A STUDY OF THE SUSCEPTIBILITY OF TADPOLES DEPRIVED OF THE PITUITARY GLAND

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PART I.

INTRODUCTORY

In the course of feeding experiments upon pituitaryless tadpoles conducted in the season of 1921 and again this year, the writer was occasionally disturbed by the loss of the pituitaryless tadpoles under conditions which did not prove fatal in nearly so high a degree to the normal tadpoles. Such, for example, was the experience when the water in which the tadpoles were living became stale. A dose of a narcotic (chloretone) which was sublethal to the normal tadpoles was frequently fatal to the pituitaryless animals. This difference in the resistance of pituitaryless and normal tadpoles to adverse conditions had been noted previously by both Allen (1,2) and Smith (3). It was at the suggestion of Dr. Allen, to whom the writer is indebted for advice and kindly criticism, that a study of the susceptibility of pituitaryless tadpoles was undertaken. In view of the fact that one of the most striking effects of pituitary removal is to reduce the growth rate (1,2,3), the writer felt called upon to note any possible relation between the length of the tadpoles at any given age and their susceptibility. A further objective was to note the effect of starvation upon the susceptibility, inasmuch as earlier experiments of the writer had indicated a difference in the survival of the pituitary-less and normal tadpoles. A lessened resistance to the toxicity of cyanides has been noted in the case of starved Planaria, Didinia, and Paramecia (4).

MATERIAL AND METHOD

The animals used for the experiments herein to be described were the larvae of Rana pipiens, hatched in the laboratory from eggs gathered in the neighborhood of Lawrence. Those from which the pituitary anlage was to be removed were broken out of their capsules at the three to five millimeter stage, before any muscular activity had commenced, making it unnecessary to use any narcotic during the operation. The pituitary anlage was removed according to the technique perfected independently in 1916 by Allen (1,2) and Smith (5). The operation involves the making of a frontal incision between the brain and the pharynx and the breaking off of the tongue of cells growing back from the buccal ectoderm and normally adherent to the ventral surface of the brain. It is performed under a comparatively low power binocular microscope by means of a fine knife ground from a needle. The striking color change from the normal black to a silvery white (1,2,3,6) is taken as an indication of the success of the operation.

Two types of control were kept. The one type consisted of perfectly normal tadpoles. These are herein designated as "controls". The other type consisted of those in which a frontal incision was made as for pituitary removal, but from which no tissue was removed. The wound was deliverately lacerated, however, to simulate as nearly as possible the surgical effect of pituitary removal. These tadpoles will be designated as "cut controls". The tadpoles from which the pituitary enlage has been removed are spoken of as "pituitaryless". The abbreviations for these three types are "c", for the controls, "cc" for the cut controls, and "p" for the pituitaryless. For the indicator of susceptibility, weak concentrations of KCN were used. The poison was applied by immersing the tadpoles in solutions of the proper concentration, and then each animal was observed every half hour, or in some cases every quarter hour. The point at which the heart was not seen to contract for five minutes during which time it was watched constantly was called the death point of the animal. Narcotization took place within an hour or so in the concentrations of KCN used, and after that the animals could readily be overturned and the heart activity clearly seen thru the ventral body wall.

The most favorable concentration of the KCN was determined by trial. The concentration of the poison must be sufficiently high to kill within a few hours, or a varying amount of acclimation may take place within the tissues, which would postpone the death point and probably reverse the readings of the susceptibility (Child,7). It was found that concentrations higher than M/8000 killed very rapidly - too rapidly to give dependable results. Most of the animals were killed in a solution of M/8000 strength, but the data seem to indicate that it would have been better to have worked with a solution even less strong than that. Attention will be called to the fact that some of the starved tadpoles were killed in a solution of M/16000 strength. All the solutions were made up in distilled water. That the change from the tap water in which they were reared to the distilled did not itself kill the animals is shown by the fact that pituitaryless and control tadpoles in pure distilled water were alive after twenty-two days. The animals to be observed were put into the solution in covered Stender dishes. The number of animals which were killed at one time was small enough that each one could be given the necessary attention to determine accurately the death point. For each new group of tadpoles to be killed, the solution was always mixed up fresh from a stock solution made up of M/100 strength and kept on hand in a stoppered flask. The KCN used was the pure fused product prepared by Mallinckrodt.

EXPERIMENTAL

Fifteen separate groups of tadpoles were used, totalling two hundred and seventy eight. Of these, one hundred and twelve were those from which the pituitary had been removed, eighty-three were those in which a frontal incision had been made (the so-called cut controls), and eighty-three were normal unoperated controls. These fifteen groups are divisable and subdivisable as follows:

Group I - Fed tadpoles killed in M/8000 KCN

1 a - Fed controls

2 a - Fed cut controls

3 a - Fed pituitaryless

1 b - Fed controls

2 b - Fed cut controls

3 b - Fed pituitaryless

Group II - Fed and Semi-starved tadpoles killed in M/8000 KCN

1 - Semi-starved controls

2 - Semi-starved cut controls

3 - Fed pituitaryless

Group III- Starved tadpoles killed in M/8000 KCN

1 - Starved controls

2 - Starved cut controls

3 - Starved pituitaryless

Group IV- Starved tadpoles killed in M/16000 KCN

1 - Starved controls

2 - Starved cut controls

3 - Starved pituitaryless

Where abbreviations are necessary or convenient, fed controls will be designated as "fc", fed cut controls will be designated as "fcc", and fed pituitaryless will be designated as "fp". Similarly, semistarved tadpoles will be designated as "ssc" and "sscc", and starved tadpoles will be designated as "sc", "scc", and "sp". When particular groups are referred to, the proper Roman numeral will be affixed to the symbol.

The tadpoles of section a and section b of Group I were from the same stock, and were treated similarly except that those in a were killed and measured approximately five days younger than those in b. Table I shows that the control tadpoles in a are on the average shorter than those in b. Graph I shows very clearly that this shorter length varies only insignificantly from being directly proportional to the lesser age. Table II shows the same situation to prevail in the case of the cut controls of sections a and b, and Graph II bears out the relation by displaying again an insignificant variation from the direct proportion. The approximation of the direct proportion is not quite so close as it is with the controls, but it is to be expected that in those animals which have undergone an operation there will be a wider degree of variation than in the perfectly normal animals, and the numbers of animals here used were not sufficient to concentrate the means in coincidental lines. That the same thing is true of the pituitaryless tadpoles is shown in Table III and Graph III. Here again the variation is wider than in the case of the controls, and even wider than in the case of the cut controls, but here again there has been an operation, probably somewhat more severe than that of the cut controls, so that the variation may well be greater, due to the almost unavoidable slight differences in the individual operations, and the numbers are again too small to give an absolutely accurate proportion. As a matter of fact, in this case of least accurate proportions, it can be shown by biometrical methods that the distribution of the death points is such that this deviation from the direct proportion can be attibuted to random sampling, the difference being but slightly less than three times as great as the probable error in the difference.

In view of the very apparent accurate proportion between homologous tadpoles of the two sections, we are justified in massing them together to obtain a more accurate mean from the larger number of animals considered. Section 1-a and section 1-b are therefore hereafter considered as one group, and so also sections 2-a and 2-b, and sections 3-a and 3-b.

Table V gives the figures for the lengths of all the tadpoles, controls, cut controls, and pituitaryless, of group I, and a graphic comparison of the three types with respect to length is given in graph V. It is clear from the table and the graph that at a given age (during the period before the changes incident to metamorphosis upset the normal curve) the pituitaryless tadpoles are shorter in body length than either a normal control or a cut control. The data also show that the cut control is longer than the control.

The relation of the three types in group I is not the same with respect to susceptibility in KCN as it is with respect to length. The cut controls were the first to die. The controls lived slightly longer, and the pituitaryless somewhat longer than the controls. The figures for the time of dying in the poison are given in Table VI, and the situation is portrayed graphically in Graph VI. An analysis of the distribution of the death points is given in Graph VI a. This comparison of susceptibility is worthless in view of what is revealed by Graph VI a, which is that the first and third quartile point of the control group include the first and third quartile point of the other two groups, and the population of all three groups is very widely scattered.

The conditions in the case of Group II are altogether changed. Here the pituitaryless tadpoles were fed normally, but the controls and the cut controls were partially starved. Between the fed pituitaryless of group I and group II, the difference is again only one of age. Table IV records the length and age of pituitaryless tadpoles of group II as compared with the figures for group I, and Graph IV shows that the proportion between length and age is true, and therefore that the two groups are similar. In view of the wide discrepancy in their ages, however, it is desirable to treat of the two groups separately, especially since the pituitaryless tadpoles of group II are to be contrasted with an altogether different type of control and cut control.

Since the pituitaryless specimens of groups I and II are found to be entirely comparable, the controls and cut controls of group II are comparable to the controls and cut controls of group I for an absolute comparison, which is made in Tables VII, and VIII, and in Graphs VII and VIII.

The controls and cut controls of this group were not fed as much as they could or would have eaten. The purpose of this semi-starving was to rear tadpoles which would be smaller than the pituitaryless tadpoles of the same age while at the same time not reducing the vitality as in the completely starved tadpoles. Table IX and Graph IX show the results of this experiment with respect to length, and show that the partial starvation of the controls and the cut controls had the desired effect of reducing the proportional length to a figure below that of the normally fed pituitaryless specimens, in spite of the fact that the pituitaryless specimens are smaller than normally fed control and cut control tadpoles of the same age. The semi-starved tadpoles are not so small, however, as they would have been if they had been completely starved. Swingle (8) is authority for the statement that the starved tadpole is less than half the length of the normal on the seventeenth day.

