THE IMBIBITION OF WATER BY FROGS

T H E S I S

THE ACTION

OF THE PREPARATIONS FROM THE POSTERIOR LOBE OF THE PITUITARY GLAND UPON THE IMBIBITION OF WATER BY FROGS

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During the past fifteen years several investigators have shown that commercial or laboratory preparations of the posterior lobe of the pituitary gland when injected into frogs give rise to a transient increase in weight.

The weight changes observed vary somewhat in magnitude and duration, due probably to seasonal variations in susceptibility, to differences in the species employed, and to the amount of active substance injected. However, the general sequence of events consists in a rather sudden increase in weight, reaching a maximum usually in from one to five hours, followed by a more gradual return to the normal weight in about eight to twenty-four hours, though increases lasting for several days have been reported.

It is a matter of interest to know which constituent of the posterior lobe is responsible for these weight changes. Brunn (1), on observing that pituglandol gave about twice as great an increase as did corresponding doses of two other preparations (hypophysin and pituitrin), suggested that the effect paralleled the intermediate lobe content. Unfortunately he gives no data concerning the relative intermediate lobe contents of the three preparations. Bělehrádek and Huxley (2), who compared the effects of pituitrin on larval and metamorphosed Amblystoma, observed that the weight changes coincided with the melanophore changes. Steggerda (3) considered that the increase in weight might be due to an increased skin permeability brought about in some way by the expansion of the melanophores; this would indicate that the melanophore-dilating constituent is the responsible agent.

In 1930, Heller (4) studied the effects of the pressor and oxytocic preparations and found that the oxytocic fraction caused a greater increase in weight than the pressor. This was confirmed by Steggerda (5) who reported also that the activity of pituitrin was intermediate to those of its two components; unfortunately he has expressed his doses in cubic centimeters instead of units and as the preparations employed are put up in various strengths, one can only guess at the relative quantities of the constituents which he used. Novelli (6), on the other hand, using toads, found the oxytocic substance to be only a third as effective as the pressor. As all three of these investigators obtained an effect with the oxytocic preparations, it would seem that the melanophore hormone plays no part in causing the increase in weight in frogs, since it is not contained in this fraction.

Since pituitary extracts have been shown to have an antidiuretic effect in unanethetized mammals, a similar action in amphibia would explain the weight increases; absorption through the skin might not be compensated by excretion by the kidneys. This hypothesis receives some support from the perfusion experiments of Brunn (1) and the observations of Richards and Schmidt (7). Brunn perfused the isolated kidney from the renal artery and noted a constriction of the glomerular vessels and a diminution of urine secretion when pituitrin was added to the perfusing Ringer. Richards and Schmidt, by direct microscopic examination, found that constrictor doses of pituitrin greatly diminished the number of active glomeruli, while smaller

doses gave an increase. They suggest that minute amounts affect only the more sensitive efferent vessels, causing a rise of glomerular pressure and consequently an increased urine output, while larger doses constrict the afferent vessels as well and so reduce the blood flow through the kidneys.

However, later experiments on the intact frog have shown that apparently the action of pituitary extracts does not involve the kidneys. Fromherz (8) did report a slight antidiuresis, but since the salt concentration of the urine was unaffected, he concluded that the effect was not analogous to that obtained in the higher vertebrates. Brunn (1) extirpated the kidneys and still obtained an increase in weight with injections of pituitary. In another series of experiments he tied off the cloaca, injected pituitrin and withdrew the urine at intervals. The volumes obtained from these and from saline-injected controls were comparable.

Burgess, Harvey and Marshall (9) catheterized the bladders of Rana catesbiana under conditions of normal urine flow and increased urine flow produced by the injection of water into a lymph sac. They found that in neither case did from 0.05 to 2 units per kg. of pitressin or pituitrin have any effect on the urine output. They state that larger doses produced toxic effects, though Heller (10) and Novelli (6) injected 20 units of pituitrin and 50 mg. per 100 gm. of the standard powder, respectively, with no deleterious effects; it is possible that the smaller frogs (esculenta and leptodactylus) which they used are not so sensitive.

Both Biasotti (11) and Steggerda (3) employed another method to demonstrate the absence of any antidiuretic effect. Each took two series of frogs. Half of the first series received an injection of pituitrin and the other half the same volume of saline to serve as controls. In the second series the cloacas were tied off and again half were injected with pituitary while half served as saline controls. The results showed that the normal controls underwent no change in weight. The other three lots all increased, but not to the same extent. Those with tied-off cloacas which had received pituitrin showed by far the greatest increase. Next came the normal pituitary-injected frogs, and finally the controls with tied cloacas. If the weight increasesafter pituitary are due to an antidiuretic effect, one would expect to find no difference between the injected and the control frogs with tied cloacas.

The foregoing review of the literature of the subject in question seems to show on the whole that both the pressor and the oxytocic constituents of the pituitary are involved in the action of pituitary extract upon water imbibition in amphibia, or that a third substance contaminates both. Were the pressor or the oxytocic substance responsible, the active one should be about twenty times as active as the other, since each preparation is contaminated to the extent of approximately five per cent by the other.

About two years ago a new method of separating the pressor and oxytocic substances of the pituitary was developed

in this laboratory (12). By studying the action of these substances in parallel with pitressin and pitocin, it was hoped that it would be possible to determine which of the suggested explanations was most likely true, it being assumed that because of the different procedure used in the new method of separation the third substance - if such a substance exists - would be distributed in different proportions in the pressor and oxytocic fractions, or might even be absent from one or both. On the other hand, if no difference could be detected between the actions of the two pairs of preparations, the conclusion that each has some action of the kind in question would be strengthened. Because of the results obtained in these comparisons, the action of pituitrin was also compared with that of the oxytocic constituent.

In some of the early experiments it appeared that sex influenced the action of the substances studied. Hence this question was investigated in some detail.

The circumstance that a crude preparation was available which was strong in the melanophore-dilating action but weak as concerns vascular and oxytocic actions made it possible to examine its effect also upon the weight of frogs.

Finally, it was considered desirable to determine the effect of pituitary extract upon the elimination of previously injected water. One would expect the elimination to be slowed, and this is indeed the case.

METHOD

The experiments were performed between November and March, using Rana pipiens averaging 40 gm. in weight. During the experiment and for the preceding 12 or 36 hours, the animals were kept in individual cages immersed in water to a depth of about one inch and at room temperature (18-20° C). The solutions to be injected were so diluted with 0.9% sodium chloride solution that 0.5 cc. contained the required number of units. The injections were made into the dorsal lymph sac. The frogs were weighed immediately after the injection and at half-hour intervals until their weights approached normal again. Previous to the injection and to each subsequent weighing, the frogs were dried with a cloth and squeezed gently to expel any urine.

Some 15 frogs were used for each experiment. The average weight at each weighing was calculated from the individual weights, so that the changes could be expressed as the average increase or decrease in percentage of the original weight, or rather the weight immediately after the injection. From the data graphs were constructed, the abscissas being the time in hours and the ordinates the change in weight per cent.

The solutions used were pituitrin, pitocin and pitressin (the Parke, Davis & Co. preparations of the whole lobe, of the oxytocic fraction and of the pressor fraction respectively), and two laboratory preparations, postlobin-O (212), an oxytocic preparation, and postlobin-V (120), a pressor preparation. The melanophore preparation used was obtained as a by-product in separating postlobin-O and postlobin-V and was contaminated to some extent with both these substances.

RESULTS

The results taken as a whole show that both of the known constituents of the posterior lobe, and of course the whole posterior lobe extract, give rise to an increase in weight when injected into the dorsal lymph sac of frogs. The increases vary directly with the strength and the nature of the preparations used and contrast strikingly with the immediate decreases which follow the injection of saline alone (see plate 3).

In practically every instance the rate of increase was decidedly slower for the first half-hour after injection. This is specially noticeable when small doses are given, in which case there may even be an initial loss of weight. This may be the result of defecation caused by the action of pituitary extracts on the gut, and indeed Bělehrádek and Huxley noted such an occurrence in their experiments with Amblystoma. However, this explanation seems rather improbable in the case of the hibernating frog, in which the alimentary tract is probably empty. It seems more likely that slow absorption from the lymph sacs causes the delay.

