Comparative effects of fish and beef pituitary on water imbibition and retention in Rana pipiens

Edgar A. Lazo-Wasem

The University of Montana

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Comparative Effects

of

Fish and Beef Pituitary on Water Imbibition

and Retention in Rana pipiens.

by

Edgar A. Lazo-Wasem


Presented in partial fulfillment of the requirements
for the degree of Master of Arts in Zoology.

Montana State University

1951

Approved:

George J. Wessel
Chairman, Board of Examiners

W. P. Clark
Dean, Graduate School
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Introduction

The diuretic and antidiuretic effects of the posterior lobe of the hypophysis on the mammalian kidney are well known (Bagbee and Kamm, 1928; Van Dyke, 1936). In addition to, and probably distinct from this reaction, it has been found that injection of posterior lobe extracts into frogs produces a temporary gain in weight due to the absorption of water (Brann, 1921; Kaller, 1931; Boyd and Brown, 1938). Furthermore, it has been shown that there are at least two principles (pitocin and pitressin) from the posterior lobe of the bovine hypophysis which cause an uptake and retention of water frequently exceeding 15 per cent of the body weight of the leopard frog (Steggerda, 1931, 1937; Steggerda and Essex, 1934; Oldham, 1936). This investigation was designed to determine whether or not the teleost pituitary exerts the same effect in this respect on an amphibian, *Rana pipiens*, as does the beef gland.
Review of Literature

It has been shown that the posterior lobe of the pituitary gland contains two active principles: an oxytocic (pitocin) which causes contractions of the uterus, and a pressor (pitressin) which raises blood pressure (Bugbee and Kamm, 1928). The unfractioned extract is commercially referred to as pituitrin. The two active principles have been separated and obtained in the form of white, stable, water soluble powders of great potency. Their chemical nature seems to be protein; all the various effects of pituitrin are exerted only by fractions which contain proteins (Mitchell, 1946). Their properties, by the use of ultracentrifuge and electrophoresis, are those of a substance with a molecular weight exceeding 30,000 and an isoelectric point at pH 4.8. The fact that some hormones are proteins presents a problem in the general physiology of the cell, because cell membranes appear to be typically impermeable to proteins. The escape of protein hormones from the producing cells and their entrance into cells upon which they exert their action remains to be explained. Activity at cell surfaces is one obvious possibility, although Mitchell points out that the relative low molecular weights of these hormones "are hardly low enough to ensure diffusion through cell membranes." The fact that it is possible to prepare several of these hormones from urine suggests that the kidney cells, at least, are permeable to them.
Although the extracts of the posterior lobe (pars nervosa) which exhibit vasopressor, antidiuretic, and oxytocic activities are generally assumed to be produced in that lobe, investigators have not been unmindful of the unsatisfactory experimental and cytological evidence upon which the assumption is based. The cells of the pars nervosa, states O'Connor (1947), "are few and have little in common with secreting cells elsewhere in the body to suggest for them a secretory function."

Bargmann and Scharrer (1951) reviewed the evidence, both cytological and experimental, that has accumulated in support of the concept that these hormones are most probably produced by nerve cells in the hypothalamus of the brain. Neurosecretion in the hypothalamus, first reported only for the nucleus preopticus of various teleost fishes, has in the course of time been extended to include the nuclei supraopticus and paraventricularis of mammals.

Although the problem of where and how these protein hormones of the posterior lobe are produced is yet to be solved, it remains a fact that its extracts exhibit vasopressor, antidiuretic and oxytocic activities. In addition, it has a marked effect on the water metabolism of amphibians. Bruna (1921), who first studied this subject, found that frogs treated with neurohypophyseal extracts, increased their weight 15 per cent or more in from 5 to 10 hours. He did not attribute this effect to a renal cause analogous to diuresis inhibition in mammals, as the effect was observed after nephrectomy. Boyd and Maek (1940), who devised a method for determining the volume of an unknown pituitary extract which contained one frog unit of water retention principle, reported that "pituitrin may alter the ability of extrarenal tissues to hold water, especially in frogs in which renal loss is relatively
insignificant." From studies by Brunn and others, it may be concluded
the effect is due to a change in the physiology of the skin (Adolph,
1925; Heller, 1930b and c; Biasotti, 1923; Jungmann and Bernhardt, 1923;
Steggerda, 1931; and Oldham, 1936). Collin and Drouet (1932) doubted
that the effect could be produced, but the consensus is to the contrary.

