University of New Mexico
UNM Digital Repository

Biology ETDs | Electronic Theses and Dissertations

5-27-1961

A Comparative Study of the Antidiuretic Hormone Bio-Assay Using Three Species of Bufo

Jeanne Marie Jordan

Follow this and additional works at: https://digitalrepository.unm.edu/biol_etds

 Part of the Biology Commons

Recommended Citation
Jordan, Jeanne Marie. "A Comparative Study of the Antidiuretic Hormone Bio-Assay Using Three Species of Bufo." (1961).
https://digitalrepository.unm.edu/biol_etds/183

This Thesis is brought to you for free and open access by the Electronic Theses and Dissertations at UNM Digital Repository. It has been accepted for inclusion in Biology ETDs by an authorized administrator of UNM Digital Repository. For more information, please contact disc@unm.edu.

UNIVERSITY OF NEW MEXICO-UNIVERSITY LIBRARIES
A14429 085672

378.789
Un3Ojor
1961
cop. 2

A COMPARATIVE STUDY OF THE ANTIDIURETIC HORMONE BIO-ASSAY

JORDAN

THE LIBRARY
UNIVERSITY OF NEW MEXICO

Call No.
378.789
Un30jor
1961
cop.2

Accession
Number
274129

UNIVERSITY OF NEW MEXICO LIBRARY

MANUSCRIPT THESES

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the University of New Mexico Library are open for inspection, but are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages may be copied only with the permission of the authors, and proper credit must be given in subsequent written or published work. Extensive copying or publication of the thesis in whole or in part requires also the consent of the Dean of the Graduate School of the University of New Mexico.

This thesis by ..Jeanne. Marie. Jordan..... has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

A Library which borrows this thesis for use by its patrons is expected to secure the signature of each user.

NAME AND ADDRESS	DATE

UNIVERSITY OF NEW MEXICO LIBRARY

MANUSCRIPT THESES

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the University of New Mexico Library are open for inspection, but are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages may be copied only with the permission of the authors, and proper credit must be given in subsequent written or published work. Extensive copying or publication of the thesis in whole or in part requires also the consent of the Dean of the Graduate School of the University of New Mexico.

This thesis by . . Jeanne Marie Jordan . . . . . has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

A Library which borrows this thesis for use by its patrons is expected to secure the signature of each user.

NAME AND ADDRESS DATE

A COMPARATIVE STUDY OF THE ANTIDIURETIC HORMONE BIO-ASSAY USING THREE SPECIES OF BUFO

by

JEANNE MARIE JORDAN

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Biology

The University of New Mexico

1961

This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

E. L. Castetter
Dean

May 27, 1961
Date

Thesis committee

M. L. Fisher
Chairman

J. Dudley

E. W. Rypka

378.789
Un30jor
1961
Cop.2

ACKNOWLEDGEMENTS

I wish to express appreciation to my major professor, Dr. M. L. Riedesel, for his advise and guidance during this study. I thank Drs. E. W. Rypka, J. S. Findley, J. K. Lauber, and W. J. Eversole for the help they gave me in this work. I also thank Parke-Davis and Co. for the supply of Pitressin.

274129

I wish to express appreciation to my major professors, Dr. [illegible] Riedesel, for his advice and guidance during [illegible] years, [illegible] Dr. E. W. Hyde, J. S. Findley, J. C. Hunter, [illegible] C. Swanson for [illegible] help they gave me in this work. I also thank [illegible] for the supply of Fibressin.

TABLE OF CONTENTS

CHAPTER		PAGE
I	INTRODUCTION	1
II	LITERATURE SURVEY	3
III	MATERIALS AND METHODS	8
IV	RESULTS	15
V	DISCUSSION	22
VI	CONCLUSIONS	27
	LITERATURE CITED	28
	TABLES	30
	FIGURES	35

TABLE OF TABLES

TABLE		PAGE
I	CHANGE IN BODY WEIGHT AFTER INJECTION OF PITRESSIN	30
II	LEVELS OF SIGNIFICANCE OF SPECIES RESPONSE TO DILUTIONS OF PITRESSIN	31
III	LEVELS OF SIGNIFICANCE COMPARING SPECIES AT SPECIFIC DOSAGES	32
IV	WEIGHT LOSS DURING IMMERSION IN CHLORINE-FREE WATER (WEIGHT LOSS EXPRESSED IN % OF INITIAL WEIGHT BEFORE URINE REMOVAL)	33
V	WEIGHT LOSS DURING IMMERSION IN CHLORINE-FREE WATER (WEIGHT LOSS EXPRESSED IN % OF INITIAL WEIGHT AFTER URINE REMOVAL)	34

TABLE OF FIGURES

FIGURE		PAGE
1	WEIGHT VARIATION IN B. VALLICEPS	35
2	WEIGHT GAIN 1 HOUR AFTER INJECTION OF SALINE OR PITRESSIN	36
3	WEIGHT GAIN 2 HOURS AFTER INJECTION OF SALINE OR PITRESSIN	37
4	WEIGHT GAIN 3 HOURS AFTER INJECTION OF SALINE OR PITRESSIN	38
5	WEIGHT GAIN 4 HOURS AFTER INJECTION OF SALINE OR PITRESSIN	39
6	WEIGHT GAIN 4 HOURS AFTER INJECTION OF 0.7% SALINE	40
7	WEIGHT GAIN 4 HOURS AFTER INJECTION OF 1 MICROUNIT OF PITRESSIN	41
8	WEIGHT GAIN 4 HOURS AFTER INJECTION OF 10 MICROUNITS OF PITRESSIN	42
9	WEIGHT GAIN 4 HOURS AFTER INJECTION OF 100 MICROUNITS OF PITRESSIN	43
10	WEIGHT GAIN 4 HOURS AFTER INJECTION OF 1000 MICROUNITS OF PITRESSIN	44
11	WEIGHT GAIN 4 HOURS AFTER INJECTION OF 10,000 MICROUNITS OF PITRESSIN	45
12 -	WEIGHT GAIN DURING IMMERSION IN CHLORINE-FREE WATER (ANIMALS WEIGHED BEFORE URINE WAS REMOVED)	46
13	WEIGHT GAIN DURING IMMERSION IN CHLORINE-FREE WATER (ANIMALS WEIGHED AFTER URINE WAS REMOVED)	47

FIGURE

CHAPTER I

INTRODUCTION

The regulation of body water and electrolytes in vertebrates is partly under the influence of a complex endocrine mechanism. The principle hormone involved in mammalian water-electrolyte balance is the antidiuretic hormone, vasopressin. The present concept is that this hormone produced by neurosecretory cells in the supraoptic and paraventricular nuclei of the hypothalamus diffuses down the supra-optico-hypophysial tract and accumulates in nerve terminals around blood vessels in the posterior lobe of the pituitary. The hormone is stored in the posterior pituitary until it is released upon proper stressful stimulation. Antidiuretic hormone modifies the active process of water absorption in the distal renal tubule of the mammalian kidney. The skin of amphibians represents another target organ of antidiuretic hormone.

The importance of this hormone is demonstrated clearly by the responses to excess and deficiency. If vasopressin and water are administered to an animal hydremia results. Destruction of the posterior pituitary results in a disease, diabetes insipidus, characterized by excessive fluid loss from the body. Antidiuretic substances have been demonstrated in the urine of vertebrates during withdrawal of drinking water.

Since vasopressin has a direct effect on the body water-electrolyte balance and is an indirect cause of certain pathological

conditions resulting from an upset of this balance, it is extremely important to be able to determine the levels of this antidiuretic hormone in the blood of certain individuals. An assay sensitive and accurate to detect low levels of this hormone in the blood plasma would be of great value. Of the various assays available, the bio-assay has proved most successful. Previous evidence has suggested that toads would be the best assay animals since they are more sensitive to low levels of vasopressin than mammals or other amphibians. There is among the amphibians a direct correlation between sensitivity to the antidiuretic hormone and resistance to drying (Ewer, 1952, pp. 429-439; Steggerda, 1937, pp. 103-106; Sawyer and Sawyer, 1952, pp. 84-98).

This study was designed to (1) determine and compare the sensitivity of three species of toads of the genus Bufo to the hormone Pitressin, a commercial antidiuretic hormone, (2) determine and compare the suitability of three species of Bufo as subjects in antidiuretic hormone bio-assay, and (3) compare the physiological responses of the three species to the injected hormone.

CHAPTER II

LITERATURE SURVEY

The mammalian posterior pituitary releases two hormones, vasopressin and oxytocin. The oxytocic principle acts on smooth muscle of the uterus. Vasopressin or the commercial Pitressin has two effects, one vasopressor and one antidiuretic. Pitressin effects smooth muscle constriction of the blood vessels, especially arteries, and indirectly causes an increase in blood pressure. Pitressin produces more pronounced effects when it is injected intravenously. Pitressin has an antidiuretic effect which usually causes a retention of water and a loss of salt. This is brought about by an increase in reabsorption of water and a decrease in salt reabsorption by the kidney tubules.

The vasopressor effect of the extracts from the posterior pituitary was demonstrated by Oliver and Schafer (1895, cited in Turner, 1955, p. 423). Dale (1909, pp. 427-447) reported the oxytocic effect, contraction of uterine smooth muscle, of pituitary extract. The vasopressor and oxytocic principles were separated by Kamm (1928, pp. 573-600). The chemical structure of beef vasopressin was identified by duVigneaud (1953, pp. 4879-4880). The polypeptide hormone was synthesized by duVigneaud and his group (duVigneaud et al., 1954, pp. 4751-4752). In swine vasopressin there is a lysine amino acid in place of the arginine amino acid found in beef vasopressin.

In 1921 Brunn (1921, pp. 170-175, cited in Ewer, 1950, pp. 40-49) demonstrated a temporary increase in the body water content of frogs kept in water following injection with mammalian posterior pituitary

extract. Brunn termed this fraction the water-balance principle. He concluded that the water-balance effect was due to increased water absorption through the skin. The oxytocic fraction influences both antidiuretic response and water-balance in frogs, and since most previous work was with Rana there was a tendency to assume that the water-balance principle was a single entity more closely related to the oxytocic principle of the mammalian posterior pituitary extract. The results of a study by Ewer (1952, pp. 429-439) show that Anura vary in their sensitivity to the oxytocic and pressor fractions of mammalian extracts. Rana was more sensitive to the oxytocic fraction, Bufo was more sensitive to the vasopressor fraction, and Xenopus (an aquatic toad) was equally responsive to both. The evidence suggests that the water-balance principle is not identical in different genera of Anura, but in all cases is similar chemically to Pitocin and Pitressin. The animal's own water-balance fraction may resemble either the mammalian pressor fraction or the mammalian oxytocic fraction. The animal will respond to a greater extent to the mammalian extract most similar to that of the animal's own water-balance fraction.

Sawyer and Sawyer (1952, pp. 84-98) demonstrated the vasopressor principle, Pitressin, and not the oxytocic principle, oxytocin, accounted for the antidiuretic response of Bufo marinus. Regarding the sensitivity to Pitressin and the antidiuretic response, the toad resembles reptiles and mammals rather than frogs. The authors concluded that the toad and reptile probably depend upon glomerular antidiuresis as a physiological mechanism for the conservation of body water.

Ewer (1950, pp. 40-49) found that the water-balance effect of Pituitrin (commercial unfractionated extract of posterior pituitary) in Bufo regularis (Reuss) was the result of an increased uptake and decreased output of water. The excess absorbed water was retained in the lymph spaces and not the body tissues. He concluded that this increased absorption of water resulted directly from changes in skin permeability or indirectly as a result of skin capillary dilation. Pitressin had a greater effect on B. regularis than on Xenopus. Bufo regularis took up water more rapidly than Xenopus in response to Pitressin, which caused lymph accumulation and water reabsorption from the bladder. After desiccation, B. regularis took up water at a greatly increased rate but Xenopus did not (Ewer, 1952, pp. 429-439).

Steggerda (1937, pp. 103-106) reported Necturus was less sensitive to Pituitrin than frogs which were less sensitive than toads. Steggerda emphasized the relation of degree of water-balance response to the environmental conditions of the animal in question. Toads living in a dry environment give a greater response than the permanently aquatic vertebrates. He interprets this as suggesting that the water-balance effect is an adaptation to the more terrestrial life of some vertebrates and should not be expected in primarily aquatic vertebrates. Howes (1940, pp. 128-138) supports this idea in his study of the effect of mammalian posterior pituitary extract on the body water of several developmental stages of B. bufo. The extract had no effect until tail absorption had occurred. The water-balance effect became more pronounced with increasing development. The adult type of response was observed only when the animal entered terrestrial life. In

Ewer (1950, pp. 40-49) found that the water-balance effect of *Pituitrin* (commercial unfractionated extract of posterior pituitary) in Bufo regularis (Reuss) was the result of an increased uptake and decreased output of water. The excess absorbed water was retained in the lymph spaces and not the body tissues. He concluded that this increased absorption of water resulted directly from changes in skin permeability or indirectly as a result of skin capillary dilation. Pitressin had a greater effect on B. regularis than on Xenopus. Bufo regularis took up water more rapidly than Xenopus in response to Pitressin, which caused lymph accumulation and water reabsorption from the bladder. After desiccation, B. regularis took up water at a greatly increased rate but Xenopus did not (Ewer, 1952, pp. 429-439).

Steggerda (1937, pp. 103-106) reported Necturus was less sensitive to Pituitrin than frogs which were less sensitive than toads. Steggerda emphasized the relation of degree of water-balance response to the environmental conditions of the animal in question. Toads living in a dry environment give a greater response than the permanently aquatic vertebrates. He interprets this as suggesting that the water-balance effect is an adaptation to the more terrestrial life of some vertebrates and should not be expected in primarily aquatic vertebrates. Howes (1940, pp. 128-138) supports this idea in his study of the effect of mammalian posterior pituitary extract on the body water of several developmental stages of B. bufo. The extract had no effect until tail absorption had occurred. The water-balance effect became more pronounced with increasing development. The adult type of response was observed only when the animal entered terrestrial life. In

amphibian water-balance there is a positive correlation between tolerance to drying and sensitivity to the antidiuretic hormone.

