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The University of Michigan, Ph.D., 1972
Zoology

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# SOME ASPECTS OF CARBOHYDRATE METABOLISM AND ITS REGULATION BY ADRENALIN AND GLUCAGON IN *RANA PIPIENS*

by

Eugenia Sue Smith Farrar

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Zoology)
in The University of Michigan
1972

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# INTRODUCTION

Systematic study of the different major groups of vertebrates may be expected to reveal "adaptative radiations" in the effects and modes of action of hormones which cannot become apparent from studies of mammals only. The validity of this view is remarkably well illustrated by the diversity of biological actions of prolactin among the various groups of vertebrates (Bern et al., 1967). Snyder and Frye (1972) have shown that, although growth hormone is an important hormone in the regulation of carbohydrate, fat and protein metabolism in frogs, the specific effects of this hormone are quite different from those seen in mammals. Similarly, although the vertebrate antidiuretic hormones have the common feature of promoting water conservation in the terrestrial groups, the exact manner by which this is accomplished varies significantly (Sawyer, 1966). Thus, there is an element of truth to the familiar statement that it is not hormones which have evolved, but the uses to which they are put.

Several observations encourage us to expect that amphibians may show specializations in the regulation of carbohydrate metabolism which are related to specialized aspects of their biology, such as life cycle, diet, behavior patterns, metabolic rate, heterothermy, and so on. Among

the more important of these specialized aspects are the following: (1) amphibians typically maintain lower blood glucose levels and higher glycogen levels than are found in mammals or other vertebrates (Wright, 1959; Smith, 1950); (2) amphibians tolerate very long periods of fasting during which they maintain essentially normal blood glucose and very substantial glycogen stores (Frye and Farrar, unpublished data; Rose, 1966); (3) amphibians show significant changes in blood glucose values and in glucose and insulin tolerance between the larval and the postmetamorphic stages of the life cycle (Frye, 1964; Bartell, 1969).

The levels of carbohydrates and related substances in tissues are determined by the balance between the rates of intake and de novo synthesis on the one hand, and the rates of utilization on the other. In mammals, it is well established that under a given set of circumstances, the levels of glucose and glycogen, are in large part determined by the balanced interactions of hormones which influence one or another of the above mentioned processes. It is therefore reasonable to postulate that the regulation of carbohydrate at different absolute levels of concentration in different species may in part be achieved by differences, either qualitative or quantitative, in the effects of hormones on metabolism. This includes not only the possibility that the spectrum of kinds of hormones or the

relative amounts of each of several hormones may differ in different species. In mammals both adrenalin and glucagon play key roles in regulating blood glucose levels and metabolic utilization of glucose through their effects upon glycogenolysis in liver. Adrenalin also causes glycogenolysis in muscle. Consequently, they not only influence blood glucose levels, but also blood lactate and tissue glycogen levels. It thus seemed possible that qualitative or quantitative differences in response to adrenalin and glucagon might contribute to the differences in concentrations of glucose and glycogen that are observed between amphibians and mammals.

There is not sufficient information at present to describe completely the role of hormones in the regulation of carbohydrate metabolism in amphibians. However, certain pertinent evidence does exist. The importance of insulin as a hypoglycemic agent is reasonably clear: insulin-producing beta cells are histochemically identifiable in amphibian pancreas (Frye, 1958 and 1964), and hyperglycemia occurs after pancreatectomy in toads (Penhos and Lavintman, 1964) and salamanders (Bartell, 1969). Bovine insulin depresses blood glucose levels in frogs and salamanders (Smith, 1953; Bartell, 1969). However, the role of insulin in glucose uptake by tissues, or glycogen synthesis has not been investigated in amphibians. Most of the mammalian hormones which cause hyperglycemia are implicated in the

regulation of carbohydrate metabolism in amphibia. Toad pituitary extracts contain substances which maintain blood glucose levels in hypophysectomized toads (Houssay and Biasotti, 1930); mammalian adrenocorticotropin and growth hormone also show this effect (Hermansen and Jorgensen, 1969). Glucocorticoids have been identified in extracts of amphibian adrenals (Chester Jones, 1959); hydrocortisone and aldosterone cause an increase in blood glucose and liver glycogen in Rana temporaria (Bergerhoff and Hanke, 1967), and beta cell degranulation in Taricha torosa (Wurster and Miller, 1960). Adrenalin has been extracted from frog adrenals (West, 1951), and several studies demonstrate hyperglycemic and glycogenolytic response to adrenalin in frogs, toads, and salamanders (Wright, 1959; Tindal, 1956; Hermansen and Jorgensen, 1969; Matei-Vladescue, 1965). In frogs there is a positive relationship between thyroxine stimulated increase in metabolism and glycogen utilization (McNabb, 1969).

Of the hormones which are known to induce hyperglycemia in mammals, glucagon is the only one whose presence is uncertain in amphibians. Wurster and Miller (1960) failed to observe alpha cells and glucagon induced hyperglycemia in several species of urodeles. Epple (1966) has demonstrated alpha cells in some urodeles, but they are comparatively scarce and have not been shown to be analogous to mammalian

alpha cells. Glucagon has not been extracted or assayed in any amphibian species.

The general occurrence of low blood glucose values, and high tissue glycogen levels in amphibians raises questions about the comparative role of these hyperglycemia-inducing hormones in amphibian carbohydrate metabolism. Are they secreted under conditions which elicit their secretion in mammals? Are the normal levels of carbohydrate possibly related to low physiological levels of one or more these hormones, or possibly to absence of glucagon? Do these hormones have the same general kinds of effects upon glycogenesis, glucogenesis, glycogenolysis, and blood glucose as those observed in mammals? How does target system sensitivity compare to that of mammals? What is the quantitative balance, in terms of relative effects upon the various metabolic pathways, between insulin and these "insulin antagonistic" hormones? To what extent are changes in the rates of secretion and/or activities of these hormones responsible for the differences in blood glucose levels that exist between the larval and the postmetamorphic phases of the amphibian life history?

The specific objective of this investigation, stated in the light of the above remarks, was to characterize some responses of frogs and tadpoles to adrenalin and glucagon, as a basis for evaluating the possible role of these two

hormones in controlling the characteristic carbohydrate levels seen in amphibians. In addition to analysis of the hormonal effects, it became necessary to examine in detail the normal carbohydrate levels and the effects of experimental manipulation on them in order to establish a base line from which to evaluate experimental effects.

Part I of this thesis describes some basic carbohydrate physiology in Rana pipiens, and problems involved in making standardized measurements. Part II deals with adult and tadpole responses to adrenalin and glucagon in vivo and in vitro.

# CHAPTER I. MATERIALS AND METHODS

## Animal Source and Maintenance

Male Rana pipiens weighing 30 to 80 grams were obtained from the Nasco supply company of Oshkosh, Wisconsin. These animals were collected from the Red River valley of North Dakota and were used in all the hormone injection experiments. However, because animals from this locality were unavailable during mid-summer liver slice experiments were conducted on Rana pipiens from the southwestern United States. Leopard frogs from Whitmore Lake, Michigan were used in some seasonal and fasting studies for comparisons with frogs from North Dakota. Tadpoles were reared from the laboratory fertilization of eggs obtained from North Dakota frogs.

Frogs were maintained in large closed tanks equipped with running tap water. The temperature was kept between 15°C and 17°C throughout the year by the use of a temperature control valve on the waterline. Air temperature was consistently 19°C. The tanks were fitted with fluorescent lights on a 12 hour light:12 hour dark cycle.

Frogs were force fed 1 to 2 grams of beef liver twice a week. They were fasted for 3 to 5 days prior to each experiment unless otherwise specified. When they were not in experimental use frogs were given crickets (Acheta

domesticus*) as a dietary supplement. All animals were kept under laboratory conditions for two weeks prior to their use in experiments unless specified to the contrary.

Tadpoles were reared in enamel pans containing dechlorinated water. They were fed on spinach which was boiled, well rinsed and drained. Tadpoles were maintained at a water temperature of 20°C to 22°C under a 12 hour light:12 hour dark cycle.

## Anesthetics

The most satisfactory method of anesthetizing the frogs was to immerse them in 0.5% Tricaine methane sulfonate (MS-222, Sandoz Pharmaceutical Co; or Finquel, Ayerst Laboratories). Anesthesia was necessary prior to intravenous injection, blood sampling by heart puncture, and tissue removal. Immersion in the anesthetic gave a much longer lasting effect than injection of the anesthetic. Animals lost most of the reflex response to a toe pinch after immersion for 10 to 15 minutes and remained immobile for at least an hour after they were removed from the anesthetic. Tadpoles were immersed in 0.03% Finquel for 2 to 3 minutes, or until a slight nudge failed to induce a swimming reflex, before collecting tissues and injection.

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*Obtained from Dr. George Nace, University of Michigan Amphibian Facility.

## Injections

Intravenous injections were made into the femoral vein of anesthetized frogs. For measuring and delivering the material to be injected a very small glass needle was glued into a section of a 0.10 ml glass pipette calibrated in 0.01 ml divisions. The fluid was forced through the needle into the vein by forced air regulated by a finger tip control outlet on the rubber tubing.

Frogs were injected intraperitoneally in the lower abdomen using disposable syringes fitted with 25-gauge needles. Tadpoles were injected intraperitoneally with 0.05 ml microliter syringe fitted with a 30-gauge needle inserted through the anterior abdominal wall via the operculum.

Adrenalin (Parke-Davis 1 mg/ml, as the hydrochloride) was injected either without further dilution or after dilution with amphibian Ringers solution. Injection volumes were less than 0.10 ml for adults and less than 0.01 ml for tadpoles. Doses of 50 µg/kg to 1 mg/kg were used. All adrenalin solutions were kept on ice and were discarded after 6 hours; no discoloration indicative of oxidation (Sokal and Sarcione, 1959) was ever observed in these solutions.