Although both the oxytocic and the pressor principle cause the
imbibition of water in frogs, Oldham (1936) has shown that the potency
of the oxytocic substance is between 3 to 5 times that of the pressor.
Heller (1930c) also reported a significant difference in favor of the
oxytocic principle. However, Novelli (1932), who worked with Bufo
arenarum, reported the pressor factor as being three times more potent
than the oxytocic.

The smallest dose of pituitrin which causes the inhibition of
water loss is evidently about the same as that which produces the max-
imum uptake of water by frogs. In this respect pitressin and pitocin
are equally effective in inhibiting loss of water, but neither one is
as effective as pituitrin (Boyd and White, 1938). The unfractioned
extract completely inhibits the loss of added distilled water for three
hours (Boyd and White, 1939). The fact that induced water retention
is not due to an antidiuretic effect was originally reported by Brunn
(1921) after reproducing the effect in nephrectomized frogs, and was
confirmed by Steggerda (1931), who demonstrated that injected frogs
with tied cloacas still increased 15 per cent more in weight than did
control frogs which also had their cloacas tied.

The accepted explanation of a change in the physiology of the skin
as being the main cause by which pituitrin induces imbibition and reten-
tion of water in the frog, seems to be that such an extract alters the
permeability of the skin of frogs, allowing the uptake of more than the usual amount of water. Heller (1930a) found that the liver and gastrocnemius muscles could, if isolated, be made to increase or decrease 6 per cent of their own weight. He concluded that the excess water in a treated frog was stored in the muscles, the liver, and subcutaneous spaces. This seems to be in agreement with reports by Adolph (1925) and Boyd and White (1938). Heller (1930b) also reported that three or four weeks after denervation of the gastrocnemius muscle of a frog, the muscle seemed to gradually lose its power to take up water.

The hormone apparently not only increases the absorption of water, but also functions to retain it. Pituitrin injected specimens kept in a dry environment after removal from water lose less water than untreated animals, provided that evaporation from the skin is not excessive such as occurs in warm sunlight or under a forced draft from a fan (Boyd and White, 1938).

Passage of water through isolated frog skin indicates that the control for the passage of water resides within the skin. Freshly isolated frog skin initially gains or loses water in proportion to the square root of the time elapsed after immersion. Circulation of blood does not seem to affect the passage of water through the skin, since when the circulation is stopped altogether, the rates of osmosis into or out of intact frogs were the same as for normal intact animals (Adolph, 1931). On the other hand, in skinless frogs the rate at which water is taken up or given off is proportional to the concentration of sodium chloride in the medium (Adolph, 1930).

The presence of the intact pituitary gland in the frog appears to be essential for pituitrin to bring about the characteristic weight
increase. James and Steggerda (1935) treated hypophysectomized frogs with pituitrin one to four weeks after operation, and found that such frogs did not show any increase in weight. Control frogs similarly treated increased 18 per cent in weight.

There are a number of conditions which influence the effectiveness of injected posterior lobes on the uptake of water by frogs. Boyd and Brown's (1938) experiments dealt with light, temperature, salt concentration, and pH of the medium. They concluded that the factors which seemed to have little or no effect on the reaction, were: Difference in species within the genus Rana (Steggerda in 1937, reported a variation to occur between Rana pipiens and Rana clamitans), body weight, sex, volume of water in the frog bath, and the mode of injection (subcutaneous, intramuscular, or directly into the dorsal lymph sac). However, they reported that a decrease in temperature prolongs the duration and increases the height of the reaction, that the extent of the reaction varies inversely as intensity of the light, and that concentrations of sodium chloride, potassium chloride, and sodium phosphates greater than 0.4 per cent in the frog bath inhibited the reaction. The optimum pH is 7.0. Boyd and Mack (1940) added to the above factors while working on a method to assay pituitary water retention principle. They found that air in motion and that repeated dosages lessened the effect, and that some individuals were "non-reacting." A seasonal variation in the susceptibility of frogs to the preparations had been reported previously by Oldham (1936); the peak of susceptibility occurring during the summer months. This is in agreement with reports by the pioneers of this work; Brunn (1921) and Heller (1930b).
Evidence presented in this review of literature points out that neurohypophysial extracts exert their action by altering the permeability of the skin, that such permeability is increased only in a one-way direction, that this change in the skin only occurs in the presence of the intact pituitary, and that there exist numerous environmental factors which govern the amount and rate of water imbibition and retention.
Materials and Methods

