Feeding Experiments on Tadpoles.

I. The influence of specific organs given as food on growth and differentiation.

A contribution to the knowledge of organs with internal secretion.

By

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[From the department of histology, German University of Prague. Director: Prof. ALFRED KOHN [1]).]

With plate IX.

Eingegangen am 11. Juli 1912.

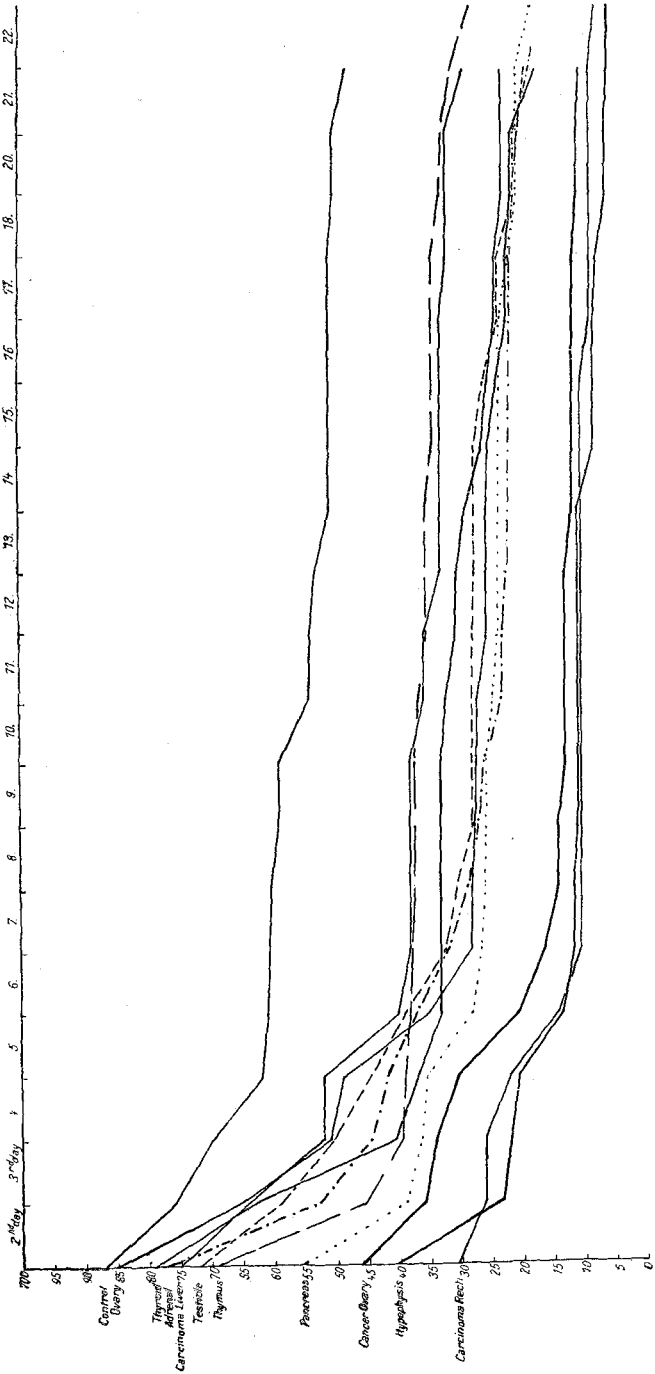
During a stay in the Zoological Station of Naples, spring 1910, an attempt was made to study the influence of various organic extracts on the development of fish (*Belone*, *Gobius* etc.) and amphibian (*Rana*) eggs. The substances used were extracts from mammalian tissues, viz. thyroid, thymus, testicle, ovary, hypophysis, adrenal, pancreas, cancer of ovary, carcinoma of liver and carcinoma of rectum. Different quantities of the extracts, in corresponding degrees of concentration, were added to the sea-water, containing the recently fertilized eggs. The eggs were kept for various lengths of time — up to 20 hours — in this mixture and afterwards transferred into pure sea-water. In every case an influence upon the developing eggs was noticeable; and the disturbances of the normal development caused by the various substances were different. The difficulties met in these experiments were unusually great, partly on account of the rapid decomposition of the extracts brought from New

[1]) My best thanks are due to Prof. KOHN for permitting me to work in his laboratory, furnishing ample material and giving me out of his enormous experience valuable help and advice as well as for carefully revising the manuscript.

York — on board ship they were kept in cold storage, but there was no ice-box in the laboratory — and partly because in that season it was very difficult to get sufficient material; in two months *Belone* eggs were brought to the laboratory only twice. In spite of these inconveniences a great number of eggs were kept under observation up to the time of hatching. Yet the work could not be carried on systematically with sufficient repetitions and control experiments. Besides it was not to be expected that the influences of the various organs on development would allow of any conclusions as to the function of the respective organs, viz. thyroid gland etc. It was more likely that the disturbances of the normal development were of a general type caused perhaps by the change in the osmotic pressure of the surrounding medium etc.

To show that the influences of the various substances upon development were actually different, the following table may be given. It cannot, of course, be used for any generalizations; for it is the result of only one experiment on *Belone* eggs. The curves show the respective percentages of the living and developing eggs after a certain number of days; viz. of each 100 eggs there were on the second day still living: in the control 87, after addition of ovary exctract 85, etc. to . . . . carcinoma recti extract 30; at the beginning of the third day 76, 65, ... 26, etc. After the first day (each 100) a different decline of the curves is already visible. A certain percentage of the eggs, 13 of the control, died, since naturally not all eggs were equally able to develop, others were not fertilized etc. For this reason it may be that on the second day the curves rather run parallel. A striking decline of all curves with the exception of the control and thymus curves appears on the fifth day. On this day in normal development, the heart begins to beat. Many eggs which up to that time remained alive, although probably with diminished energy, seem not to have been able to survive this critical point, the starting of pulsation. From the sixth day on the curves run more or less parallel. The control shows after this time a comparatively strong decline. An explanation for this phenomenon may be that under the influence of the extracts the more feeble eggs were killed in the earlier stages of development, while in pure sea-water they were able to go on developing for some time before their vitality was exhausted.

Table I.


2nd day
3rd day
Control Ovary 85
Thyroid 80
Adrenal
Carcinoma Liver 75
Testicle
Thymus 70
Pancreas 55
Cancer Ovary 45
Hypophysis 40
Carcinoma Rect. 30

Later on the advice of Prof. KOHN, the various organs have been applied as fresh feeding material. The tadpoles of *Rana temporaria* and *Rana esculenta* were chosen for the experiments. These feeding experiments carried on during the summer of 1911 in the histological laboratory in Prague gave some very interesting results.