With respect to the time which the semi-starved controls and cut controls remained alive in the KCN, the figures are striking. Table X and XI show the comparison of the semi-starved controls and cut controls of Group II with the homologous fed tadpoles of Group I. The fed tadpoles in each case died in considerably less time. When compared with the fed pituitaryless tadpoles of the Group II, an equally striking result is seen. Table XII and Graph X show that the fed pituitaryless tadpoles died first by a significant margin over the cut controls and a wide margin over the control tadpoles.

The third group of these tadpoles was completely starved during their entire life. They were killed at the same concentration of KCN as both of the former groups, i.e., M/8000. A considerable number of tadpoles of exactly the same stock were killed at lower concentrations. In comparing the relative lengths of these tadpoles, the length figures for all are included, whereas in treating with the hours of life in the poison, the animals in the different concentrations are considered separately. None of the tadpoles used in the complete starvation experiment were of the same stock as those used in groups I and II, so the third group can be compared with the first two only in a general way. Table XIII and Graph XII show that as in the case of the fed tadpoles, the pituitaryless are the shortest type in the group. In this case, the controls and the cut controls are of exactly the same length.

With regard to susceptibility to KCN of the starved tadpoles, it is apparent from Table XIV and Graph XIII that the concentration M/8000 was too strong to allow of an accurate differentiation between the types. The results obtained at the high concentration are significant, however, in view of the results obtained at lower concentration. At the concentration of M/8000 there is not a significant difference between the length of life of the pituitaryless and the control tadpoles, but between the control and the cut control the difference is large enough to consider, and it is even greater between the pituitaryless and the cut control. The first to die were the pituitaryless, followed at an insignificant interval by the controls, and at a significant interval by the cut controls.

In group IV the concentration of the KCN was reduced to M/16000, and the results with respect to susceptibility are the same, relatively, as those obtained in group III. The differences are all made to stand out more clearly because of the fact that at the lower concentration the animals of all types lived about twice as long as they did in the higher concentration. Table XV and Graph XIV show that the pituitaryless died first, the controls followed considerably later, and then the cut controls considerably after the controls.

The general summary shows that with respect to length (Table XVI) the pituitaryless tadpoles are consistently smaller than the controls and cut controls except in the case of group II where the controls and cut controls were partially starved. In the first two groups the cut controls are longer than the controls, while in the combined third and fourth group the mean length of the controls is just the same as the mean length of the cut controls. With respect to the hours of life in KCN (Table XVII), the pituitaryless tadpoles are seen to have died more quickly than the controls and cut controls in the last three groups, while in the first group, that of the normally fed tadpoles, the pituitaryless tadpoles lived the longest. In the first group, the differences between the three types are, however, less marked than in any of the other groups. In the first group the cut controls died before the controls, while in the other three groups the controls died more quickly than the cut controls. The summary is given graphically in Graph XV.

DISCUSSION AND CONCLUSIONS

The purpose of running the double controls on all the experiments was to ascertain that the effects noted in the case of the pituitaryless tadpoles were not due merely to the mechanical effect of the operation. The experiments show that the behavior of the cut control is somewhat inconsistent, but that the tendency is for the cut controls to remain with the controls rather than to remain with the pituitaryless, both as regards length and susceptibility. As a matter of fact, in most of the cases, the cut controls are on the opposite side of the controls from the pituitaryless in respect to their susceptibility, that is to say, the susceptibility is lessened by the operation rather than speeded up. This might be due to an overgrowth of the pituitary gland as a result of the injury to it in the operation, indicating that hypopituitarism and hyperpituitarism manifest themselves in reciprocal results.

The remarks concerning the effect of pituitary removal are therefore made with the conviction that the results of the operation are actually due to the absence of the gland and not to the operation itself.

It is unfortunate that the figures for the susceptibility of the normally fed tadpoles should be the ones which in themselves cannot be considered of any significance in the comparison of pituitary-less and normal specimens. Perhaps it is to be expected that any group of tadpoles which are allowed to eat as they will, will vary in the condition of their bodies through a greater range than will those in which the conditions are prescribed by rigid control of the diet, On the other hand, it may be that the scattering of the population of the fed tadpoles is due to faulty technique in the experiments. The correction here would be the further reduction of the concentration of the KCN. In the case of the starved tadpoles, the reduction of the concentration had the effect of spreading out the different types without greatly increasing the range through which each group varied within itself. This possibility is made a probability by a comparison of the length relations existing among the starved tadpoles and among those normally fed. The pituitaryless tadpoles are markedly shorter in each case. In the case of the starved tadpoles, this shortened length is coincident with the increased susceptibility. The very marked difference in the length of the normally fed tadpoles might be taken to indicate that there is a difference in the susceptibility which has been hidden by the technique rather than that the tadpoles are actually similar. The settlement of this point will be the objective in further experimentation.

The writer is somewhat at a loss to account for the relations of susceptibility in group II. The fed pituitaryless tadpoles behaved about as the fed pituitaryless tadpoles of group I, and their position can be considered normal for fed pituitaryless tadpoles killed in M/8000 KCN. The semi-starved controls and cut controls, however, lived far beyond anything attained by the normally fed controls and cut controls killed at the same concentration. If they were actually suffering from the effects of starvation, we should expect a lessened resistance, as in the case of the completely starved animals.

If one is willing to accept the authority of Child that susceptibility is dependent upon metabolic rate, a fairly satisfactory explanation can be offered, but with disproving of Child's work this explanation is put on an unsound basis. In the case of human beings, at least, it is known that starvation decreases basal metabolism (9). Supposing this to be true in the frog larvae, the decrease in food supply would be responded to by a decreased basal metabolism and lessened susceptibility. This condition is expressed by the semi-starved controls and cut controls. The animals were getting enough food, however, to forestall the condition of lessened resistance which is dependent, not upon an increased metabolism, but upon a decreased vitality found in completely starved animals. This decreased vitality may be due to the consumption of the reserve food supply in the body and of the tissues themselves. The semi-starved tadpoles do not reach this emergency, so their normal vitality is retained, and the resistance to poison is augmented by the lessened basal metabolism.

The results in the third and fourth parts of the experiment are very marked and are considered to be the most reliable demonstrations of the entire experiment. As was stated earlier in the paper, it is reasonable to assume that the results obtained in group III, although they are not very significant in themselves, are a true indication of the conditions prevailing, since the relations revealed are the same as those which are brought out more clearly when the action of the poison is less drastic. It is assumed to be true that the effect of starvation is the same upon pituitaryless tadpoles and controls so far as susceptibility to KCN is concerned, although the possibility is granted that there may be a direct association between starvation and susceptibility with or without the presence of the pituitary gland.

In the second part of this paper, it is shown that the removal of the pituitary gland has a direct effect on the survival of the tadpoles.

A suggestive situation is found when the death point of the individual starved tadpole is plotted against their length (Graphs XVI and XVII), the concentration of the poison being the same for all tadpoles on the graph. In the case of both those animals which were killed in M/8000 and M/16000 KCN, it is noticeable that there is a marked tendency among the tadpoles of all three types alike to increase the number of hours of life in KCN as their length increases. This would seem to indicate that both time and length were being influenced by the same factor. It bears out even within the separate groups the condition that is observed in dealing with the whole groups, which is that the shorter tadpoles, which are the pituitaryless, are the first to die, followed by the longer controls and cut controls. This situation does not show clearly within the separate groups of ~~fed~~ tadpoles.

SUMMARY

The evidence given by these experiments is in perfect accord with the statements made by previous writers that the removal of the pituitary gland lowers the growth rate of the tadpoles. In the case of the starved tadpoles the conclusion is thoroughly justified that the removal of the pituitary gland causes an increased susceptibility to KCN. The evidence is less conclusive in the case of the fed tadpoles, but it is probable that the great difference in the susceptibility of the fed pituitaryless tadpoles and the semi-starved tadpoles of group II represents both the effect of partial starvation and a type difference similar to the difference between starved pituitaryless and starved control tadpoles.

PART II.

THE SURVIVAL AND ACTIVITY OF STARVED PITUITARYLESS TADPOLES

The purpose of this experiment was to see if there could be found any significant difference in the survival and activity of pituitaryless and cut control tadpoles which were being allowed to starve to death. Thirteen pituitaryless and thirteen cut control tadpoles were put together in a clean dish as soon as the color sign had indicated those in which the pituitary removal was successful. No food was given to these animals at any time, but the food supply within the body was sufficient to prolong life for a considerable period. The dish was kept clean by frequent changing of the water, and the debris in the dish was removed with a siphon several times a day throughout the experiment. As each animal died, the body was removed and the trunk length was recorded.

The criterion of the activity of the tadpoles was the response evoked by attempting to roll the animal onto its back with a blunt needle. If the animal remained on its back for a period of fifteen seconds, it was given a negative rating. If it succeeded in righting itself within the quarter minute, or if five attempts to upset it were futile, it was given a positive rating.

The figures for the size and survival of the tadpoles are given in Table XVIII and are compared graphically in Graph XVIII. The comparison of activity is shown in Table XIX.

