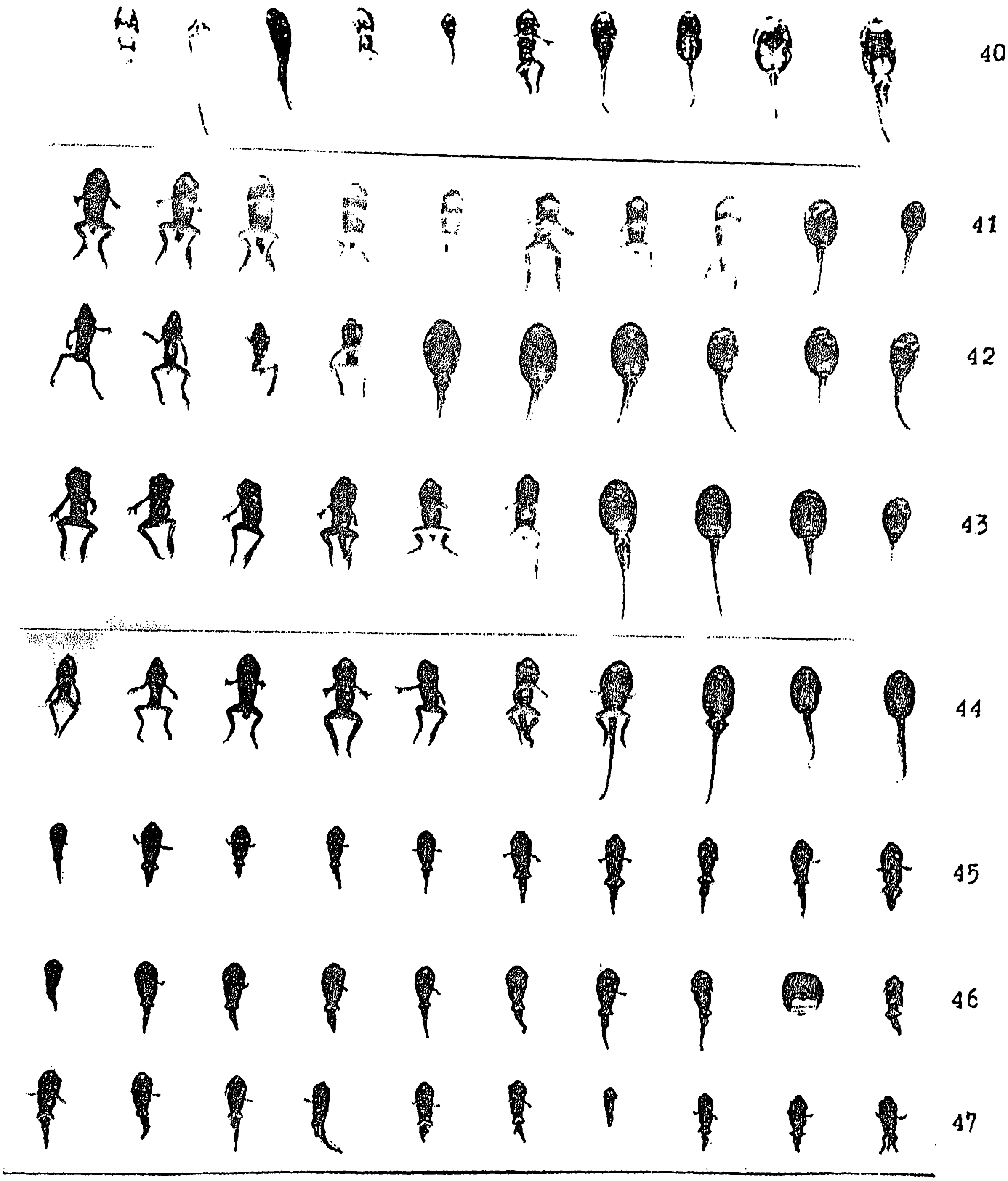


PLATE V

Rana pipiens

Fig. 48. Specimens in experiment GA, control, fed beef.

Fig. 49. Specimens in experiment GB, fed calf thyroid.

Fig. 50. Specimens in experiment GC, fed raw beef thyroid.

Fig. 51. Specimens in experiment GD, fed cooked beef thyroid.

Fig. 52. Specimens in experiment GE, fed pig thyroid.