Heller (1945, pp. 147-157) lists three mechanisms which may explain the temporary increase in body water in amphibians injected with posterior pituitary extracts and kept in water: (1) an increase in water uptake through the skin, (2) inhibition of renal water excretion, and (3) increased water-binding power of the tissues. Sawyer (1956, pp. 44-59) lists four ways amphibians respond to dehydration or injection of antidiuretic hormone: (1) increased water absorption through the skin, (2) constriction of afferent arterioles to reduce glomerular filtration, (3) water reabsorption by the bladder, and (4) tubular reabsorption. Only a limited amount of water may be reabsorbed by the urinary system since toads cannot excrete urine hypertonic to the blood plasma and some water must be lost with salt excretion.

Toads have been used as assay animals by many groups studying the water uptake produced by extracts from the posterior pituitary of toads. Ewer (1952, pp. 429-439) reported B. regularis and B. carens showed large weight increases after injection of toad posterior pituitary extract, but Xenopus did not. The frog posterior pituitary released its hormone in response to dehydration and the hormone increased the rate of water absorption by the skin of dehydrated frogs (Levinsky and Sawyer, 1952, pp. 110-116; 1953, pp. 272-274).

Buchborn (1955, pp. 614-625) appears to have been the first to use toads as assay animals in determining levels of mammalian antidiuretic hormone. He found that Bufo bufo was sensitive to so little as 10 microunits of Pitressin per cc of solution, and he was able to plot

amphibian water-balance [illegible] regulation necessary to [illegible] to drying and possibility to the invasion of [illegible].

Heller (1945, pp. 167-174) lists three mechanisms which may explain the temporary increase in body weight in amphibians injected with posterior pituitary extracts and kept in water: (1) an increase in water uptake through the skin, (2) inhibition of renal water excretion, and (3) increased water-binding power of the tissues. Sawyer (1951, pp. 44-47) lists four ways amphibians respond to dehydration or injection of antidiuretic hormone: (1) increased water absorption through the skin, (2) constriction of afferent arterioles to reduce glomerular filtration, (3) water reabsorption by the bladder, and (4) tubular reabsorption. Only a limited amount of water may be reabsorbed by the urinary system since anurans cannot excrete urine hypertonic to the blood plasma and some water must be lost with salt excretion.

Toads have been used as assay animals for some years in studying the water uptake produced by extracts from the posterior pituitary gland. Ewer (1952, pp. 429-439) reported B. regularis and B. carens showed large weight increases after injection of toad posterior pituitary extract, but Xenopus did not. The frog posterior pituitary releases its hormone in response to dehydration and the hormone increases the rate of water absorption by the skin of dehydrated frogs (Levinsky and Sawyer, 1952, pp. 110-116; 1953, pp. 272-276).

Buchborn (1955, pp. 311-325) was the first to use toads as assay animals in determining levels of the mammalian antidiuretic hormone. He found that Bufo bufo was sensitive to as little as 10 microunits of [illegible] and he [illegible].

semilogarithmically an S-shaped dose-response curve with constant slope between 10 and 1500 microunits of antidiuretic hormone (Buchborn, 1957, pp. 375-379).

Further work has been done on the physiological response of B. marinus to Pitressin. Leaf (1960, pp. 175-189) demonstrated that the addition of 100 mU. of Pitressin increased the permeability of the toad bladder to urea by as much as 40 times, increased the permeability to sodium, and increased water permeability by a factor of two. The hormone acted on the mucosal diffusion barrier (single layer of epithelial cells) to increase permeability. The hormone had to be added to the serosal layer. Vasopressin caused a passive but selective permeability increase of the bladder of B. marinus to urea (Maffly, 1960, pp. 630-641). Larger doses of vasopressin caused sodium loss through the skin (Bentley, 1957, pp. 126-134). Vasopressin is the hormone responsible for the water movement through the bladder of B. marinus. This movement is dependent on an intact oxygen and glucose supply plus certain enzymatic systems (Bentley, 1958, pp. 201-207).

CHAPTER III

MATERIALS AND METHODS

The toads used in this bio-assay were adult males. Animals in any stage of the molting process were not used. The Bufo valliceps (Carolina Biological Supply Co., Elon College, North Carolina) ranged in size from 15 to 40 grams. Bufo marinus (Quivira Specialties Co., Topeka, Kansas) weighed from 105 to 220 grams, and B. woodhousei from 30 to 115 grams. The B. woodhousei specimens were collected locally in the fall from the Tijeras Canyon Creek 10 miles east of Albuquerque, Bernalillo Co., New Mexico.

Identification of the species was made on cranial crests, skin coloration, parotid glands, and texture of the skin. Errors in identification may have occured regarding B. valliceps because of similarities of species, however the species of B. woodhousei and B. marinus were clearly identified. The B. valliceps specimens had been erroneously identified by the supply house as B. americanus.

The animals were kept in dark animal quarters air-conditioned at 21 degrees centigrade. They were placed in large tanks, each equipped with a drain. Shallow pans were located on the bottom of the tanks. Into these pans a fresh supply of water constantly dripped from an over-head pipe. The tanks were rinsed out daily and scrubbed once a week. It is necessary to keep the containers clean to prevent the spread of disease, especially red leg which is so contagious and fatal to most of those infected.

CHAPTER III

MATERIALS AND METHODS

The toads used in this bio-assay were adult males. Animals in any stage of the molting process were not used. The Bufo valliceps (Carolina Biological Supply Co., Elon College, North Carolina) ranged in size from 15 to 40 grams. Bufo punctatus (Quivira Specialties Co., Topeka, Kansas) weighed from 105 to 220 grams, and B. woodhousei from 30 to 115 grams. The B. woodhousei specimens were collected locally in the fall from the Tijeras Canyon Creek 10 miles east of Albuquerque, Bernalillo Co., New Mexico.

Identification of the species was made on cranial crests, skin coloration, parotid glands, and texture of the skin. Errors in identification may have occured regarding B. valliceps because of similarities of species, however the species of B. woodhousei and B. punctatus were clearly identified. The B. valliceps specimens had been erroneously identified by the supply house as B. americanus.

The animals were kept in dark animal quarters air-conditioned at 21 degrees centigrade. They were placed in large tanks, each equipped with a drain. Shallow pans were located on the bottom of the tanks. Into these pans a fresh supply of water constantly dripped from an over-head pipe. The tanks were rinsed out daily and scrubbed once a week. It is necessary to keep the containers clean to prevent the spread of disease, especially red leg which is so contagious and fatal to most of those infected.

Bufo marinus and B. woodhousei were marked either by Michel 14 mm metal wound clips attached to their skin on the extreme posterior dorsal surface or by numbered plastic bird bands placed around their legs. Bufo valliceps was kept in a separate tank in which separate containers were placed, one for each animal.

The animals were force fed small chunks of beef liver every two weeks. The meat, about one square centimeter in size, was placed on the end of a small spoon which was then inserted between the jaws of the animal. When the jaws were opened the meat was forced into the mouth. The subsequent stimulation of the animal's tongue caused the animal to swallow the food. The animals were not fed for four days prior to experimentation to prevent changes in body weight due to the loss of waste food material.

The procedure followed in the bio-assay was adapted by Lauber (1958, pp. 25-30) from the original work of Buchborn (1955, pp. 614-625). The animals were removed from the animal quarters and brought to the laboratory 12 hours prior to experimentation and placed together in a large container containing aged water approximately three inches deep. Tap water was allowed to stand in containers for at least 24 hours in order to decrease the chlorine content before the water was used. The animals were left undisturbed in the aged water over night for a 12 hour period. In this way it could be assumed that at the beginning of the experiment the animals were in equilibrium with the water.

At the end of 12 hours the animals were removed from the soaking containers and placed in individual glass jars. The urine was removed from each animal prior to experimentation. To remove the urine the animal was held on its back in the left hand with the thumb on the

ventral surface near the region of the pelvic girdle. A 500 lambda pipette, held between the thumb and forefinger of the right hand, was inserted into the cloaca. The third finger of the right hand was pressed against the dorsal surface of the animal. A slight downward movement of the pipette to either side was enough to cause the cloacal spincter muscle to relax and the urine to be voided. Occasionally it was necessary to stretch the cloacal opening several different times in order to remove all of the urine from the bladder.

During the experiment the animals were submerged in brown glass vessels containing chlorine-free water. The individual containers used for the experiment were made from the bottom half of brown glass gallon jars. The dark color of the glass decreased the amount of light in the container, thus decreasing the chance of any indirect effects on the animal because of the stimulation of the intermediate lobe of the pituitary which regulates the degree of pigmentation. The dark color also decreased the likelyhood of the animals being disturbed or excited. The glass jars were filled with enough aged water to cover the animal's body, but at the same time it allowed them to rest on the bottom of the jar. It is important to use enough water to submerge the animal. Some of the animals, especially B. valliceps, attempted to stand on their hind legs and as a result only their lower legs were in the water. In cases such as this it was necessary to add more water to the jar. It was necessary to cover the jars with a piece of plywood and to place weights on the lids to prevent the animals from jumping out of the jar or being disturbed by movements from above. Enough open space was allowed for an adequate air supply. The water temp-

ventral surface near the region of the pelvic girdle. The glass pipette, held between the thumb and forefinger of the right hand, was inserted into the cloaca. The third finger of the right hand was pressed against the dorsal surface of the animal. A slight forward movement of the pipette to either side was enough to cause the cloacal sphincter muscle to relax and the urine to be voided. Occasionally it was necessary to stretch the cloacal opening several different times in order to remove all of the urine from the bladder.

During the experiment the animals were placed in brown glass vessels containing chlorine-free water. The individual containers used for the experiment were made from the bottom half of brown glass gallon jars. The dark color of the glass decreased the amount of light in the container, thus decreasing the chances for any undesirable effects on the animal because of the stimulation of the intermediate lobe of the pituitary which regulates some degree of pigmentation. The dark color also decreased the likelihood of the animals being frightened or excited. The glass jars were filled with enough tap water to cover the animal's body, but at the same time it allowed them to rest on the bottom of the jar. It is important to have enough water to submerge the animal. Some of the animals, especially [illegible], attempted to stand on their hind legs and as a result only their lower legs were in the water. In cases such as this it was necessary to add more water to the jar. It was necessary to cover the jar with a piece of cloth and to place weights on the cloth to prevent the animals from jumping out of the jar or being disturbed by movements from above. Enough open space was allowed for an adequate air supply. The water being

erature varied from 19 to 22 degrees centigrade from one experiment to another but remained constant during each experiment. The room temperature was normally 24 degrees centigrade plus or minus one degree.

After being placed into jars the animals were weighed at hourly intervals for three hours. After each weighing the urine was removed and the animals were returned to the jars. Each weighing represented the body weight plus the urine accumulated during the previous hour. Before each weighing all excess water on the animal was blotted with absorbent toweling. The animals were weighed on a Mettler balance to the nearest 0.1 gram.

After the third weighing each animal was injected with the test solution equivalent to 5% of its body weight. The amount of the injection was determined by taking an average of the three previous weighings. The solutions were injected slowly into the dorsal lymph sac by inserting a 23 gauge, one inch needle under the skin of the medial surface of the left thigh and extending the needle into the lymph sac. The skin of the thigh was easy to penetrate with the needle and there was little chance of losing any of the solution by leakage out the puncture wound. During the injection the animals were held dorsal side up in the left hand. The right leg of the animal was held down by the palm of the left hand and the left leg was held between the thumb and forefinger of the left hand. The control group was injected with 0.7% NaCl solution equivalent to 5% of the body weight as determined by the average weight of the three previous weighings.

The various dosages of the hormone were made by serially diluting

erature varied from 19 to 22 degrees centigrade from one experiment to another but remained constant during each experiment. The room temperature was normally 24 degrees centigrade plus or minus one degree.

After being placed into jars the animals were weighed at hourly intervals for three hours. After each weighing the water was changed and the animals were returned to the jars. Each weighing represented the body weight plus the water ingested during the previous hour. Before each weighing all excess water on the animal was blotted with absorbent toweling. The animals were weighed on a torsion balance to the nearest 0.1 gram.

After the third weighing each animal was injected with the test solution equivalent to 5% of its body weight. The amount of the injection was determined by taking an average of the three previous weighings. The solutions were injected slowly into the dorsal lymph sac by inserting a 27 gauge, one inch needle under the skin of the medial surface of the left thigh, then forcing the needle into the lymph sac. The skin of the thigh was easy to penetrate with the needle and there was little chance of losing any of the solution by leakage out the puncture wound. During the injection the animals were held dorsal side up in the left hand. The right leg of the animal was held down by the palm of the left hand and the left leg was held between the thumb and forefinger of the left hand. The control group was injected with 0.7% NaCl solution equivalent to 5% of the body weight as determined by the average weight of the three previous weighings.

The various dosages of the hormones were made by serially diluting

ampoules of Pitressin* (Parke-Davis Co.) in 0.7% NaCl solution. The dosage range is given in microunits, each microunit being equivalent to one millionth of an International Unit. A fresh refrigerated ampoule was used for each dilution. No attempt was made to keep the solutions longer than a few days.

Immediately after injection the animals were returned to the jars and weighed at hourly intervals. The weighing procedure was the same as before injection. Excess water was blotted, the animal weighed, and urine removed. Four such hourly weighings were made. The experiment terminated with the fourth weighing. The protocol of the bio-assay experiment may be summarized as follows:

1. Bring animals from animal quarters to laboratory.
2. Animals placed in a container containing chlorine-free water for 12 hours.
3. Animals removed from containers, dried, and weighed.
4. Urine removed.
5. Animals placed in individual glass jars containing chlorine-free water for 1 hour.
6. Animals removed from jars, dried, and weighed.
7. Urine removed.
8. Steps 5-7 repeated twice.
9. Animals injected with the test solution.
10. Steps 5-7 repeated four times.
11. Animals returned to animal quarters for a two week period.