DISCUSSION

The results obtained in this experiment are very clear. In the first place, it is to be noted that the pituitaryless tadpoles are shorter than the cut control tadpoles when they died. This figure cannot be taken to mean much, however, as it is not known whether they were shrinking or still growing after the twenty-fourth day, when the first death occurred. More significant is the second observation, namely, that the pituitaryless tadpoles showed a tendency to die earlier after the operation than the cut controls. The fact that the last pituitaryless specimen and the last three cut controls lived so much longer than the others is probably an indication that they were guilty of having eaten the body of the one cut control animal that disappeared over night, obtaining therefrom sufficient nourishment to prolong their lives the several days. The extreme longevity of these four animals throws the numerical ratio of the survival of the pituitaryless to the cut control tadpoles somewhat unjustly in favor of the longer life of the cut controls, but examination of the graph shows that even disregarding the extreme figures, the pituitaryless tadpoles are concentrated around an earlier death. The mean survival of the animals might be more accurately approximated if the four last animals were considered to have died on the thirtieth day, when the lost body was consumed. Even this manipulation would show the pituitaryless specimen dying ahead of the cut controls. From this we can conclude that the wasting of the body proceeds more rapidly in starved pituitaryless tadpoles than in starved cut control tadpoles.

The comparison of the activity of the starved tadpoles is almost equally interesting. Beginning on the day of the first death in the group, daily tests were made which consistently showed that more of the cut control tadpoles were able to right themselves when overturned than of the pituitaryless tadpoles. This would indicate that in spite of the fact that the pituitaryless tadpoles died before the cut controls, there was far less expenditure of muscular effort and hence a minimum of body fuel consumption is represented in muscular activity.

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TABLE I.

LENGTH AND AGE OF CONTROL TADPOLES. GROUP I

	LENGTH IN MMS.		AGE IN DAYS AFTER OPERATION	
	Group I-a-1	Group I-b-1	Group I-a-1	Group I-B-1
1	6.8	8.4	23	31
2	8.3	7.6	24	31
3	7.3	8.0	25	31
4	8.0	7.6	30	32
5	7.9	8.7	30	32
6	8.2	7.2	30	32
7	8.7	7.9	31	32
8	8.5	9.1	31	32
9	7.8	9.0	31	32
10	8.8	7.6	32	33
11	8.5	9.2	32	33
12	7.6	8.3	32	33
13	7.0	8.2	23	33
14	7.2	8.1	24	33
15	7.0	7.4	25	33
16		8.7		34
17		9.9		34
18		10.0		34
19		9.1		34
20		7.3		34
21		9.1		34
22		8.4		34
23		8.3		34
24		8.2		34
25		8.0		34
26		8.4		34
27		7.4		34
28		9.5		34
29		8.6		34
30		8.5		34
31		7.5		34
32		8.0		34
Mean	7.84	8.38	28.2	33.1

TABLE II

LENGTH AND AGE OF CUT CONTROL TADPOLES. GROUP I

	LENGTH IN MMS.		AGE IN DAYS AFTER OPERATION	
	Group I-a-2	Group I-b-2	Group I-a-2	Group I-b-2
1	7.0	8.0	23	31
2	8.5	8.6	24	31
3	8.2	7.3	25	31
4	8.5	8.5	30	32
5	8.6	8.3	30	31
6	8.7	8.0	30	32
7	9.1	8.4	31	32
8	8.8	9.2	31	32
9	8.0	8.4	31	32
10	10.0	8.6	32	32
11	8.9	8.3	32	33
12	8.4	8.2	32	33
13	7.2	9.2	23	33
14	7.3	10.0	24	33
15	7.8	8.2	25	33
16		11.0		33
17		7.7		33
18		8.8		33
19		8.4		33
20		8.8		33
21		8.9		33
22		8.8		33
23		8.6		33
24		8.0		33
25		9.1		33
26		7.7		33
27		8.4		33
28		8.9		33
29		9.0		33
30		9.5		33
31		9.4		33
32		8.7		33
Mean	8.33	8.66	28.2	32.6

TABLE III.

LENGTH AND AGE OF PITUITARYLESS TADPOLES. GROUP I.

	LENGTH IN MMS.		AGE IN DAYS AFTER OPERATION	
	Group I-a-3	Group I-b-3	Group I-a-2	Group I-b-2
1	7.6	7.2	23	31
2	7.3	8.3	23	31
3	8.0	8.0	24	31
4	8.0	8.1	24	31
5	8.0	9.0	25	31
6	7.0	7.0	25	31
7	7.6	8.2	30	32
8	7.3	9.5	30	32
9	8.0	7.9	30	32
10	8.3	7.9	30	32
11	8.3	7.0	30	31
12	8.5	7.4	30	32
13	9.5	7.3	31	32
14	7.2	8.0	31	32
15	7.7	8.9	31	32
16	7.5	6.9	31	32
17	7.2	7.4	31	32
18	7.0	7.0	31	32
19	7.2	9.5	32	32
20	6.4	8.1	32	33
21	7.3	7.4	32	33
22	11.1	7.8	32	33
23	6.9	6.7	32	33
24	6.0	7.0	32	33
25	6.8	8.0	23	33
26	7.2	6.0	23	33
27	8.0	6.4	24	33
28	7.2	5.5	24	33
29	7.8		30	
30	7.8		30	
Mean	7.72	7.62	28.5	32.1

TABLE IV.

LENGTH AND AGE OF PITUITARYLESS TADPOLES. GROUPS I AND II

	LENGTH IN MMS.		AGE IN DAYS AFTER OPERATION	
	Group I (a and b)	Group II-3	Group I (a and b)	Group II-3
1		6.8		23
2		5.6		23
3	See Table III	7.1	See Table III	23
4		6.8		23
5		5.6		23
6		6.5		23
7		7.0		23
8		6.0		23
9		5.4		23
10		7.0		23
11		6.7		23
12		7.5		23
13		6.5		23
14		7.9		23
15		7.7		23
16		5.0		23
17		7.1		23
18		6.4		23
19		6.4		22
20		6.7		22
21		7.0		22
22		8.0		22
23		7.9		22
24		7.1		22
Mean	7.67	6.74	30.2	22.9

TABLE V.

LENGTH OF FED TADPOLES IN MMS. GROUP I

	p	cc	c
1	7.6	7.0	6.8
2	7.3	8.5	8.3
3	8.0	8.2	7.3
4	8.0	8.5	8.0
5	8.0	8.6	7.9
6	7.0	8.7	8.2
7	7.6	9.1	8.7
8	7.3	8.8	8.5
9	8.0	8.0	7.8
10	8.3	10.0	8.8
11	8.3	8.9	8.5
12	8.5	8.4	7.6
13	9.5	7.2	7.0
14	9.2	7.3	7.2
15	7.7	7.8	7.0
16	7.5	8.0	8.4
17	7.2	8.6	7.6
18	7.0	7.3	8.0
19	7.2	8.5	7.6
20	6.4	8.3	8.7
21	7.3	8.0	7.2
22	11.1	8.4	7.9
23	6.9	9.2	9.1
24	6.0	8.4	9.0
25	6.8	8.6	7.6
26	7.2	8.3	9.2
27	8.0	8.2	8.3
28	7.2	9.2	8.2
29	7.8	10.0	8.1
30	7.8	8.2	7.4
31	7.2	11.0	8.7
32	8.3	7.7	9.9
33	8.0	8.8	10.0
34	8.1	8.4	9.1
35	9.0	8.8	7.3

(Table continued - following page)

TABLE V - continued

	p	cc	c
36	7.0	8.9	9.1
37	8.2	8.8	8.4
38	9.5	8.6	8.3
39	7.9	8.0	9.2
40	7.9	9.1	8.0
41	7.0	7.7	8.4
42	7.4	8.4	7.4
43	7.3	8.9	9.5
44	8.0	9.0	8.6
45	8.9	9.5	8.5
46	6.9	9.4	7.5
47	7.4	8.7	8.0
48	7.0		
49	9.5		
50	8.1		
51	7.4		
52	7.8		
53	6.7		
54	7.0		
55	8.0		
56	6.0		
57	6.4		
58	5.5		
Mean	7.67	8.55	8.21

TABLE VI.

HOURS ALIVE IN KCN, FED TADPOLES, GROUP I

	p	cc	c
1	5.00	9.00	9.00
2	7.25	4.25	4.25
3	4.75	8.75	9.25
4	3.75	4.75	3.75
5	5.25	3.25	3.75
6	6.25	8.25	3.25
7	3.25	3.00	3.50
8	4.75	3.00	3.00
9	3.25	3.00	3.50
10	5.75	4.50	4.00
11	3.25	3.50	4.00
12	4.75	5.50	6.00
13	2.50	5.50	5.00
14	3.50	4.50	4.50
15	4.00	6.00	5.50
16	3.50	4.50	5.00
17	4.50	6.00	4.50
18	3.50	3.00	3.50
19	4.50	4.50	4.00
20	6.00	5.00	3.50
21	6.00	4.00	3.50
22	4.00	5.50	6.00
23	5.00	5.00	3.50
24	4.00	6.00	3.50
25	8.00	8.00	4.50
26	7.00	2.50	4.50
27	7.50	3.50	3.50
28	8.50	3.50	3.00
29	4.50	3.50	3.50
30	7.50	3.50	3.50
31	5.00	3.50	3.50
32	5.50	4.00	4.00
33	4.00	4.00	4.00

(Table continued - following page)