Crystalline ox glucagon (Eli Lilly Co.) was dissolved in diluent, which contained lactose and phenol

as a preservative, and diluted in Ringers. No injections exceeded 0.2 ml for adults or 0.02 ml for tadpoles. Doses of glucagon ranged from 50 µg/kg to 2 mg/kg. Solutions were made up fresh weekly and kept under refrigeration. In this condition glucagon will remain potent for at least 30 days (Technical Bulletin PA 2317 AMP, Eli Lilly Co.).

Controls for the hormone experiments were given placebo injections of amphibian Ringers of pH equivalent to that of the adrenalin solutions and glucagon diluent.

## Tissue Sampling Procedures

Animals were anesthetized and washed in tap water. For blood removal, a small incision was made ventral to the heart and the heart punctured with a fine glass needle. Blood flow into the needle was assisted by suction from a mouth tube. No anticoagulant was necessary in the case of frog blood. Tadpole blood was collected in glass capillaries containing sodium citrate crystals. Generally 150 µl of frog blood and 100 µl of tadpole blood were taken for analysis. For glucose and lactate determination two measured aliquots of blood were transferred to tubes of deproteinizing solutions. Following this procedure the frog's incisions could be sutured and the animals used again at a later date. Usually, however, liver and muscle samples for glycogen analysis were also collected at the time of blood

sampling. Mid portions of the right gastrocnemius muscle and tips of all three liver lobes were rapidly (within 30 seconds) removed, blotted, weighed, and placed in hot 30% KOH for digestion. The entire tadpole liver was analyzed. Between 50 mg and 500 mg of tissue was digested in 1 ml of KOH.

## Analysis of Blood Glucose and Reducing Substances

### Blood glucose analysis

Blood glucose was analyzed by the glucose oxidase technique (Glucostat, Worthington Biochem. Corp.). A 50 µl blood sample was mixed with 1 ml of deproteinizing solution (15 parts 0.08 N NaOH, 4 parts 5% $ZnSO_4$) and the deproteinized supernatant analyzed for glucose. One half ml quantities of deproteinized supernatant were transferred to matched 12 by 75 mm test tubes. Two ml of glucostat-chromogen reagent were added to each tube. After 30 minutes incubation at 37°C the reaction in each tube was terminated with a drop of 6 N HCl. Optical density was read against a reagent blank in a Coleman Junior colorimeter at 401 nm. Glucose concentration was determined from glucose standards diluted in the deproteinizing solution and handled identically to the blood samples. Glucose concentrations were expressed as mg/100 cc of blood or mg %.

### Folin-Malmros blood sugar analysis

Blood sugar was determined by the Folin-Malmros technique as modified by Horvath and Knehr (1941). One hundred µl of

blood were deproteinized in 10 ml of tungstic acid deproteinizing solution and centrifuged. Four ml quantities of deproteinized supernatant were transferred to 25 ml calibrated test tubes. Two ml of potassium ferricyanide (0.4%) and 1 ml of a sodium cyanide (0.25%)- sodium carbonate (1.6%) mixture were added. The tubes were then boiled for 20 minutes and cooled under running water for 3 minutes. Five ml of ferric ammonium sulfate solution were added and mixed. The tubes were left for 1-2 minutes and then diluted to 25 ml. They were then allowed to stand for 10 minutes and optical density was read within 30 minutes at 465 nm on a Coleman Junior colorimeter. Sugar concentrations were determined from a glucose standard curve which was linear between 20 and 100 µg.

Total reducing carbohydrates

Blood reducing carbohydrates were analyzed by the phenol sulfuric acid method of DuBois et al. (1956). Blood was deproteinized in zinc hydroxide, as in the blood glucose analysis. To 0.5 ml of deproteinized supernatant was added 0.5 ml distilled water, 0.05 ml of 80% phenol and 2.5 ml of concentrated sulfuric acid. Color was allowed to develop for 30 minutes and optical density read at 490 nm on a Coleman Junior colorimeter. Carbohydrate concentrations were determined from a glucose standard curve which was linear between 5 and 50 µgm.

## Blood Lactate Analysis

Blood lactate was analyzed enzymatically using the lactate dehydrogenase method of Scholz et al. (1959). A 50 µl blood sample was deproteinized with 0.15 ml of 6% tungstic acid and centrifuged. Fifty µl of supernatant were drawn off in a calibrated capillary tube and incubated with 1 ml of buffer containing 0.5 M glycine and 0.4 M hydrazine at pH 9, 15 µl of a lactic dehydrogenase suspension having an activity of 1500 units/mg, and 100 µl of 0.027 M NAD at 25°C for one hour. (All reagents were supplied in kit form from Boehringer Mannheim Corp.). Optical density of blood sample tubes and lactate standards were read against distilled water and reagent blanks at 340 nm in a Beckman DU spectrophotometer. Lactate concentrations were determined from L-lactate standard curves. Standards were made prior to each analysis from frozen 1 N lactate solutions by diluting to a final concentration of 90 µg/ml.

## Glycogen Analysis

Liver and muscle samples were digested in hot 30% KOH and glycogen precipitated with 95% ethanol according to the technique of Good et al. (1933). The samples were refrigerated overnight at 5°C, and then centrifuged for 15 minutes in an International clinical centrifuge. The supernatant was removed, the pellet resuspended in 1 to 2 ml of water and the glycogen precipitated with ethanol.

The sample was then recentrifuged, and the pellet washed with 95% ethanol. It was then dissolved and diluted to 50 ml with distilled water. Aliquots containing 10 to 50 µgm of glycogen were analyzed for total reducing carbohydrate using the phenol sulfuric acid method (Montgomery, 1957). Glycogen concentrations were expressed as grams/100 grams of fresh liver sample weight or gm %.

## Liver Slice Techniques

Livers were rapidly removed from frogs whose spinal cords had been transected. Livers were placed in iced Ringers and sliced free hand. Slices were blotted, weighed and assigned to control or experimental flasks. Less than 5 minutes transpired between liver removal and slice incubation. The slices weighed between 60 and 100 mg. Two incubation media were tried: sodium chloride (0.75%) buffered with 0.1 M potassium phosphate of pH 7.0, and amphibian Ringers buffered with 0.1 M sodium bicarbonate of pH 7.8. Slices were incubated in 25 ml flasks containing 2 ml of medium at 25°C in a water bath shaker. Hormones were added to the medium in 0.05 ml of solvent just prior to adding the liver slice. After an incubation period of 30 minutes to one hour with and without hormone the slices were removed, 50 µl of the supernatant deproteinized, and glucose content analyzed as described for blood glucose. Usually 10 flasks, 2 controls and 2 flasks each of 4 hormone

doses, could be obtained from each frog; 6 frogs were used per experiment. Tadpole liver slices were incubated as just described. About 10 to 12 stage XII-XV tadpole (staged by the standard series of metamorphic stages of Taylor and Kollros, 1946) livers were required for each 10 flasks. The tadpoles were immobilized by transection of the spinal cord or were precooled in ice water prior to the transection. Then livers were removed and placed in iced Ringers. Slices from 10 livers were mixed, blotted and weighed, and assigned to flasks. Each flask received 20 to 80 mg of tissue.

## Statistics

Standard statistical methods (Snedecor, 1956) were used to obtain means, standard errors, and t values. The Mann-Whitney U test (Siegel, 1956) was used in comparing groups with sample sizes of less than twenty. Means of groups larger than twenty and all groups from liver slice experiments were compared by using Student's T test. In tests of significance a probability of 5% or less was considered to be significant.

## CHAPTER II. DETERMINATION OF NORMAL OR STANDARDIZED CARBOHYDRATE LEVELS

Because reported amphibian fasting blood glucose levels are apparently much lower and more variable than the values observed in other vertebrates, some doubt concerning the reliability of previously reported data exists. Levels of glucose, glycogen and lactate are readily subject to variation due to a variety of causes, not all of which have been taken into consideration by investigators in this field. This undoubtedly accounts for a significant portion of the variation and inconsistency in reported values, especially within the same species, but possibly also between species (Table 1). A major objective of this thesis, therefore, has been to identify and control some of the factors which can lead to variations in carbohydrate levels in order to standardize conditions for evaluating hormone effects on carbohydrate levels as reported in Part Two. The factors taken into consideration in this study include the reliability of the analytical methods used, the effects of handling, anesthesia, activity and fasting, seasonal variation, and developmental changes.