There was a very marked difference in the reactions of the individual frogs, this being the reason why a fairly large group of frogs was used for each experiment and the average change determined. But even then it is questionable if the average of such a group is strictly accurate. Not only do the frogs increase to different extents, but some may start to lose weight before the others.

When considering the effects it is necessary to bear in mind the limiting factor of a maximal dose. Thus, as is pointed

out in the section on the relative activities of the pressor and oxytocic preparations, one cannot draw any conclusions from the similarity in behavior of 0.25 unit of the oxytocic preparations and 2 units of the pressor, since the large dose in the latter case undoubtedly gives a maximal effect (compare 10A and 11A, plate 1, and 8A and 9A, plate 2).

Four different lots of frogs were used, distinguished in the diagrams as A, B, C and D. Of the first lot, females only were used, while in the second lot a few males were included in the last few experiments. No systematic check was kept, but at the time no difference was apparent between the two sexes. In lots C and D, half males and half females were used and the results of the two sexes were plotted separately. As may be seen from any of the diagrams marked C and D, there is a striking difference between the two sexes which cannot be accounted for by the differences in their average weights alone. However, though the females are much less sensitive the effects of different doses vary with them as they do with the males.

Further differences were noted between experiments performed in different months. The increases in weight noted between November and December were much lower than those for February and March. The first lot of frogs gave the lowest increases of all.

COMPARISON OF THE ACTIVITIES OF PITOCIN AND POSTLOBIN-O

A series of experiments was performed using equivalent doses of pitocin and postlobin-O in order to ascertain if there was any difference between these two oxytocic preparations with regard to their effects upon the water balance. The results are shown in the figures on plate 1. The doses employed were 0.05, 0.1 and 0.25 unit respectively. The intensity of action varied with the dosage but the results obtained with the two preparations were similar. Consequently if a separate substance, contained in the posterior lobe of the pituitary gland, is responsible for the increase in weight noted, this substance must contaminate both oxytocic preparations to the same extent. This is regarded as unlikely in view of the very different technics employed in the preparation of these two oxytocic substances. The action may be attributed with more probability to the oxytocic substance itself.

COMPARISON OF THE ACTIVITIES OF PITRESSIN AND POSTLOBIN-V

In another series of experiments the actions of pitressin and postlobin-V were compared. The results are shown in the figures on plate 2. In figures 8A and 9A the doses employed (2 units) were rather large, so that it is likely that a maximal effect was obtained in both cases, but even when smaller doses were used (0.05 and 0.25 unit) the behavior of the two preparations was very similar, pitressin being slightly stronger in action in the former case and postlobin-V in the latter.

The remarks made in the preceding section concerning the possibility of a substance separate from the oxytocic constituent of the lobe being responsible for the action are applicable in the case of the pressor substance also. Hence it is assumed that the action here described is due to the pressor substance itself.

COMPARISON OF THE ACTIVITIES OF OXYTOCIC AND PRESSOR PREPARATIONS

It may be seen from the results recorded in the two preceding sections that there is a considerable difference quantitatively in the action of pitocin and postlobin-O on the one hand and pitressin and postlobin-V on the other. Thus 0.05 unit of either of the oxytocic preparations gives rise to a definite though small increase in weight (up to 0.5 per cent), while similar doses of pressor materials give rise to no increase. Nevertheless these small pressor doses appear to have some effect, as may be seen by contrasting the delayed loss of weight in these instances with the rapid fall which follows the injection of sodium chloride solution alone (see plate 3, figures 6B and 10B).

It was considered desirable to determine the ratio of activity between pressor and oxytocic preparations. Figure 10A ~~and 13A~~ on plate 1 shows that 0.25 unit of pitocin gives rise to an increase in weight of about 2.7 per cent, while that obtained with 0.25 unit of pitressin is only 1 per cent (plate 2, figure 13A). Furthermore it can be seen that the frogs receiving pitressin start to lose weight an hour before those receiving pitocin.

There is a strong resemblance between the curves representing the effects of two pressor units (plate 2) and 0.25 oxytocic unit (plate 1). This would mean that the oxytocic constituent was about eight times as active as the pressor. This value is probably too high, since doses as large as two units of pitressin or postlobin-V very likely give maximal effects. However, plate 1 (figures 5A and 7A) and plate 2 (figures 13A and 15A) show that the oxytocic preparations are more than two and one half times as effective as the pressor. That the ratio lies in the neighborhood of 1:3 or 1:4 is seen from later experiments (compare plates 4, 5 and 6). Thus 0.25 unit of postlobin-O (figures 1C and 4C) is more effective than 0.75 unit of postlobin-V (figures 2C and 5C) though less effective than 1.25 units pitocin (figures 3C and 6C), while 0.25 unit (figures 9C and 12C) is more effective than 0.5 unit of pitressin (13C and 14C) but less effective than 1.0 unit (figures 8C and 11C).

THE ACTIVITY OF PITUITRIN AS COMPARED WITH THE ACTIVITIES OF ITS PRESSOR AND OXYTOCIC CONSTITUENTS

Since pituitrin contains both pressor and oxytocic constituents in equal proportions, one would expect it to be more effective than either preparation alone, except possibly in the case of small doses when differences are not so easily discernible. It was considered desirable to subject this inference to experiment.

Doses of 0.05 and 0.1 unit of pituitrin in some cases seemed to be no more active than similar doses of pitocin (see

plate 7, figures 4A and 6A, and plate 1, figures 2A, 3A and 5A). When the dose was increased to 0.25 unit the pituitrin gave a greater and more lasting effect (see plate 7, figure 12A and plate 1, figure 10A; also plate 7, figures 7C and 10C, and plate 4, figures 9C and 12C).

However, in the case of frogs from lot D which were more responsive than those employed in the earlier experiments, even with 0.1 unit doses, pituitrin was more effective than postlobin-O (see plate 8, figures 3D and 4D and figures 1D and 2D).

INFLUENCE OF SEX ON THE RESPONSE TO PITUITARY PREPARATIONS

The same dose of either a pressor or an oxytocic preparation seems to produce a greater increase in weight in males than in females. This is illustrated in plate 4, which concerns the action of the oxytocic substances, and in plates 5 and 6, which concern the pressor substances. A similar difference is shown by pituitrin (see plate 7, figures 7C and 10C, and plate 8 figures 3D and 4D). The literature contains no reference to this sex factor. If it is not taken into consideration considerable confusion can result.

THE ACTION OF THE MELANOPHORE HORMONE

The melanophore preparation used was first compared with a whole posterior lobe extract to determine its melanophore activity in units. It was found that 0.5 cc. contained 0.07 unit. This quantity caused no increase in weight, as may be seen in figures 1E and 2E on plate 9. Since 0.05 unit of oxy-

tocic substance causes a definite increase in weight, it may be concluded that the melanophore constituent has little or no action. In these experiments the melanophores were fully expanded. The fact that the oxytocic constituent has no action upon melanophores and yet causes weight increases is additional evidence that the melanophores are not concerned in the weight increases.

THE RETARDING ACTION OF PITUITARY UPON THE ELIMINATION OF WATER

The foregoing experiments indicate that the oxytocic and pressor constituents of the pituitary gland increase the imbibition of water. They should also reduce the rate at which water injected into a lymph sac is eliminated.

Five cc. of distilled water containing 0.2 unit of pituitrin were injected into the lymph spaces and the changes in weight were compared with those of controls which received water alone.

As may be seen from plate 10 (figs. 1F and 2F) those receiving pituitrin actually increased in weight while the controls fell off rapidly.

The experiment was repeated, using 0.1 unit of pitocin, and this time the frogs were weighed just before and immediately after the injection, so the starting point of the curves on plate 8 (fig. 1G) represents the percentage increase in weight caused by the injected water. Mechanical loss is unavoidable but it can easily be seen that pitocin retarded the return to

normal, and if additional small amounts were given at intervals the elimination of water ceased and the frogs increased in weight, falling off again when the injections were stopped.