TABLE VI. - continued

	p	cc	c
34	4.50	4.00	4.00
35	5.00	4.00	4.50
36	5.50	4.50	5.00
37	4.00	4.50	5.00
38	4.00	4.50	6.00
39	4.00	4.50	6.50
40	4.00	4.50	6.50
41	4.00	5.00	7.00
42	3.50	5.00	7.50
43	3.00	5.50	8.50
44	5.00	6.00	9.50
45	6.00		
46	6.00		
47	4.50		
48	4.50		
49	5.50		
50	3.50		
51	7.50		
52	11.50		
Mean	5.024	4.733	4.846

TABLE VII

LENGTH AND AGE OF FED AND SEMI-STARVED CONTROL TADPOLES

	LENGTH IN MMS		AGE IN DAYS AFTER OPERATION	
	Group I-1	Group II-1	Group I-1	Group II-1
1	See Table V	6.6	See Table I	23
2		6.2		23
3		6.1		23
4		6.4		23
5		5.9		23
6		5.7		23
7		6.2		23
8		6.5		23
9		6.4		23
10		5.9		23
11		5.2		23
12		6.8		23
13		6.0		23
14		5.8		23
15		5.9		23
16		6.2		23
17		6.2		23
18		5.6		23
19		8.3		21
20		6.1		21
21		6.4		21
Mean	8.21	6.18	31.5	22.7

TABLE VIII

LENGTH AND AGE OF FED AND SEMI-STARVED CUT CONTROLS

	LENGTH IN MMS.		AGE IN DAYS AFTER OPERATION	
	Group I-2	Group II-2	Group I-2	Group II-2
1	See Table V.	6.3	See Table II	22
2		6.4		22
3		6.0		22
4		6.8		22
5		6.5		22
6		6.1		22
7		6.2		22
8		6.6		22
9		6.4		22
10		6.1		22
11		6.1		22
12		6.6		22
13		7.0		22
14		6.1		22
15		6.1		22
16		6.5		22
17		6.8		22
18		6.3		22
19		6.3		21
20		6.1		21
21		6.1		21
Mean	8.55	6.35	31.2	21.9

TABLE IX

LENGTH OF TADPOLES IN GROUP II

	p	cc	c
	See Table IV	See Table VIII	See Table VII
Mean	6.74	6.35	6.18

TABLE X

HOURS ALIVE IN KCN. FED CONTROLS AND SEMI-STARVED CONTROLS

	Group I-1	Group II-1
1	See Table VI	5.50
2		5.50
3		6.00
4		6.00
5		7.00
6		7.50
7		7.50
8		7.50
9		8.00
10		8.00
11		8.00
12		8.00
13		8.50
14		8.50
15		8.50
16		8.50
17		10.00
18		10.00
19		5.50
20		4.50
21		4.50
Mean	4.85	7.29

TABLE XI

HOURS ALIVE IN KCN. FED CUT CONTROLS AND SEMI-STARVED CUT CONTROLS

	Group I-2	Group II-2
1	See Table VI	4.50
2		4.50
3		4.50
4		4.50
5		4.50
6		4.50
7		5.50
8		6.00
9		6.00
10		6.50
11		7.00
12		7.00
13		7.00
14		7.00
15		7.00
16		7.00
17		8.00
18		8.50
19		4.50
20		4.50
21		4.50
Mean	4.73	5.87

TABLE XII

HOURS ALIVE IN KCN. GROUP II PITUITARYLESS AND GROUP II SEMI-STARVED CONTROL AND SEMI-STARVED CUT CONTROL

	p	cc	c
1	3.00	See Table XI	See Table X
2	4.00		
3	4.00		
4	4.00		
5	4.50		
6	5.00		
7	5.00		
8	5.50		
9	5.50		
10	5.50		
11	5.50		
12	5.50		
13	6.00		
14	6.50		
15	7.00		
16	7.50		
17	8.00		
18	8.50		
19	4.50		
20	5.00		
21	5.00		
22	4.00		
23	6.00		
24	4.50		
Mean	5.44	5.87	7.29

TABLE XIII

LENGTH OF STARVED TADPOLES

	p	cc	c
1	5.4	6.0	5.7
2	5.6	6.0	5.5
3	5.5	6.2	5.6
4	5.0	5.7	5.8
5	5.1	5.5	5.9
6	5.4	5.2	5.1
7	5.0	5.5	5.4
8	5.8	5.2	5.2
9	5.4	5.5	5.5
10	5.0	5.4	5.9
11	5.0	5.0	5.5
12	5.1	5.2	5.3
13	5.1	5.0	5.5
14	5.2	5.4	5.1
15	5.0	5.2	5.1
16	4.8		
17	5.0		
18	5.0		
19	5.2		
20	5.0		
21	4.9		
22	5.0		
23	5.4		
24	5.4		
25	4.8		
26	5.0		
27	5.0		
28	5.8		
29	4.9		
30	5.2		
Mean	5.17	5.47	5.47

TABLE XIV

HOURS ALIVE IN KCN. STARVED TADPOLES IN M/8000 KCN, GROUP III.

	p	cc	c
1	2.25	2.75	2.75
2	2.00	2.50	4.00
3	3.00	2.50	2.00
4	3.00	5.00	2.50
5	2.75		
6	4.50		
7	2.00		
8	2.50		
Mean	2.75	3.19	2.81

TABLE XV.

HOURS ALIVE IN KCN - STARVED TADPOLES
IN M/16000 KCN., GROUP IV

	p	cc	c
1	4.00	7.25	7.25
2	9.75	8.25	7.25
3	2.75	7.50	5.00
4	2.25	7.50	7.00
5	5.00	6.50	7.50
6	2.50	6.50	6.00
7	3.00		
8	6.75		
9	3.50		
10	3.50		
11	6.50		
12	4.00		
Mean	4.46	7.25	6.67

TABLE XVI.

GENERAL SUMMARY - LENGTH IN MMS.

	p	cc	c
Group I (Fed Tadpoles)	7.67	8.55	8.21
Group II (Fed p, semi-starved cc, semistarved c)	6.74	6.35	6.18
Groups III and IV (Starved tadpoles)	5.17	5.47	5.47

TABLE XVII.

GENERAL SUMMARY -- HOURS ALIVE IN KCN

	p	cc	c
Group I (Fed tadpoles)	5.02	4.73	4.85
Group II (Fed p, semi-starved cc, semistarved c)	5.44	5.87	7.29
Group III (Starved tadpoles in M/8000 KCN)	2.75	3.19	2.81
Group IV (Starved tadpoles in M/16000 KCN)	4.46	7.25	6.67

TABLE XVIII

THE SIZE AND SURVIVAL OF STARVED TADPOLES

	LENGTH IN MMS		AGE IN DAYS AFTER OPERATION	
	p	cc	p	cc
1	3.6	3.7	27	24
2	3.5	3.8	27	27
3	3.2	3.8	27	27
4	3.1	3.5	27	28
5	3.1	3.3	28	28
6	3.4	3.5	28	29
7	3.4	3.8	28	29
8	3.4	3.9	28	30
9	3.4	3.6	28	32
10	3.5	lost	30	lost
11	3.5	3.8	30	45
12	3.9	4.0	33	46
13	3.7	4.1	48	47
Mean	3.44	3.73	29.9	32.7

TABLE XIX

BEHAVIOR OF STARVED TADPOLES

Pituitaryless

	Days After Operation																								
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	o
2	x	x	-	x	-	-	-	-	-	o															
3	x	-	-	-	-	-	o																		
4	x	-	-	-	-	-	o																		
5	-	-	-	-	o																				
6	-	-	-	-	o																				
7	-	-	-	-	o																				
8	-	-	-	-	o																				
9	-	-	-	-	o																				
10	-	-	-	-	o																				
11	-	-	-	o																					
12	-	-	-	o																					
13	-	-	-	o																					

Cut Control

	Days after Operation																								
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	-	o	
2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	-	-	-	o	
3	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	x	-	-	-	-	o			
4	x	x	x	x	x	x	x	x	x	x	x	x	o												
5	x	x	x	x	x	x	x	-	o																
6	x	x	x	-	-	-	o																		
7	x	x	x	-	-	o																			
8	x	x	-	-	o																				
9	x	x	-	-	o																				
10	x	--	-	-	o																				
11	-	-	-	o																					
12	-	-	-	o																					
13	-	o																							

x = positive response. - = negative response. o = dead.

GRAPH I.

Showing the approximate direct proportion between age and length of normally fed control tadpoles.