Medium sized *Rana pipiens* were obtained from dealers in biological supplies at Wisconsin and Illinois. They were kept in a metal tank (about 1 x 1 x 2 meters) with enough tap water to cover them, and with occasional rocks on which they could come out of the water. No frogs were utilized in any of the experiments until they had been acclimated two or more days to this environment. Since the frogs were received during the time of the year in which they hibernate under normal conditions, no attempt was made to feed them before or during the experiments.

Both fresh and acetone desiccated pituitaries were used. The latter was previously prepared by Dr. G. F. Weisel of the University of Montana, by grinding either whole fish or whole beef pituitary in a mortar, and adding enough acetone from time to time to remove all of the fat present. The powdered extract was then air-dried and sealed in ampoules under reduced pressure. Desiccated extracts of fish and beef brain were prepared in a similar way for treatment of controls. Desiccated fish extracts were made from barracuda (*Sphyraena argentea*) from the coast of California, while fresh fish pituitary was obtained from spawning sockeye salmon (*Oncorhynchus nerka*) from Flathead Lake, Montana. Pituitary glands and portions of brain from the salmon were frozen in liquid carbon dioxide at the time of their removal and kept in that state until immediately before being used, when they were thawed and then macerated by cutting them into ten to fifteen parts.
The general procedure followed in treating the frogs was similar to that of Steggerda (1937) and Boyd and Brown (1938). The frogs were placed in individual jars and each jar was numbered and labeled with the substance to be injected. The glass jars, in which the frogs were kept during experimentation, were twenty-five centimeters in height and fifteen centimeters in diameter. Enough water, about three hundred cubic centimeters, was poured into each jar to cover all of the body surface but the nostrils and eyes. Tap water was used as it more closely approximates the water of their natural habitat than does distilled water. The water was aired for about twenty-four hours previous to being used, since it was believed that in this way the excess chlorine would escape. The jars were covered with cardboard tops to minimize or completely avoid air currents. Porous cardboard was used in order to allow for the passage of the necessary oxygen. About twenty-four hours before the start of the experiment, the frogs were placed in the jars to allow for acclimatization. No attempt was made at this time to sex the animals.

Desiccated extracts, as well as fresh pituitary and their respective control solutions, were suspended in 0.5 cubic centimeter of cold-blooded Ringer's solution. Dosages of acetone desiccated extracts were 4 milligrams in weight, as determined by a chainomatic analytical balance.

One barracuda pituitary equals 4.2 milligrams, which consequently means that slightly less than one barracuda pituitary was injected when using 4.0 milligrams as the dosage for desiccated extracts (Weisel, 1950). Although two salmon glands were injected in experimenting with fresh pituitary, a size comparison of both glands shows that one
harrasuda pituitary represents slightly more material than two salmon pituitaries. Controls were treated with comparable amounts of desiccated and fresh fish brain and desiccated beef brain.

In all cases, injections were made directly into the dorsal lymph sac with one milliliter syringes. Immediately after injection, the frog was dried with paper towels and as much of its urine expelled as possible by pressure on the abdomen. The frog was then weighed by placing it in a metal container with a glass top, which container and top were re-weighed immediately after with what water was left in it after the frog was removed. It was believed that in this way the exact weight of each frog at weighing was more accurately determined, than by determining the weight of the empty container alone before the experiment. The balance used in weighing the injected frogs was accurate to three-tenths of a gram.