TABLE 1

NORMAL BLOOD SUGAR OR GLUCOSE LEVELS OF SEVERAL AMPHIBIAN SPECIES

| Species | Mean Blood Sugar mg% ± S.E. (Range) | Number of Animals | Carbohydrate Measured | Comments | Reference |
|---|---|---|---|---|---|
| Rana pipiens | 16.3 ± 1.3 (--) | 18 | glucose | Bled from tongue of Finquel anesthetized frogs | Mathews and Zaentz, 1963 |
| Rana pipiens | 60, fall<br>52, winter<br>(--) | 30<br>43 | reducing substances | Bled from heart of pithed frogs | Mizell, 1965 |
| Rana pipiens | 65-85 (--) | -- | reducing substances | Bled from conus after pithing | Seiden, 1945 |
| Rana catesbeiana | 13.5 (0-70) | 154 | reducing sugar | " | Wright, 1959 |
| Rana temporaria | 38 ± 1.4 (--) | 60-70 | reducing sugar | Bled from heart of pithed frogs | Smith, 1954 |

TABLE 1 (Continued)

| Species | Mean Blood Sugar mg% ± S.E. (Range) | Number of Animals | Carbohydrate Measured | Comments | Reference |
|---|---|---|---|---|---|
| Rana tigrina | 33.0 (--) | 25 | reducing sugar | Bled from conus of pithed frogs | Rangnekar and Sabnis, 1964 |
| Bufo bufo | 22.1 ± 1.4 (--) | 10 | glucose | Bled from decapitation wound | Hermansen and Jorgensen, 1969 |
| Taricha torosa | 25.0 (9-58) | 54 | reducing sugar | " | Wurster and Miller, 1960 |
| Ambystoma tigrinum | 27.8 ± 0.8 (7-71) | 198 | glucose | Bled from heart of Finquel anesthetized salamanders | Bartell, 1969 |

## Evaluation of Analytical Methods

The reliability of the analytical procedures was assessed in three ways. First, experiments testing for recovery of known amounts of glycogen, glucose, and lactate were carried out. Second, glucose analysis was tested by determining glucose levels in the laboratory rat. Third, the possibility that sugars other than glucose contribute significantly to the circulating carbohydrate level was examined. Aliquots of pooled blood samples were analyzed by three different methods: the glucose oxidase method for glucose, the Folin-Malmros method for reducing substances, and the phenol sulfuric acid method for reducing carbohydrates.

### Accuracy of assays in recovering known quantities of glucose, lactate, and glycogen

Glucose and lactate were analyzed in six aliquots from pooled frog blood and known amounts of glucose or lactate standard solutions were added to equivalent aliquots of the same blood. The samples were deproteinized and the concentrations determined by the glucose oxidase and the lactic dehydrogenase techniques. Table 2 shows the mean glucose and lactate concentrations obtained and compared with the expected means. The per cent recoveries of the means were: blood glucose, 104 and 97% for the low and high standard respectively; lactate, 92 and 91% for the low and high

TABLE 2

RECOVERY OF ADDED GLUCOSE, LACTATE, AND GLYCOGEN

A. Blood glucose

| Sample # | Blood Glucose | Mean | Expected Mean | Recovery |
|---|---|---|---|---|
| Blood alone: | | | | |
| 1 | 22.6 | | | |
| 2 | 20.8 | | | |
| 3 | 21.3 | | | |
| 4 | 21.7 | | | |
| 5 | 28.6 | | | |
| 6 | 22.2 | 22.9 | | |
| Blood plus glucose to make 10 mg% | | | | |
| 1 | 34.3 | | | |
| 2 | 35.6 | | | |
| 3 | 36.1 | | | |
| 4 | 32.6 | | | |
| 5 | 33.0 | | | |
| 6 | 33.4 | 34.2 | 32.9 | 104% |
| Blood plus glucose to make 20 mg% | | | | |
| 1 | 43.0 | | | |
| 2 | 41.3 | | | |
| 3 | 41.8 | | | |
| 4 | 41.3 | | | |
| 5 | 39.5 | 41.4 | 42.9 | 97% |

B. Blood lactate

| Sample # | Lactate µg/tube | Mean | Expected Mean | Recovery |
|---|---|---|---|---|
| 1 | 3.5 | | | |
| 2 | 3.4 | | | |
| 3 | 3.4 | | | |
| 4 | 3.8 | | | |
| 5 | 3.9 | | | |
| 6 | 3.7 | 3.6 | | |
| Blood plus 1.6 µg/tube: | | | | |
| 1 | 4.8 | | | |
| 2 | 4.5 | | | |
| 3 | 4.9 | | | |
| 5 | 4.7 | | | |
| 6 | 4.9 | 4.7 | 5.2 | 92% |

TABLE 2--Continued

B. Blood lactate--Continued

| Sample # | Lactate µg/tube | Mean | Expected Mean | Recovery |
|---|---|---|---|---|
| Blood plus 3.1 µg/tube: | | | | |
| 1 | 6.1 | | | |
| 2 | 6.4 | | | |
| 3 | 6.7 | | | |
| 4 | 7.3 | | | |
| 5 | 6.1 | | | |
| 6 | 6.4 | 6.5 | 6.7 | 91% |

C. Liver glycogen

| Sample # | Glycogen µg/tube | Mean | Expected Mean | Recovery |
|---|---|---|---|---|
| 1 | 10.6 | | | |
| 2 | 10.9 | | | |
| 3 | 10.3 | | | |
| 4 | 10.6 | | | |
| 5 | 10.3 | | | |
| 6 | 10.9 | 10.6 | | |
| Homogenate plus 5 µg/tube | | | | |
| 1 | 14.6 | | | |
| 2 | 15.0 | | | |
| 3 | 14.6 | | | |
| 4 | 14.6 | | | |
| 5 | 14.3 | | | |
| 6 | 14.9 | 14.7 | 15.6 | 94% |
| Homogenate plus 10 µg/tube | | | | |
| 1 | 18.8 | | | |
| 2 | 19.8 | | | |
| 3 | 20.1 | | | |
| 4 | 20.6 | | | |
| 5 | 20.0 | | | |
| 6 | 20.0 | 19.9 | 20.6 | 97% |

standards.

Glycogen recovery was determined on portions of liver homogenate to which different amounts of purified glycogen standards had been added. Recovery was 94% in the low standard and 97% in the high standard (Table 2).

Determination of rat blood glucose and lactate

Rat blood glucose and lactate concentrations were determined for four rats fed ad libitum and bled from the tail vein. Blood glucose as determined by the glucose oxidase method, varied from 101 to 121 mg%, lactate from 15.0 to 19.0 mg% (Table 3). These levels are well within the range of commonly reported levels for the fed rat. Blood glucose ranged between 80 and 118 mg% and lactate between 15 and 20 mg% in the data of Bloom and Russell (1955). This information, coupled with the recovery of glucose and lactate added to frog blood, indicates that the assays used in this study are valid.

TABLE 3

BLOOD GLUCOSE AND LACTATE DETERMINATIONS ON RAT BLOOD

| Animal # | Blood glucose | Blood lactate |
|---|---|---|
| 1 | 119 mg% | 17.3 mg% |
| 2 | 121 | 15.0 |
| 3 | 116 | 19.0 |
| 4 | 101 | 19.2 |

Comparison of the Glucose oxidase, Folin-Malmros, and phenol-sulfuric acid assays of blood glucose and sugar

Blood from 6 frogs was pooled and aliquots analyzed for blood glucose, using the glucose oxidase technique after zinc hydroxide deproteinization, and for reducing substances, using the Folin-Malmros method after tungstic acid deproteinization. Another blood pool from 6 frogs was analyzed by both glucose oxidase and the phenol sulfuric acid technique for reducing carbohydrates.

TABLE 4

COMPARISON OF GLUCOSE, REDUCING SUBSTANCES AND REDUCING CARBOHYDRATES OF FROG BLOOD

| Frog # | Blood glucose (Glucose Oxidase) mg% | | Blood reducing substances (Folin-Malmros) mg% | | Blood reducing carbohydrates (phenol-sulfuric acid) mg% | |
|---|---|---|---|---|---|---|
| 1 | 21.0 | | 42.9 | | | |
| 2 | 22.5 | | 35.0 | | | |
| 3 | 24.0 | | 50.0 | | | |
| 4 | 22.5 | | 29.6 | | | |
| 5 | 27.5 | | 30.8 | | | |
| 6 | 24.0 | | 32.9 | | | |
| Means: | | 23.6 | | 36.7 | p=.001* | |
| 7 | 26.8 | | | | 30.4 | |
| 8 | 30.0 | | | | 34.4 | |
| 9 | 24.0 | | | | 30.4 | |
| 10 | 27.5 | | | | 30.4 | |
| 11 | 21.5 | | | | 19.6 | |
| 12 | 35.8 | | | | 29.2 | |
| Means: | | 27.6 | p=0.45 | | | 29.1 |

*p = probability of higher U in Mann Whitney U test.

Data listed in Table 4 indicate that while blood glucose values determined by the glucose oxidase method did not differ from total reducing carbohydrate measured by the phenol-sulfuric acid method, significantly higher values were obtained when the Folin-Malmros technique was applied. The average difference between glucose and Folin-Malmros "sugar" levels was 13.1 mg%, an increase of 55% over initial levels.

What accounts for this difference? The amount of circulating reducing carbohydrates other than glucose are not responsible, since the phenol sulfuric acid method indicated little non-glucose material was present. This method would detect not only reducing sugars but also oligosaccharides, polysaccharides or derivatives with free or potentially free reducing groups (DuBois et al., 1956). Since the Folin-Malmros technique employs deproteinization with tungstic acid it seems likely that reducing substances present in the plasma or released from red blood cells during deproteinization might be responsible. Fales et al. (1961) reported that techniques involving the use of tungstic acid often also measure saccharoids which are abundant in red blood cells, thus giving spuriously high blood sugar measurements. Reducing compounds such as ascorbic acid, cysteine, and glutathione could contribute also. Reports in the literature in which harsh deproteinization procedures were utilized during the glucose

analysis may be suspect. It is interesting in this regard that two reports of blood "glucose" values for Rana pipiens of over 50 mg% both utilized the Folin-Malmros technique (Mizell, 1965; Seiden, 1945; see Table 1).