Group I-a-1 (o)

Group I-b-1 (x)

Note: The slight discrepancy of the lines (they do not run quite through the center of distribution) is due to the fact that they are constructed as straight lines from the starting point to the numerical mean, whereas they actually should curve to follow the distribution. The starting point for all the growth lines is taken to be the day of the operation, for up to that time the development of the animals has been similar. These comments apply to all the graphs in which length is plotted against age

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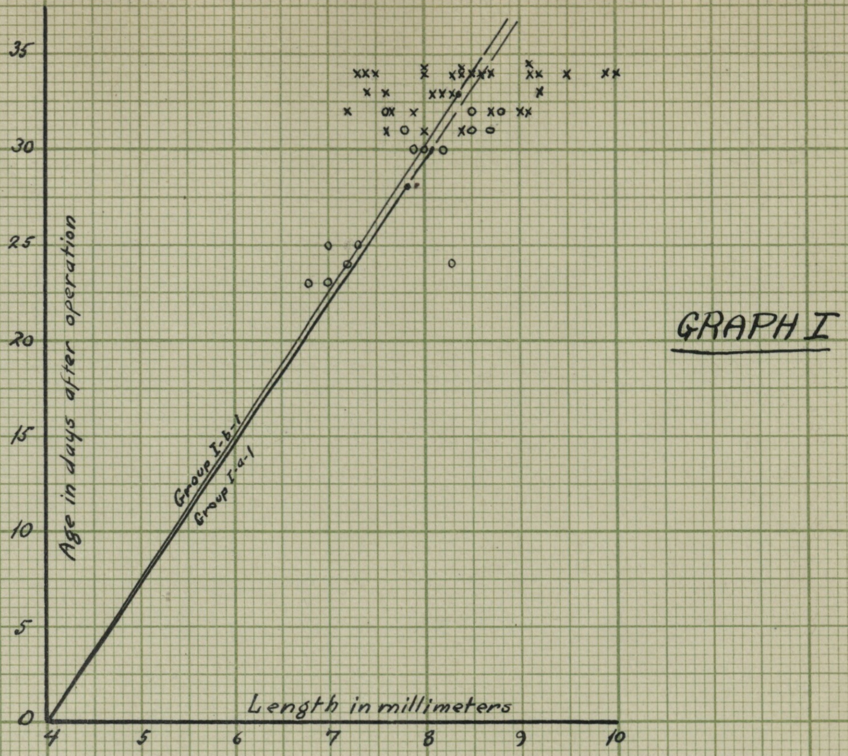
GRAPH II

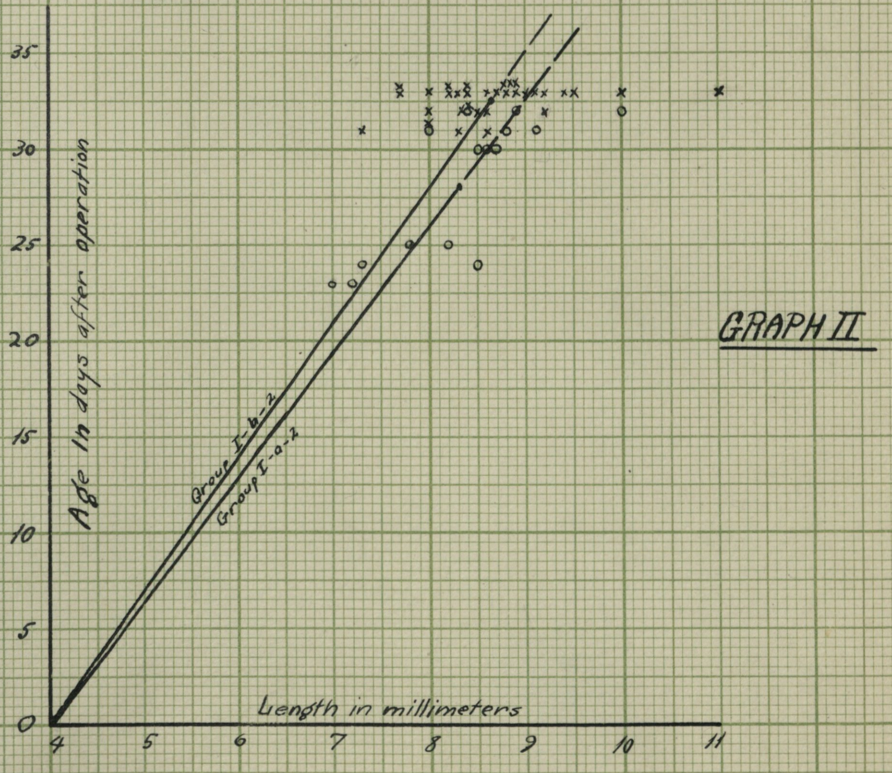
Showing the approximate direct proportion between age and length of normally fed cut control tadpoles.

Group I-a-2 (o)

Group I-b-2 (x)

See note with Graph I.


GRAPH I
Age in days after operation
Length in millimeters
Group I-b-1
Group I-a-1
0
5
10
15
20
25
30
35
4
5
6
7
8
9
10


GRAPH II
Age in days after operation
Length in millimeters
Group I-b-2
Group I-a-2
0
5
10
15
20
25
30
35
4
5
6
7
8
9
10
11

GRAPH III.

Showing the approximate direct proportion between the age and length of normally fed pituitaryless tadpoles.

Group I-a-3 (0)

Group I-b-3 (x)

See note with Graph I.

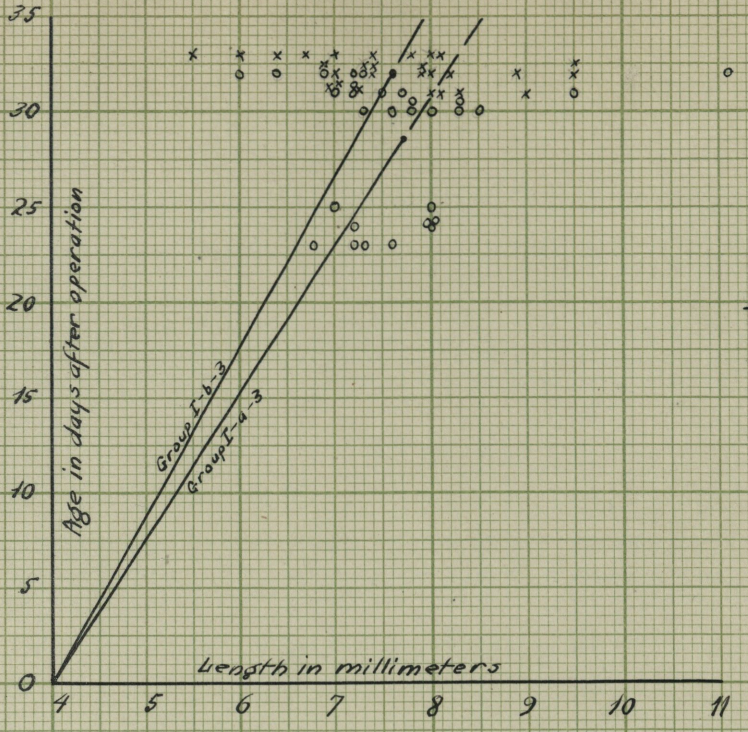
GRAPH IV.

Showing that the difference between the lengths of the fed pituitaryless tadpoles of groups I and II is due only to a difference in age.

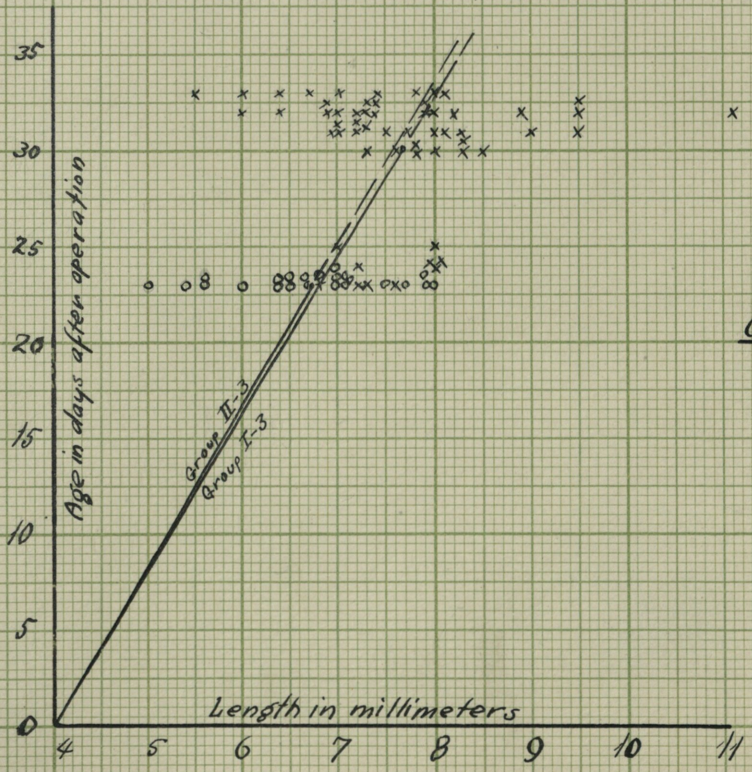
Group I (x)

Group II (o)

See note with Graph I.



GRAPH III



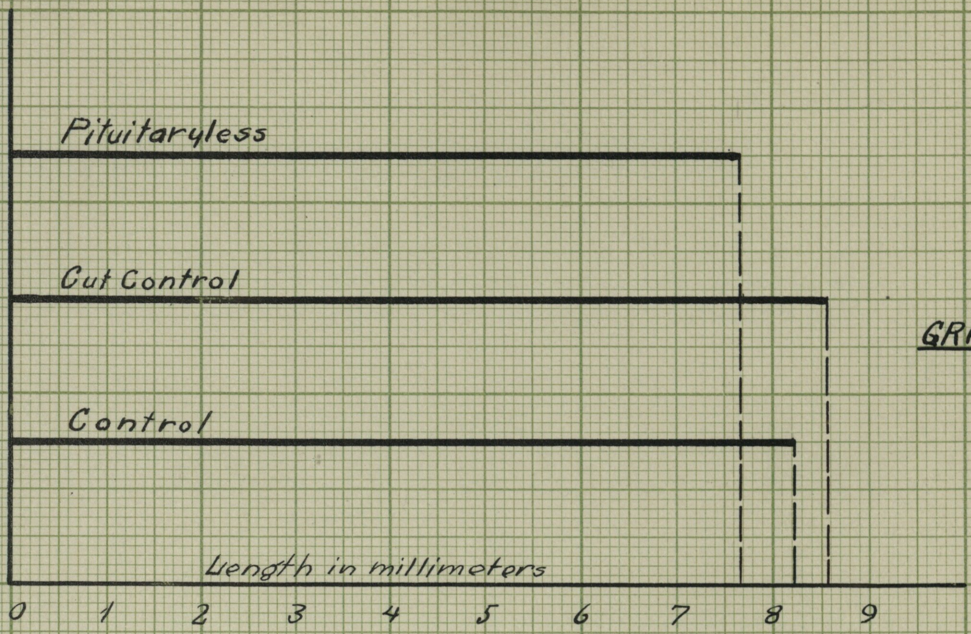
GRAPH IV

GRAPH V.