At least two weeks elapsed before an individual animal was again an experimental subject. In most cases 10 animals of each species were used for each dosage.

---

*Note: The Pitressin (stock 3-161-10) used was an aqueous solution of the pressor and antidiuretic principle of the posterior pituitary gland substantially free from the oxytocic principle and each ampoule contained 20 pressor units per cc. The Pitressin was made from a combination of beef and hog extract.

The surface area was determined for each animal based on the third pre-injection weight using the formula:

$$SA = 9W^{2/3}$$

(Spector, 1956, p. 258). The weight gain per 100 cm^2 was determined for each hour after injection by the formula:

$$\frac{W' - W}{SA} \times 100$$

where W' equals the third weight just prior to injection and W equals the post-injection weight at hourly intervals. An average was then taken for the ten cases.

Statistical analysis was applied to determine the significance of the data. The variances of the samples were assumed equal and the Null Hypothesis to be tested was that the true means of the samples were equal. The formula used for the "t" test was:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where $\bar{x}_1$ equals the mean of the first sample, $\bar{x}_2$ the mean of the second sample, s the standard deviation, n_1 the number of cases in the first sample, and n_2 the number of cases in the second sample. The formula used for variance was:

$$s^2 = \frac{\Sigma(x - \bar{x}_1)^2 + \Sigma(x - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

A value for p of 0.05 or less was considered significant. P is the percent probably that the value of the two means of the samples were be equal. The p value was determined from tables (Bailey, 1959, p. 193).

The effect of soaking was determined for the three species. The animals were moved from the animal quarters to the laboratory and weighed immediately. This weight was used as the normal base weight. The urine was then removed and the animals weighed. This weight was used as the base weight after urine removal. The animals were immediately placed in individual containers of chlorine-free water for a 12 hour period. At the end of this time the animals were removed from the container and weighed, the urine removed and the animals weighed, and replaced into the containers. This was repeated at hourly intervals for the next three hours. The protocol may be summarized as follows:

1. Bring animals from animal quarters to laboratory.
2. Animals weighed.
3. Urine removed.
4. Animals weighed.
5. Animals placed in separate containers containing chlorine-free water for 12 hours.
6. Animals removed from container and weighed.
7. Urine removed.
8. Animals weighed.
9. Animals returned to container for 1 hour.
10. Animals removed from container and weighed.
11. Urine removed.
12. Animals weighed.
13. Steps 9-12 repeated twice.

Percent weight loss was calculated for each weighing based on the normal base weight and the percent weight loss after urine removal was calculated for each weighing based on the base weight after urine removal.

CHAPTER IV

RESULTS

The variables investigated include species, dosage level, and time. The following results describe significant differences in the response of the three species to the antidiuretic hormone. Time and dosage level were significant factors in most of the experiments.

Animals were kept in good nutritional state by force feeding beef liver. Bufo valliceps maintained relatively constant weight over a period of three months (Figure 1). Bufo woodhousei did not maintain a constant weight. Several of the animals died, possibly from improper diet. Many of the males died at the end of two months in the laboratory. Some females had to be sustituted as assay animals, but no significant difference was found between sexes in response to Pitressin.

The responses to Pitressin injection are shown in Tables I, II, and III and in Figures 2 through 11. In Figures 2 through 5 the weight gained in grams per 100 square centimeters body surface area is plotted against the dosage of Pitressin. Weight gain at hourly intervals increased with higher dose levels (Figures 2, 3, 4, 5). The difference in dosage response was greatest four hours after injection of the Pitressin (Figure 5). At the fourth hour the average responses of B. marinus and B. valliceps were in direct relationship to the dosage given. Bufo marinus responded to the lower dosages of one microunit and 10 microunits of Pitressin to a greater degree than the controls, 2.23 and 3.52 grams/100cm^2 surface area respectively (Table I). The degree of the average response was less for the higher dosages of 100; 1,000; and 10,000 microunits; 3.93, 4.94,

and 5.50 grams respectively (Figure 5). The reverse was true for B. valliceps. In the latter case the degree of the response was less for the lower dosages of one and 10 microunits (0.56 and 1.61 respectively) and greater for the higher dosages of 100; 1,000; and 10,000 microunits (2.70, 3.92, and 5.18 respectively). At 10,000 microunits the response of B. valliceps approximated that of B. marinus. At the fourth hour B. woodhousei, like B. marinus, showed a greater response to lower dosages of one and 10 microunits (0.9 and 3.01 grams), but at 100 microunits there was a decline in response (1.45 grams). At 1,000 and 10,000 microunits (3.80 and 3.83 grams) there was again an increase in response, but neither was significantly higher than the response to 10 microunits.

As early as the first hour after injection variations in response of each species to the dosage range could be observed (Figure 2). In B. marinus the response to one microunit (2.91 grams) was significant but the responses to the following dosage levels was essentially equivalent (3.10, 2.81, 2.81, and 2.81 grams). At the first hour after injection the response of B. valliceps and B. woodhousei were similar.

At the second hour the response of B. marinus to 100; 1,000; and 10,000 microunits was nearly equivalent (3.28, 3.57, and 3.76 grams), while that of B. valliceps was greatly increased at 1,000 and 10,000 microunits (2.67 and 3.04 grams). Bufo woodhousei exhibited responses which prevailed during the last three weighings in relation to the other species. Bufo woodhousei like B. marinus was more sensitive to the lower levels of one and 10 microunits (1.45 and 2.40 grams) with the

maximum response at 10 microunits. There was decline in response to 100; 1,000; and 10,000 microunits (2.16, 2.15, and 2.02). The response to 1,000 and 10,000 microunits increased at the third hour to 2.84 and 3.03 grams respectively and this increase persisted to the fourth hour as stated above.

Statistical analysis of the data obtained on specimens of B. marinus demonstrated significant differences in the responses to most dosages of Pitressin and B. valliceps demonstrated significant differences in the responses to all dosages above one microunit. Table II summarizes the levels of significance for the data in Figures 2 through 5. The average response of B. marinus to one microunit was greater than the average response to saline (p equals 0.01). The response to 10 microunits was greater than the response to saline (p equals 0.001). The response to 1,000 microunits was greater than that to 100 microunits (p equals 0.05). The response to 10,000 microunits was greater than that to 100 microunits (p equals 0.01). The average response of B. valliceps to one microunit was not significantly greater than that to saline. The response to 10 microunits was greater than that to saline (p equals 0.02). The response to 100 microunits was greater than that to 10 microunits (p equals 0.05), the response to 1,000 microunits was greater than that to 100 microunits (p equals 0.05), and the response to 10,000 microunits was greater than that to 1,000 microunits (p equals 0.05). The response of B. woodhousei to one microunit was not significant but the response to 10 microunits was significant (p equals 0.001). The response to 100 microunits was greater than the response to 10 microunits (p equals 0.02). The response to 1,000 microunits was greater than that

maximum response to 10 microunits. There was a decline in response to 100, 1,000, and 10,000 microunits (2.16, 2.45, and 2.62). The response to 1,000 and 10,000 microunits increased in the third hour to 2.84 and 3.03 grams respectively, and this increase persisted into the fourth hour as stated above.

Statistical analysis of the data obtained from the responses of *P. maxima* demonstrated significant differences in the responses to most doses of Pitressin and *B. williamsi* demonstrated significant differences in the responses to all dosages above one microunit. Table II summarizes the levels of significance for the data in figures [illegible] through [illegible]. The average response of *P. maxima* to one microunit was greater than the average response to saline (p equals 0.01). The response to 10 microunits was greater than the response to saline (p equals 0.001). The response to 1,000 microunits was greater than that to 100 microunits (p equals 0.05). The response to 10,000 microunits was greater than that to 100 microunits (p equals 0.02). The average response of *B. williamsi* to one microunit was not significantly greater than that to saline. The response to 10 microunits was greater than that to saline (p equals 0.02). The response to 100 microunits was greater than that to 10 microunits (p equals 0.05); the response to 1,000 microunits was greater than that to 100 microunits (p equals 0.05), and the response to 10,000 microunits was greater than that to 1,000 microunits (p equals 0.05). The response of *B. [illegible]* to one microunit was not significantly greater than that to saline; the response to 10 microunits was significantly (p equals 0.001). The response to 100 microunits was greater than the response to 10 microunits (p equals 0.02). The response to 1,000 microunits was greater than the

to 100 microunits (p equals 0.02) but not significantly greater than that to 10 microunits. The response to 10,000 microunits was greater than that to 100 microunits (p equals 0.01) but not greater than that to 10 microunits.

Figures 6 through 11 express the mean weight gain in grams per $100cm^2$ of surface area plotted against time. The variation in response of the three species is evident by comparing the data in Figures 6 through 11. The response to saline over the four hour period was similar for all three species. The first hour represented the maximum response of B. woodhousei (1.56 grams) and B. valliceps (1.19 grams) while the second hour represented the maximum response of B. marinus (1.95 grams). The average response of all three species declined the third hour and further declined the fourth hour after weighing (Figure 6). The first hour represented the maximum response of B. valliceps (1.53 grams) and B. marinus (2.91 grams) to one microunit injections, but B. woodhousei did not reach its maximum response until the second hour (1.45 grams) as seen in Figure 7. Bufo marinus had a maximum response to 10 microunits at the second weighing (3.76 grams) and B. valliceps had a maximum response at the first weighing (1.98 grams). The response of both species leveled off after the second hour. Bufo woodhousei differed in its response to 10 microunits as it did to one microunit with its maximal response at the last hour (3.01 grams) as seen in Figure 8. At the 100 microunit level and greater dosages there was a drop in response of B. woodhousei and an increase in response of B. valliceps. The maximum response for B. woodhousei at 100 microunits was at the first hour (2.16 grams) with a constant decline for subsequent weighings. The maximum response was not

until the third hour for B. valliceps (2.81 grams) and the fourth hour for B. marinus (3.93 grams) as seen in Figure 9. The response of the three species to 1,000 microunits increased with each succeeding hour. The maximum responses for B. marinus, B. valliceps, and B. woodhousei was at the fourth hour (4.94, 3.92, and 3.80 grams respectively) as seen in Figure 10. The maximum responses of the three species to 10,000 microunits was at the same time as that of 1,000 microunits but the increase was greatest for B. valliceps (5.18 grams) and less for B. woodhousei (3.83 grams) and B. marinus (5.50 grams) shown in Figure 11.

The significance of the species mean differences was determined for each dose level and the results are shown in Table III. Significant differences could be found among the species at several hourly periods depending on the dose level. However, the second hour was chosen for species comparison for all doses because the greatest differences among the species was observed at this hour. No significant differences were found in the mean response to saline. There was a significant difference between B. marinus and B. valliceps for all Pitressin dosages (p equals 0.02, 0.002, 0.02, 0.02, and 0.05 for one microunit through 10,000 microunits respectively). Significant differences between B. marinus and B. woodhousei were found at 10 microunits (p equals 0.01), 100 microunits (p equals 0.02), 1,000 microunits (p equals 0.001), and at 10,000 microunits (p equals 0.001). Significant differences between B. valliceps and B. woodhousei were found only at 10 microunits (p equals 0.05) and 10,000 microunits (p equals 0.05).

Pitressin decreased the urine production in all animals. The amount of urine voided after injection of 100 microunits decreased.

Little or no urine could be removed in any of the three species after two hours with 1,000 and 10,000 microunit injections.

The results of the soaking experiment are shown in Figures 12 and 13 and Tables IV and V. The animals were soaked in chlorine-free water for 12 hours and weighed at the 13th through 15th hours after soaking. Bufo valliceps and B. woodhousei store a greater quantity of urine (12.05 and 11.82 grams/100 grams body weight respectively) than does B. marinus (8.17 grams). During the 12 hour soaking period B. valliceps regained 1.57 grams, B. woodhousei 4.98, and B. marinus 1.39 grams. The body weight plus urine remained relatively constant for the three hours after soaking. At the third hour the mean loss for B. valliceps was 12.71 grams, B. woodhousei 11.56 grams, and B. marinus 9.89 grams. This represents the time when the animals were at equilibrium with the water. The percent loss in body weight was determined after the 12 hour soaking period for each species based on the initial weight after urine removal. After the first hour post-soaking period the loss remained constant. B. woodhousei lost 2.10 grams/100 grams body weight, B. marinus 2.88 grams, and B. valliceps 3.85 grams.

On the basis of resistance to disease and ease of maintaining a good nutritional state, B. marinus is preferable to B. valliceps and B. woodhousei as a bio-assay animal. Bufo marinus was more resistant to red leg and skin injuries. Whereas B. marinus was more excitable and difficult to inject initially, with constant handling B. marinus became docile. This is significant because there is greater diuresis in disturbed toads (Jorgensen, et al., 1956, pp. 306-309).

Attempts were made to use two other species, B. terrestris, and B. debilis, but these animals were too small for experimental use. The animal could not be weighed accurately and it was difficult to remove urine. Also animals from both species died soon from unknown causes.

CHAPTER V

DISCUSSION

The results of the experiment indicate that B. marinus and B. valliceps show differential responses to various doses of Pitressin; the response increases as the dosage of the hormone is increased. Although the responses of B. valliceps are less than those of B. marinus for each dose level, the response to the various dosages are significantly higher with increased concentration. This factor may make B. valliceps more valuable as an assay animal for levels of more than 10 microunits. The results of the experiment indicate that B. marinus is more sensitive to Pitressin at all test levels than B. valliceps or B. woodhousei, and B. woodhousei is intermediate in sensitivity at the lower dosages (10 microunits) but is less sensitive than B. valliceps at the higher levels of 1,000 and 10,000 microunits (Figure 5).