The range in environmental temperatures was obtained by an Eimer and Amend maximum-minimum thermometer, while water temperatures were recorded with a seventy-six centimeter immersion thermometer. Temperature could not be ideally controlled, as it was in Boyd's and Mack's (1940) experiments. Water temperatures varied from eighteen to twenty-five degrees centigrade, but with no greater change than five-tenths degree centigrade in any two-hour period throughout the seven months in which the experiments were carried out. Boyd and Brown (1938) found that a temperature decrease prolongs and increases the reaction.

Light was controlled as much as possible by the use of constant artificial light, although, according to Boyd and Smith (1938), it is doubtful that light affects the action of the principles involved.
Background-coloration was not controlled as it has been shown to have no effect (Staggerda and Jones, 1935). Previous experiments (Boyd and Brown, 1938) show that there is no difference in the water uptake between the sexes, so no assortment was made, although the sex was determined by autopsies or, when possible, by the enlargement of the thumb pads of the males at the termination of the experiment.

Most of the test animals were utilized twice. Those frogs which had been treated with pituitary were reinjected a week later with one of the control solutions, and vice versa in the case of animals which were originally injected with control solutions. It was believed that by doing so, more accurate evidence would be provided in support of the peculiar effects of pituitary extracts, as the variability in individual reaction would be included in both experimental and control groups.

It was noticed during the preliminary work that in order to accurately weigh each frog at hourly intervals the number in each series should not exceed ten. Therefore, each series usually consisted of ten frogs. For every three frogs injected with pituitary, at least one was injected with a control solution. In each series of desiccated extracts run, there were usually three or four frogs injected with fish pituitary, an identical number with beef pituitary, while the remainder served as controls treated with either brain or pure Ringer's solution.

The total number of frogs used in these experiments was 218. About forty frogs were injected with 8.0 milligrams of desiccated extracts when the first trials were run, but since twelve of these animals died during the investigation, it was concluded that the amount of extract injected had to be reduced. Since 4.0 milligrams of the extracts seemed to produce a significant degree of weight change with a negligible
degree of mortality, this dosage was used for all experimental animals, and only these are included in the results.

Fifty frogs were each injected with two whole fresh salmon pituitaries previously macerated and suspended in 0.5 cubic centimeter of Ringer's solution, while nineteen frogs were injected with fresh salmon brain (a volume approximately equivalent to that of two glands) also previously macerated and suspended in Ringer's. Twenty-one other specimens were injected with 0.5 cubic centimeter pure Ringer's solution as an additional control.

Forty-four frogs were treated with acetone-desiccated barracuda pituitary and the same number with acetone-desiccated beef pituitary. For controls, twenty were injected with acetone-desiccated barracuda brain and another twenty with a similar extract of beef brain. All of these extracts were given in doses of 4.0 milligrams suspended in 0.5 cubic centimeter of Ringer's.

Weight changes of injected frogs, taken at regular intervals, were figured on a percentage basis. Since some of the values obtained were negative (in the case of frogs showing a decrease in weight as compared to their weight at the time of injection), it was necessary to code all numbers in order to have only positive values when making the calculations.

First, the arithmetical mean was computed for each sample group. The standard deviation was computed by taking the quadratic mean of the deviations from the arithmetic mean (Arkin and Colton, 1939), using the conventional formula

\[ \sigma = \sqrt{\frac{(x^2)\Sigma}{N}} \]

where:

\[ \sigma = \text{standard deviation} \]
\[ x = \text{deviations from arithmetic mean} \]
\[ N = \text{total number of items (if } N \text{ was smaller than 30, } N-1 \text{ was used)} \]

The standard error of the mean (standard deviation of the distribution of the means of samples) was calculated by dividing the standard deviation by the square root of the total number of samples in the group, thus

\[ \sigma_x = \frac{\sigma}{\sqrt{N}} \]

Again \( N-1 \) was used, if \( N \) was smaller than 30.

The statistical comparisons between means were made by the method of Rice and Leraas (1936), by which method any difference between two means which is greater than two times the sum of the standard errors of the two respective means is considered to be significant.