## The Effect of Handling, Pithing, and Anesthesia on Blood Glucose and Lactate

It is probable that disturbance and excitation of frogs during experimental manipulation results in changes in blood glucose and lactate and liver glycogen. Chavin (1964) has found that even minor disturbance of goldfish results in profound and prolonged hyperglycemia. Anesthesia is a necessary part of most experiments which is also very likely to induce changes in the substances being studied. Pithing is another commonly used method for immobilization of frogs during sampling, but the effects of pithing upon blood glucose and lactate have not been studied. Because it is necessary to use one or more of these manipulations in sampling, it is difficult to ascertain what are "normal" values. It is likely that the lowest and most uniform values are closest to the normal resting levels and procedures producing these levels are the most desirable. The purpose of the experiments reported in this section was to evaluate the effects of handling, pithing and anesthesia upon blood glucose and lactate.

Comparison of blood glucose levels of pithed and Finquel anesthetized frogs

Six frogs were doubly pithed and blood was taken from the heart within 30 seconds. Six other frogs were anesthetized by immersion in 0.5% Finquel for 10 minutes and blood removed from the heart as usual. The blood glucose values are given in Table 5. The groups are not significantly different ($p=0.47$) but the variation is considerably greater with pithing. Consequently, individual values obtained from pithed animals are not reliable, and pithing should be avoided as a method of immobilization.

TABLE 5

COMPARISON OF BLOOD GLUCOSE VALUES OBTAINED FROM PITHED AND ANESTHETIZED FROGS

| | Blood Glucose mg%: | |
|---|---|---|
| | Pithed | Anesthetized |
| | 10.5 | 16.5 |
| | 7.5 | 14.6 |
| | 25.0 | 12.0 |
| | 5.6 | 17.8 |
| | 23.5 | 14.0 |
| | 35.4 | 12.5 |
| Mean: | 17.9 | 14.6 |

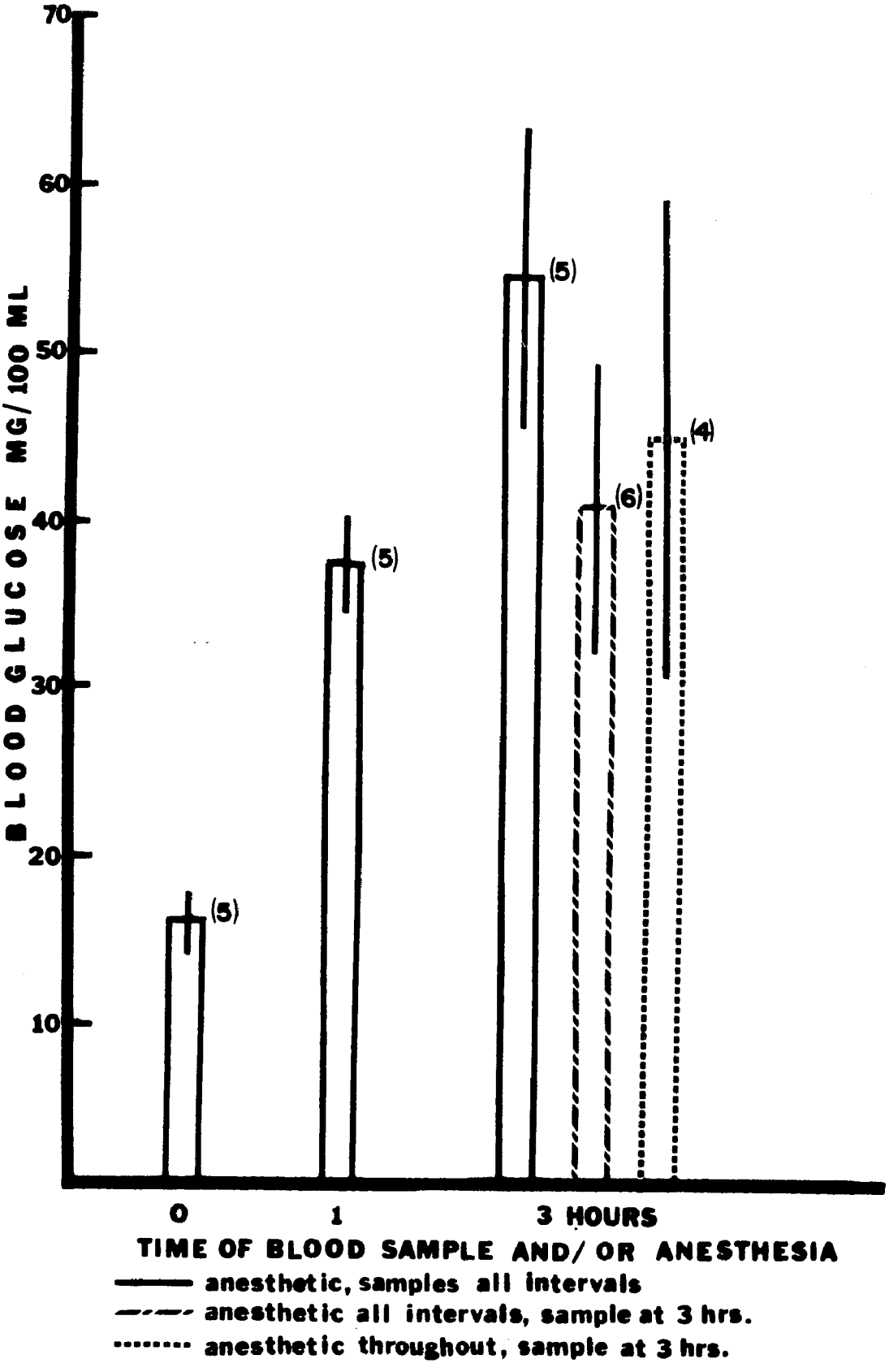
Effects of anesthetic dose, repeated anesthesia, blood sampling, and handling upon blood glucose

Repeated sampling at successive time intervals is a desirable, if not essential, procedure in studies of hormone

effects upon tissue levels of glucose, lactate and glycogen. This requires repeated anesthetization or continual anesthesia. It was discovered early in the course of this work that distinct increases in blood glucose and lactate occurred during repeated sampling, even in placebo injected animals. An experiment was done in which the frequency and interval of anesthetization and/or blood sampling were varied in an effort to discover the cause for this change. One group of animals was anesthetized in 0.5% Finquel and blood samples taken at 0, 1, and 3 hours. A second group was anesthetized at each of these time intervals, but blood was drawn only at 3 hours. A third group was initially anesthetized in 0.5% Finquel and then kept under continuous anesthetic in 0.06% Finquel for the remainder of the experiment. This concentration was sufficient to prevent arousal during the three hour interval. At the end of this time blood was withdrawn.

Figure 1 shows the results of this experiment. Blood glucose levels of all three groups at 3 hours were 2 to 3 times higher than at zero time, suggesting that the anesthetic alone can cause hyperglycemia whether applied repeatedly or continuously. Blood withdrawal or arousal are not necessary for this effect. Consequently, continuous or repeated anesthesia must either be avoided, or very carefully taken into account during experiments designed to

FIGURE 1 - Effect of Multiple and Prolonged Anesthetic Application on Blood Glucose (means $\pm$ standard errors of data in Appendix I. A. 1-3, ( ) = number of animals in each group)


70
60
50
40
30
20
10
BLOOD GLUCOSE MG/100 ML
(5)
(5)
(5)
(6)
(4)
0
1
3 HOURS
TIME OF BLOOD SAMPLE AND/OR ANESTHESIA
anesthetic, samples all intervals
anesthetic all intervals, sample at 3 hrs.
anesthetic throughout, sample at 3 hrs.

test the effects of hormones on carbohydrate levels.

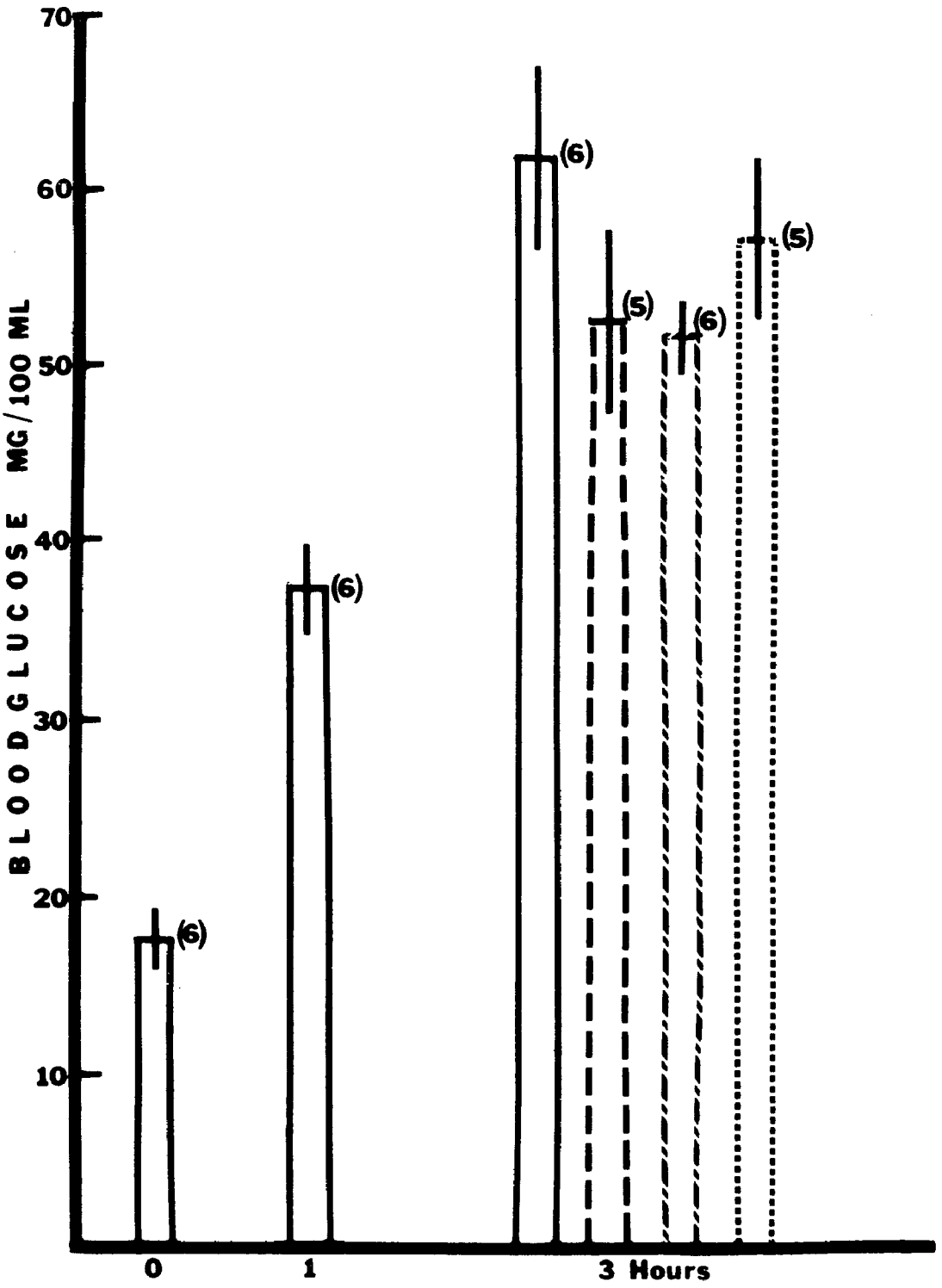
In order to evaluate the effect of repeated capture and handling upon the hyperglycemic response of animals during repeated sampling, an experiment was done in which frogs were anesthetized without handling. Frogs were placed in containers with measured amounts of water several hours prior to the experiment and Finquel added to the water without disturbing them. As can be seen from Figure 2, merely repeatedly anesthetizing animals in this manner resulted in a mean blood sugar concentration of 52 mg% compared to an initial level of 17.6 mg% (see Appendix I.A.4. for raw data). Hyperglycemia accompanying repeated blood sampling is apparent at 1 and 3 hours when this method of anesthesia is used also. Lowering the dose of Finquel to 0.125%, the lowest dose which would effectively immobilize these animals, failed to alleviate the problem (Figure 2).