The results of species sensitivity correlates with the differences observed in the thickness of the skin and the soaking experiment. Bufo marinus has a very thick skin which is usually thought to be an indication of tolerance to drying (Noble, 1939, p. 140). Bufo valliceps and B. woodhousei had skin intermediate in thickness, and in some cases the skin of B. woodhousei appeared to be somewhat thinner and smoother than that of B. valliceps. The skin of B. woodhousei and B. valliceps was a great deal thinner and smoother than the skin of B. marinus which made B. valliceps and B. woodhousei easier to inject than B. marinus.

It is difficult to report the exact habitat of the three species, but the geographical range may give some indication of the macrohabitat which is somewhat different for the three species. Bufo marinus is found in the coastal regions of Mexico, Central America, and South America to Patagonia (Wright and Wright, 1949, p. 188). Bufo valliceps is found in Louisiana, eastern and south-central Texas, and the east coast of Mexico to Costa Rica (Wright and Wright, 1949, p. 203). Bufo woodhousei is found in southeast Oregon, Idaho, Montana, South Dakota, western Iowa and Missouri, Kansas, Oklahoma, Texas, Arizona, New Mexico, Nevada, Utah, and Wyoming (Wright and Wright, 1949, p. 206). Bufo woodhousei has a very diverse habitat and is normally found in a drier environment than that of the laboratory where they were kept for several months during experimentation. It is possible that due to this change in the environment the water-balance mechanisms of the animal may have been disturbed. Increased environmental humidity may have resulted in decreased sensitivity of the toads whose sensitivity to Pitressin may be normally high.

Bufo marinus was significantly more sensitive than B. valliceps to all dosages of Pitressin. It would be a decided advantage to have a physiological mechanism sensitive to an antidiuretic hormone which could be released during periods of dryness and dehydration. Increase in hormonal influence on the water and electrolyte balance represents an advantage or actual necessity in a xeric environment.

During the hydration experiment where the animals were soaked for 12 hours in chlorine-free water, B. marinus apparently did not produce and store as much urine as did B. woodhousei or B. valliceps. The range

of net loss after urine removal was greatest for B. woodhousei (11.82 - 2.10 grams). The range for B. valliceps was 12.01 - 3.85, and for B. marinus the range was 8.19 - 2.88 grams. This may indicate that in B. marinus the antidiuretic hormone exerts more control over the water balance mechanism than in B. woodhousei or B. valliceps. In B. marinus there appeared to be greater reabsorption of water from the bladder so that at any one time a limited amount of water loss could be expected due to urine excretion. B. woodhousei gained more weight during the 12 hour soaking period indicating a greater increase in water absorption than B. marinus or B. valliceps.

The greatest response to the hormone occurred four hours after injection. There is evidence from the hour of greatest response that the hormone was absorbed more readily from the lymph sac of B. valliceps and to a lesser extent B. marinus, while in B. woodhousei the absorption rate is slower. At the same time it appears from the data, represented in Figures 7 through 11 that the hormone is more readily metabolized or excreted by the kidneys of B. valliceps. At every dosage level except 10,000 microunits the rate of weight change is decreasing by the fourth hour. The rate of hormone metabolism and excretion is slower in B. marinus at levels of one and 10 microunits as evidenced by the slight decrease in weight at the third and fourth hours. At the 100 and 1,000 microunit level B. marinus is still gaining weight at a rapid rate.

The results indicate that B. woodhousei was more sensitive than B. valliceps to injections of one and 10 microunits. Bufo woodhousei absorbed the hormone more slowly since its peak response comes at a

later hour. At the 10 through 10,000 microunit level the situation was reversed and B. valliceps had a greater response to the hormone and at an increased rate. The delay seen in B. woodhousei may be due to slower absorption of the hormone from the lymph sac or to a delayed response of the target organ, the skin. This may be due to the fact that the skin permeability of B. woodhousei has a limited response to Pitressin and after the level of 10 microunits no increase in permeability is evident. The animal may be subject to fatigue at this level. The data supports this fact because even at the fourth hour after injection of 10,000 microunits the increase in body weight of B. woodhousei is not significant over the increase at 10 microunits. The difficulty in maintaining B. woodhousei may have been a factor in determining the response of these animals to the hormone. Whereas in B. valliceps the hormone is absorbed faster but is still effective in causing an increased response with increased dosage.

The data from the saline controls suggests the 0.7% NaCl solution used in the injection of Pitressin may have been hypertonic to the plasma of B. woodhousei, isotonic with that of B. valliceps, and hypotonic to the plasma of B. marinus. This last observation is supported by Lauber (1958, p. 50) who determined the freezing point of B. marinus plasma and found the salinity to be .9% NaCl. Buchborn (1957, pp. 375 - 379) stated that 0.6% NaCl was slightly hypotonic to the plasma of B. bufo. Injected with 0.7% saline solution B. woodhousei lost weight and B. valliceps stayed the same. This may have some taxonomic and evolutionary significance. The more terrestrial types are more independent from the aquatic habitat and therefore are considered more advanced. Water

later hour. At the 10 through 10,000 microunit level the condition was reversed and B. valliceps had a greater response to the hormone and at an increased rate. The delay seen in B. woodhousei may be due to slower absorption of the hormone from the lymph sac or to a delayed response of the target organs, the skin. This may be due to the fact that the skin permeability of B. woodhousei has a limited response to Pitressin and after the level of 10 microunits no increase in permeability is evident. The animal may be unable to exceed this level. The data supports this fact because even at the fourth hour after injection of 10,000 microunits the increase in weight gain of B. woodhousei is not significant over the increase at 10 microunits. The difficulty in maintaining B. woodhousei may have been a factor in determining the response of these animals to the hormone. Whereas in B. valliceps the hormone is absorbed faster but is still effective in causing an increased response with increased dosage.

The data from the saline control suggests the 0.75% NaCl solution used in the injection of Pitressin may have been hypertonic to the plasma of B. woodhousei, isotonic with that of B. valliceps and hypotonic to the plasma of B. marinus. The latter observation is supported by Krogh (1958, p. 50) who determined the freezing point of B. marinus plasma and found the salinity to be 0.9% NaCl. [illegible] (19[illegible], pp. [illegible]) stated that 0.6% NaCl was slightly hypotonic to the plasma of R. pipa. Injected with 0.75 saline solution B. woodhousei decreased and B. valliceps stayed the same. This may have some research and survival significance. The more terrestrial toads are more independent from the aquatic habitat and therefore are [illegible] more [illegible].

conservation and increased salt loss become the major problems. Difference in saline concentration of the plasma may reflect adaptation to a terrestrial life.

The influence of two additional factors on the assay should be investigated for possible differences in physiological responses of the three species: (1) urine output should be measured after each weighing to detect difference in the water reabsorption by the bladder, (2) the concentration of sodium chloride should be determined for the toad plasma and sodium concentration of the water used in the experiment since sodium concentration may effect water transport.

CHAPTER VI

CONCLUSIONS

1. Analysis of the data obtained in this study indicates that B. marinus was more sensitive to the hormone Pitressin than B. valliceps or B. woodhousei at the 1; 10; 100; 1,000; and 10,000 microunit levels.

2. There was significant response of B. marinus to one microunit and in B. valliceps and B. woodhousei there was a significant response to 10 microunits compared with the saline control.

3. Bufo valliceps demonstrated the greatest differential response from one dosage to another. There was a significant increase in response to each increase in hormone concentration.

4. Bufo valliceps absorbed the hormone more readily and excreted it faster than B. marinus or B. woodhousei.

5. Bufo woodhousei was sensitive to dosages of one microunit and 10 microunits but not to those of 100 microunits or more. This indicates the extent of permeability of the skin was at a maximum at 10 microunit injections and there was fatigue of the target organ with subsequent dosage increase.

6. The target organ for the hormone appears to be the skin since decreased urine production was similar in all three species.

7. The differential responses of the three species correlated with variance in skin thickness, and response to soaking in chlorine-free water.

8. The response of the animal to soaking in water indicates that B. marinus had a better hydration regulating mechanism.

LITERATURE CITED

Bailey, Norman T. J. 1959. Statistical methods in biology. English Universities Press Ltd., London. 199 p.

Bentley, P. J. 1957. The effects of vasopressin on water uptake of the toad, Bufo marinus, while bathed in different hypotonic solutions. J. of Endo. 16: 126-134.

Bentley, P. J. 1958. The effects of neurohypophysial extracts on water transfer across the wall of the isolated urinary bladder of the toad, Bufo marinus. J. of Endo. 17: 201-209.

Buchborn, Eberhard. 1955. Ein quantitativer biologischer andiuretin-(vasopressin-) Nachweis an der Kröte. Zeitschr. Ges. Exptl. Med. 125: 614-625.

Buchborn, Eberhard. 1957. Plasma levels of antidiuretic hormone and serum osmolarity in normal human adults. Endocrinology 61: 375-379.

Dale, H. H. 1909. The action of extracts of the pituitary body. Biochem. Jour. 4: 427-447.

Ewer, R. F. 1950. The effect of pituitrin injection on the water balance of Bufo regularis Reuss. Jour. Exptl. Biol. 47: 40-49.

Ewer, R. F. 1952. The effects of posterior pituitary extracts on water balance in Bufo carens and Xenopus laevus, together with some general consideration of anuran water economy. Jour. Exptl. Biol. 29: 429-439.

Heller, H. 1945. The effect of neurohypophyseal extracts on water balance of lower vertebrates. Biol. Rev. 20: 147-157.

Howes, N. H. 1940. The response of the water regulating mechanism of developmental stages of the common toad Bufo bufo bufo (L.) to treatment with extracts of the posterior lobe of the pituitary body. Jour. Exptl. Biol. 17: 128-138.

Jorgensen, C. Barker, and Per Rosenkilde. 1956. Relative effectiveness of dehydration and neurohypophysial extracts in enhancing water absorption in toads and frogs. Biol. Bull. 110: 306-309.

Kamm, Oliver, T. B. Aldrich, I. W. Grote, L. W. Rowe, and E. P. Bugbee. 1928. The active principles of the posterior lobe of the pituitary gland. I. The demonstration of the presence of two active principles. II. The separation of the two principles and their concentration in the form of potent solid preparation. Jour. Amer. Chem. Soc. 50: 573-600.

LITERATURE CITED

Lauber, Jean Kautz. 1958. A bio-assay and histochemical study of antidiuretic hormone in normal and adrenalectomized rats. Ph.D. Thesis. Univ. of New Mexico, Albuquerque, New Mexico, 100 p.

Leaf, Alexander. 1960. Some actions of Neurohypophyseal hormones on a living membrane. Jour. Gen. Physiol. 43: 175-189.

Levinsky, Norman G., and Wilbur H. Sawyer. 1952. Influence of the adenohypophysis on the frog water-balance response. Endocrinology. 51: 110-116.

Levinsky, Norman G., and Wilbur H. Sawyer. 1953. Significance of the neurohypophysis in regulation of fluid balance in the frog. Proc. Soc. Exptl. Biol. and Med. 82: 272-274.

Maffly, Roy H., Richard M. Hays, Ezra Yamdin, and Alexander Leaf. 1960. The effect of neurohypophysial hormones on the permeability of the toad bladder to urea. Jour. Clin. Invest. 39: 630-641.

Noble, G. Kingsley. 1931. The biology of the amphibia. Dover Publications, Inc., New York. 577 p.

Sawyer, W. H. 1956. The hormonal control of water and salt-electrolyte metabolism with special reference to the amphibia. Mem. Soc. Endo. No. 5: 44-59.

Sawyer, W. H., and M. K. Sawyer. 1952. Adaptive responses to neurohypophyseal fractions in vertebrates. Physiol. Zool. 25: 84-98.

Spector, William S. 1956. Handbook of biological data. W. B. Saunders Co., Philadelphia. 258 p.

Steggerda, F. R. 1937. Comparative study of water metabolism in amphibians injected with pituitrin. Proc. Soc. Exptl. Biol. and Med. 36: 103-106.

Turner, C. Donnell. 1955. General endocrinology. Second edition. W. B. Saunders, Philadelphia. 423 p.

Du Vigneaud, Vincent, Charlotte Ressler, John M. Swan, Carleton W. Roberts, Panayotis G. Katsoyannis, and Samuel Gordon. 1953. The synthesis of an octapeptide amide with the hormonal activity of oxytocin. Jour. Amer. Chem. Soc. 73: 4879-4880.

Du Vigneaud, Vincent, Duane T. Gish, and Panayotis G. Katsoyannis. 1954. A synthetic preparation possessing biological properties associated with arginine-vasopressine. Jour. Amer. Chem. Soc. 76: 4751-4752.

Wright, Albert Hazen, and Anna Allen Wright. 1949. Handbook of frogs and toads of the United States and Canada. Comstock Publishing Co. Inc., Ithaca, New York. 640 p.