Graphically presented results were drawn according to the method of Rice and Leraas (1936), except that, as a measure of dispersion, the standard deviation was plotted additionally, as recommended by Hubbs and Perlmutter (1942).
Results

A striking difference was exhibited by the group of frogs injected with fresh salmon pituitary as compared with the two control groups injected with either pure Ringer's solution or fresh salmon brain. The arithmetical mean of the group injected with fresh salmon pituitary exhibited a maximum weight increase of 16.0 per cent, four hours after injection. This rapid increase was followed by a prolonged weight decrease phase down to 2.8 per cent, thirty-six hours after injection. This arrival of the mean weight changes of fresh pituitary injected animals to 2.8 per cent may be considered as a return to normal weight, since the normal weight variation for this group of frogs had been previously determined to be plus or minus 4.2 per cent. Such determination had been made by weighing nine uninjected frogs every two hours for seventy-two hours under environmental conditions as similar as possible to those which experimental animals were subjected.

The group injected with pure Ringer's solution showed an average maximum weight increase of 1.0 per cent, two hours after injection. This group exhibited a maximum decrease in weight of 1.9 per cent after twelve hours, followed by a stationary decrease of 1.8 per cent, twenty-four to thirty-six hours after injection. At no time did this group show an increase in weight other than at the second hour following the time of injection.

Average weight changes exhibited by specimens which were treated with fresh salmon brain showed a maximum weight increase of only 0.1 per
cent, two hours after injection. A sharp decrease of 2.1 per cent at ten hours, 2.0 per cent at twelve hours, and a maximum decrease in weight of 3.6 per cent at twenty-four hours followed. When this experiment was discontinued thirty-six hours following the time of injection, this control group showed an average weight decrease of 2.4 per cent (Fig. 1).

A statistical analysis of the weight changes induced by fresh salmon pituitary showed a markedly significant difference between them and the controls. While at no time did the two control groups show any significant difference from each other, those injected with fresh pituitary exhibited a significant difference from either control group for thirty-six hours. Furthermore, the maximum range of the group treated with pituitary seemed to increase or decrease following the same pattern of change that the mean exhibited (Table I and Fig. 2).

The series of frogs injected with acetone desiccated extracts consisted partly of specimens from Oshkosh, Wisconsin, and partly of specimens from Chicago, Illinois. Natural fluctuations of uninjected specimens determined previous to the experiments exhibited a plus or minus 2.7 per cent variation for the Chicago frogs, while uninjected specimens from Wisconsin showed a weight variation of plus or minus 4.2 per cent. Inasmuch as these natural fluctuations did not show a significant difference when studied statistically, both groups of frogs were treated together, and the results which follow are made up of combined specimens from both localities.

Since the group of twenty-one controls injected with 0.5 cubic centimeter of cold-blooded physiological saline was partly conducted with groups of specimens injected with fresh pituitary, and partly with
Fig. 1. — Graph of mean weight changes of frogs injected with fresh salmon pituitary as compared with those injected with a comparable amount of fresh salmon brain and with pure Ringer's solution.
<table>
<thead>
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<td></td>
<td></td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>24</td>
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<tr>
<td>Pure Ringer's Solution</td>
<td>Mean of % weight change</td>
<td>1.00</td>
<td>.20</td>
<td>-1.10</td>
<td>-1.20</td>
<td>-1.60</td>
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<td>Standard deviation</td>
<td>2.92</td>
<td>3.75</td>
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<td>4.62</td>
<td>3.78</td>
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<td>Pure Ringer's Solution</td>
<td>Standard error</td>
<td>.65</td>
<td>.84</td>
<td>1.01</td>
<td>.92</td>
<td>.97</td>
<td>.85</td>
<td>1.17</td>
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<tr>
<td>Fresh Salmon Brain</td>
<td>Mean of % weight change</td>
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<td>-1.50</td>
<td>-1.60</td>
<td>-2.00</td>
<td>-2.10</td>
<td>-2.00</td>
<td>-3.60</td>
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<td>Standard deviation</td>
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<td>3.93</td>
<td>4.54</td>
<td>4.25</td>
<td>4.35</td>
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<tr>
<td>Fresh Salmon Brain</td>
<td>Standard error</td>
<td>.74</td>
<td>.95</td>
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<td>1.02</td>
<td>1.05</td>
<td>1.20</td>
<td>1.37</td>
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<tr>
<td>Fresh Salmon Pituitary</td>
<td>Mean of % weight change</td>
<td>11.00</td>
<td>16.00</td>
<td>15.10</td>
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<td>10.00</td>
<td>7.90</td>
<td>5.00</td>
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<tr>
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<td>Standard deviation</td>
<td>6.72</td>
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<td>Fresh Salmon Pituitary</td>
<td>Standard error</td>
<td>.99</td>
<td>1.23</td>
<td>1.39</td>
<td>1.36</td>
<td>1.17</td>
<td>1.04</td>
<td>1.10</td>
</tr>
</tbody>
</table>