SUMMARY

The two oxytocic preparations from the posterior lobe of the pituitary gland, pitocin and postlobin-O, behave similarly as regards their effect on water balance in the frog. Likewise the two pressor preparations, pitressin and postlobin-V exert equal effects, but the action is not so great as that of the oxytocic preparations. However, the ratio of activity between the two (3:1 or 4:1) renders unlikely the possibility of the effect of one being due to contamination with the other.

Pituitrin is more effective than either pitocin or pitressin, while the melanophore constituent is quite ineffective.

There is a considerable difference between the behavior of the two sexes, the males being far more responsive than the females. This appears to be independent of the nature of the preparation used.

The elimination of large volumes of water is noticeably retarded when small doses of pituitrin or pitocin are added to the injected water.

I wish to thank Professor R.L. Stehle for his many helpful criticisms during the course of this work.

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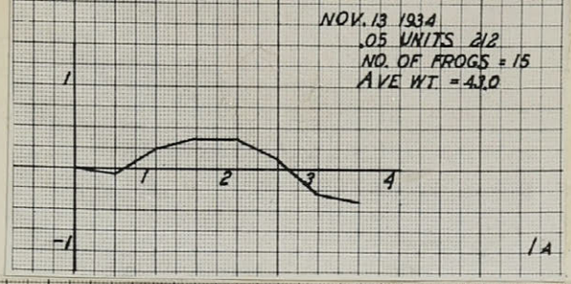
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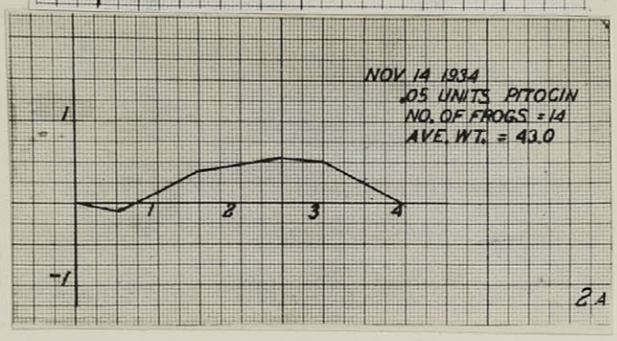
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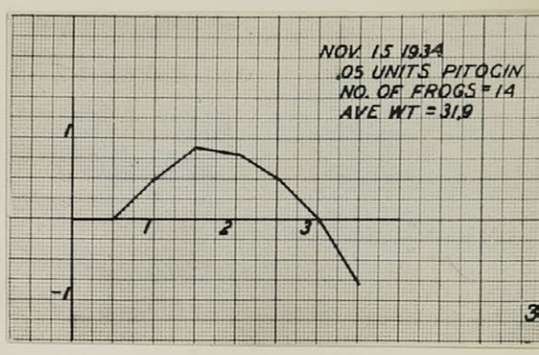