The tadpoles were kept in bowls, each containing 15 to 20 individuals, and were fed three or four times a week on the different organs. *Rana temporaria* was used in two sets of different ages. As food were tried: thyroid, liver, adrenal, hypophysis, and muscle from horse, thymus from calf, testicle and ovary from dog or cat. Some organs from rabbits and pigs also were given. The origin of the organs used apparently made no difference in their action. The food was put into the water and was ravenously taken by the animals. With each experiment one group was left unfed as control to test how much nourishment the animals could take from the tap-water which in Prague is very rich on organisms. The water was changed daily, on hot days sometimes twice. This frequent change of water as well as the accumulation of the products of metabolism in the water between the changes may have exerted some influence on the development, yet the prevailing conditions were the same for all animals. Unfortunately it was found impossible to carry a constant current of air or water through the great number of dishes. It was also found impossible on account of the artificial feeding to keep plants in the dishes. The feeding was continued till shortly after the appearance of the fore legs, then the animals cease to take food for some time on account of the transformation of the jaws, and since breathing through the lungs begins the metamorphosed animals leave the water and look for fresh food.

Experiment I.

Rana temporaria in two sets of different sizes. Fig. 1 *a*—*e*, 2 *a*—*e*. Original size on May 23rd 1911 of set I 1.1—1.7 cm, of set II 1.8—2.3 cm.

The differences in size of the animals of the same set diminish somewhat with continued feeding, so that the deviations from a mean become less obvious. The following table gives the sizes of the different groups 26 days after the beginning of the experiment:

		set I	set II
May 23.	Original size, average . . .	1.4 cm	2.05 cm
June 18.	Average size		
	control unfed	2.2 -	3.0 -
	liver	3.6 -	4.1 -
	muscle	3.5 -	3.9 -
	thymus	3.6 -	4.1 -
	adrenal	2.8 -	3.9 -
	hypophysis	2.8 -	— -
	testis	2.5 -	3.5 -
	ovary	2.1 -	3.3 -
June 14.	thyroid	1.1 -	1.8 -

Testis and ovary could not be fed regularly on account of the material lacking, so that these animals are not much ahead of the unfed control.

The thyroid fed animals had died on June 16 as fully developed frogs, though dwarfs in size; therefore their measurement on June 14 is given, on which day the fore legs in set I were also noticeable.

From the notes the following data may be given [1]):

May 23. Beginning of the experiment.

May 30 (up to this day food had been given 4 times). All thyroid II show hind legs.

June 4. 3 thymus II show hind legs.

June 5. The differences in size between the individual groups become marked, thyroid I and II are smaller and thymus I and II bigger than the other animals. The differentiation (limbs, form of the body etc.) is most evident in thyroid.

June 6. More thymus II and some of the other groups grow hind legs.

June 7. The differences in size and differentiation have become more striking. Thymus II are the biggest animals, they have, however, retained the typical form of tadpoles. Thyroid II begin to grow fore legs and to reduce their tails, their bodies are markedly frog-like.

June 8. All thyroid I grow hind legs. Thyroid II have become typical, but very small frogs and begin to jump. Some adrenal I

[1]) For the sake of simplicity, the organ given is used throughout the paper as an index of the respective group. I and II mean: size I and II. For instance, liver I means: tadpoles, size I, fed on liver, etc. Thymusthyroid means: tadpoles, fed first on thymus, later on thyroid.

and II show a somewhat lighter color than the rest of the tadpoles.

June 14. Thyroid II begin to die off. Thyroid I grow fore legs and start to absorb their tails.

June 16. Thyroid I die off. 2 thymus II grow fore legs — i. e. 9 days later than thyroid II. The lighter coloring of the adrenal I and II becomes more evident.

June 18. Thymus I and II are the biggest tadpoles of each set. The difference in length between thymus I and II and liver I and II is not marked, yet the thymus tadpoles are broader, have stronger legs etc.

June 20. The liver show a greenish coloring. Some thymus I and liver I grow hind legs, i. e. 12 days later than thyroid I.

June 23. Thymus II seem to show a retarded differentiation being behind liver II, muscle II and adrenal II.

June 25. This difference becomes more evident. All adrenal tadpoles show a color markedly lighter than that of the rest of the groups.

June 27. Thymus I are much bigger, yet far less differentiated than liver I and muscle I.

June 29. For several days no more of the thymus II have grown fore legs, while more and more of the liver II, muscle II, adrenal II and even some of the poorly fed testicle II and ovary II have done so.

July 2. Thymus I and II are very big and their color is very dark. No more thymus II develop fore legs, while of liver II, muscle II and adrenal II there is only one in each group without fore legs. Even of the poorly fed testis II and ovary II there are only 3, respectively 5 without them. Adrenal I grow hind legs, muscle I fore legs.

July 3. All liver II, muscle II and adrenal II tadpoles have grown fore legs.

July 8. The adrenal II frogs which on July 3 had been taken out of the water and placed on sand are now just as dark as the frogs of the other groups. There is only 1 of liver I and 3 of muscle I without fore legs, while there are still 9 of thymus II and 10 of thymus I without them, though the latter tadpoles are much bigger than the former.

July 9. Hypophysis I and testis I grow hind legs, adrenal I are the largest, almost as big as thymus I.

July 11. Adrenal I grow fore legs.
July 13. There are only 2 of muscle I without fore legs, while there are still 7 of thymus I, and 5 of thymus II lacking fore legs.
July 17. Some hypophysis I and testis I grow fore legs, the last muscle I grows fore legs, while there are still 3 of thymus I and 3 of thymus II without them. Hypophysis I tadpoles gradually become rather transparent, especially their heads. They show on the right side of their body a greenish swelling beneath the skin. The green color is also seen through on the right side of their bodies, yet there is no swelling there. The tails of some are twisted in a peculiar manner.
July 21. Hypophysis I begin to die one after the other without completing their metamorphosis. There are still 3 thymus I and 3 thymus II without fore legs, their bodies assume a very irregular shape and become very broad and bloated.
July 26. The last adrenal I grows fore legs.
Aug. 3. 1 more thymus I grows fore legs, there remain 2 without fore legs.
Aug. 4. 1 more thymus I grows fore legs, there remains 1 without them, 2 thymus II die without fore legs. One only survives.
Aug. 5. The last thymus I and thymus II die without fore legs.
Aug. 17. Some of the unfed control grow hind legs.

It is evident from the data just given that the thyroid and thymus tadpoles (Fig. 1*a*—*b*, 2*a*—*b*) reacted most peculiarly to their specific foods, while the liver, muscle and adrenal animals showed a more indifferent behavior. However, the very light coloring of the adrenal was striking as compared with the very dark color of the thymus and the dark greenish one of the liver tadpoles. It is highly probable that the light color of the adrenal is not the result of the feeding with adrenal, but was merely a contraction of the pigment cells due to the contents of the chromaffine cells going into solution (adrenalin reaction). The gradually developing transparency of the hypophysis fed tadpoles must also be mentioned as well as the fact that most of them died without completing their metamorphosis. The nature of the green swelling in their abdomen can only be determined by microscopic examination.

The results of the testis and ovary feeding are inconclusive since regular feeding was impossible on account of the difficulties met with in providing the food. The behavior of the other groups,

however, is characteristic, and there is also a definite control given, as the experiments were conducted on two different sets with corresponding results.