**TABLE I**

Weight Changes of Frogs Injected with Fresh Pituitary Extracts Compared with Controls
Fig. 2. - Graph of weight changes of frogs injected with fresh salmon pituitary as compared with those injected with a comparable amount of fresh salmon brain and with pure Ringer’s solution, drawn according to the method of Hubbs and Perlmutter (1942). The range in this measurement for each group is indicated by the length of the light line; the position of the mean is shown by a crossbar; the open rectangle marks off two times the standard error on each side of the mean; and the heavy line extends for one standard deviation on each side of the mean.
PERCENT OF WEIGHT CHANGE

2

4

6

8

10

12

24

36

Ringers

Brain

Pituitary

HOURS

0 5 10 15 20 25 30 35
those of animals injected with desiccated extracts, the same control

group was used to compare with those treated with the desiccated

pituitary as well as those which had received the fresh gland.

The results obtained from twenty frogs each receiving 4.0 milli-

grams of desiccated beef brain, and twenty more treated with the same

amount of desiccated barracuda brain, may be described as a fluctua-

tion within normal limits of untreated animals.

The mean weight changes of the groups injected with desiccated

beef and desiccated barracuda pituitary seem to follow the same pattern,

although the pattern of the latter is an enlarged image of that of the

former. Mean weight changes of barracuda pituitary treated animals

showed a rapid increase to 18.9 per cent four hours after the time of

injection. A decrease followed this rise, until forty-eight hours

after injection this group showed only 5.5 per cent of weight increase.

The experiments were discontinued at this time as the weight increment

was not statistically significant when compared to the natural fluctu-

ations of uninjected specimens.

The mean weight changes of frogs receiving beef pituitary exhibited

a maximum weight increase of 8.1 per cent two hours after injection,

this being earlier than the time at which the barracuda extract showed

its maximum effect. This rapid increase, caused by beef pituitary,

was followed by a more gradual decrease back to normal nine hours

later, which again is considerably more rapid than the time in which

the effect of the barracuda extract seemed to lose its effectiveness.

Following the arrival to normal weight in ten to twelve hours after

injection, the beef pituitary injected specimens had a slight to

pronounced decrease below normal weight; and towards the end of the
experiment, these animals seemed to merely have a normal fluctuation in weight.

A statistical analysis of the weight changes induced by beef and fish gland extracts showed that the rise was significantly different from those of the control groups beginning at one hour after injection. The two pituitary extracts, however, did not show a difference from each other at this time. From the second to the tenth hour after injection, increases in weight caused by barracuda pituitary were found to be statistically greater than those caused by beef pituitary. Both were significantly different from the weight changes of groups injected with control suspensions. From the twelfth hour after injection until the end of the experiment, the weights of animals treated with fish pituitary remained above those of the control groups. The beef pituitary extract, apparently having lost its effectiveness, did not maintain a significant weight change after the tenth hour (Figs. 3 and 4; Table II).
Fig. 3. - Graph of mean weight changes of frogs injected with acetone desiccated barracuda pituitary extracts and beef pituitary extracts as compared with those injected with an equivalent amount of acetone desiccated barracuda brain extracts, beef brain extracts and with pure Ringer's solution.
Fig. 4. - Graph of weight changes of frogs injected with acetone desiccated barracuda pituitary extracts and beef pituitary extracts as compared with those injected with an equivalent amount of acetone desiccated barracuda brain extracts, beef brain extracts and with pure Ringer's solution, drawn according to the method of Hubbe and Perlmutter (1942).
### TABLE II