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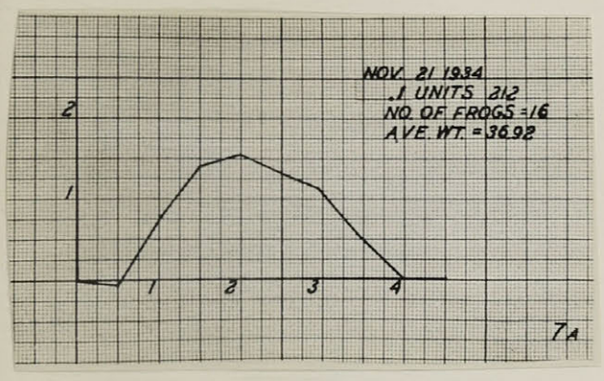
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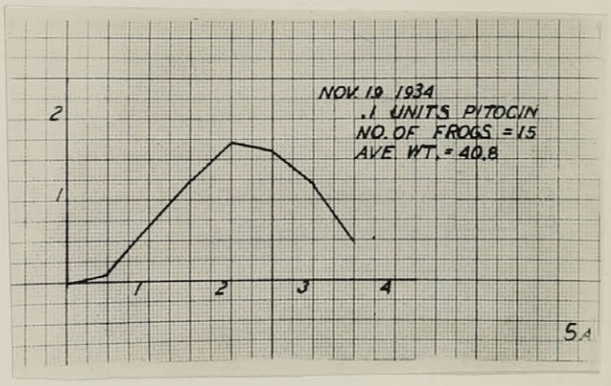
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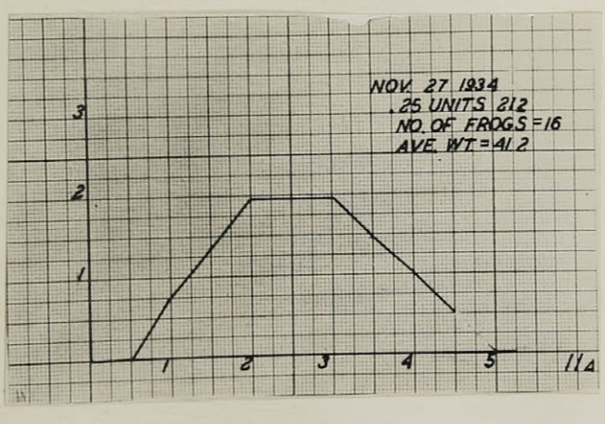
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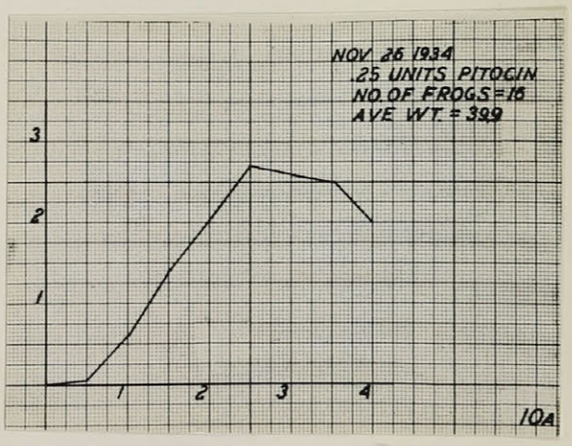
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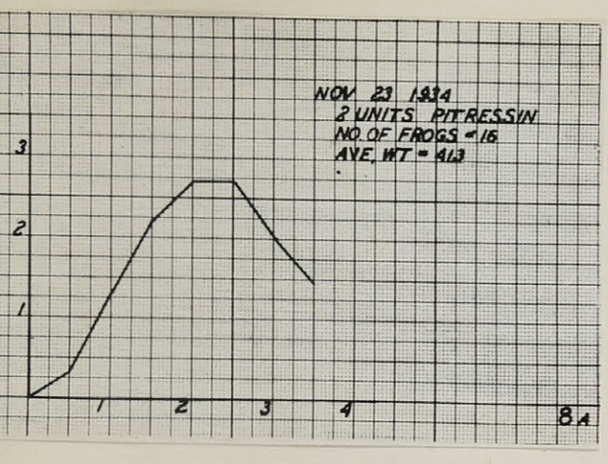
PLATE 1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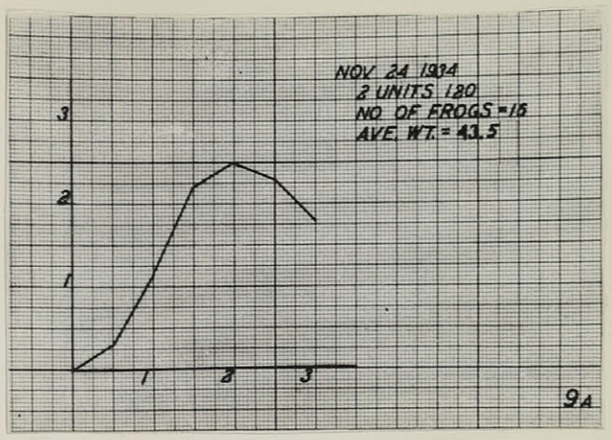
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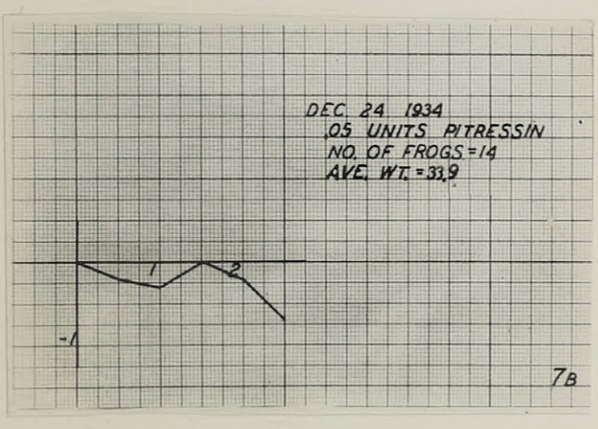
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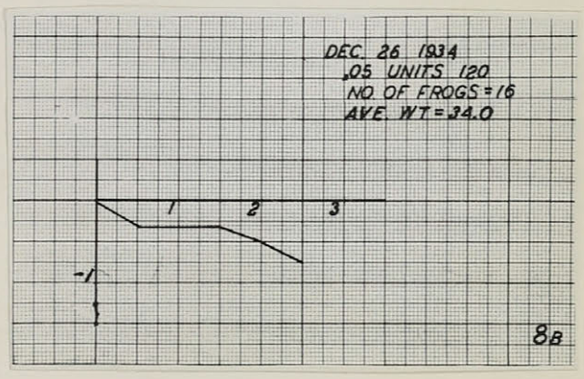
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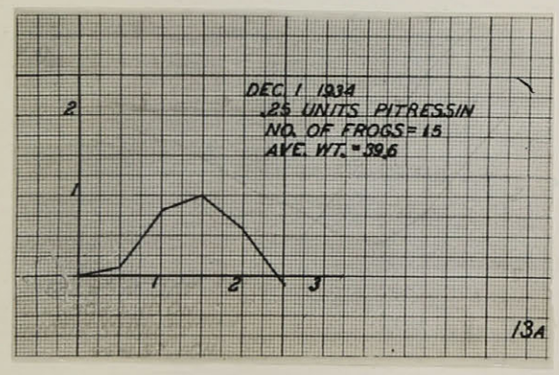
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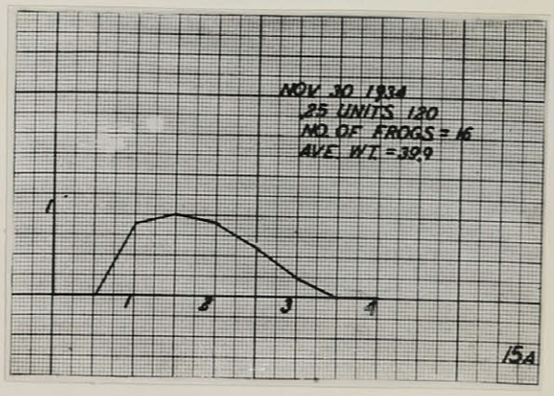
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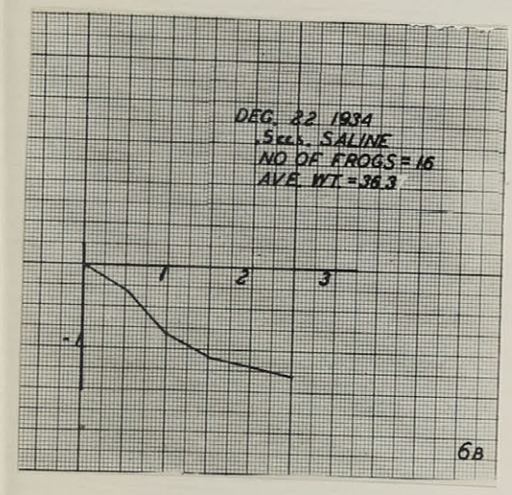
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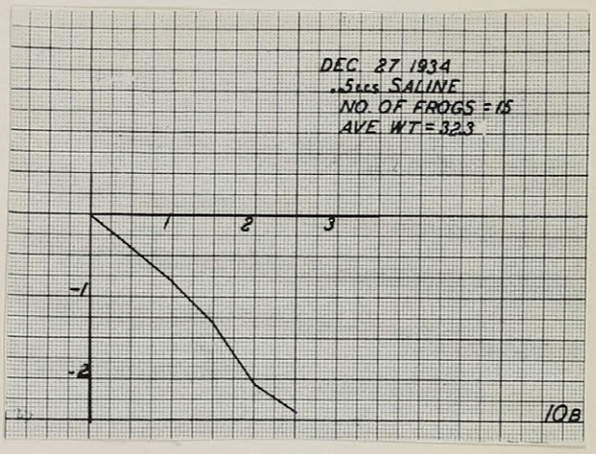
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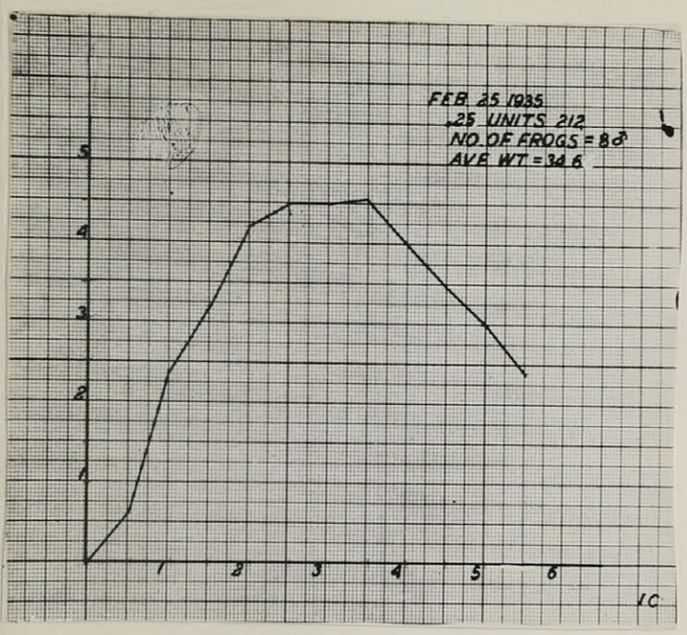
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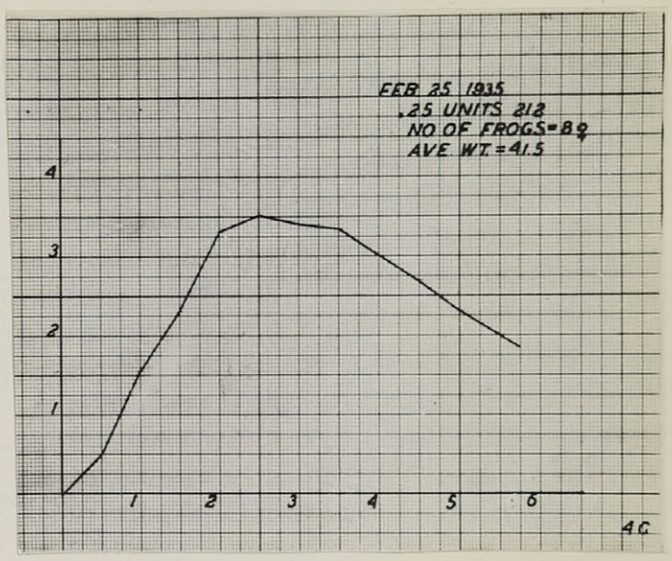
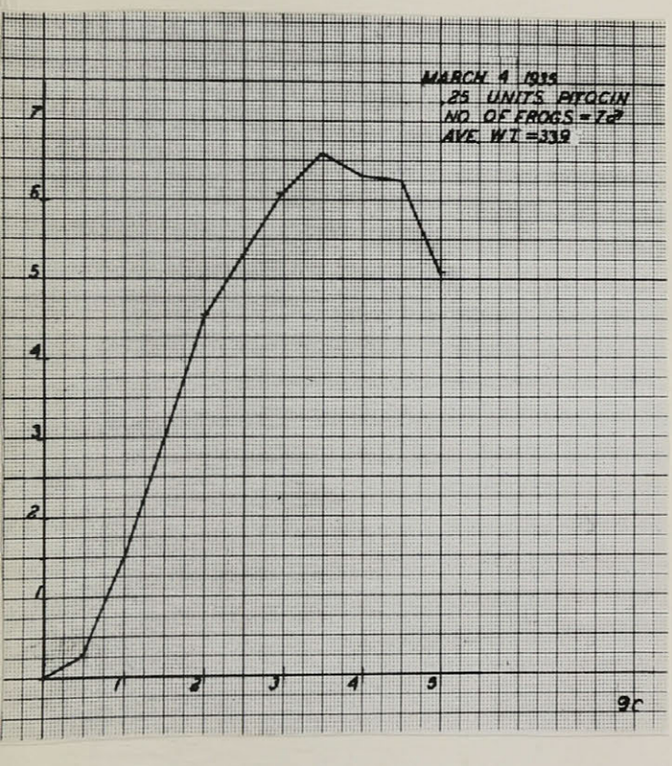
AVE WT = 34.6
1C