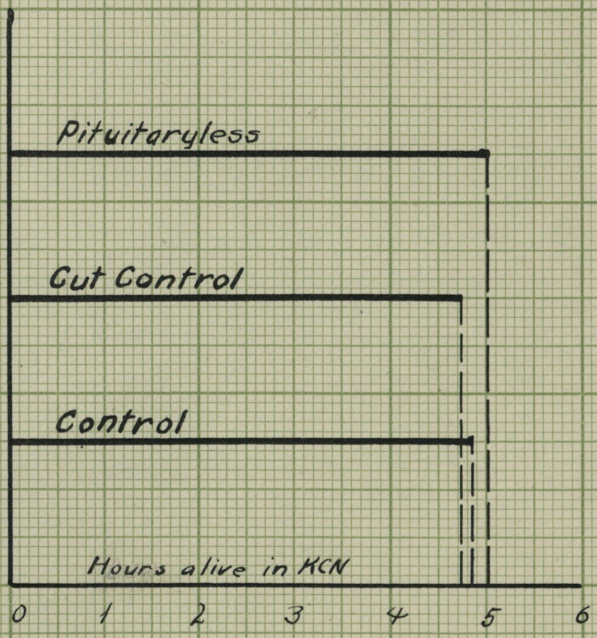
A comparison of the length of the fed pituitaryless, fed control, and fed cut control tadpoles of group I.

GRAPH VI.

A comparison of the number of hours during which the fed pituitaryless, control and cut control tadpoles of group 1 were alive in M/8000 KCN.



GRAPH V



GRAPH VI

GRAPH VI a.

Showing the distribution of the death points of the fed pituitaryless, control, and cut control tadpoles killed in M/8000 KCN. The vertical lines represent the first and third quartile points.


0 1 2 3 4 5 6 7 8 9 10 11 12
Hours alive in KCN
Pituitaryless
Cut Control
Control

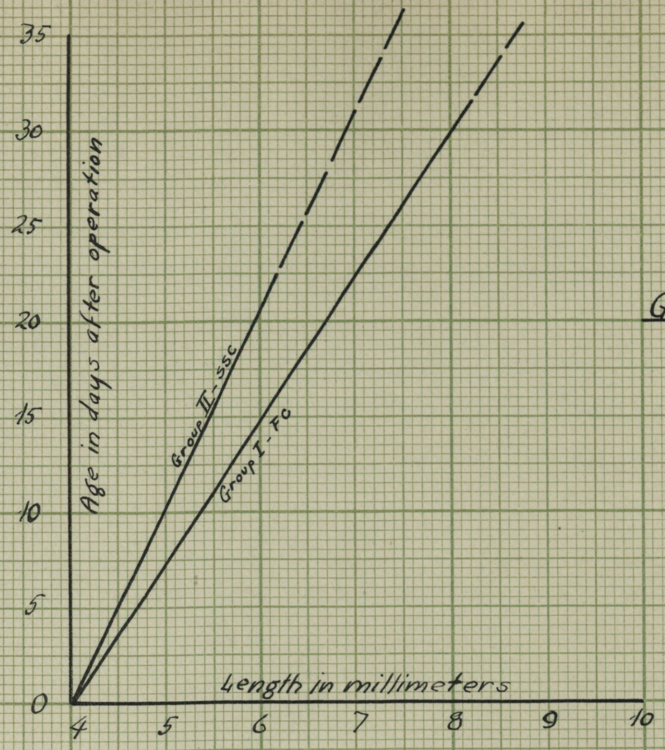
GRAPH VI a

GRAPH VII.

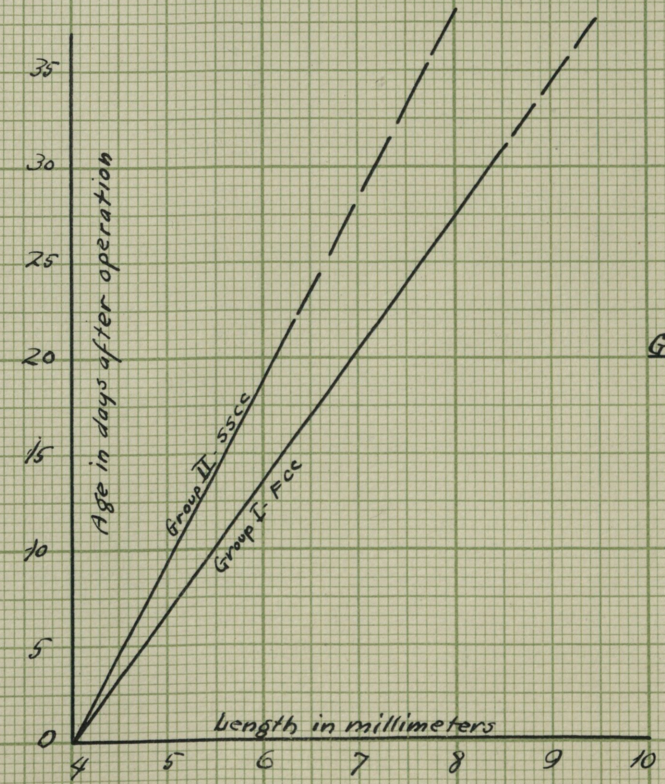
A comparison of the fed controls of group I with the semi-starved control of group II. The lines represent the mean approximate growth curve.

GRAPH VIII

A comparison of the fed cut controls of group I with the semi-starved cut controls of group II. The lines represent the mean approximate growth curves.



GRAPH VII



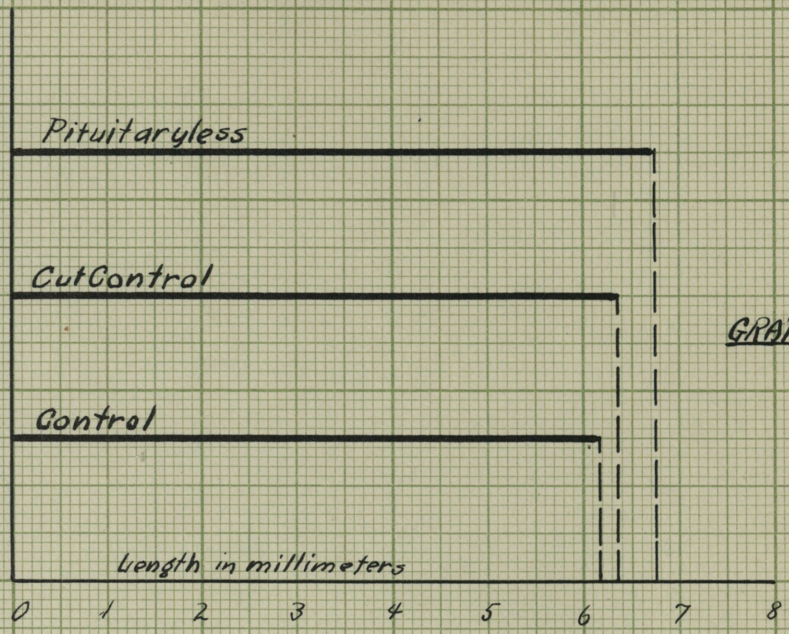
GRAPH VIII

GRAPH IX

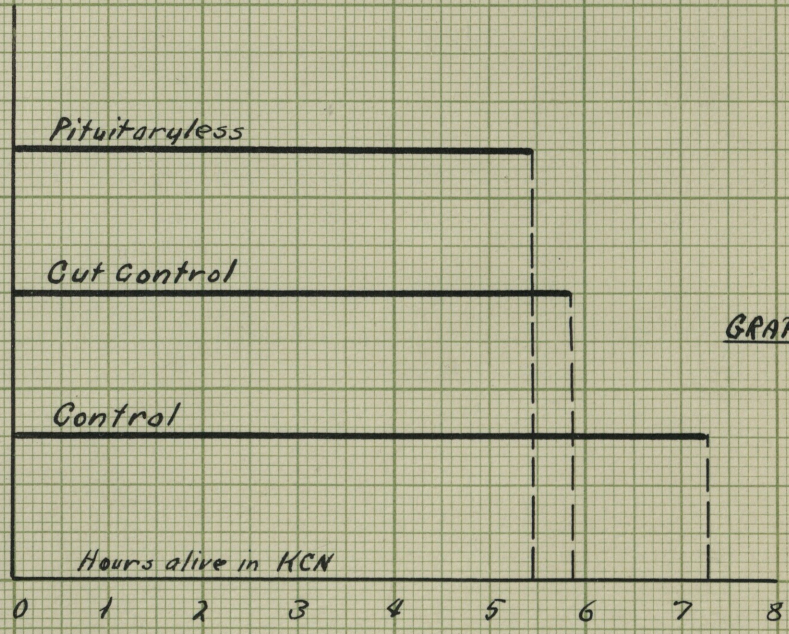
Showing the comparative length of the fed pituitaryless, semi-starved control, and semi-starved cut control tadpoles, of group II.