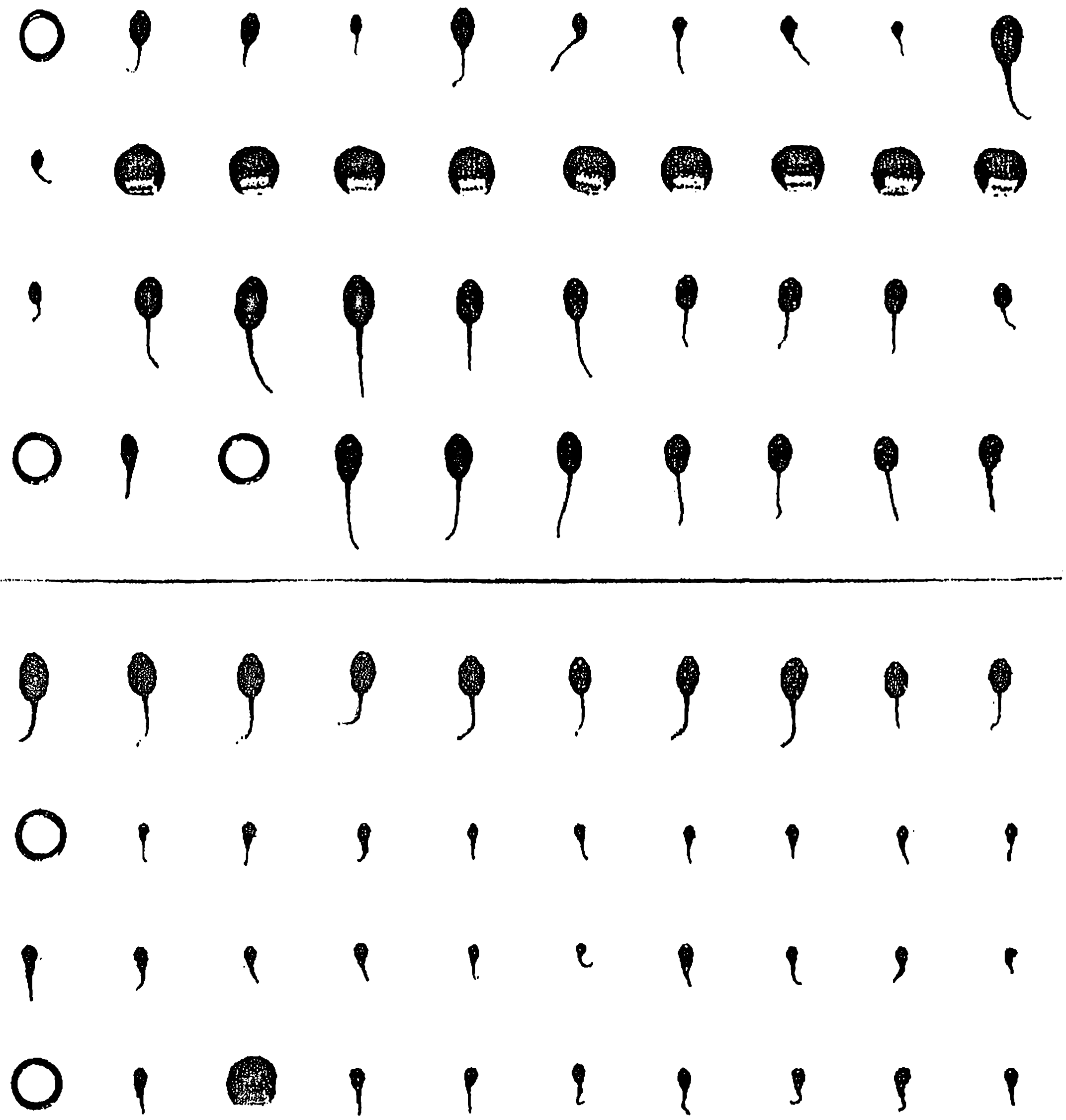
Fig. 53. Specimens in experiment GF, fed dog thyroid.

Fig. 54. Specimens in experiment GG, fed Parke Davis and Company's thyroid.

Fig. 55. Specimens in experiment GH, fed Armour and Company's thyroid.

The rings in Figs. 48, 51, 53 and 55 represent specimens that died and were consumed by the other individuals, leaving no trace. The lead slugs in Figs. 49 and 55 represent specimens that accidently dried up in transit and could not be photographed.

Plate V



48

49

50

51

52

53

54

55

PLATE VI

Rana pipiens

Fig. 56. Specimens in experiment HA, control, fed beef. This experiment was also the control for series G and is, therefore, a duplicate of Fig. 48.

Fig. 57. Specimens in experiment HB, fed calf thyroid.

Fig. 58. Specimens in experiment HC, fed raw beef thyroid.

Fig. 59. Specimens in experiment HD, fed cooked beef thyroid.

Fig. 60. Specimens in experiment HE, fed pig thyroid.