Blood lactate was not determined in all the experiments, but consistent hyperlactacidemia occurred in situations in which hyperglycemia was also evident. For example, repeated anesthetizations in 0.5% Finquel at zero time, 1 and 3 hours produced an increase from an initial level of 19.5 mg% to 49.3 mg% at 3 hours (Appendix I.B.).

Mechanism of the effect of Finquel on glucose and lactate

There are two major mechanisms by which the anesthetic could be causing increased blood glucose and lactate:

FIGURE 2 - Effect of Anesthesia Without Handling and of Different Doses of Finquel on Blood Glucose (means ± standard errors of data in Appendix I. A. 4 and 5, ( ) = number of animals in each group)


70
60
50
40
30
20
10
BLOOD GLUCOSE MG/100 ML
(6)
(6)
(6)
(5)
(6)
(5)
0
1
3 Hours
TIME OF BLOOD SAMPLE AND/OR ANESTHESIA
0.5% Finquel all intervals, samples all intervals
0.5% Finquel all intervals, sample at 3 hours
0.25% Finquel " " , " " "
0.125% Finquel " " , " " "

(1) a direct effect of Finquel on glycogenolysis in liver and skeletal muscle, and (2) stimulation of humoral or neural activity which activate glycogenolysis; for example, secretion of adrenalin.

The possibility of a direct effect of Finquel on glycogenolysis was tested by incubating liver slices in vitro with the anesthetic. Finquel concentrations of 0.015, 0.03, and 0.06% were prepared in amphibian Ringers incubation medium. Control flasks contained identical incubation media but without Finquel. Slices were incubated at 25°C for 30 minutes (Table 6). None of the Finquel treated

TABLE 6

EFFECT OF FINQUEL ON GLUCOSE RELEASE FROM FROG LIVER SLICES

| Glucose release in µg/mg/30 min | | | | | |
|---|---|---|---|---|---|
| Saline Controls | | | 0.06% Finquel | 0.03% Finquel | 0.015% Finquel |
| 2.1 | 1.6 | 1.9 | 1.6 | 1.9 | 1.8 |
| 0.9 | 1.8 | 1.3 | 1.0 | 2.7 | 1.7 |
| 0.9 | 1.5 | 2.7 | 1.4 | 1.5 | 1.4 |
| 1.2 | 1.7 | 0.6 | 2.0 | 1.1 | 1.8 |
| 1.1 | 1.0 | 1.1 | 1.6 | 1.7 | 1.1 |
| 0.8 | | | | | |
| Means: 1.4 ± 0.47 | | | 1.5 ± 0.16 | 1.8 ± 0.27 | 1.6 ± 0.13 |

groups had significantly elevated glucose release rates. However, it is known that a glycogenolytic agent, adrenalin, stimulates glucose release in frog liver slices (Tindal,

1956). Thus, it is likely that the effect of Finquel is indirect. It is possible that higher anesthetic doses would have stimulated increased glucose release in liver slices. However, it seems unlikely that the internal concentrations of Finquel could exceed 0.06% following immersion of frogs in concentrations of 0.125 to 0.5%, the doses which induced hyperglycemia.

The possibility that Finquel induces hyperglycemia by eliciting adrenalin release was tested by the use of 2 blocking agents. Dihydroergotamine was chosen as a β-adrenergic blocking agent because of its ability to inhibit adrenalin stimulated hyperglycemia in rats, dogs, cats, and rabbits (Himms-Hagen, 1967). Hexamethonium was chosen as a ganglionic blocking agent (ibid.) because, if effective in inhibiting hyperglycemia due to medullary release of adrenalin associated with anesthesia, it could be used in adrenalin injection experiments. The drugs were injected intraperitoneally at doses at 1, 10, or 25 mg/kg for dihydroergotamine and of 25, 50, or 100 mg/kg hexamethonium 30 minutes prior to initial anesthesia. Animals were then reanesthetized and blood withdrawn at 0, 1, and 3 hours or at 3 hours only. No dose of either dihydroergotamine (Figure 3) or hexamethonium (Figure 4) prevented hyperglycemia at 3 hours. Hexamethonium (100 mg/kg) did not prevent increased lactate at 3 hours (Figure 5).

FIGURE 3 - Effect of Dihydroergotamine and Finquel Anesthesia on Blood Glucose (means ± standard errors of data in Appendix I. A. 6., ( ) = number of animals in each group)
Treatment: Animals were injected at zero time and anesthetized at 0, 1, and 3 hours. Blood was sampled at 0 and 3 hours.

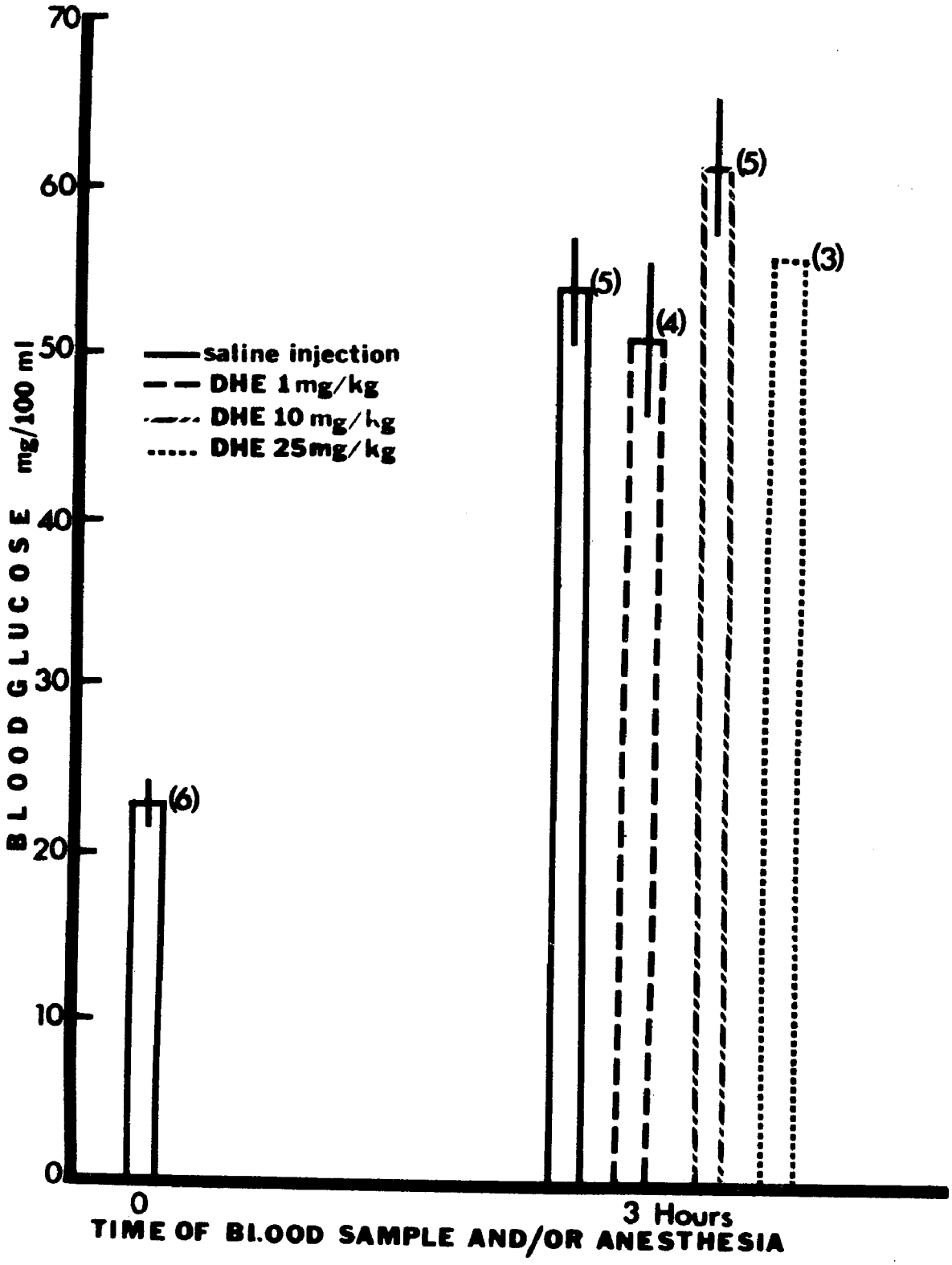

70
60
50
40
30
20
10
0
BLOOD GLUCOSE mg/100 ml
saline injection
DHE 1 mg/kg
DHE 10 mg/kg
DHE 25mg/kg
(6)
(5)
(4)
(5)
(3)
0
3 Hours
TIME OF BLOOD SAMPLE AND/OR ANESTHESIA