The quickest results were seen in the thyroid groups. While an increase in body size was lacking, the differentiation of the body was extremely rapid, both hind and fore legs appeared earlier than in any other group and the animals metamorphosed long before those fed on other substances. It was peculiar that every change in the body form set in almost simultaneously in all the animals of one set, so that the corresponding stages of development were reached within 24 hours or less; for instance, all had their hind or fore legs come out on the same day etc. In no other group could such a uniform development be observed. The only explanation of this can be the increased velocity of the differentiation processes. In the other groups only a few animals at first began to grow hind or fore legs, and often many weeks elapsed before all the others had reached the same stage in development. This is the natural course of events, since *a priori* not all tadpoles possessed the same vitality. The thyroid food, however, enacted such a strong accelerating influence on the body differentiation that the differences in time which existed in the development of the individual tadpoles were so reduced, that they hardly remained noticeable. The greatest difference in time, between the slowest and the most rapid differentiation of thyroid tadpoles of one set was less than one day.

The difference in time between the thyroid groups and those fed on other substances was as might be expected greater, the longer the treatment lasted. For instance, while only 5 days (May 30—June 4) lie between the appearance of the hind legs in thyroid II and thymus II, this interval in set I — I is the younger set, therefore was fed longer — is 12 days (June 8—June 20).

The precocious body differentiation of the thyroid fed tadpoles did not allow the animals to continue their growth, the result of the metamorphosis were therefore extremely small (pigmy-) frogs (Fig. 1*a*, 2*a*).

The feeding with thymus showed an influence on the development of the tadpoles, exactly the opposite of that caused by the thyroid diet. Its consequence was a prolonged increase in size beyond the normal, the metamorphosis, however, was much retarded or not completed at all as the animals died before that time. With this retarded development the individual differences, of course, were much

emphasized. Not all individuals of one set grew their hind or fore limbs on the same day, as in the thyroid groups, but there were intervals of days and weeks between the corresponding stages in different individuals. Those tadpoles that possessed the least amount of vital energy had to be fed longest. They were, therefore, longest under the influence of the retarding food.

The later an organ develops in normal ontogeny, for instance fore legs later than hind ones, the more its appearance was postponed by the thymus and accelerated by the thyroid diet. The hind legs of thyroid II and thymus II appeared at an interval of 5 days (May 30—June 4), for the fore legs the interval was 9 days (June 7—June 16). This can also be expressed in the opposite way: the younger a tadpole is at the beginning of the feeding, the greater is the retarding influence on development by the thymus treatment and the accelerating influence by the thyroid treatment. For instance, in the appearance of the hind limbs in thyroid II and thymus II (older set) the difference in time is only 5 days (May 30—June 4), while in thyroid I and thymus I (younger set) 12 days (June 8—June 20). In this comparison those thymus fed tadpoles with quickest differentiation, about 10%, were chosen. If all the thymus tadpoles were considered, the average difference in time would be much greater; for thymus I and II, although fed regularly and abundantly, needed about two months before all had completed their development, while all the other groups had metamorphosed long before.

Thus the influence of the thymus food was such that in the beginning it caused a rapid increase in body size, going beyond the normal, while later on it postponed the metamorphosis extremely. The color of the animals became very dark during the experiment. Those tadpoles most backward in development showed a clumsy bloated shape.

Experiment II.

A group of *Rana temporaria* tadpoles, originally selected for ovary feeding, had been fed only twice, May 23 and May 25, with that substance, after this time, up to the start of experiment II, July 6, through 43 days, they had starved. The short feeding of ovary was of so little influence, that these animals differed in no respect from the unfed control tadpoles (Fig. 3). From July 6 on a part of these tadpoles were fed on thyroid, another part on thymus. The differences in the results were most evident (Fig. 6*a*, *b*) and corresponded to those of experiment I.

The diary reads as follows:

July 6. Start of the experiment. Average size 2.75 cm.

July 9. (after 3 days only) thyroid grow hind legs.

July 11. A difference in the sizes of thyroid and thymus is noticeable. 2 thymus grow hind legs.

July 12. Size of thyroid 2.6 cm, of thymus 3.2 cm. Thyroid assume frog-shape.

July 13. Thyroid grow fore legs and swim on their back.

July 14. Thyroid have completed their metamorphosis and begin to die.

July 17. Thymus are very big, entire length 3.7 cm (body 1.3 cm, tail 2.4 cm) and are very dark colored.

July 18. 2 thymus are still without hind legs. From to-day on these two will be fed on thyroid, so that the experiment now runs thus:

Thymus.	Thymusthyroid.
July 21.	After 3 days only! Appearance of hind legs.
July 22.	Body assumes frog-shape. The dark (thymus) color has disappeared.
July 23.	Fore legs! The animals swim on their back.
	Length of body 0.8 cm 0.9 cm - - tail 1,3 - 1.3 - entire length 2.1 cm 2.2 cm
July 24. Not until to-day 2 of this group grow fore legs, although on July 17 they were so much further along than those in the right column.	
Aug. 5. The last one completes its metamorphosis, 22 days later than thyroid, and 13 days later than thymusthyroid.	

Thus experiment II ends with the same results as experiment I. The feeding on thyroid causes an extremely rapid differentiation of the body with a complete suppression of growth (compare Fig. 3 and 6*a*), the feeding on thymus furthers the growth (Fig. 6*b*), but retards the differentiation. This is most strikingly seen in the sub-

experiment described in the right column. Those tadpoles which were backward most on July 17 metamorphose after having been fed on thyroid for only 5 days, sooner than those farthest along in development, which remained on thymus.

In this experiment the influence of the thyroid food made itself manifest after only 3 days. The reason for this might be that the animals had starved through 43 days and had thus become older without being able to develop. They were, one might say, in a condition of latent overripeness and the first application of food rapidly caused a further development.

Experiment III.

Taken alone experiment III would not allow of any conclusions, since it was done with only a few animals. Yet the results attained are absolutely the same as those of experiments I and II, and therefore furnish a confirmation of the latter.

Some control animals of experiment I which had been starving since the first feeding, May 23, through 51 days, were fed on thyroid from July 13, others on liver.

The experiment ran as follows:

Thyroid.	Liver.
July 13. Average size 2.4—2.6 cm	
July 18. Hind legs appear, tail gets shorter, body assumes frog-shape	
length of body 0.7 cm	0.9 cm
- - tail 0.8 -	1.9 -
entire length 1.5 cm	2.8 cm
July 19. They swim on their back.	
July 20. Fore legs just noticeable. They die off.	
July 25.	hind legs appear (7 days later than in thyroid!).
Aug. 16.	fore legs appear (17 days later than in thyroid!).

Experiment III again shows the extremely strong influence of the thyroid food in accelerating the development as compared with an indifferent food, as liver can be regarded. At the same time it confirms the above statement, that the differences in time between

corresponding stages of development become greater the later an organ appears in normal ontogeny. The time between the appearance of the hind legs in thyroid and liver was an interval of 7 days, while the interval between the appearance of the fore legs in the two sets was 17 days.