FEB 25 1935
.25 UNITS 212
NO OF FROGS = 8♀
AVE WT = 41.5
4C


MARCH 4 1935
.25 UNITS PITOCIN
NO OF FROGS = 7♂
AVE WT = 33.9
9C

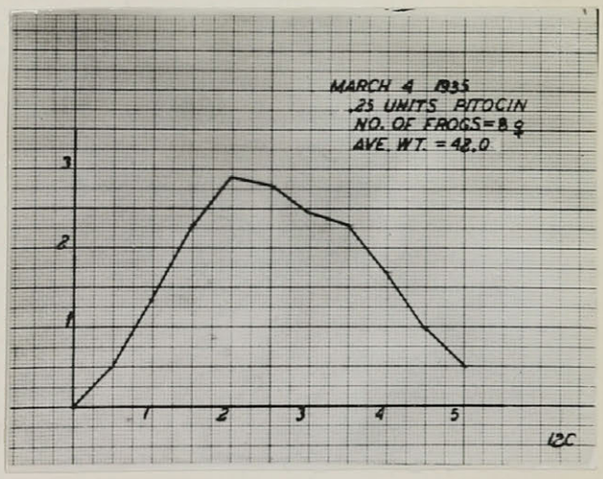
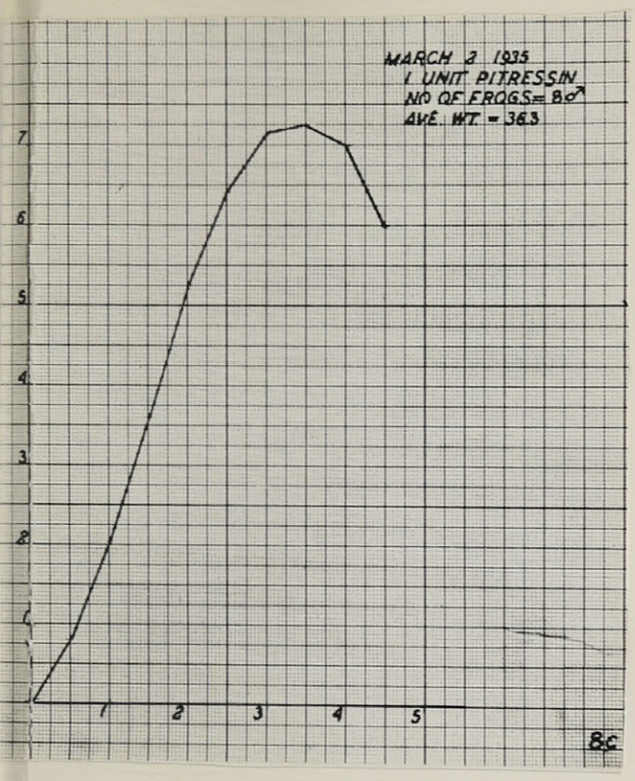
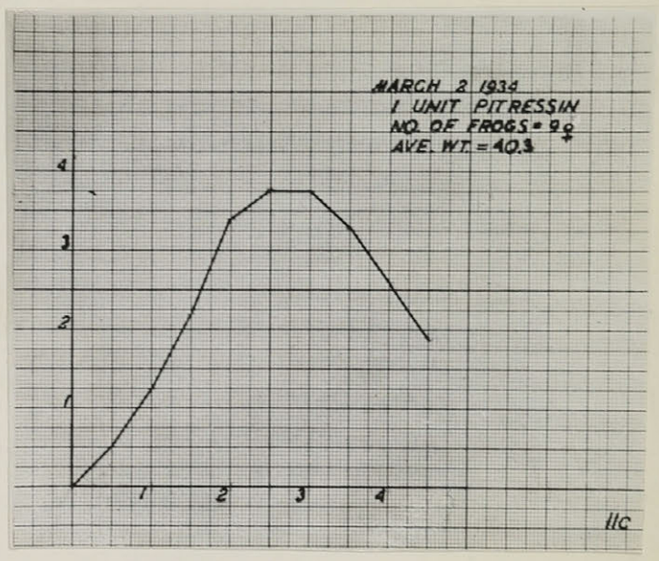
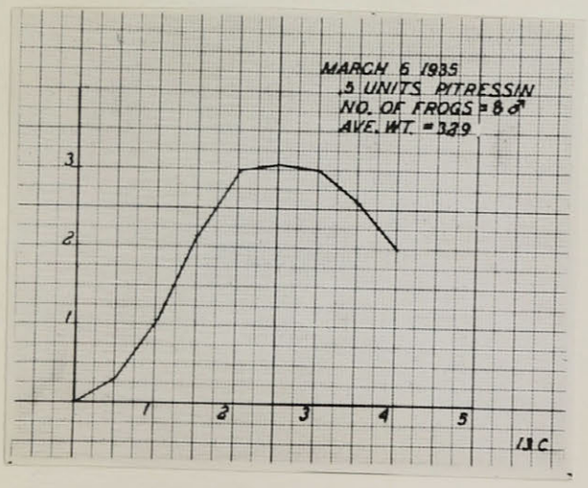