FIGURE 4 - Effect of Hexamethonium and Finquel Anesthesia on Blood Glucose (means ± standard errors of data in Appendix I. A. 7., ( ) = number of animals in each group)
Treatment: Animals were injected at zero time, and anesthetized at 0, 1, and 3 hours. Blood samples from animals given 25 and 50 mg/kg were taken at 1 hour and those from animals given 100 mg/kg at 3 hours

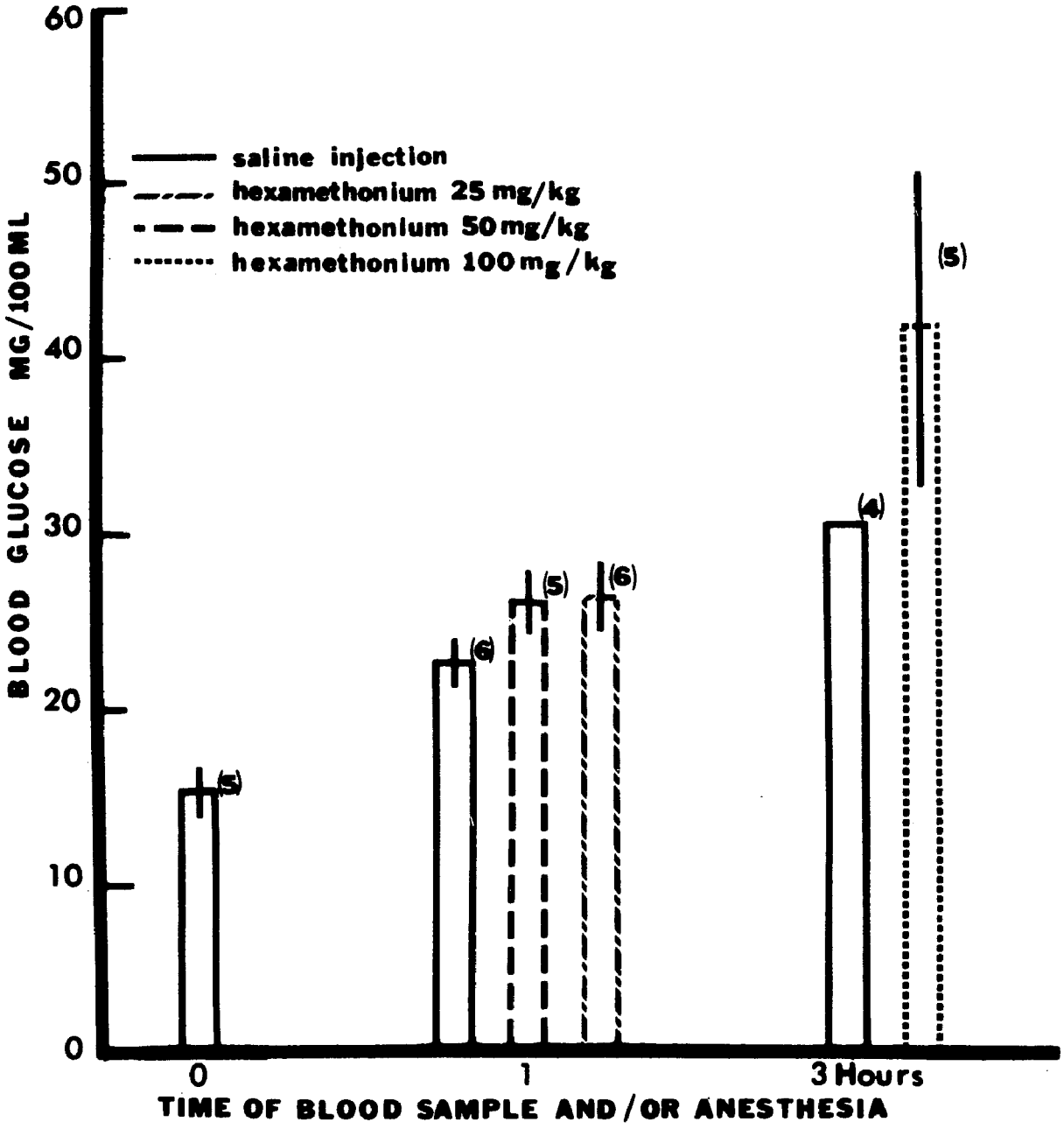
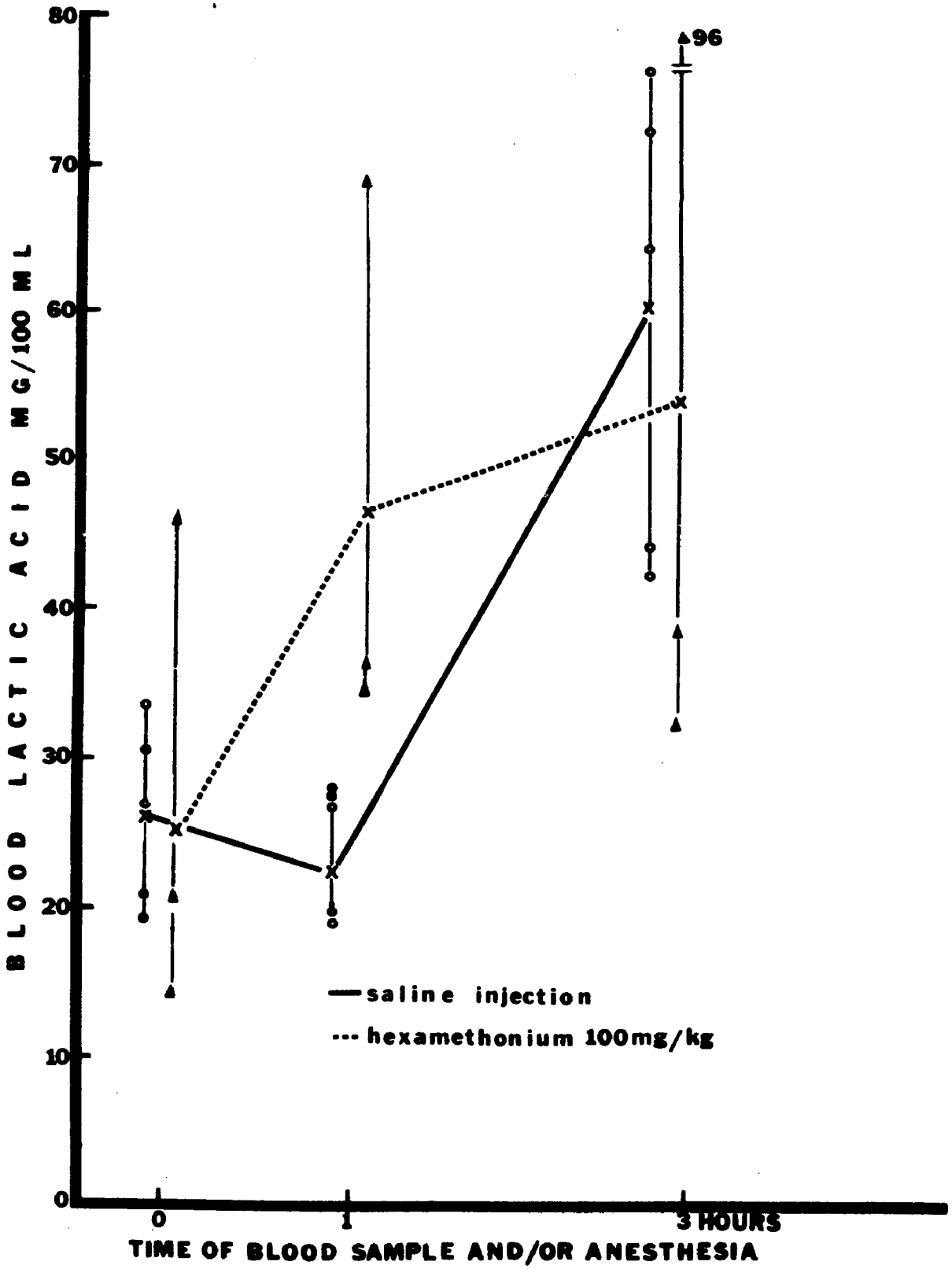

60
50
40
30
20
10
0
BLOOD GLUCOSE MG/100ML
saline injection
hexamethonium 25 mg/kg
hexamethonium 50 mg/kg
hexamethonium 100 mg/kg
(5)
(6)
(5)
(6)
(4)
(5)
0
1
3 Hours
TIME OF BLOOD SAMPLE AND/OR ANESTHESIA

FIGURE 5 - Effect of Hexamethonium and Finquel Anesthesia on Blood Lactate (X=mean of each group, other symbols represent 1 blood sample in Appendix I. B.)