**Weight Changes of Frogs Injected with Acetone Desiccated Pituitary Extracts Compared with Controls**

<table>
<thead>
<tr>
<th></th>
<th>HOURS AFTER INJECTION</th>
</tr>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>Pituitary</td>
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<tr>
<td>Standard deviation</td>
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<tr>
<td>Standard error</td>
<td>.62</td>
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<tr>
<td><strong>Beef</strong></td>
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<tr>
<td>Pituitary</td>
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<tr>
<td><strong>Barracuda</strong></td>
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<tr>
<td>Brain</td>
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<td>20 specimens</td>
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<td><strong>Beef</strong></td>
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<tr>
<td>Brain</td>
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<td><strong>Pure Ringer's</strong></td>
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Discussion

As previously indicated, there exists enough evidence to substantiate the belief that weight changes like the ones induced in these experiments are attributed to an alteration in the permeability of the skin (Brunn, 1921; Adolph, 1925).

Unlike most of the literature cited hereto, weight changes of treated frogs in the present investigation were induced by whole pituitary, rather than by specific neurohypophysial fractions. It was believed that for the purpose of comparing the effects of beef and fish pituitary, whole pituitary would satisfactorily show whether or not a difference exists between two extracts which come from widely separated groups of vertebrates. The small size and united lobes of the pituitary body of fishes rendered impractical any attempt to segregate the different lobes of this gland. Haller (1930b) stated that anterior lobe pituitary extracts do not bring about any weight increases due to water uptake in frogs. He stated that the effect of posterior lobe pituitary hormones was not affected by the presence of anterior lobe extracts. As for the intermediate lobe, Oldham (1936) reported that the melanophore-dilating hormone (intermedin) does not enter in the reactions produced by neurohypophysial extracts.

It is apparent that increases and decreases in weight like the ones shown by the two control groups seem to represent a random fluctuation, rather than to follow a regular pattern. These changes may possibly be attributed partly to retention or loss of urine which was
not entirely controlled during the experiments, and it may be partly attributed to the normal variation of uninjected frogs previously discussed.

On the other hand, the series injected with pituitary exhibited a definite pattern, namely, a rapid increase in weight in about four hours after injection, followed by a very gradual decrease back to normal in about thirty-six hours.

The fact that the weights of control specimens always showed a lower average towards the end of the experimental period as compared with the early hours of the experiment may be explained by the fact that original weights were determined immediately after injection of extracts suspended in one-half cubic centimeter of saline. The complete expulsion of this liquid from the body could account for a decrease in weight of from 2.0 to 5.0 per cent, depending on the weight of the animal in question. The weight fluctuation may be partly attributed to expulsion of urine, and partly to the natural weight fluctuation typical of untreated specimens.

The similarity in potency of fresh fish pituitary and desiccated fish pituitary seems to indicate that the process of extraction did not materially change the effect of the hormone. Both exerted their maximum effect towards the fourth hour after injection. The maximum mean weight increase for specimens treated with salmon pituitary ascended to 16.0 per cent, the mean for those treated with barracuda to 18.9 per cent. Both induced the same pattern of decrease in weight after the time of maximum increase, although frogs treated with fresh salmon gland attained normal weight in thirty-six hours, whereas those which received the desiccated barracuda material remained above normal weight for forty-eight hours.
The amounts of fresh and extracted fish pituitary were considered approximately equivalent. The difference in peak effects and duration between them may be explained in terms of absorption. Specimens injected with two fresh glands dissected right after experimentation still contained a large part of the injected salmon gland, which apparently remains unabsorbed, suggesting that not all of the active principles were absorbed. Individual variations and experimental errors also have to be taken into consideration, as well as specific differences between the salmon and the barracuda.

There are three marked differences between frogs treated with desiccated beef extracts as compared to those which received the desiccated fish pituitary. (1) The beef material induced its peak effect within two hours after injection, whereas the fish gland required four hours. (2) The fish extract caused a greater maximum weight gain. (3) The fish extract had a more prolonged effect.

The first difference in particular may partly be explained by a dissimilarity in the size of the particles of the injected powders. Inasmuch as the fish material was the coarser of the two, it may have been absorbed more slowly. Furthermore, individual frogs injected with either fish or beef pituitary reached their peak weight within the rather wide range of one to ten hours, a variability previously noted by Brunn (1921), Heller (1930b; 1930c), and others cited. However, it is believed that in this experiment a large enough sample was used to make such variability in reaction of individuals negligible when they were treated together statistically.