MARCH 4 1935
.25 UNITS PITOCIN
NO. OF FROGS = 8♀
AVE. WT. = 42.0
12C

PLATE 4


MARCH 2 1935
1 UNIT PITRESSIN
NO OF FROGS = 8♂
AVE. WT = 36.3
7
6
5
4
3
2
1
1
2
3
4
5
8C


MARCH 2 1934
1 UNIT PITRESSIN
NO. OF FROGS = 9♀
AVE. WT. = 40.3
4
3
2
1
1
2
3
4
11C


MARCH 6 1935
.5 UNITS PITRESSIN
NO. OF FROGS = 8♂
AVE. WT. = 32.9
3
2
1
1
2
3
4
5
13C

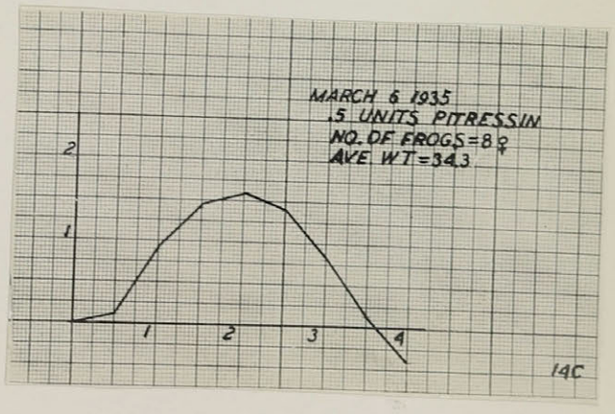
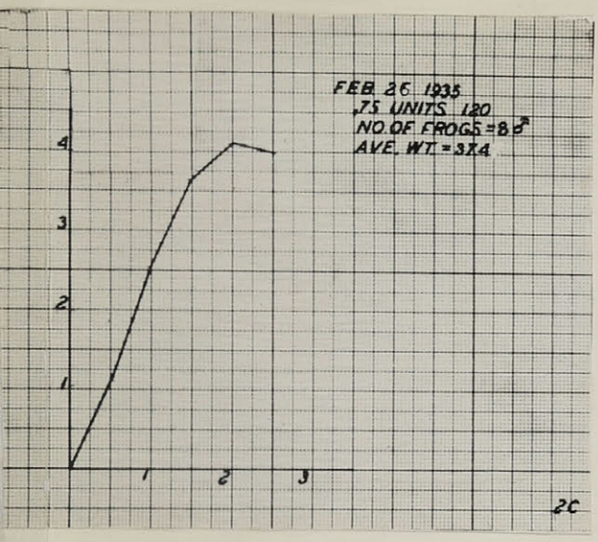
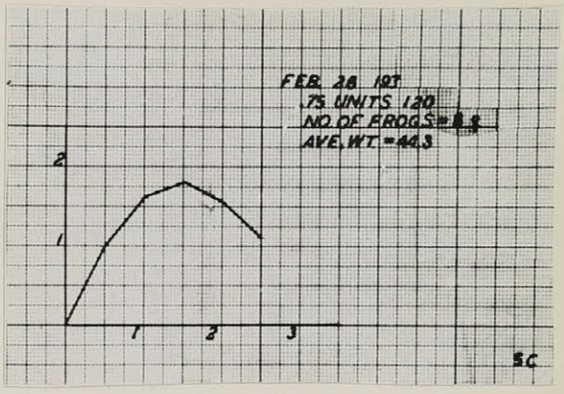
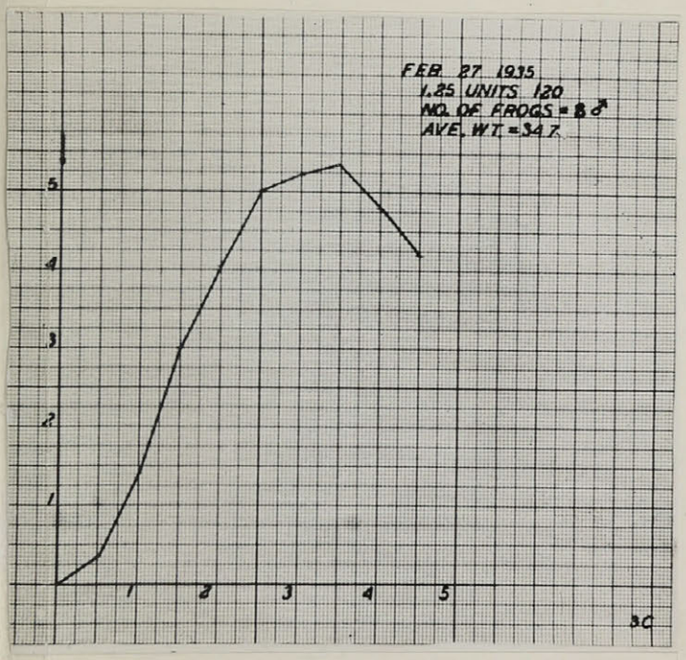

MARCH 6 1935
.5 UNITS PITRESSIN
NO. OF FROGS = 8♀
AVE. WT = 34.3
2
1
1
2
3
4
14C

PLATE 5


FEB. 26 1935
.75 UNITS 120
NO. OF FROGS = 8 ♂
AVE. WT. = 3.14
2C


FEB. 28 1935
.75 UNITS 120
NO. OF FROGS = 8 ♀
AVE. WT. = 44.3
5C


FEB. 27 1935
1.25 UNITS 120
NO. OF FROGS = 8 ♂
AVE. WT. = 34.7
3C

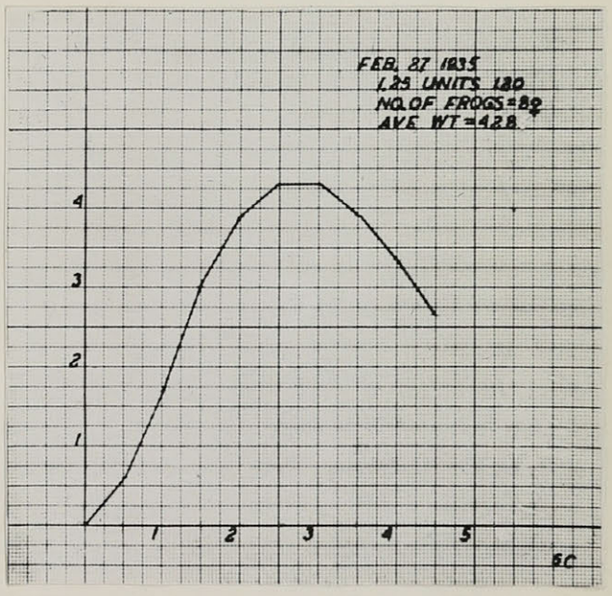
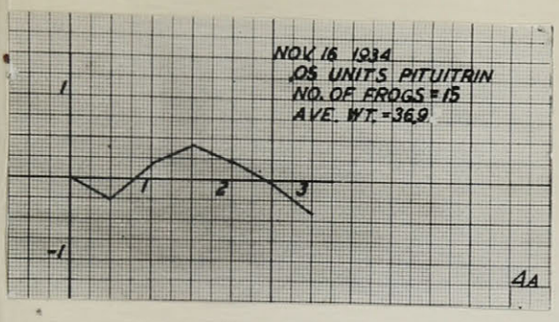
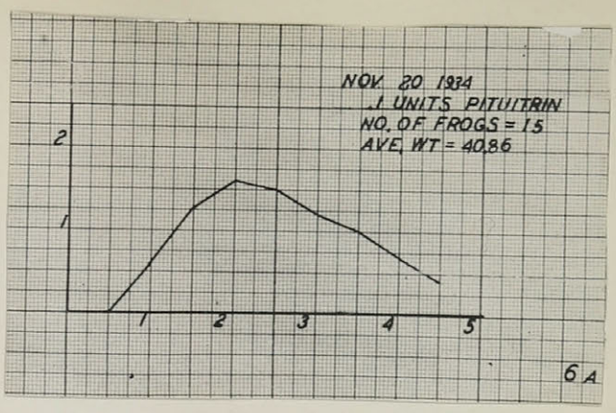
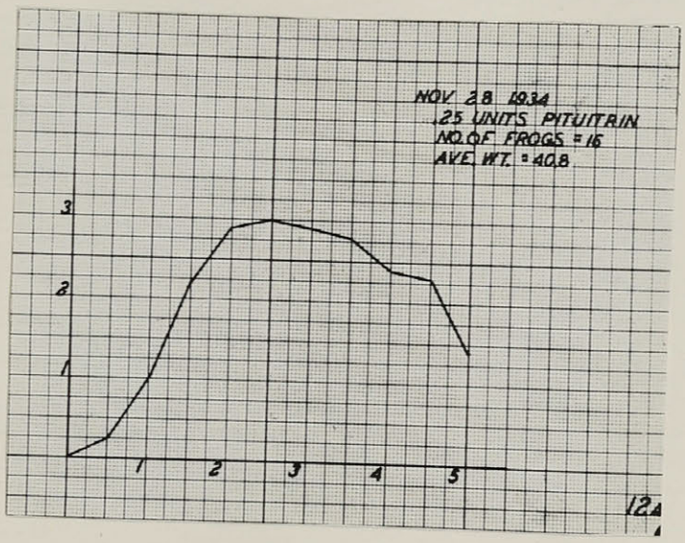
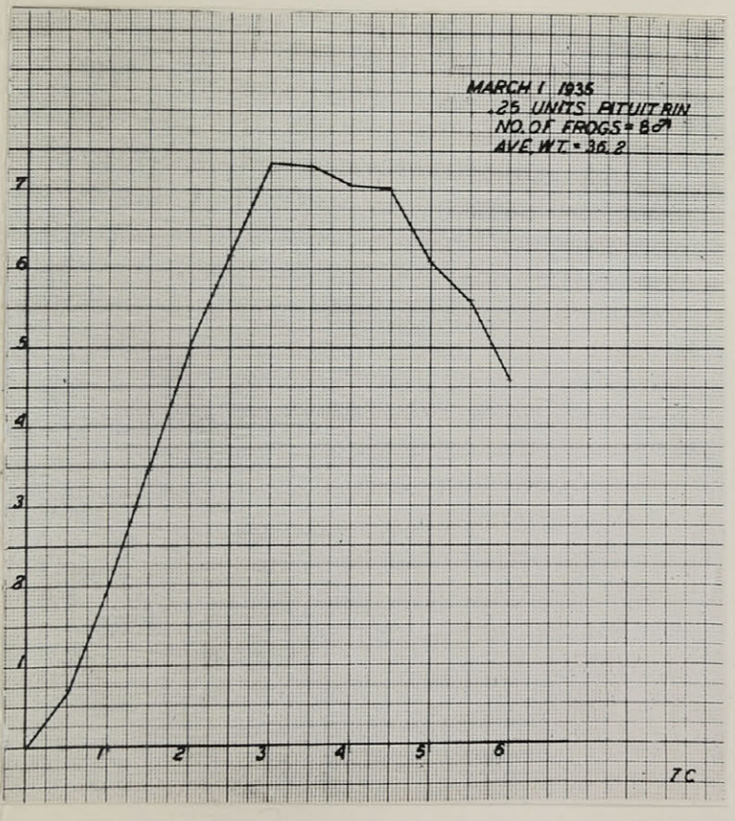