To gain a further control of the results of experiments I—III on *Rana temporaria*, a similar set of experiments was repeated on *Rana esculenta*.

Experiment IV.

Tadpoles of *Rana esculenta* were fed in groups of 20 on thyroid, thymus and liver and one group was left unfed. For the feeding on thyroid 3 groups of different sizes were used, the smallest ones in group I, the largest ones in III. Group II as well as the liver, thymus and control groups consisted of tadpoles of the intermediate size.

The differences in size at various times of the experiment are given in the following table:

		Control	Liver	Thymus	Thyroid I	Thyroid II	Thyroid III
July 6.	Size at the start of the experiment	cm 2.5—3.0			2.1	cm 2.7	3.3
July 17.	Length of body [1]	1.0	1.2	1.4	0.7	1.0	1.2
	- - tail	1.5	1.7	1.7	1.1	1.4	1.8
	entire length	2.5	2.9	3.1	1.8	2.4	3.0
July 21.	Length of body	1.0	1.2	1.4	0.7	1.0	1.1
	- - tail	1.5	1.7	1.8	1.0	1.1	1.6
	entire length	2.5	2.9	3.2	1.7	2.1	2.7
July 31.	Length of body	1.0	1.2	1.4			
	- - tail	1.5	1.8	2.1			
	entire length	2.5	3.0	3.5			
	breadth of body	—	0.6	0.7			
Aug. 10.	Length of body		1.2	1.5			
	- - tail		1.9	2.3			
	entire length		3.1	3.8			
	breadth of body		0.7	0.8			
	- - tail		0.4	0.7			

[1] At the beginning of the experiments only the entire length of the animals was measured. Later it was found better to determine the lengths of the body and tail separately.

	Liver	Thymus		
Aug. 16. Length of body	1.2	1.5		
- - tail	2.0	2.5		
entire length	3.2	4.0		
breadth of body	0.7	0.9		
		a	b	
Aug. 29. Length of body	1.3	1.6	1.9	a = smallest
- - tail	2.1	2.6	3.2	b = biggest
entire length	3.3	4.2	5.1	
breadth of body	0.7	0.9	1.0	

From the record of the experiment may be mentioned:

July 16. Liver are big and show a greenish color. Thymus are very big and dark. All thyroid II and III grow hind legs.

July 17. Thyroid I grow hind legs. Some thyroid II and III show buds of fore legs, thyroid II breathe very rapidly and swim on their backs. The bodies of all thyroid I—III assume frog-shape.

July 20. Thyroid II begin to die, they have typical frog-shape. Thyroid III swim on their backs.

July 21. All thyroid II are dead.

July 23. Thyroid I and III begin to die.

July 24. All are dead. Their bodies are typically frog-like.

July 31. The unfed control begin to die. The thymus lose their dark color and become lighter than liver.

Aug. 13. Liver begin to grow hind legs, 28 days later than thyroid.

Aug 15. Thymus begin to grow hind legs, 30 days later than thyroid.

Aug. 13—Sept. 15. Liver die one after the other without completing their metamorphosis.

Sept. 5—Sept. 7. Thymus die one after the other without completing their metamorphosis.

This experiment has therefore given the same results as those attained on *Rana temp.* The effect of the thyroid diet is again striking.

At present it is not clear, why the liver and thymus tadpoles in spite of the continual feeding (July 6—Sept. 7, Sept. 15) did not complete their metamorphosis, but died before. The only respect in which the *Rana esculenta* experiments differed from those on *temporaria* was the higher temperature of the water and air. The former were undertaken during the hottest period of the summer of 1911, while the latter had been completed before that time. However, it is unlikely that the rise in the temperature itself should have enacted such a retarding influence on the development of the tadpoles. One

should rather expect the contrary. Although the high temperature may not be directly injurious, it may indirectly create unfavorable conditions for artificially rearing the animals. The water in which the tadpoles were kept contained a large amount of organic substances constantly undergoing decomposition much more rapidly than on cooler days. Therefore the accelerating influence of the higher temperature may well have been counteracted by this process.

Still another reason may account for the delay in development. *Rana esculenta* is less fit than *temporaria* to be reared under artificial surroundings, therefore in general less resistent to aquarium conditions. Furthermore, it sometimes happens that under apparently favorable conditions *esculenta* tadpoles do not complete their metamorphosis before the following spring. BARFURTH and TORNIER state that overfeeding may postpone the metamorphosis.

The thyroid tadpoles did not succumb to any of the above mentioned influences. This can easily be explained by the fact that the thyroid treatment did not have to last very long on account of the immensely accelerating influence of that food.

During the first half of the experiment the thymus fed animals showed the same dark pigmentation as the *temporaria* did, later on this dark color disappeared and they became even lighter than the liver fed tadpoles.

Experiment V.

The aim of this experiment was to study the influence that a sudden change in the food given would have on the development of *Rana esculenta* tadpoles. For this purpose a set of animals which had been fed on liver since July 6 was on July 21 put on thymus-, another set on thyroid diet. The same was done with thymus fed animals which were put on liver and thymus respectively. Thyroid fed tadpoles for feeding on liver and thymus unfortunately could not be used. With other animals it was tried, however, to stop the rapid progress in differentiation after thyroid diet by giving liver or thymus, but without results.

a. Liver fed tadpoles, put on thymus or thyroid diet on July 21.

	Liverthymus.	Liverthyroid.
Average size:		
July 21. Length of body	1.2 cm	1.2 cm
- - tail	1.7 -	1.7 -
entire length	2.9 cm	2.9 cm

Liverthymus.	Liverthyroid.
July 24.	After 3 days feeding! hind legs appear.
July 27.	Frog-shape is noticeable.
Length of body 1.2 cm	1.1 cm
- - tail 2.1 -	1.6 -
entire length 3.3 cm	2.7 cm
July 31. Length of body 1.3 cm	1.0 cm
- - tail 2.3 -	1.4 -
entire length 3.6 cm	2.4 cm
breadth of body 0.75 -	0.6 -, length of legs 0.3 cm.
	Swim on the back, air vesicles in the gill region, begin to die off.
(continued unter c.)	

b. Thymus fed tadpoles, put on liver or thyroid diet on July 21.

Thymusliver.	Thymusthyroid.
July 21. Length of body 1.4 cm	1.4 cm
- - tail 1.8 -	1.8 -
entire length 3.2 cm	3.2 cm
July 24.	After 3 days feeding! hind legs appear.
July 27.	Frog-shape is noticeable.
Length of body 1.4 cm	1.3 cm
- - tail 2.1 -	1.8 -
entire length 3.5 cm	3.1 cm
July 30. Length of body 1.4 cm	1.1 cm
- - tail 2.1 -	1.5 -
entire length 3.5 cm	2.6 cm
breadth of body 0.7 -	0,6 -, length of legs 0.6 cm.
	Swim on the back, air vesicles in the gill region.
Aug. 2.	Begin to die. A few have the buds of the fore legs out.
Aug. 3.	The last ones die.
	Length of body 1.0 cm
	- - tail 1.5 -
	entire length 2.5 cm
	breadth 0.6 -
(continued under c.)	length of legs 0.6 -

c. Continuation of the left columns of the above tables. A comparison of liverthymus and thymusliver.