The second and third differences strongly suggest that the teleost pituitary is more potent in its effect to cause water absorption and
retention in amphibians than is the bovine pituitary. In an equal amount of extract, there must either be a greater concentration of the principle (principles?) involved in the fish gland, or the factors concerned in the pituitaries from the two widely separated vertebrates may differ basically.

The teleost extracts were from a marine species. There is certainly the possibility that such fish, which live in a hypertonic medium, possess a hormonal mechanism that can greatly inhibit water loss. In this respect it would be interesting to compare the effect of glands from marine and fresh-water species.
Summary and Conclusions

1. Frogs injected with bovine and fish pituitary extracts show a rapid increase in weight followed by a prolonged phase of weight decrease back to normal. These weight changes are attributed to the uptake and temporary retention of water in the subcutaneous spaces, muscles, and some organs like the liver.

2. The uptake of water is believed to be caused by effects of the oxytocic and the pressor principles of the posterior lobe of the pituitary on the permeability of the skin.

3. Frogs injected with two fresh salmon pituitaries exhibited a rapid increase to 16.0 per cent of their original weight in four hours, followed by a prolonged decrease back to normal in thirty-six hours. Control frogs injected with salmon brain and Ringer's solution did not show any weight changes outside their normal range.

4. Frogs injected with 4.0 milligrams of acetone desiccated bovine pituitary or with an identical amount of acetone desiccated barracuda pituitary, exhibited marked weight changes when compared with controls injected with similar amounts of beef brain, barracuda brain, and Ringer's solution.

5. A statistical analysis of weight changes induced by barracuda and by beef pituitary extracts shows a highly significant difference between the two; the pituitary extracts of fish induced a higher and more prolonged weight increase. Frogs injected with beef
pituitary showed a rapid increase to 8.1 per cent in one hour, followed by a return to normal in about ten hours, whereas those treated with barracuda pituitary exhibited an increase to 18.9 per cent in four hours, followed by a prolonged weight decrease back to normal in about forty-eight hours.

6. It is believed that the greater potency of the fish pituitary may be due to a difference in the concentration of the principles involved, or the principles themselves may differ basically in the two extracts.

7. Since marine fishes were used as the source of desiccated fish pituitary, there is the possibility that these animals may possess some special principle by which they can inhibit excessive water loss while inhabiting a hypertonic medium.

8. The principles of the pituitary body of all vertebrate classes should not always be looked upon as identical.
Acknowledgements

During the course of this investigation, I received valuable assistance from many persons. I especially want to express my indebtedness to Dr. George F. Weisel under whose direction this study was carried out and who gave me counsel and assistance throughout the period of experimentation and during the preparation of this paper. Drs. Ludvig G. Browman and Philip L. Wright gave me advice and help on numerous problems throughout the study. My wife spent many hours calculating most of the statistical data presented here.

Also, I am indebted to Summer S. Dow, Charles D. Haynes, Jr., and Walt Them for aid in photography, making of charts, and lettering of the same, respectively.
Literature Cited


Heller, J., 1930(c) Über die Wirkung der getrennten Hypophysenhinterlappenhormone auf die Wasseraufnahme beim Frosch. *Arch. exp. Path. Pharmak.*, 157: 323-329.

Hubbs, C. L. and A. Perlmutter, 1942 Biometric comparison of several samples, with particular reference to racial investigations. *Amer. Nat.*, 76: 582-592.


Fig. 5. - Photographs of specimens injected with 4 milligrams of acetone desiccated hirracusa pituitary extract and with 4 milligrams of acetone desiccated hirracusa brain. The card in front of each specimen indicates the weight of the animal at the time the picture was taken; the watch to the left gives the actual time. The specimens were replaced in their water bath immediately after the taking of each picture.

Note the darkening of the skin in the case of the hirracusa pituitary injected specimen, which, according to Weisb (1950), is due to a distal migration of the pigment in the melanophores.

a. Immediately after injection.

b. Two and one-half hours after injection.

c. Six hours after injection.