TABLE I

CHANGE IN BODY WEIGHT AFTER INJECTION OF PITRESSIN

Number of animals	Pitressin Dosage in microunits	Hourly Response 1	2	3	4
	B. marinus				
9	saline	1.73	1.95	1.13	0.93
9	1	2.91	2.45	2.42	2.23
10	10	3.10	3.76	3.58	3.52
10	100	2.81	3.28	3.46	3.93
10	1000	2.81	3.57	4.30	4.94
10	10000	2.81	3.76	4.62	5.50
	B. valliceps				
10	saline	1.19	1.14	0.53	0.10
10	1	1.53	1.24	0.64	0.56
6	10	1.98	1.66	1.74	1.61
10	100	1.92	2.48	2.81	2.70
10	1000	2.02	2.67	3.44	3.92
9	10000	2.16	3.04	4.18	5.18
	B. woodhousei				
10	saline	1.56	0.94	0.11	0.39
9	1	1.31	1.45	1.22	0.90
10	10	1.92	2.40	2.88	3.01
10	100	2.16	2.16	1.83	1.45
10	1000	1.62	2.15	2.84	3.80
9	10000	0.94	2.02	3.03	3.83

TABLE II

LEVELS OF SIGNIFICANCE
OF SPECIES RESPONSE TO DILUTIONS OF PITRESSIN

Dosage Comparison	B. marinus	B. valliceps	B. woodhousei
1 microunit vs saline	P - 0.01	*NS	0.10
10 microunits vs saline	0.001	0.02	
10 microunits vs 1 microunit	0.10		0.001
100 microunits vs 1 microunit	0.002		
100 microunits vs 10 microunits	*NS	0.05	0.02
1000 microunits vs 10 microunits			0.10
1000 microunits vs 100 microunits	0.05	0.05	0.002
10000 microunits vs 10 microunits			0.10
10000 microunits vs 100 microunits	0.01		0.01
10000 microunits vs 1000 microunits	*NS	0.05	

*Not Significant

TABLE III

LEVELS OF SIGNIFICANCE
COMPARING SPECIES AT SPECIFIC DOSAGES

Dosage in microunits	*B. marinus* vs *B. valliceps*	*B. marinus* vs *B. woodhousei*	*B. valliceps* vs *B. woodhousei*
saline	0.10	0.10	NS
1	0.02	NS	NS
10	0.002	0.01	0.05
100	0.02	0.02	NS
1000	0.02	0.001	0.10
10000	0.05	0.001	0.05

TABLE IV

WEIGHT LOSS DURING IMMERSION IN CHLORINE-FREE WATER
(WEIGHT LOSS EXPRESSED IN % OF INITIAL WEIGHT
BEFORE URINE REMOVED)

	B. marinus	B. valliceps	B. woodhousei
After Initial Urine Removal	8.19%	12.05%	11.82%
After 12 Hours Soaking (urine not removed)	6.80	10.48	6.84
After 13 Hours Soaking (urine not removed)	9.81	13.73	11.52
After 14 Hours Soaking (urine not removed)	10.02	12.34	11.68
After 15 Hours Soaking (urine not removed)	9.89	12.71	11.56

TABLE V

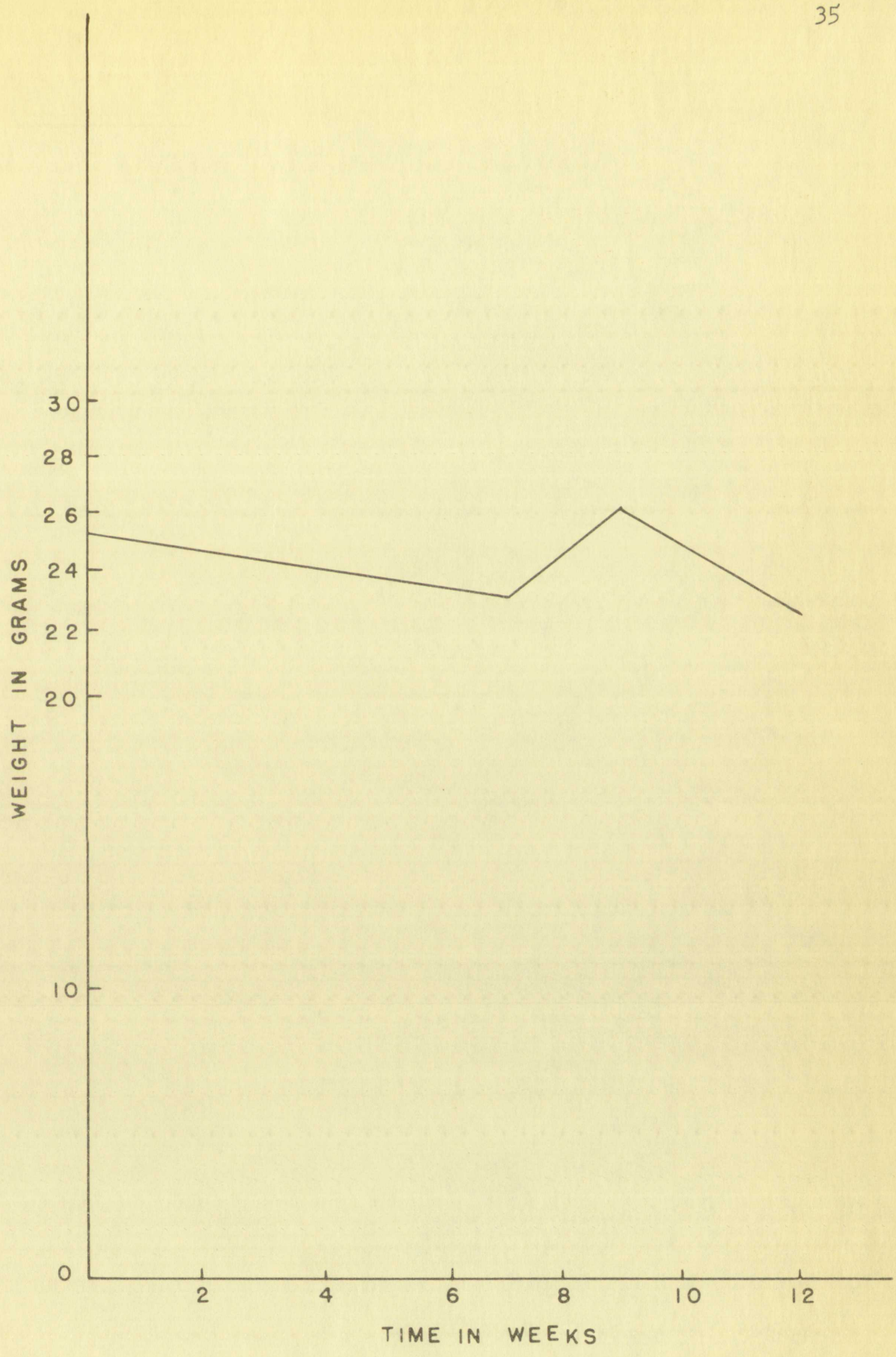
WEIGHT LOSS DURING IMMERSION IN CHLORINE-FREE WATER
(WEIGHT LOSS EXPRESSED IN % OF INITIAL WEIGHT AFTER URINE REMOVAL)

Time	B. marinus	B. valliceps	B. woodhousei
After 12 Hours Soaking (urine removed)	2.08%	3.84%	1.34%
After 13 Hours Soaking (urine removed)	2.91	4.38	2.14
After 14 Hours Soaking (urine removed	2.76	4.00	2.00
After 15 Hours Soaking (urine removed)	2.88	3.85	2.10

TABLE V

WEIGHT LOSS DURING IMMERSION IN CHLORINE-FREE WATER
(WEIGHT LOSS EXPRESSED IN % OF INITIAL WEIGHT AFTER URINE REMOVAL)

Time	B. marinus	B. vallicepe	B. woodbroweri
After 12 Hours Soaking (urine removed)	2.08%	3.8%	1.3%
After 13 Hours Soaking (urine removed)	2.91	4.38	2.1%
After 14 Hours Soaking (urine removed	2.76	4.09	2.00
After 15 Hours Soaking (urine removed)	2.88	3.85	2.10