	Liverthymus.	Thymusliver.
July 21.	Length of body 1.2 cm	1.4 cm
	- - tail 1.7 -	1.8 -
	entire length 2.9 cm	3.2 cm
July 27.	Length of body 1.2 cm	1.4 cm
	- - tail 2.1 -	2.1 -
	entire length 3.3 cm	3.5 cm
July 31.	Length of body 1.3 cm	1.4 cm
	- - tail 2.3 -	2.2 -
	entire length 3.6 cm	3.6 cm
	breadth of body 0.75 -	0.7 -
Aug. 5.	Length of body 1.45 cm	1.4 cm
	- - tail 2.5 -	2.3 -
	entire length 3.95 cm	3.7 cm
	breadth of body 0.8 -	0.7 -
Aug. 10.	Length of body 1.5 cm	1.4 cm
	- - tail 2.6 -	2.3 -
	entire length 4.1 cm	3.7 cm
	breadth of body 0.8 -	0.7 -
Aug. 13.	Hind legs appear, 20 days later than in thyroid (compare the right columns of a and b).	
Aug. 16.	Length of body 1.6 cm	1.4 cm
	- - tail 2.8 -	2.3 -
	entire length 4.4 cm	3.7 cm
	breadth of body 0.8 -	0.7 -
Sept. 2.		Begin to die without developing fore legs.
Sept. 20.	Begin to die without developing fore legs.	

Experiment V shows that a thyroid diet, started even at an advanced stage of differentiation and after other substances have been fed, is able to influence the further development intensely. It seems of little importance, which substances were fed before the thyroid, except that the relative sizes are different. The liverthyroid went almost parallel with the thymusthyroid (Fig. 8*a*, *b*). Some minute differences, however, were noticeable, yet further experiments with a combined

diet will have to determine their importance. Some of the thymus-thyroid, for instance, showed buds of the fore legs, while the liver-thyroid died before that stage showing characteristic responses to thyroid. These features as swimming on the back, formation of air bubbles in the gill region and others will be discussed later.

The liverthyroid and thymusthyroid were far ahead of the liver-thymus and thymusliver and also of the liver and thymus of experiment IV.

The liverthymus and the thymusliver ran almost parallel with the exception that the liverthymus grew bigger than the thymus-liver. The thymus diet, therefore, furthers growth even, when it is given at an advanced stage of differentiation, but apparently less than when given to younger animals.

The following comparison of tables IV and V is interesting: liverthymus Vc become gradually larger than liver IV, thymusliver Vc smaller than thymus IV. The thymus food thus seems to act differently at different ages, and it may be possible to find a time or stage for its optimum influence such as is also surmised for the thyroid diet.

In liver IV the hind legs appeared on August 13, in thymus IV on August 15. This difference in time is rather small and further experiments must show, whether or not it is significant. At any rate, this observation agrees with those made on *Rana temporaria*, which showed that the thymus food retarded the development. Liverthymus V and thymusliver V grew their hind legs on August 13, i. e. on the same day as liver IV. Thus the partial feeding on thymus seems not to have caused the same delay in development as the exclusive thymus diet. However, a difference of only 2 days, observed on one set of animals does not allow of conclusions.

Experiment VI.

This experiment can be regarded as a supplement to experiment V, at the same time it furnishes a further confirmation of the results of former thyroid feedings. Tadpoles that had been fed on liver and thymus 15 days longer than the corresponding groups of experiment V, thus were 15 days older, were put on the thyroid diet on August 5.

This last experiment shows that the thyroid when food given even at a very advanced stage of differentiation can cause an accelerated development. 5 days after the beginning of the experiment hind legs appear, this is still 3 and 5 days sooner than in the control animals liver IV and thymus IV. The effect of the previous feeding on different

	Liverthyroid.	Thymusthyroid.
Aug. 5.	Length of body 1.2 cm	1.4 cm
	- - tail 1.9 -	2.2 -
	entire length 3.1 cm	3.6 cm
	breadth of body 0.6 -	0.8 -
Aug. 10.	Length of body 1.2 cm	1.3 cm
	- - tail 1.8 -	2.1 -
	breadth of body 0.6 cm	0.7 cm

Hind legs appear after 5 days feeding. (The liver IV and thymus IV do not grow them until August 13 and August 15.)

Aug. 12. Frog-shape is noticeable.
Aug. 14. Swim on the back.
Aug. 15. 2 grow fore legs.
Aug. 16. These two (a, b) die, the rest grow fore legs

	a	b	rest	
length of body	0.9	1.05	0.9 cm	1.15 cm
- - tail	1.2	1.0	1.2 -	1.7 -
entire length	2.1	2.05	2.1 cm	2.85 cm
breadth of body	0.5 cm			0.7 -

Aug. 17. Begin to die off. Swim on the back.
Aug. 18. 1 grows fore legs.
Aug. 19. Last ones die. Last ones die.

	smallest	largest	smallest	largest
length of body	0.8 cm	0,9 cm	1.0 cm	1.1 cm
- - tail	0.6 -	0.9 -	1.2 -	1.4 -
entire length	1.4 cm	1.8 cm	2.2 cm	2.5 cm
breadth of body	0.5 -	0.5 -	0.65 -	0.7 -

substances before the thyroid diet here also manifests itself in the different sizes of the animals. During the entire experiment thymusthyroid remain bigger than liverthyroid; on the other hand liverthyroid develop quicker than thymusthyroid, which is suggested also by experiment V. If further experiments of this kind give similar results, we shall have additional evidence, that thymus food postpones the metamorphosis. In fact, at the beginning of experiment VI the liver IV must have been ahead of thymus IV, although macroscopically the difference was not evident; for liver IV grew their hind limbs on August 13 and thymus IV on August 15.

General discussion.

The most striking and at the same time unquestionable results were attained by thyroid feeding. They were the same in all experiments. The influence of the thyroid food was such that it stopped any further growth but on the contrary led to an abnormal diminution of the size in the animals treated, while simultaneously it accelerated the differentiation of the body immensely and brought it to a premature end. It was of little importance, at which stage of differentiation the thyroid diet began or which kind of food had been given before. Under all circumstances the influence of the thyroid food became noticeable in a very short time.

This influence must have been very strong, as can be concluded from two kinds of observations. First, within a very short time, 3—5 days, after the beginning of the experiments changes in the outer features of the animals were noticeable; second, the influence on all tadpoles of one group was uniform and rather parallel. While, for instance, in other groups not fed on thyroid the influence of the food became evident gradually, without abolishing the individual differences, so that the individuals of one group grew their hind legs, fore legs etc: one after the other, often at intervals of many days, the thyroid diet, on the other hand, brought all the animals of one group within a few hours, not more than 24, to the same stage of development. However, it cannot be said that the individual differences were entirely abolished. The measurable signs of these differences, the intervals between the corresponding phases of development, were greatly reduced since the entire period of development was much shortened.