GRAPH X.

A comparison of the number of hours during which the fed pituitaryless, semi-starved control, semi-starved cut control tadpoles were alive in M/8000 KCN.



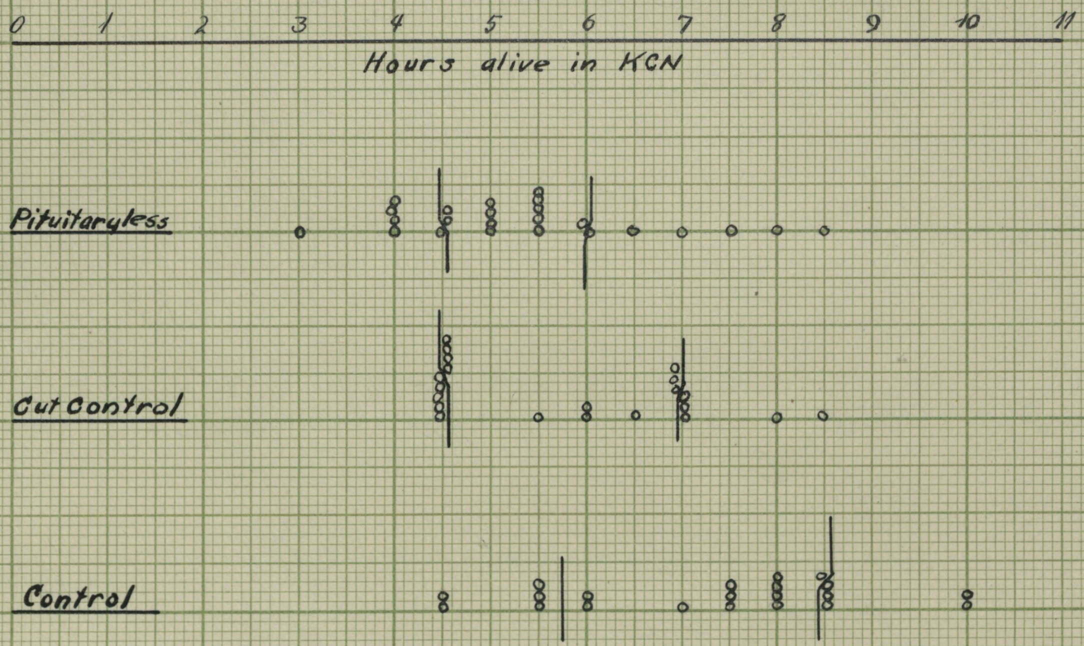
GRAPH IX



GRAPH X

GRAPH XI.

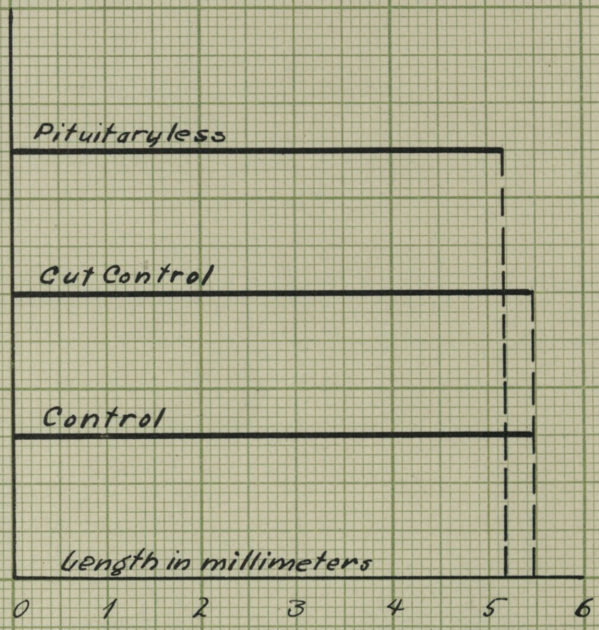
Showing the distribution of the death points of the fed pituitaryless tadpoles, semi-starved cut controls of gropu II, killed in M/8000 KCN. The vertical lines represent the first and third quartile points.


0 1 2 3 4 5 6 7 8 9 10 11
Hours alive in KCN
Pituitaryless
Cut Control
Control

GRAPH XI

GRAPH XII.

A comparison of the length of the starved pituitaryless, control, and cut control tadpoles of groups II and III combined


Pituitaryless
Cut Control
Control
Length in millimeters
0
1
2
3
4
5
6

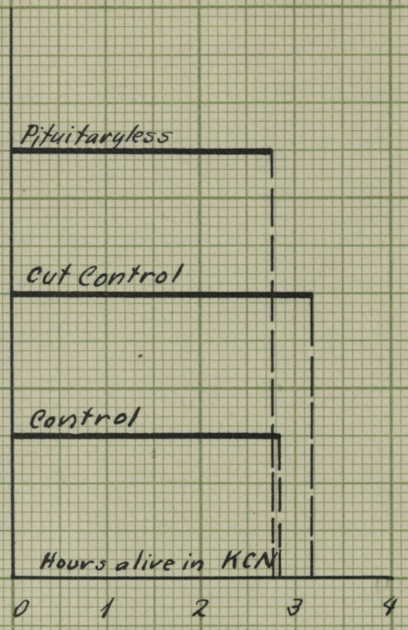
GRAPH XII

GRAPH XIII.

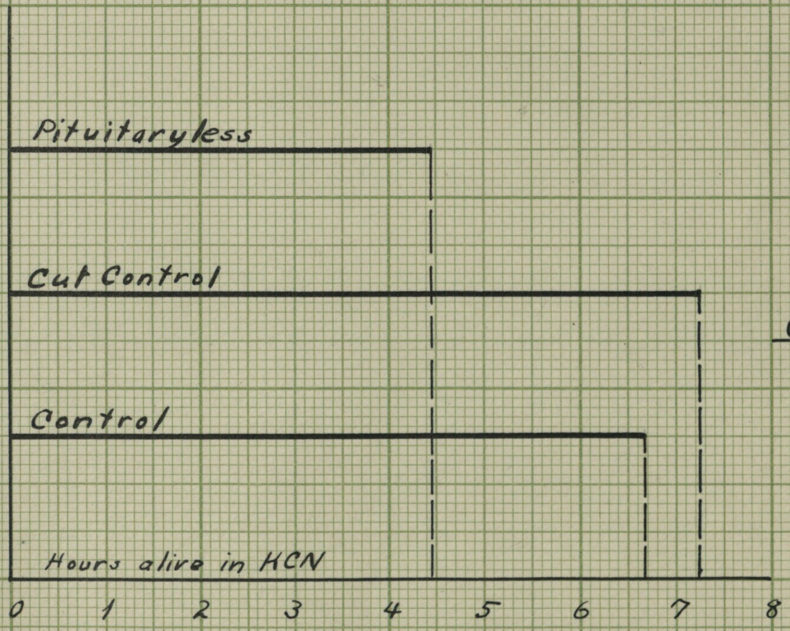
A comparison of the number of hours during which the starved pituitaryless, control, and cut control tadpoles of group III were alive in M/8000 KCN.

GRAPH XIV

A comparison of the number of hours during which the starved pituitaryless, control, and cut control tadpoles of group IV were alive in M/16000 KCN.



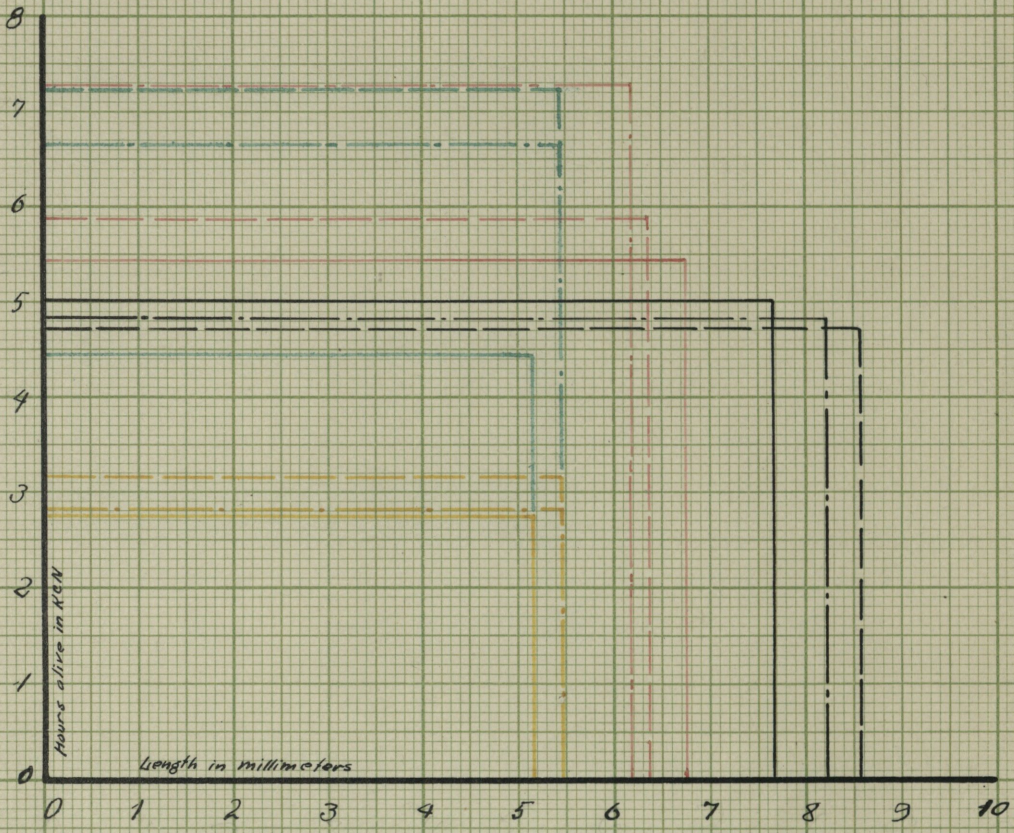
GRAPH XIII



GRAPH XIV

GRAPH XV.