Treatment: Animals were injected at zero time and anesthetized and blood sampled at 0, 1 and 3 hours.


80
70
60
50
40
30
20
10
0
96
BLOOD LACTIC ACID MG/100 ML
saline injection
hexamethonium 100mg/kg
0
1
3 HOURS
TIME OF BLOOD SAMPLE AND/OR ANESTHESIA

Effect of other anesthetics on blood glucose

Several different anesthetics were tried including nembutal and Equithesin, which contains chloral hydrate and pentobarbital. Neither was as effective an anesthetic as was Finquel. Both frequently rendered the animals groggy, but not immobile, or were lethal. Equithesin was a better anesthetic than nembutal and could be used quite well for short periods of anesthesia. An experiment was performed to compare the effects on blood glucose. Minimal doses, which would produce comparable periods of anesthesia were used. For Equithesin this dose was 4.2 ml/kg of body weight at 0, 1, and 3 hours. Equivalent hyperglycemia was present at 3 hours after Finquel (55.3 mg%) and Equithesin (49.5 mg%; see Appendix I.A.8.).

The above results indicate that multiple use of some anesthetics can cause severe hyperglycemia and lactacidemia. Attempts to reduce these effects by not handling the animals prior to anesthesia or by the use of dihydroergotamine or hexamethonium were not successful. There are some indications that the anesthetic, Finquel, does not act directly on the liver. Because at least two anesthetics with very different active components both produce comparable blood glucose and lactate increases, the stress involved with multiple anesthetization may be a general one, such as anoxia. This stress might result in nervous and

hormonal discharge, possibly of adrenalin, which would stimulate glycogenolysis. The anesthetized animals do superficially appear similar to adrenalin injected animals in that they exhibit skin vasodilatation and increased heart rates. Although the blocking agents were not effective in preventing the anesthetic effects this could have been due to incorrect doses, timing, or to species differences in responses to the inhibitors.

## Effects of Activity on Blood Glucose and Lactate

In experimental situations frogs can be very excitable and frequently jump around inside their containers for brief periods of time. Such activity might be expected to be accompanied by adrenalin release and accelerated muscle and liver glycogenolysis. Glucose utilization, on the other hand, might also be increased. It is, therefore, important to determine the effects of activity upon blood glucose and lactate before attempting to study the effect of injected hormone.

Blood was carefully removed from five frogs which had been anesthetized by immersion in 0.5% Finquel for 10 minutes. The frogs were sutured and revived; twenty four hours later the same animals were forced to hop continually for five minutes in a moist container. At this time the animals were fatigued and would not hop further. Blood

samples were again taken in an identical fashion and analyzed for glucose and lactic acid.

Table 7 shows the data obtained from this procedure. Lactate levels were tremendously elevated following this activity, averaging a nine fold increase. Blood glucose was doubled.

TABLE 7

EFFECT OF ACTIVITY ON BLOOD GLUCOSE AND LACTATE

| Frog # | Blood glucose mg% Before | After | Blood lactate mg% Before | After |
|---|---|---|---|---|
| 1 | 22.5 | 27.5 | 36.2 | 148 |
| 2 | 11.0 | 32.0 | 12.5 | 164 |
| 3 | 15.2 | 38.7 | 13.0 | 184 |
| 4 | 18.0 | 29.7 | 21.2 | 242 |
| 5 | 12.0 | 40.0 | 19.7 | 194 |
| Means: | 15.7 | 33.0 | 20.5 | 186 |

Data obtained from active animals therefore does not accurately represent basal levels of glucose or lactate, and during experimental studies the animals must remain undisturbed and in a resting state. Even so, slight activity, unnoticed by the investigator, may cause changes in lactate and glucose levels. In nature also activity is presumably associated with changes in carbohydrate levels, although the magnitude of these presumed changes may not be as great as those observed here, since in nature frogs probably seldom exercise to the point of exhaustion.

## Effects of Fasting on Carbohydrate and Fat Bodies

Amphibians are renowned for their ability to tolerate fasting. Salamanders (Amphiuma) have survived fasting in the laboratory at room temperature for up to 13 months at which time death occurred before lipid and carbohydrate deposits completely disappeared (Rose, 1966). Frogs and toads are generally not fed at biological supply houses (Gibbs et al., 1971), where they may be stored outdoors for many months prior to classroom use, but death is seldom attributed to starvation. It is evident from the seasonal habits of frogs (Table 8) that these animals, in nature, undergo periodic food abundance and scarcity. During winter the frogs are not feeding but their metabolism must be considerably lowered since their hibernation temperature is near 4°C. Active frogs must also face food shortages in both early spring and late fall.

Dietary status is extremely important in studies of mammalian carbohydrate metabolism, and is always carefully controlled and specified. This has not been true in work with amphibians. Therefore, the following experiments were designed to determine the effects of feeding and fasting on liver and muscle glycogen and blood glucose, and to indicate what standardized procedures should be adopted for experimental work.

TABLE 8

SEASONAL HABITS AND RELATIVE ENERGY STORES* IN RANA PIPIENS

| Season | Feeding Habits | Events in Life Cycle | Liver and Muscle Glycogen | Blood Glucose | Fat Body Weight |
|---|---|---|---|---|---|
| Winter (Nov.-April) | No feeding | Hibernation at 4°C | +++ | + | + |
| Spring (April-May) | Increasing | Migration to breeding waters; breeding activity | | | |
| Summer (June-Aug.) | Heavy | Egg and sperm production, growth | + | +++ | +++ |
| Autumn (Sept.-Oct.) | Decreasing | Preparation for hibernation, migration to hibernation waters | +++ | ++ | ++ |

*See Table 12 for experimental data.

Male frogs were collected at Whitmore Lake, Michigan as they emerged from the water following winter hibernation. One group of 6 animals was used for assays on arrival in the laboratory and served as the initial control group. Twelve animals were fasted in the laboratory, 6 for 9 days and 6 for 2-1/2 months. A third group of 18 frogs was fed on liver and crickets according to the usual regime for 4 months. Then 6 animals were killed at three hours, 6 at three days and 6 at five days following a force fed meal. Blood samples and liver and muscle glycogen samples were taken as in other experiments at termination. In addition fat bodies were removed and weighed.

Animals emerging from hibernation had large glycogen stores of 14.6% (Table 9). Amounts of both liver and muscle glycogen were equivalent to the amounts measured in animals just before hibernation (Table 12). Blood glucose levels of the emerging animals (27.6 mg%) were also high when compared to animals which had been in the laboratory for at least 2 weeks (19.3 mg%; Table 14). The emerging animals had no food within their digestive tracts and presumably had had nothing to eat since entering the lake in early November, but were at 4°C during this time.

After 9 days of fasting the only statistically significant drop in carbohydrate levels observed was a decrease in blood glucose to 21.8 mg%. This might represent a response

TABLE 9

LONG TERM FASTING AND ENERGY RESERVES
(MALE WHITMORE LAKE FROGS) MARCH 1969

| Body Weight (grams) | Liver Weight (grams) | Liver Glycogen (g/100 grams of liver) | Total Liver Glycogen (mg) | Muscle Glycogen (g/100g) | Blood Glucose (mg/100 cc) | Fat Body Weight (mg) |
|---|---|---|---|---|---|---|
| Initial levels, Immediately following hibernation: | | | | | | |
| 24.6 | 0.58 | 25.7 | 149.0 | 1.42 | 26.8 | 6.4 |
| 23.9 | 0.39 | 5.9 | 23.0 | 1.33 | 30.0 | 10.2 |
| 21.9 | 0.68 | 15.5 | 105.4 | 0.91 | 24.0 | 11.4 |
| 28.0 | 0.67 | 12.6 | 84.4 | 1.06 | 27.5 | 11.0 |
| 27.6 | 0.88 | 15.1 | 132.9 | 0.87 | 21.5 | 8.0 |
| 26.0 | 0.69 | 13.0 | 89.7 | 1.09 | 35.8 | 9.0 |
| Means: 25.3 | 0.65 | 14.6 | 94.9 | 1.12 | 27.6 | 9.3 |
| Fasted for 9 days in laboratory: | | | | | | |
| 29.8 | 0.90 | 12.4 | 111.6 | 1.19 | 23.6 | 50.8 |
| 29.5 | 0.87 | 10.8 | 94.0 | 1.37 | 22.5 | 31.4 |
| 22.3 | 0.44 | 7.0 | 30.8 | 0.96 | 17.7 | 00.0 |
| 22.2 | 0.73 | 11.2 | 81.8 | 1.31 | 25.8 | 13.4 |
| 27.2 | 0.72 | 13.7 | 98.6 | 1.68 | 17.0 | 17.8 |
| 26.7 | 0.69 | 7.0 | 48.3 | 1.10 | 24.5 | 14.2 |
| Means: 26.3 | 0.73 | 10.3 | 75.2 | 1.28 | 21.8 | 21.3 |
| | | | n.s. | | p=.032* | p=.032* |
| Fasted 2-1/2 months in laboratory: | | | | | | |
| 22.0 | 0.366 | 3.3 | 12.1 | 0.92 | 21.1 | 00.0 |
| 18.0 | 0.310 | 2.2 | 6.8 | 0.78 | ---- | ---- |
| 19.4 | 0.478 | 1.2 | 5.7 | 0.14 | 21.5 | 00.0 |
| 23.1 | 0.384 | 2.4 | 9.2 | 0.59 | 25.8 | 00.0 |
| Means: 20.6 | 0.385 | 2.3 | 8.84 | 0.61 | 22.8 | 00.0 |
| p=0.019* | p=0.01* | p=.005* | p=.005* | p=0.019* | n.s. | p=0.005* |

p=probability of a higher U in the Mann-Whitney U test (one tailed).
*=significant difference from initial.