One of the most peculiar features is that the time at which the feeding begins is of no importance as regards its results. The stages of development of the animals to be treated may be chosen, but always the same results will be obtained. Animals in different stages of development, others that had starved for many weeks, and still others that had before been fed on other substances were placed on thyroid diet with exactly the same results: within a few days the rapid differentiation of the body began. Thus extremely young or very old tadpoles could be forced to undergo their methamorphosis quickly. The lower and upper limit of age for the start of a successful thyroid diet will be determined later. The upper limit is probably the time shortly before completing their metamorphosis, when the tadpoles stop feeding in general. How near to the time of hatching

the lower limit can be brought further experiments will show[1]). The tadpoles that were available for the experiments here recorded had been hatched for some weeks.

The second influence of the thyroid diet, the suppression of growth, is merely the consequence of the precocious development, and this in turn seems to be caused by the well known activity of the thyroid agens to stimulate metabolism. The thyroid agens accelerates the metabolism which leads to a rapid reduction of the larval organs and thus to a premature metamorphosis. As soon as thyroid food is given the differentiation of the body begins. Hand in hand with the progressing metamorphosis goes, more than in the case in normal development, a reduction of the body mass (resorption of the tail, loss of water, therefore an increasing compactness of the body etc.) The outcome of such precocious metamorphosises are then very small (pigmy) frogs. This mass reduction was especially striking in the experiments on *Rana esculenta.*

The thyroid showed still other peculiar influences on the behavior of the tadpoles. Towards the end of the metamorphosis the animals hardly moved about in the water. They were always lying quietly, generally on their backs. When disturbed they would move for a few seconds in a somewhat convulsive manner and then drop again to the bottom of the dish, while tadpoles fed on other material would swim about for a long time. The reason for this may be that the thyroid fed tadpoles always began to reduce their tail before the extremities were at all or sufficiently strongly developed. The extremities, even if fully developed, were always extremely thin, merely thread-like (Fig. 6 *a*), and could hardly be used for swimming a long time.

At one time *Rana esculenta* tadpoles of the different groups were placed in small dishes with equal quantities of water, to which equal amounts (about 5 drops) of chloroform had been added. This was done so as to be able to photograph the animals. All tadpoles remained the same length of time in the mixture. All animals survived the narcosis very well except the thyroid fed ones which died in it.

At another time *Rana temporaria* tadpoles were taken out of the water and placed on wet filter paper to photograph them. During this procedure, which of course was somewhat rough, the thyroid

[1]) In recent experiments (1912) which will be discussed in a later paper I succeeded in forcing *Rana temporaria* tadpoles to grow fore legs as early as 15 (!) days after leaving the egg.

died, while the others stood it. Thus in different ways it was seen that the thyroid fed tadpoles possessed far less resistance against noxious influences than the others, as if the thyroid food had weakened their systems enormously. One cannot, however, speak of a poisoning of their body in the true sense, since that would not have allowed the rapid progress in development.

In the tables given above several dates are mentioned at which the animals began to swim on their backs. This, too, is one of the features observed only in thyroid tadpoles. Before the animals completed their metamorphosis, about 3—4 days previous, they began lying on their backs and floated passively on the surface of the water. They breathed very heavily and rapidly. Even when disturbed and swimming actively they did not usually turn over. It seemed as if the animals were passively forced to take this peculiar position; as under the skin in the gill region there were always one or two air bubbles visible, as if during the closure of the gill opening air had been enclosed. If the animals did not die these air bubbles were usually absorbed after which the animals assumed a normal position. It was seen that the swimming on the back always began shortly before the completion of the metamorphosis and its early appearance was watched.

The influence of the thymus diet on the development of the tadpoles was as evident as that of the thyroid, but less striking. The thymus food caused an accelerated growth beyond the normal (giant tadpoles) and at the same time it retarded or completely suppressed the differentiation of the body. In doing so individual differences were very much emphasized, so that an interval of several weeks elapsed between the metamorphosis of the first and the last tadpole, while in normal development the difference amounted to days only. The strongest tadpoles or better those which at the start of the feeding had progressed most in their development were best able to keep pace with the control. Those, however, which were backward in their development at the time the thymus diet began stayed much behind the control, since they were attacked by the thymus at a less advanced stage of differentiation, and further because they remained longest on thymus diet.

The thyroid and thymus diets were thus diametrically opposite in their influences. Their relative action, however, corresponds with the views held regarding the physiological properties of these organs.

Experiments of the kind discussed in this paper may perhaps give a direction for further studies towards a rational application of thymus and thyroid preparations.

It is not the purpose of this experimental paper to discuss the extensive literature on the functional and therapeutic importance of the organs with an internal secretion. Reference is simply made to the numerous papers in which the therapeutic value of thyroid preparations for the stimulation of metabolism and ossification, and the influence of the thymus on growth in the early periods of individual life are being discussed. A list of them will be found at the end of this paper.

Liver and muscle were about equal in their action on development and did not seem to influence especially the normal progress of differentiation. Since so far they appeared to be indifferent food stuffs the tadpoles fed on liver or muscle were regarded as a control to the other feedings. However, under natural conditions the animals have a food supply quite different from a constant meat diet, yet for various reasons it was impossible to study the development of control animals on a more vegetal or mixed diet[1]).

The tadpoles fed on adrenal[2]) developed somewhat slower than those fed on liver or muscle, otherwise quite normally. The outcome of the metamorphosises were especially large and strongly developed frogs (Fig. 5*b*).

A prolonged diet of hypophysis did not force the animals to complete their methamorphosis. They all died before that stage. No conclusions can be drawn from this fact, since these tadpoles were not fed as regularly as the others on account of great difficulties encountered in providing the food[3]). The feeding on testis and ovary was also unsatisfactory for the same reason.

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[1]) Such experiments are now being done, spring 1912, and they will be discussed in a later paper. The difference in macroscopic development between a vegetal and liver or muscle diet is slight.

[2]) Experiments on feeding adrenal cortex and medulla separately will be discussed in a later paper.

[3]) More extensive experiments on feeding the two lobes of the hypophysis will be discussed in a later paper. So far they do not confirm the above statement.

Preliminary experiments were also undertaken on *Rana esculenta* to study the influence of different diets on regenerating animals. So much can be said that of tadpoles which hat a piece of their tail amputated the thymus fed ones regenerated quickest, while the thyroid fed ones, although they did regenerate a part, showed the typical precocious metamorphosis. In one experiment the average length of the regenerated part of the tail was: in thymus 3.5 mm, in thyroid 3.2 mm, in liver 2.9 mm; in another experiment: liver 3.1 mm, thymus 4.6 mm; later liver 6 mm, thymus 9 mm. Regeneration of the tail begins even when the animals are near the point where they resorb their larval organs, otherwise the thyroid fed ones would not have regenerated. BARFURTH showed that *Rana fusca* tadpoles which metamorphosed even 2 or 3 days after the operation tried to regenerate the amputated part of their tails.