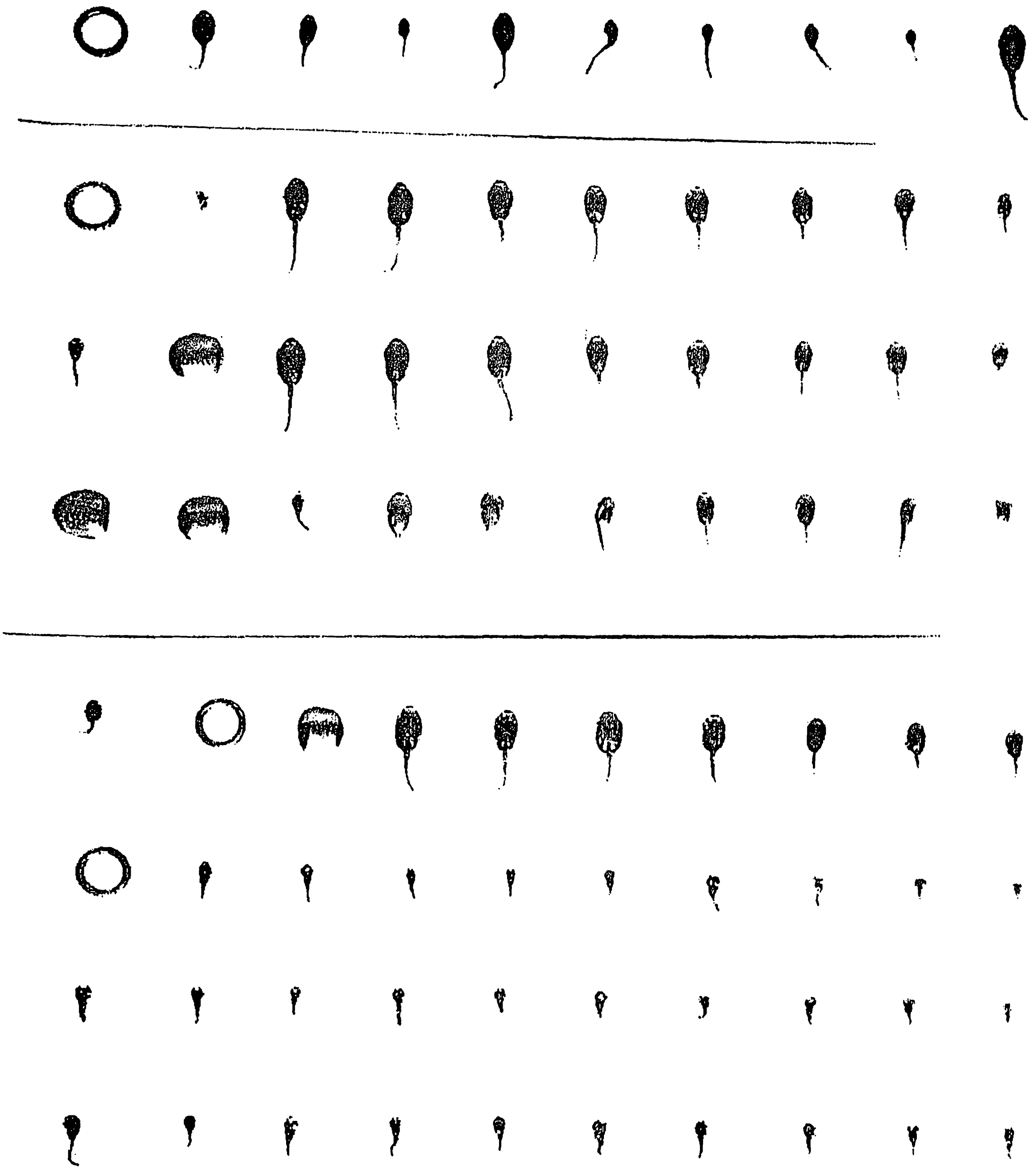
Fig. 61. Specimens in experiment HF, fed dog thyroid.

Fig. 62. Specimens in experiment HG, fed Parke Davis and Company's thyroid.

Fig. 63. Specimens in experiment HH, fed Armour and Company's thyroid.

The rings in Figs. 56, 57, 60 and 61 represent specimens that died and were consumed by the other individuals, leaving no trace. The lead slugs in Figs. 58, 59 and 60 represent specimens that accidently dried up in transit and could not be photographed.

Plate VI



56

57

58

59

60

61

62

63

PLATE VII

Rana pipiens

The first four figures, 64 to 67 inclusive, represent experiments that were carried on in containers holding 200 c.c. of water, while the last four, 68 to 71 inclusive, are photographs of similar experiments in which the amount of water was 1000 c.c.

Fig. 64. Specimens in experiment KA, control, fed beef.

Fig. 65. Specimens in experiment KF, fed dog thyroid.

Fig. 66. Specimens in experiment KG, fed Parke Davis and Company's thyroid.

Fig. 67. Specimens in experiment KH, fed Armour and Co.'s thyroid.

Fig. 68. Specimens in experiment LA, control, fed beef.

Fig. 69. Specimens in experiment LF, fed dog thyroid.

Fig. 70. Specimens in experiment LG, fed Parke Davis and Company's thyroid.

Fig. 71. Specimens in experiment LH, fed Armour and Co.'s thyroid.

In Fig. 68, the first specimen from the left was dried up.

Plate VII

64

65

66

67

68

69

70

71