WEIGHT VARIATION IN B. VALLICEPS

FIG. 1

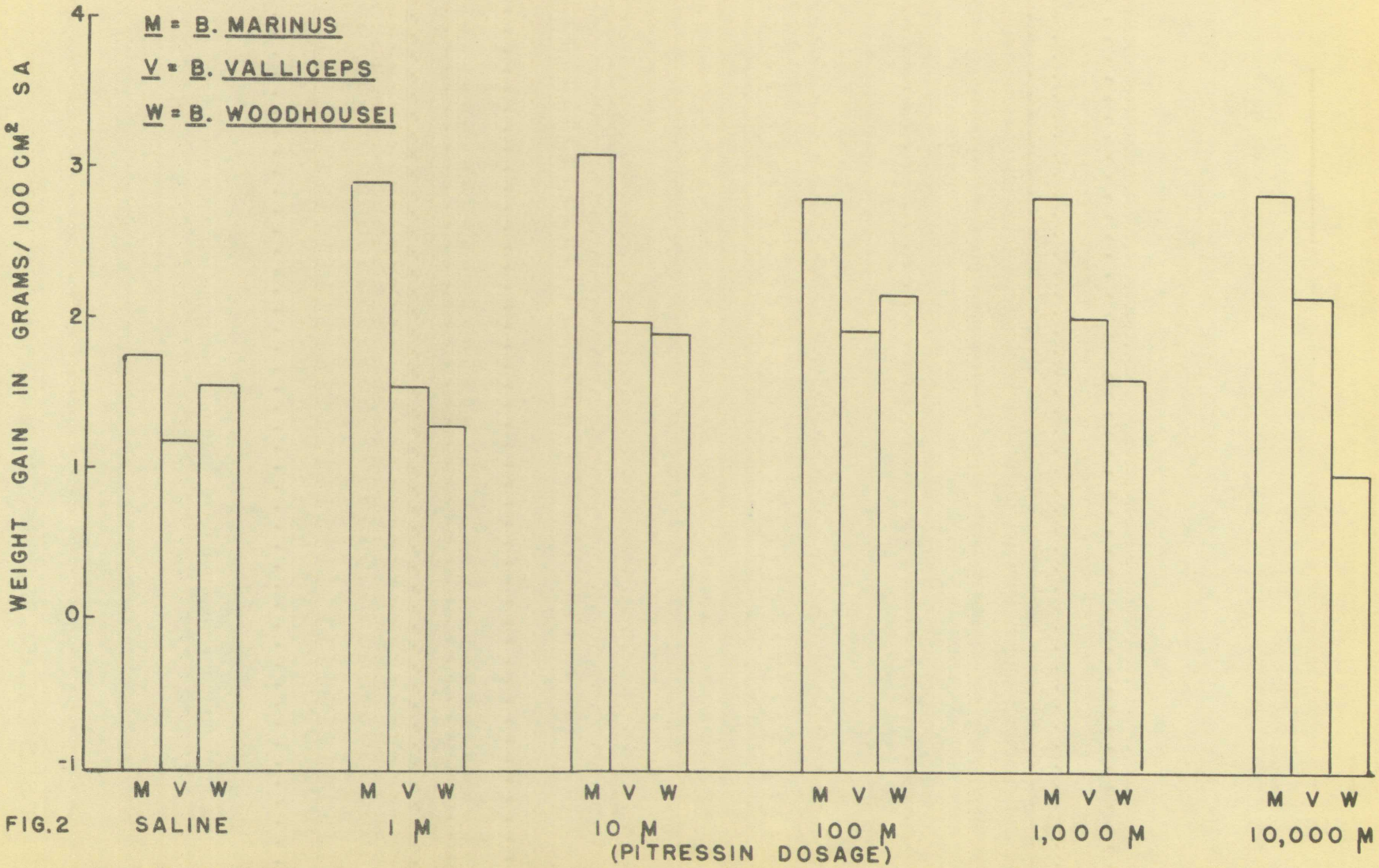


FIG. 2 WEIGHT GAIN 1 HOUR AFTER INJECTION OF SALINE OR PITRESSIN

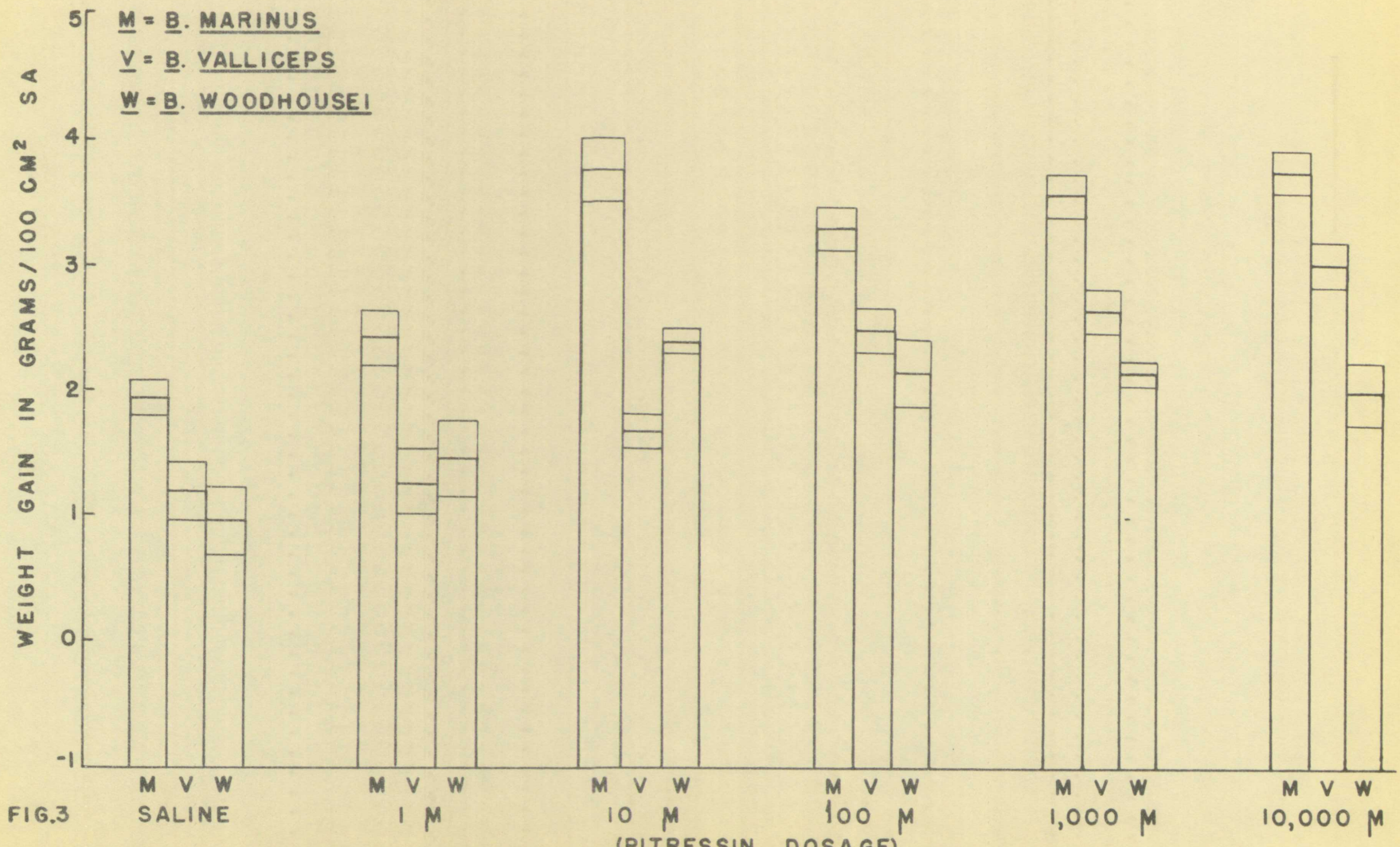


FIG.3 WEIGHT GAIN 2 HOURS AFTER INJECTION OF SALINE OR PITRESSIN

HEAVY LINE INDICATES THE MEAN VALUE OF 10 ANIMALS, THIN LINE REPRESENTS 2 STANDARD DEVIATIONS ABOVE AND BELOW THE MEAN

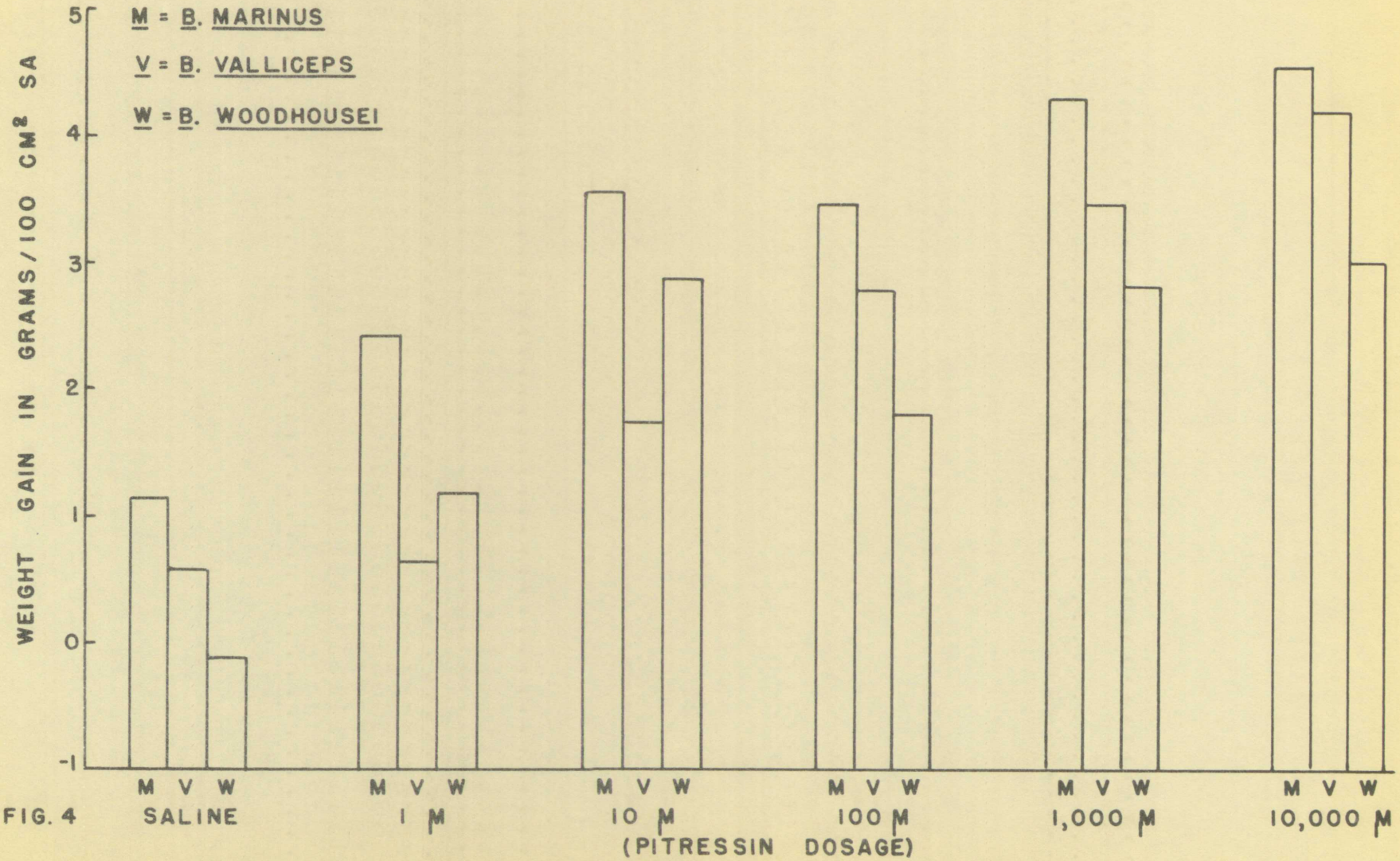


FIG. 4 WEIGHT GAIN 3 HOURS AFTER INJECTION OF SALINE OR PITRESSIN

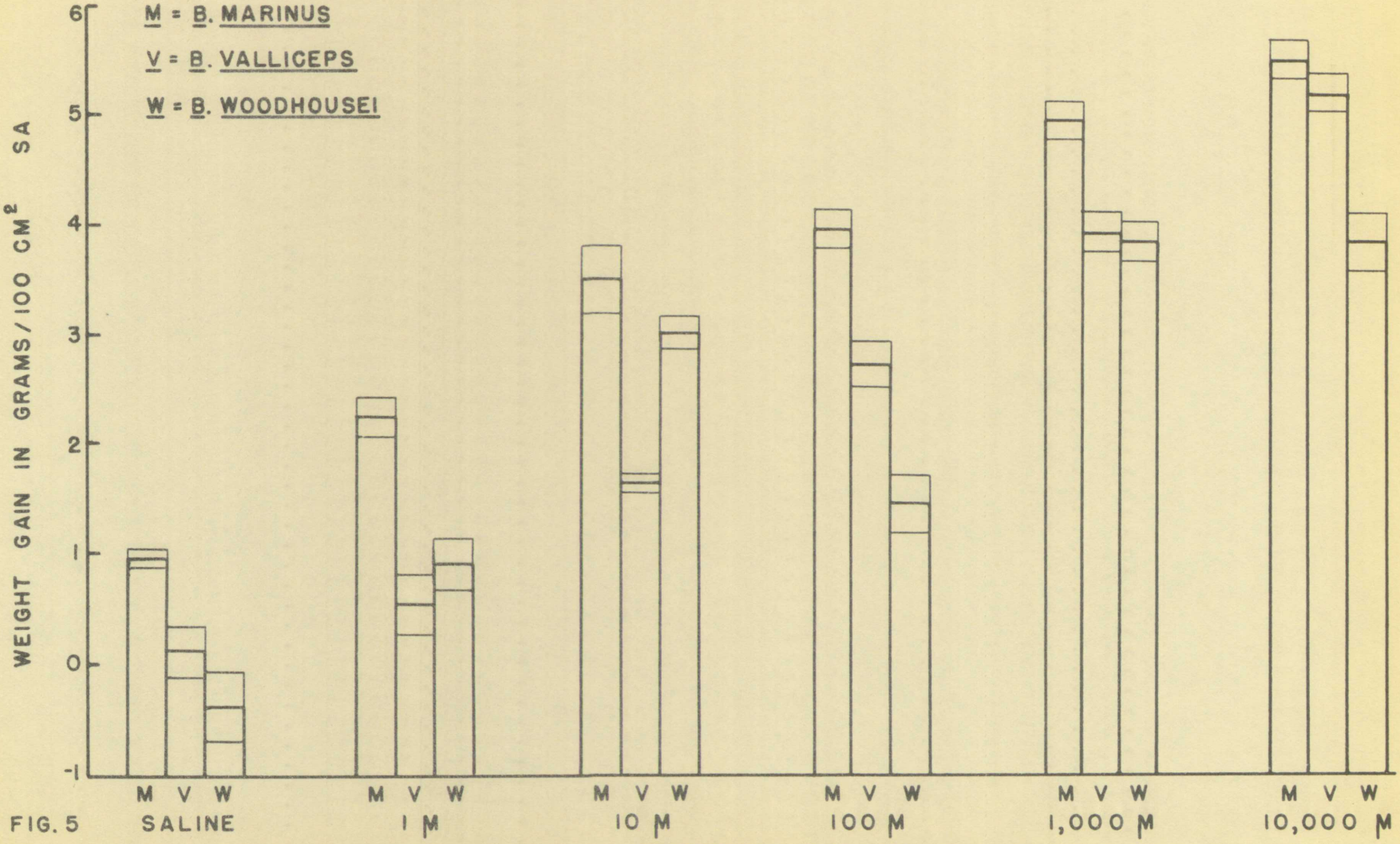
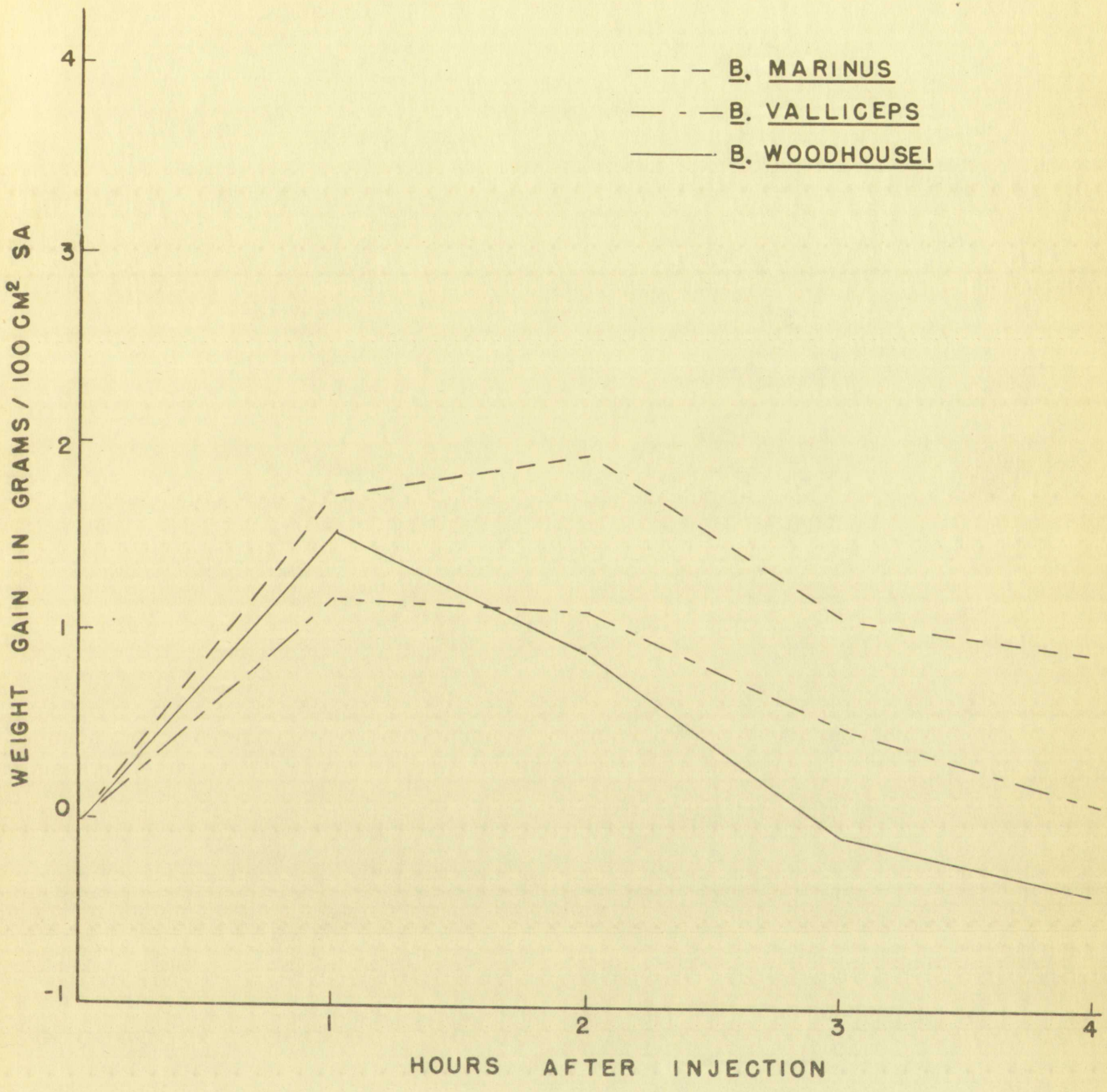