The influence of the different food stuffs on the pigmentation has been mentioned before. The animals were kept under the same conditions of light and temperature and in the same kind of dishes. The position of the dishes was changed daily in a certain rotation so that the minute differences in light and temperature were abolished as much as possible.

The liver fed tadpoles were rather dark, gradually assuming a greenish tint. The thymus fed tadpoles of *Rana temporaria* grew extremely dark with the progress of the experiments until they became almost black; those of *R. esculenta* grew dark in the beginning, later, however, they became lighter. The adrenal fed tadpoles after 3—4 weeks became extremely light in color. Those fed on hypophysis lost their pigment more and more and became almost transparent, but this may have been the consequence of the irregular feeding.

TORNIER has studied the influence of varying quantities of food on the pigmentation of *Pelobates fuscus* tadpoles and found that a minimum food ratio gives albinotic, a maximum ratio highly melanotic larvae and frogs. So the melanism of the thymus fed tadpoles may have been partly caused by an overrich diet, yet they were much darker than those in the other groups, although all were fed sufficiently well. Why the *Rana esculenta* larvae which in the first weeks of the experiments were as melanotic as the *temporaria*, later lost their dark appearance, cannot be explained at present. Very minute differences in temperature, as KAMMERER points out, may easily cause a change in pigmentation.

The very dark and the very light adrenal (cortex and medulla) tadpoles seem, roughly estimated, to have possessed equal amounts of pigment. In the thymus fed animals the pigment cells were spread out very much in a star-like manner, in the adrenal fed ones they were completely contracted. Former experiments with adrenalin would warrant the suggestion that the extract from the chromaffine cells of the medulla which dissolved in the water caused the pigment cells to contract[1]).

The histogenetic processes must have been influenced very much by the different diets. The investigation of the thyroid and thymus fed material promises especially interesting results. The report on this topic will be given later.

More experiments, especially with mixed diets, are necessary to clear up all the questions concerned in this discussion. At any rate, these experiments may open a new and extensive field of work in experimental morphology, in which success is rather certain.

At present one fact alone deserves notice, that the food stuffs given fresh were able to pass the stomach without losing at least some of their specific properties. It still remains an open question, whether their action, after they have passed the intestinal canal, is entirely the same as that which they exert as functionating organs. Before this question is solved, no conclusions can be made on the rôle of these organs in the household of the body. However, so far it has been shown that a diet on thyroid substance or the application of thyroid tablets can to a certain degree substitute the normal function of the thyroid gland. — It must also be kept in mind that mammalian organs were fed to amphibians.

Summary.

A number of mammalian organs, especially those with an internal secretion, thyroid, thymus, adrenal, testis, ovary, hypophysis, liver, muscle etc. were given as food to tadpoles of *Rana temporaria* and *esculenta*. It was seen that each organ exerted a certain influence on growth and differentiation of the animals. Most striking was the

[1]) Compare: LIEBEN, S., list of literature. Recent (1912) experiments, however, so far indicate that the feeding on adrenal cortex causes a much lighter pigmentation than an adrenal medulla diet.

influence of the thyroid food. It caused a precocious differentiation of the body, but suppressed further growth. The tadpoles began to metamorphose a few days after the first application of the thyroid and weeks before the control animals did so. The influence of the thymus was quite the opposite, especially during the first days of its application it caused a rapid growth of the animals, but postponed the final metamorphosis or suppressed it completely. The action of the other organs must be studied further before definite statements can be made. The thymus diet gave very dark, melanotic tadpoles, the adrenal diet extremely light albinos, the liver diet dark ones with a greenish tint.

Zusammenfassung.

Verschiedene Säugetierorgane, namentlich solche mit innerer Secretion, Thyreoidea, Thymus, Nebenniere, Hoden, Eierstock, Hypophyse, Leber, Muskel usw. wurden an Kaulquappen von *Rana temporaria* und *esculenta* verfüttert. Jede Fütterung übte einen andern Einfluß auf das Wachstum und die Differenzierung der Tiere aus. Äußerst auffallend war die Wirkung der Schilddrüsennahrung. Sie verursachte eine rapide Körperdifferenzierung, die zu einer vorzeitigen Metamorphose führte, wobei aber jedes Weiterwachstum unterdrückt wurde. Die Kaulquappen begannen ihre Metamorphose wenige Tage nach der ersten Schilddrüsendosis und um Wochen früher als die Kontrolltiere. Der Einfluß der Thymusnahrung war gerade entgegengesetzt. Sie bewirkte namentlich in den ersten Tagen ein schnelles Wachstum der behandelten Tiere, schob aber die Metamorphose immer weiter hinaus oder unterdrückte sie gänzlich. Der Einfluß der übrigen Organe muß noch weiter studiert werden. Die Thymusverfütterung ergab tief dunkel, fast schwarz gefärbte Quappen, die Nebenniere ganz lichte, albinotische Tiere, die Leber dunkle, mit einem Stich ins Grünliche.

List of Literature.

Barfurth, D., Versuche über die Verwandlung von Froschlarven. Arch. f. mikr. Anat. 1887. Bd. 29. S. 1.

—— Der Hunger als förderndes Prinzip in der Natur. Arch. f. mikr. Anat. 1887. Bd. 29. S. 28.

Basch, K., Beiträge zur Physiologie und Pathologie der Thymus. Jahrb. f. Kinderheilk. 1906. Bd. 64. 1908. Bd. 68.

Biedl, A., Innere Secretion. Wien-Berlin 1910.

Gudernatsch, J. F., Fütterungsversuche an Kaulquappen. Demonstr. Verh. Anat. Ges. 26. Vers. München 1912.

—— Fütterungsversuche an Kaulquappen. Vorl. Mitteil. Centralbl. f. Physiol. 1912.

Hammar, I. A., Fünfzig Jahre Thymusforschung. Ergebn. d. Anat. u. Entwicklungsgesch. 1910. Bd. 19.

Kammerer, P., Künstlicher Melanismus bei Eidechsen. Centralbl. f. Physiol. 1906. Bd. 20. S. 261.
—— Über künstliche Tiernigrinos. Verhandl. zool.-bot. Gesellsch. Wien. 1907. Bd. 57. S. 136.
—— Die Wirkung äußerer Lebensbedingungen auf die organische Variation im Lichte der experimentellen Morphologie. Arch. f. Entw.-Mech. 1910. Bd. 30. S. 379.
Lieben, S., Über die Wirkung von Extrakten chromaffinen Gewebes (Adrenalin) auf die Pigmentzellen. Centralbl. f. Physiol. Bd. 20.
Tornier, G., Nachweis über das Entstehen von Albinismus, Melanismus und Neotenie bei Fröschen. Zool. Anz. 1907. Bd. 32. S. 284.
Vincent, Swale, Internal Secretion and the Ductless Glands. Lancet. 1907.

Explanation of Figures.

Plate IX.

Fig. 1 *a—e*. *Rana temporaria* set I (smaller size), photographed June 11 1911. Natural size. *a* tadpoles fed on thyroid, already changing into frogs. Tail is shortening, fore legs appear. *b* tadpoles fed on thymus, *c* on liver, *d* on muscle, *e* on adrenal.