FEB. 27 1935
1.25 UNITS 120
NO. OF FROGS = 8 ♀
AVE. WT. = 42.8
5C

PLATE 6


NOV. 16 1934
.05 UNITS PITUITRIN
NO. OF FROGS = 15
AVE. WT. = 36.9
4A


NOV. 20 1934
.1 UNITS PITUITRIN
NO. OF FROGS = 15
AVE. WT = 40.86
6A


NOV 28 1934
.25 UNITS PITUITRIN
NO. OF FROGS = 16
AVE. WT. = 40.8
12A


MARCH 1 1935
.25 UNITS PITUITRIN
NO. OF FROGS = 8♂
AVE. WT. = 36.2
7C

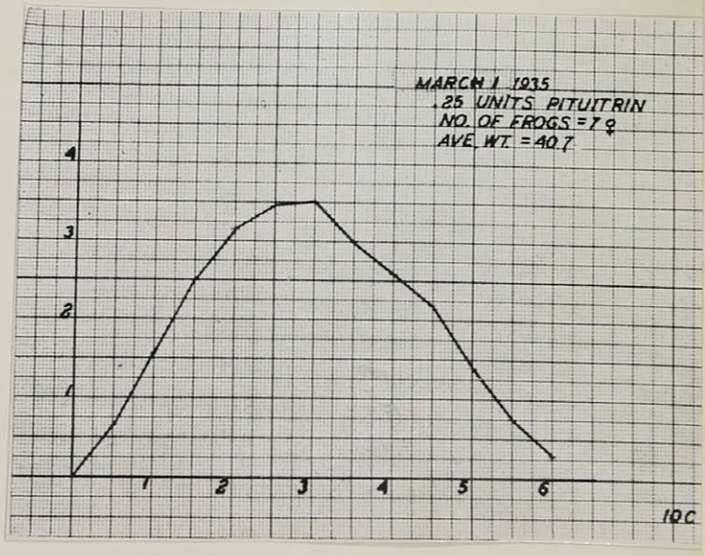
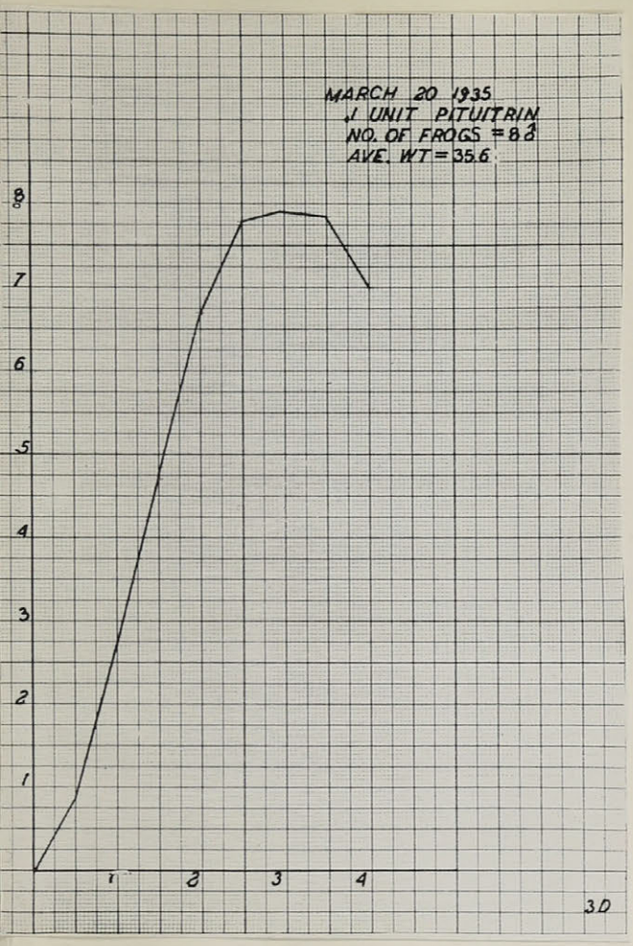
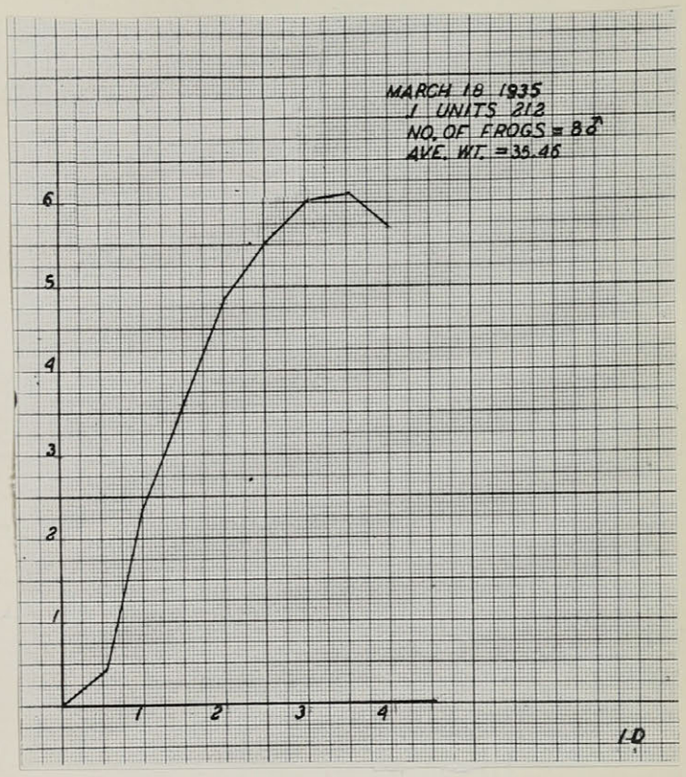
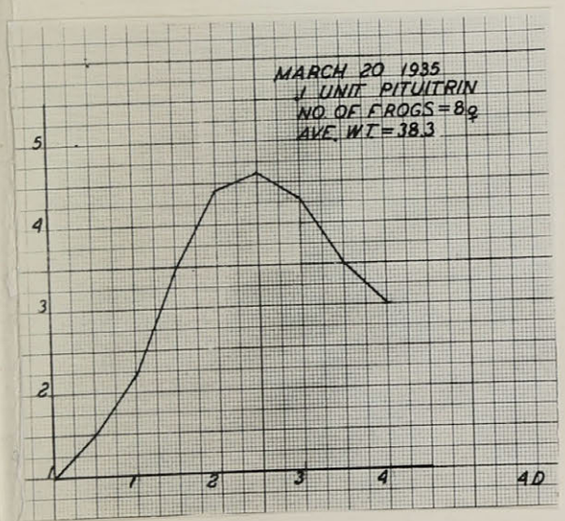