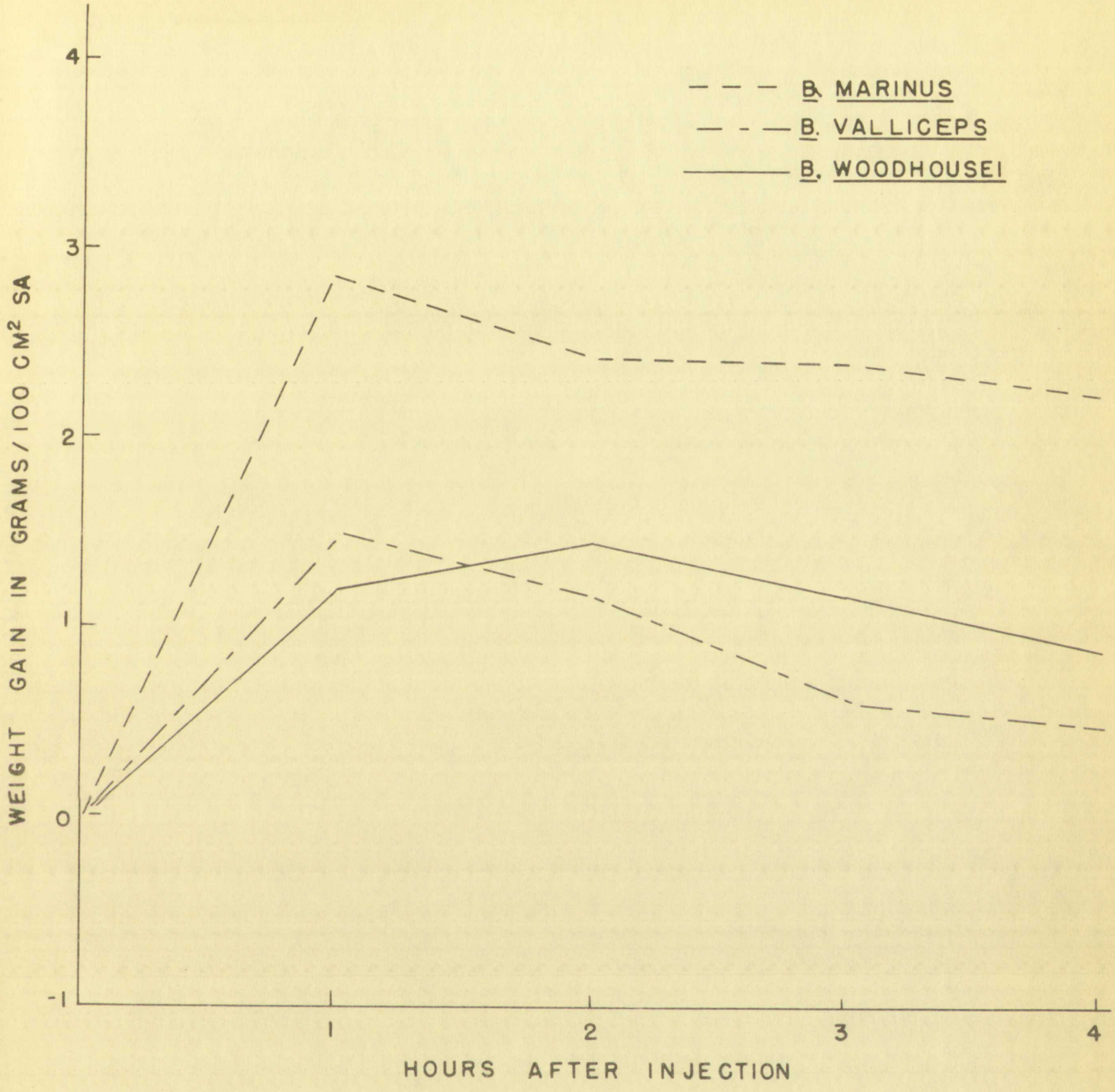
FIG. 5 WEIGHT GAIN 4 HOURS AFTER INJECTION OF SALINE OR PITRESSIN

HEAVY LINE INDICATES THE MEAN VALUE OF 10 ANIMALS, THIN LINE REPRESENTS 2 STANDARD DEVIATIONS ABOVE AND BELOW THE MEAN



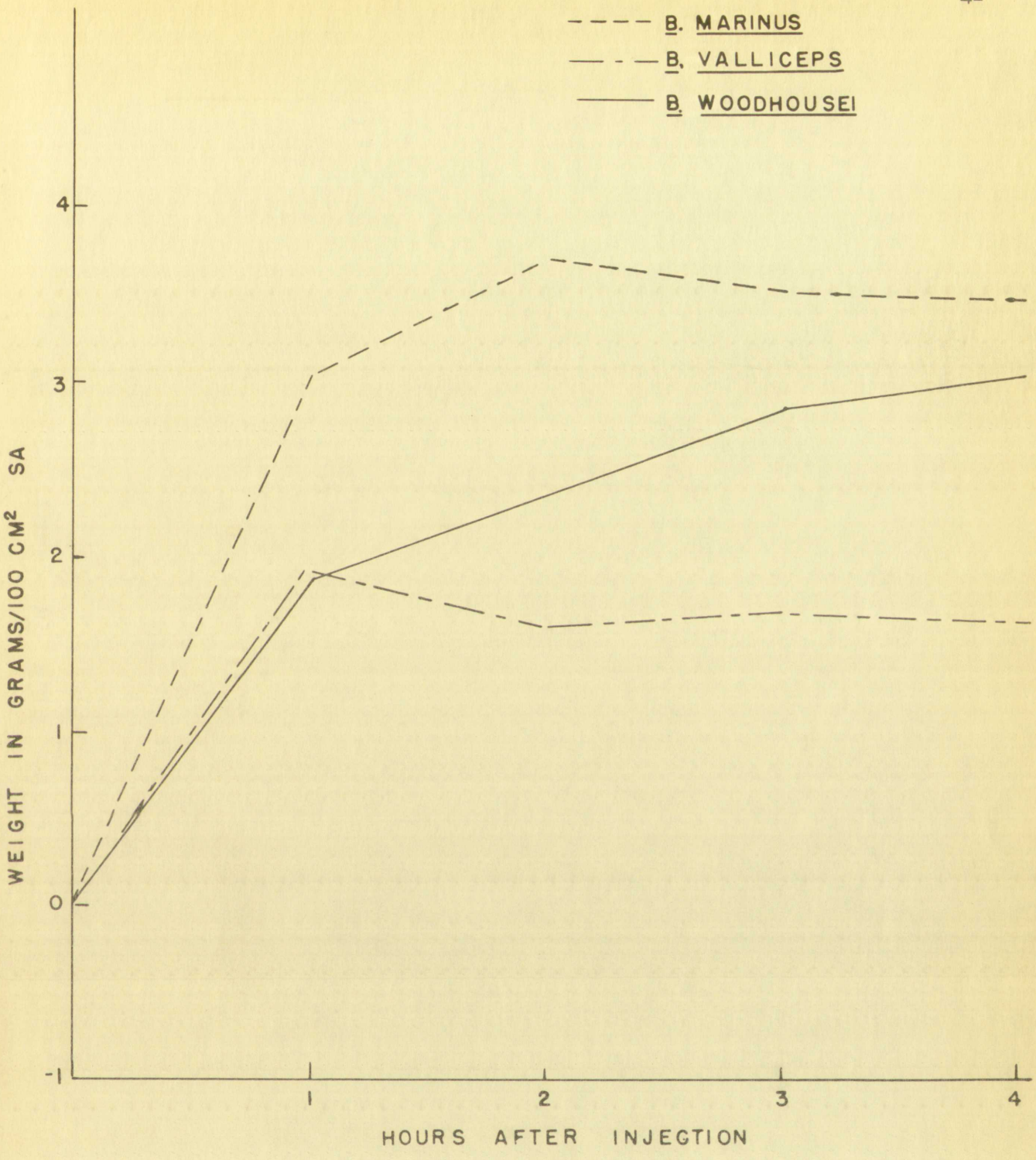
WEIGHT GAIN 4 HOURS AFTER INJECTION OF 0.7% SALINE