Fig. 2 *a—e*. *Rana temporaria* set II (larger size), photographed June 11 1911. Natural size. *a—e* as in Fig. 1. The thyroid fed tadpoles have all metamorphosed, the tail in some has almost disappeared, fore legs are well developed.

Fig. 3. *Rana temporaria* tadpoles that had been used as an unfed control in experiment I, thus starved till July 13. Photogr. July 13. Nat. size.

Fig. 4. The same tadpoles as in Fig. 3, photogr. 7 days later, July 20. *a* had been fed in the mean-time on thyroid, and are already metamorphosing into pigmy frogs. *b* had been fed on liver. These tadpoles do not metamorphose until 19 days later.

Fig. 5. *Rana temporaria* set I frogs. Photogr. July 25. Natural size. *a* fed from the beginning of experiment I on adrenal. *b* one of the tadpoles that originally had starved. Their size on July 6 was that of the tadpoles in Fig. 3. From July 6 to July 17 these tadpoles, 5 *b*, were fed on thymus, from July 18 on thyroid. On July 21 hind legs appeared, July 23 fore legs. Notice the small body and the much shortened tail of a frog metamorphosing under thyroid influence, while the adrenal frogs, 5 *a*, at the time of metamorphosis are large and still have their long tadpole tails.

Fig. 6. *Rana temporaria* set I, photogr. July 13 1911. Natural size. Animals that originally had starved. Their size on July 6 was that of the tadpoles in Fig. 3. From July 6 to July 13. *a* were fed on thyroid, *b* on thymus. *a* are changing into pigmy frogs, fore legs appear, tail shortens, *b* are still huge tadpoles. Compare also these thymus tadpoles with Fig. 4 *b* fed for the same time on liver.

The animals in Fig. 4—6 are all of the same age and the same original size, set I, but fed on different organs.

Fig. 7. *Rana esculenta*, photogr. August 9, nat. size. *a* fed on thymus since July 6, *b* fed on liver since July 6, *c* on thymus from July 6 to July 20, on liver since July 21, *d* fed on liver from July 6 to July 20, on thymus since July 21.

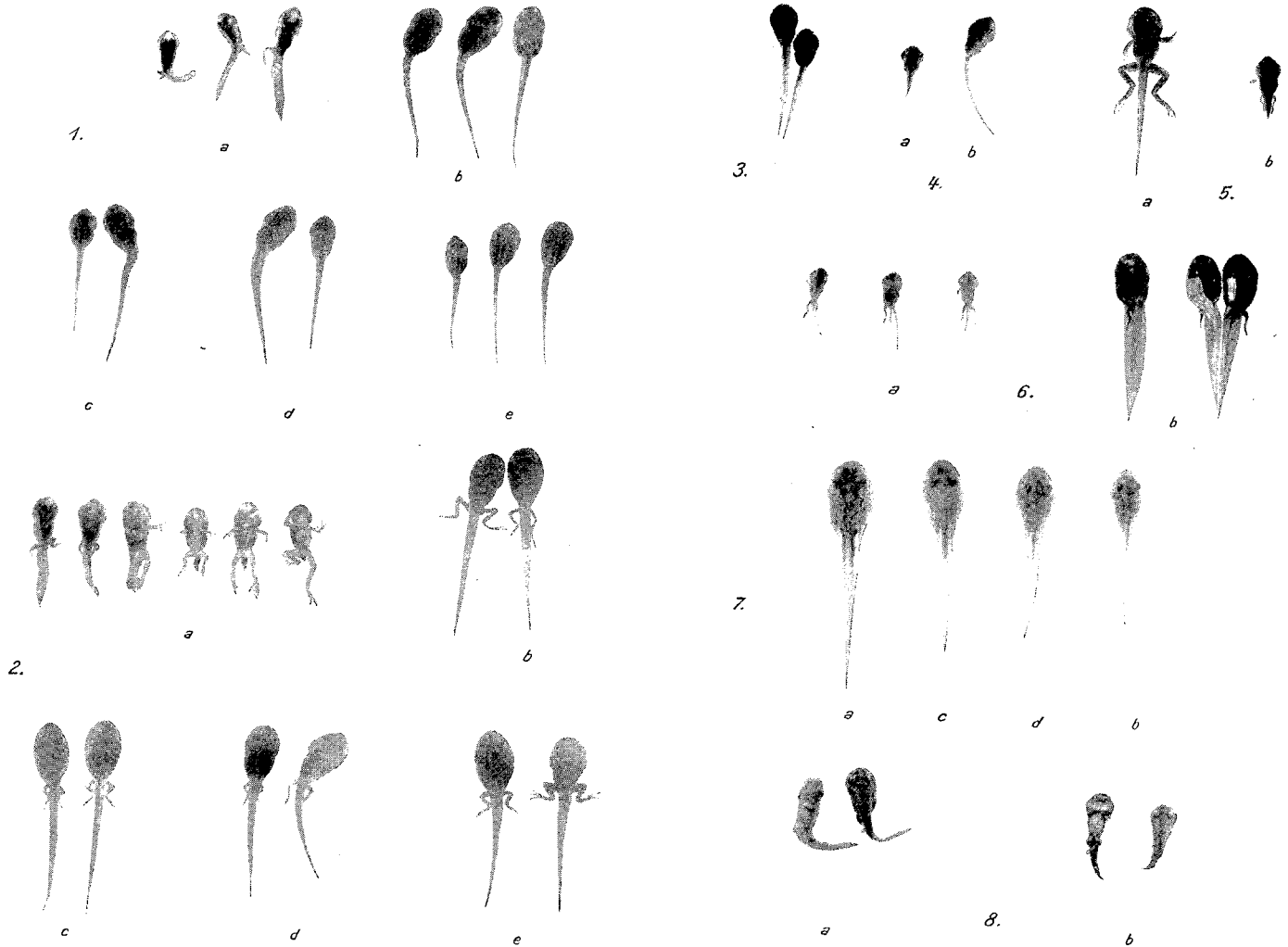
a and *c*, *d* which either entirely, *a*, or at a time of their development, *c*, *d*, were fed on thymus, are much bigger than those fed on liver only, *b*.

Fig. 8. *Rana esculenta*, photogr. August 19 1911, nat. size. The animals were dead, when being photographed, therefore the curved tails. *a* fed from July 6 to August 5 on thymus, from August 6 to August 18 on thyroid. *b* fed from July 6 to August 5 on liver, from August 6 to August 16 on thyroid.

The animals in Fig. 7 and 8 are all of the same age, but fed on different substances, and were photographed on the same day.

Anmerkung. Vorliegende Untersuchungen wurden im Sommer 1911 in meinem Institute durchgeführt. Das Manuskript war bereits im Dezember 1911 druckfertig. Eine schwere Krankheit, die mich lange zur Untätigkeit zwang, hat die Veröffentlichung verzögert.

Prag, Mai 1912. Prof. ALFRED KOHN.



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