MARCH 1 1935
.25 UNITS PITUITRIN
NO. OF FROGS = 7♀
AVE. WT. = 40.7
10C

PLATE 7


MARCH 20 1935
.1 UNIT PITUITRIN
NO. OF FROGS = 8♂
AVE. WT = 35.6
8
7
6
5
4
3
2
1
1
2
3
4
3.0


MARCH 18 1935
.1 UNITS 2.12
NO. OF FROGS = 8♂
AVE. WT. = 35.46
6
5
4
3
2
1
1
2
3
4
1.0


MARCH 20 1935
.1 UNIT PITUITRIN
NO. OF FROGS = 8♀
AVE. WT = 38.3
5
4
3
2
1
2
3
4
4.0

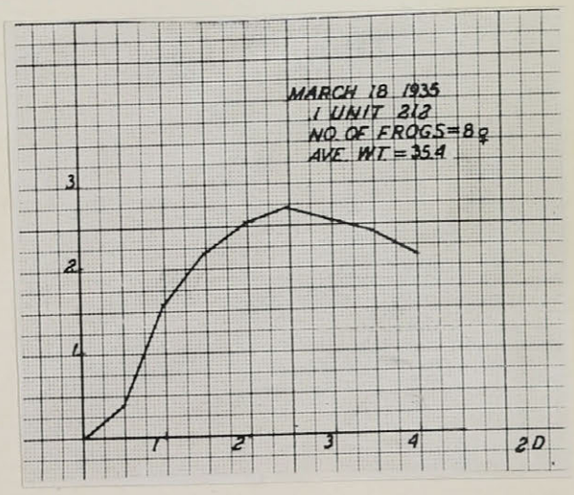
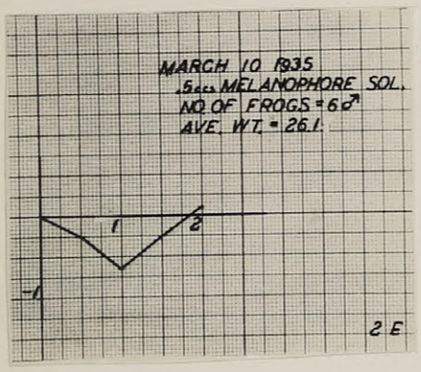

MARCH 18 1935
.1 UNIT 2.12
NO. OF FROGS = 8♀
AVE. WT = 35.4
3
2
1
1
2
3
4
2.0

PLATE 8


MARCH 10 1935
.5 cc. MELANOPHORE SOL.
NO. OF FROGS = 6 ♂
AVE. WT. = 26.1
1
2
-1
2 E

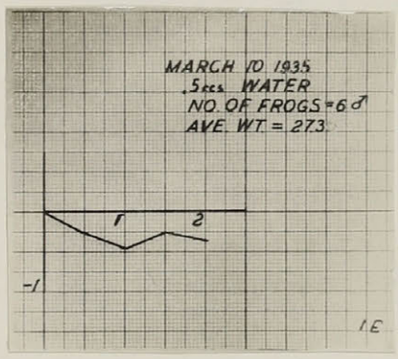
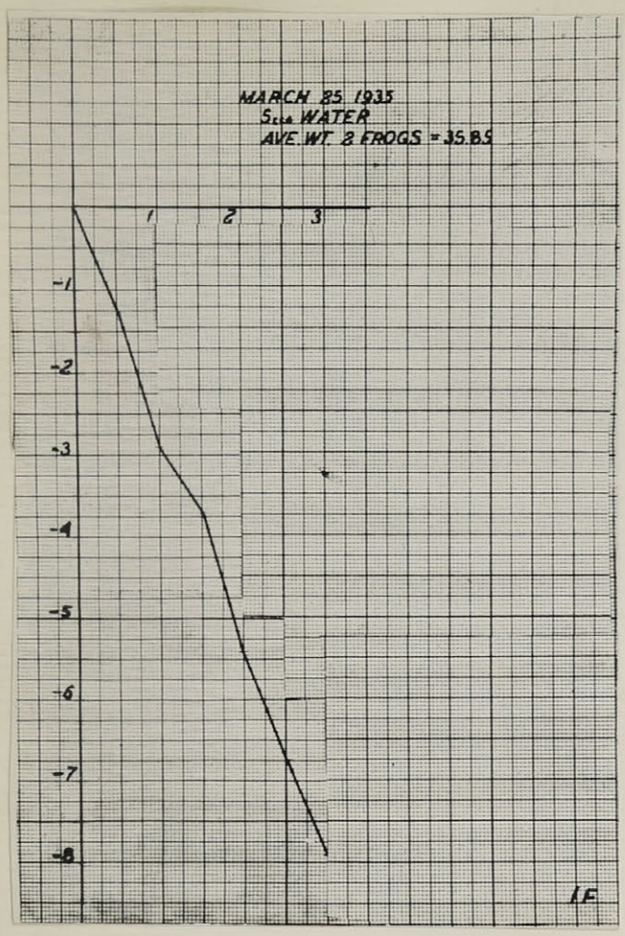
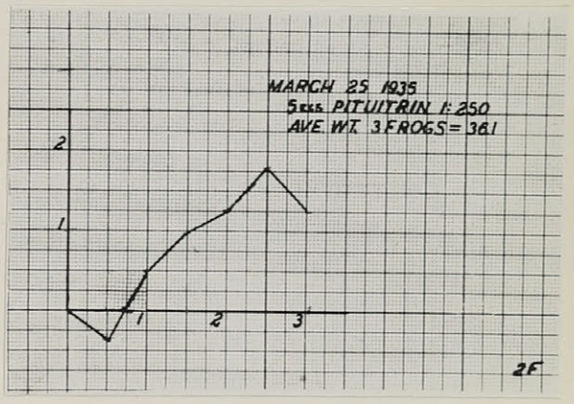

MARCH 10 1935
.5 cc. WATER
NO. OF FROGS = 6 ♂
AVE. WT. = 27.3
1
2
-1
1 E

PLATE 9


MARCH 25 1935
5 cc.s WATER
AVE. WT. 2 FROGS = 35.85
1
2
3
-1
-2
-3
-4
-5
-6
-7
-8
1F


MARCH 25 1935
5 cc.s PITUITRIN 1:250
AVE. WT. 3 FROGS = 36.1
2
1
1
2
3
2F

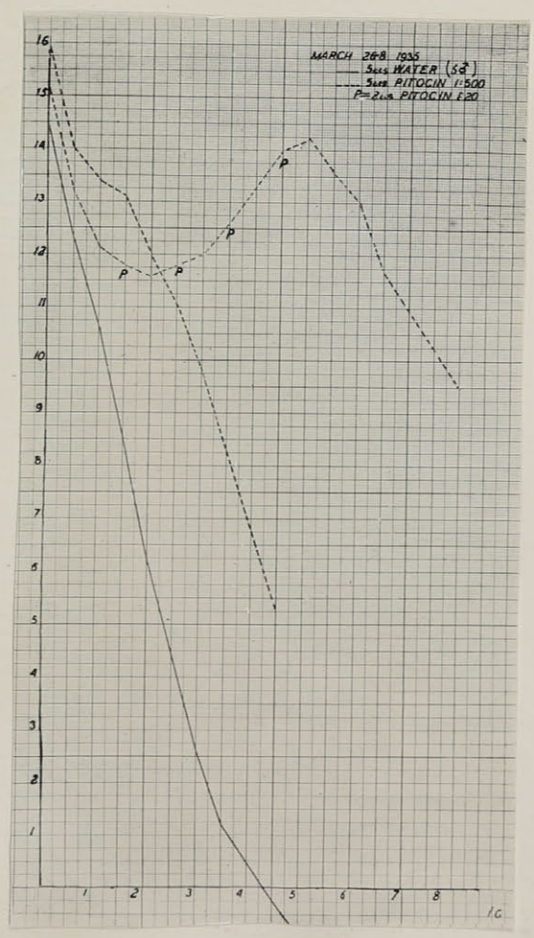

MARCH 26-8 1935
5 cc.s WATER (S²)
5 cc.s PITOCIN 1:500
P = 2 cc.s PITOCIN 1:20
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
P
P
P
P
1
2
3
4
5
6
7
8
1G

PLATE 10