FIG. 6



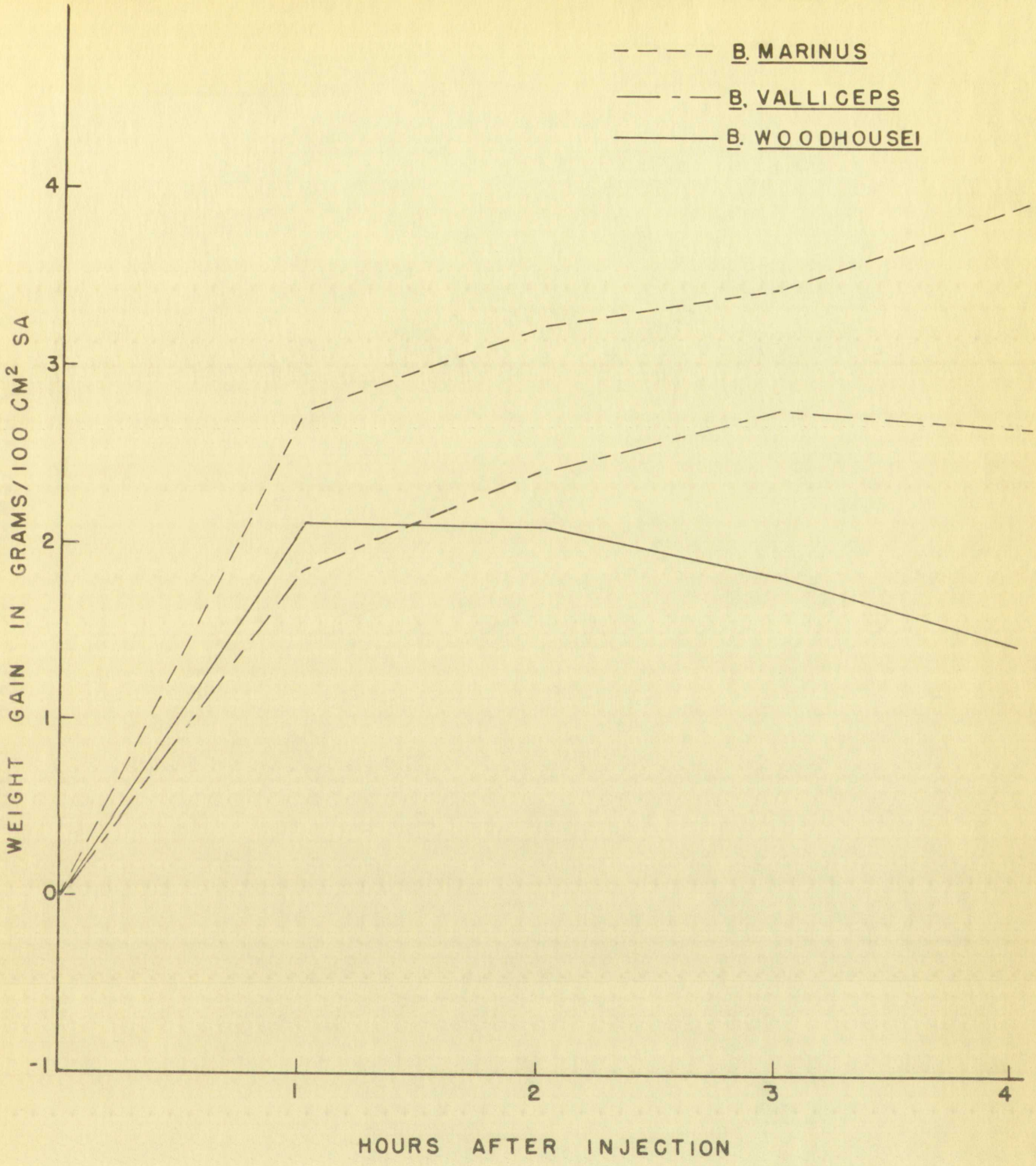
WEIGHT GAIN 4 HOURS AFTER INJECTION OF 1 MICROUNIT OF PITRESSIN

FIG. 7



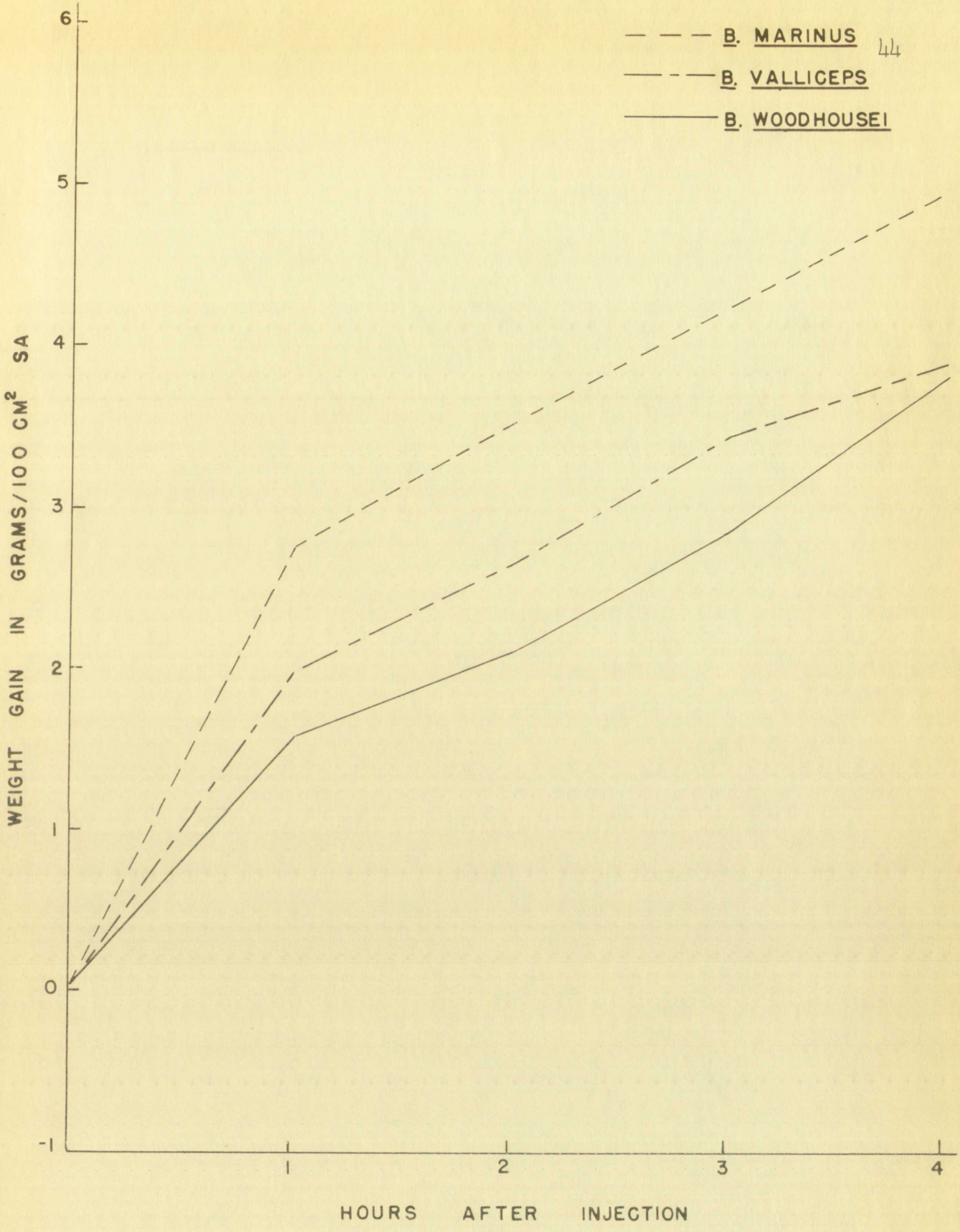
WEIGHT GAIN 4 HOURS AFTER INJECTION OF 10 MICROUNITS OF PITRESSIN

FIG. 8



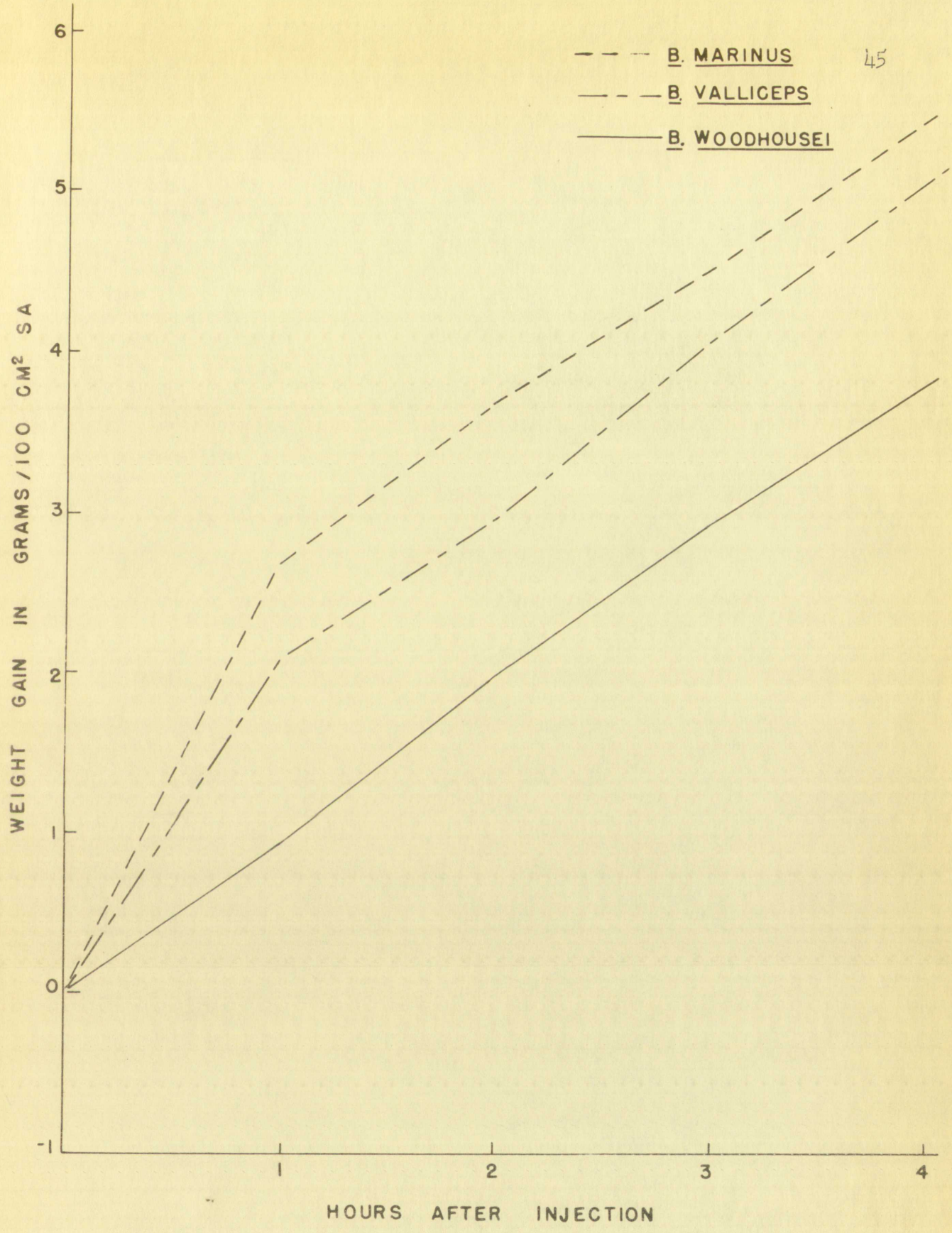
WEIGHT GAIN 4 HOURS AFTER INJECTION OF 100 MICROUNITS OF PITRESSIN

FIG. 9



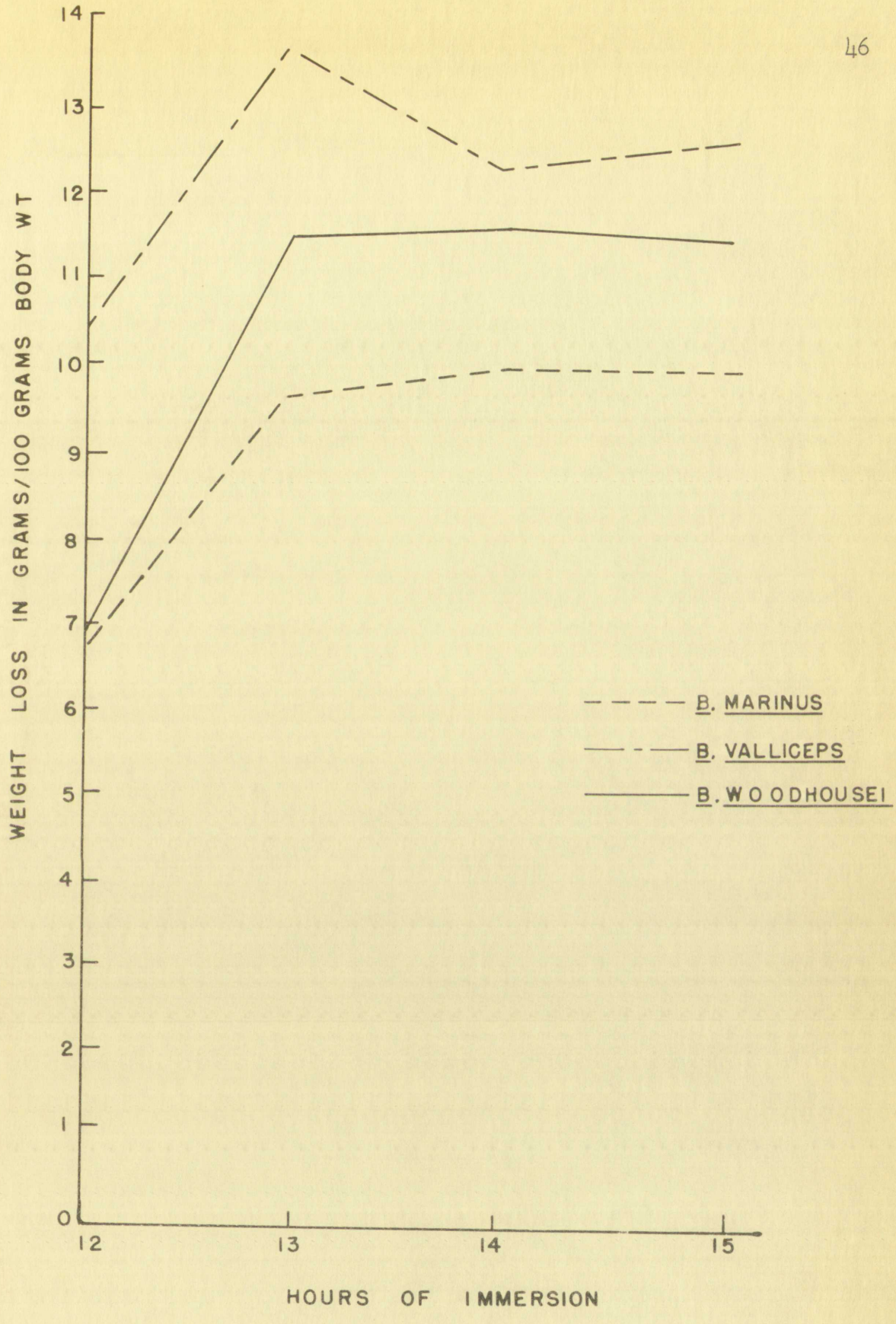
WEIGHT GAIN 4 HOURS AFTER INJECTION OF 1000 MICROUNITS OF PITRESSIN

FIG. 10



WEIGHT GAIN 4 HOURS AFTER INJECTION OF 10,000 MICROUNITS OF PITRESSIN

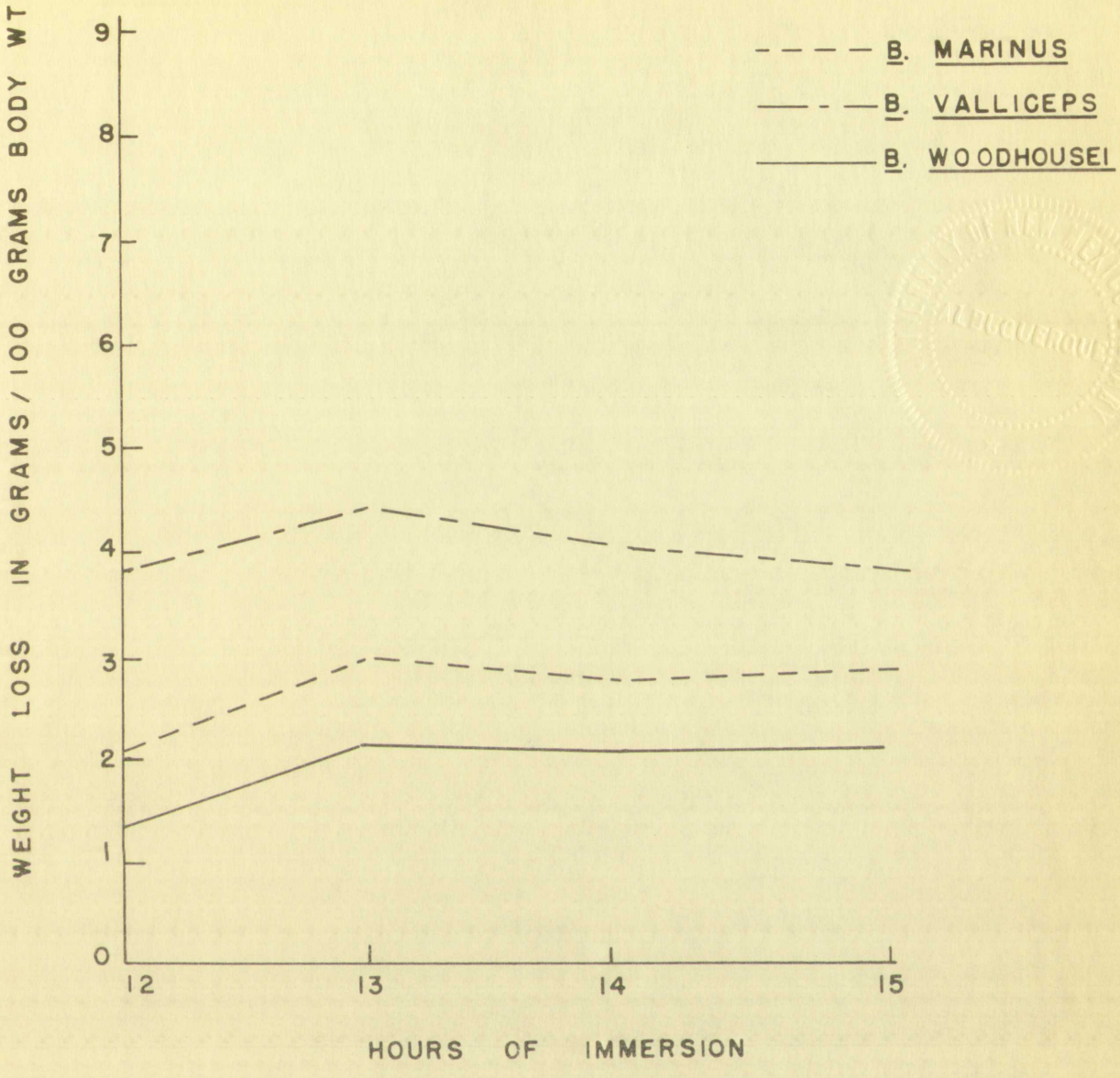
FIG. 11



WEIGHT GAIN DURING IMMERSION IN CHLORINE-FREE WATER

(ANIMALS WEIGHED BEFORE URINE WAS REMOVED)

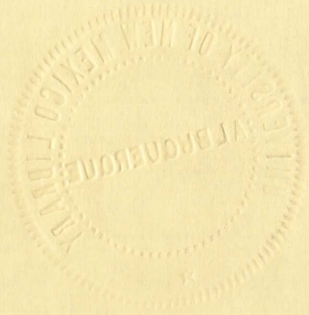
FIG. 12



WEIGHT GAIN DURING IMMERSION IN CHLORINE-FREE WATER

(ANIMALS WEIGHED AFTER URINE WAS REMOVED)

FIG. 13



IMPORTANT!

Special care should be taken to prevent loss or damage of this volume. If lost or damaged, it must be paid for at the current rate of typing.

Date ~~Loaned~~ Due			
	OCT 24 1966		
	OCT 17 REC'D		