A summary of the comparative lengths and hours of life in varying concentrations of KCN of the tadpoles of groups I, II, III, and IV. The solid line represents the pituitaryless tadpoles, the line of dashes represents the cut control tadpoles, and the line of dots and dashes represents the control tadpoles. The black lines indicate group I, the red lines indicate group II, the yellow lines indicate group III, and the green lines indicate group IV.



GRAPH XV

GRAPH XVI.

Showing the distribution with respect to length and hours of life in KCN of the starved tadpoles of group III, killed in M/8000 KCN

Pituitaryless = o

Control = x

Cut control = ⊗

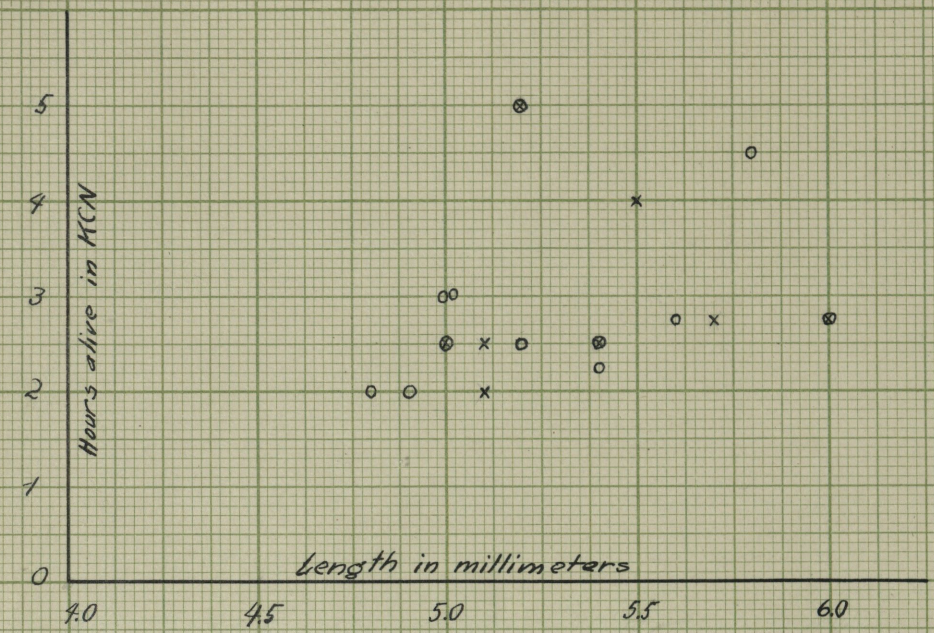
GRAPH XVII.

Showing the distribution with respect to length and hours of life in KCN of the starved tadpoles of group IV, killed in M/16000 KCN.

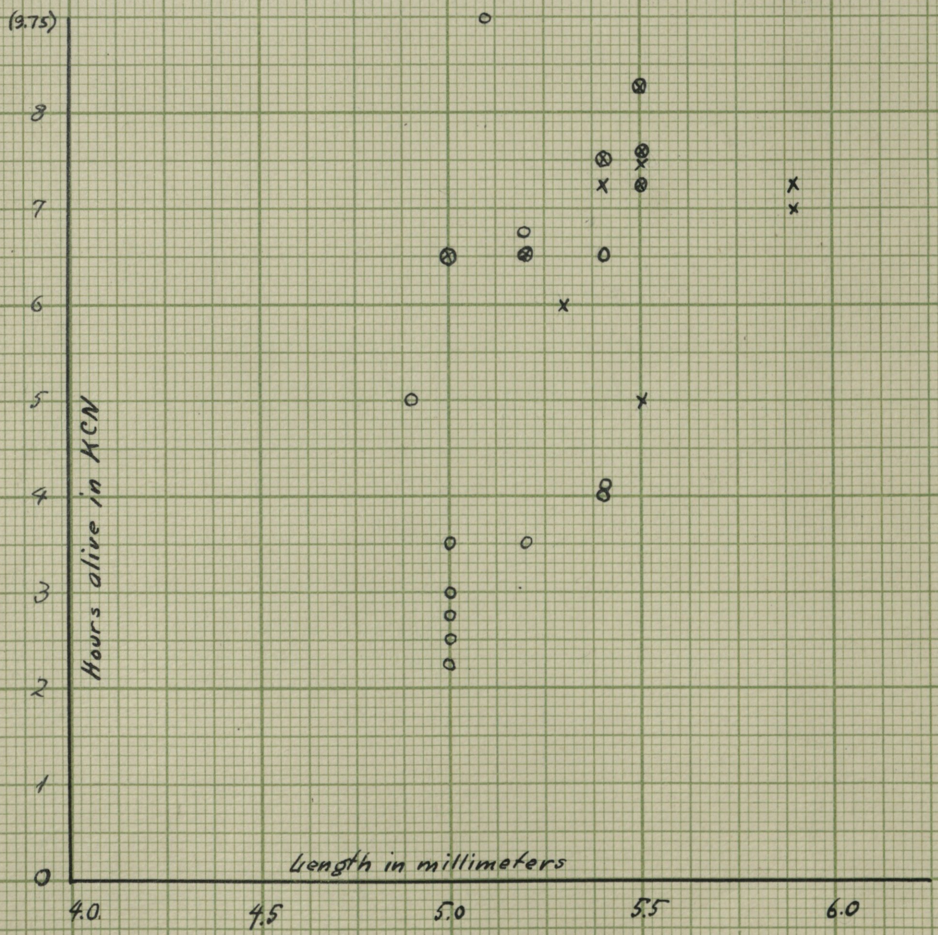
Pituitaryless = o

Control = x

Cut control = ⊗



GRAPH XVI



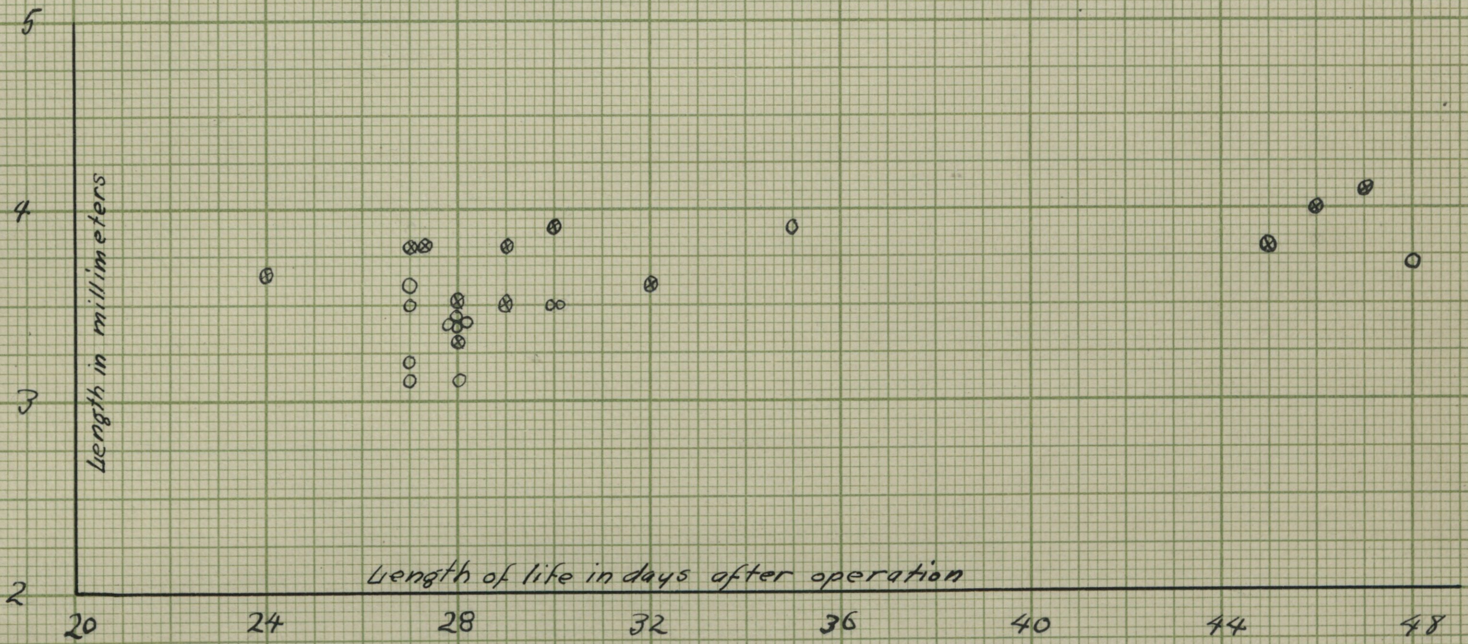
GRAPH XVII

GRAPH XVIII

Showing the distribution with respect to length and days of survival of the tadpoles of Part II.

Pituitaryless = o

Cut control = x


5
4
3
2
Length in millimeters
Length of life in days after operation
20
24
28
32
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GRAPH XVIII