TABLE 10

SHORT TERM FASTING AND ENERGY RESERVES
(MALE WHITMORE LAKE FROGS) JULY 1969

| Body Weight (grams) | Liver Weight (grams) | Liver Glycogen (g/100 grams of liver) | Total Liver Glycogen (mg) | Muscle Glycogen (g/100g) | Blood Glucose (mg/100 cc) | Fat Body Weight (mg) |
|---|---|---|---|---|---|---|
| Initial levels, fed well in lab for 4 months, sacrificed 3 hours after a meal: | | | | | | |
| 26.2 | 0.63 | 6.6 | 41.6 | 0.60 | 29.7 | 5.7 |
| 25.6 | 0.90 | 12.1 | 108.9 | 0.73 | 23.3 | 24.4 |
| 30.0 | 0.78 | 7.3 | 56.9 | 0.64 | 27.6 | 6.2 |
| 24.2 | 0.86 | 9.8 | 84.3 | 1.18 | 46.1 | 7.4 |
| 20.0 | 1.02 | 15.9 | 162.2 | 2.01 | 36.6 | 52.4 |
| Means: 25.3 | 0.84 | 10.3 | 86.5 | 1.03 | 32.6 | 19.2 |
| Fasted 3 days: | | | | | | |
| 35.4 | 0.89 | 12.9 | 114.8 | 0.91 | 21.7 | 23.2 |
| 32.4 | 1.19 | 14.7 | 175.0 | 1.93 | 12.7 | 46.5 |
| 32.1 | 1.10 | 13.0 | 143.0 | 1.05 | 11.1 | 131.0 |
| 28.8 | 1.28 | 13.7 | 175.4 | 1.62 | 8.5 | 65.7 |
| 21.1 | 0.395 | 9.0 | 35.6 | 0.51 | 23.8 | 7.2 |
| Means: 29.9 | 0.97 | 12.7 | 123.2 | 1.20 | 15.8 | 54.7 |
| p=.048* | | | n.s. | | p=0.008* | n.s. |
| Fasted 5 days: | | | | | | |
| 34.7 | 1.07 | 7.3 | 78.1 | 0.69 | 20.1 | 101.6 |
| 21.2 | 0.63 | 12.8 | 80.6 | 0.68 | 13.2 | 11.4 |
| 23.2 | 0.55 | 10.2 | 56.1 | 0.63 | 21.7 | 9.6 |
| 16.9 | 0.475 | 11.3 | 53.7 | 0.68 | 22.8 | 77.9 |
| 22.6 | 0.725 | 11.2 | 81.2 | 0.47 | 20.1 | 19.6 |
| Means: 23.7 | 0.69 | 10.6 | 74.1 | 0.63 | 19.6 | 30.1 |
| | | | n.s. | | p=.004* | n.s. |

p=probability of a higher U in the Mann-Whitney U test (one tailed)
*=significant difference from initial

to decreased stress of the laboratory situation as compared with that of recent capture and transferal to the lab or might truly represent a fasting response.

After 2-1/2 months of fasting the animals exhibited significantly lowered body weight, liver weight on both an absolute and per cent body weight basis, fat body weight, liver glycogen concentration and total amount present, and muscle glycogen concentration. However, further experiments with larger groups of animals are needed to pinpoint the time of initial glycogen losses. Blood glucose levels were not lower than after 9 days of fasting but only three samples were obtained.

After the four month feeding program carbohydrate reserves were maintained (Table 10), but not increased over those present at the time of capture (Table 9). The fed frogs, assayed only 3 hours after a force fed meal, had significantly higher blood glucose levels than those frogs fasted for 3 and 5 days. No other parameters differed in a statistically significant manner.

The ability of leopard frogs to maintain their blood glucose levels during fasting of long duration is quite apparent from these data. A definite elevation of blood glucose occurs after meals with post absorptive levels being achieved by 3 days (Table 10). No further decrease in blood glucose levels was observed in extended fasts

(Table 9). Since blood glucose levels are post absorptive and liver glycogen levels apparently stable between 3 and 5 days of fasting, this fasting interval was chosen for use in other frog experiments.

Frog liver and muscle glycogen are not depleted by short term fasting but are depleted by long term fasting. After 2-1/2 months of fasting liver and muscle glycogen concentrations are not greatly different from those of non-fasted rats (Table 11). This comparison may be misleading

TABLE 11

GLYCOGEN LEVELS OF FASTED FROGS (2-1/2 MONTHS) AND NON FASTED RATS

| Animal | Liver Glycogen % | Muscle Glycogen % | Reference |
|---|---|---|---|
| Leopard frog | 2.3 | 0.6 | This study |
| Laboratory rat | - | 0.50-0.64 | Bloom and Russell, (1955) |
| Laboratory rat | 3.49 | 0.64 | Sokal and Sarcione, (1959) |

to some extent because fasted frogs have greatly reduced liver size (Table 9) and, therefore, reduced total liver glycogen content. It is not clear to what degree this is also true for the rat. The most obvious contrast is in the

speed of liver glycogen depletion in the fasting mammal as compared to the frog. Long et al. (1940) reported a 25% decrease in rat liver glycogen concentration during a 24 hour fast. Even considering the slower metabolism of frogs at the lower experimental temperatures, unusual toleration for fasting seems to occur in these animals.

The observation that frogs fasted for 9 days or more maintain relatively high levels of blood glucose and liver and muscle glycogen suggest that either there is considerable glucose sparing by fat utilization or that substantial gluconeogenesis is occurring. This is exemplified by the results of the following calculation. Using data in Table 10 for fed frogs, the total kilocalories available in stored glycogen can be approximated, assuming a caloric content of 4.2 kilocalories per gram of carbohydrate and 50 per cent of the body weight to consist of skeletal muscle. The total liver glycogen is 0.087 grams and the total muscle glycogen is 0.130 grams, thus there would be 0.217 grams X 4.2 C/gm or 0.9 kilocalories in stored glycogen. The daily caloric requirement at 18-25°C can be estimated to be 0.042 liters/day, from oxygen consumption data of Fromm and Johnson (1955) and McNabb (1969) and the respiratory quotient to be 0.82 (Fromm and Johnson, 1955). Assuming a caloric equivalent of 4.7 kilocalories/liter of oxygen consumed, the daily frog energy requirement can be estimated at 0.17 kilocalories. Thus, for a 25.3 gram frog there is only enough

glycogen stored at this time to last around 5 days, yet, in this experiment no drop in glycogen was seen at 5 days. Considerable amounts of other substrates must be utilized during fasting. Since the fat bodies were very small in the animals used in this study (less than 30 mg in most cases) and since body wastage was very apparent in the 20% decrease in body weight of the 2-1/2 month fasted animals, gluconeogenesis from tissue protein must have been an important energy source.

## Seasonal and Geographic Variation in Frog Carbohydrate Reserves

Rana pipiens is widely distributed geographically and exhibits considerable morphological and physiological variation (Moore, 1944,1967; Littlejohn and Oldham, 1968). Within a geographic area the animals are adapted physiologically to the fluctuating environment. In northern United States extreme seasonal variations result. Seasonal differences in oxygen consumption and respiratory quotients (Fromm and Johnson, 1955), and in energy reserves including liver glycogen, blood glucose, and fat body weight have been observed frequently (Mizell, 1964, 1954; Table 8). Frogs from widely separated geographic areas are not likely to be in phase seasonally. Moreover, genetic differences have developed in various geographic populations (Moore, 1967) compounding the possible sources of physiological

variation.

Although the occurrence of seasonal patterns in blood glucose and liver glycogen has been previously investigated, this phenomenon was reexamined in the present study. I have also attempted to answer the following questions: (1) Do these seasonal changes observed in freshly captured frogs continue under relatively constant environmental conditions in the laboratory? (2) To what extent do differences in blood glucose and liver and muscle glycogen levels occur in animals collected from different localities?

## Seasonal variation in carbohydrate and fat bodies in North Dakota frogs

North Dakota frogs were collected during late July and September and were shipped immediately. Other frogs collected in October hibernated through the winter months in outdoor tanks until they were shipped in March. Some of the frogs arriving in September were maintained in the laboratory until the following summer. Other animals were used within one week of arrival in the laboratory. Comparisons were made only between post absorptive animals. At the time of study 6 animals were weighed, anesthetized, then tissue samples taken. The fat bodies and gonads were also weighed, and observations made as to nutritional and reproductive status. The studies were conducted in March, August, and October.

Both liver and muscle glycogen were low in August, while blood glucose and fat bodies were highest at this time (Table 12). Liver glycogen varied from 6.3 gm% and 54 mg total in August to 12.5 gm% and 199 mg total in October. Muscle glycogen concentration varied from 0.7 gm% in August to 1.6 gm% in October. Glycogen levels were higher in March than in mid-summer. Blood glucose exhibited a seasonal difference of 6 mg% between winter and summer. Fat bodies showed 10 fold changes in weight with season, weighing on the average 8.5 mg/gm in August and only 0.8 mg/gm in March. No significant differences in carbohydrate levels were found between October and March animals (Table 12). However, the fat bodies were much smaller in the late March frogs than in the October frogs. August frogs were statistically different from March frogs in all parameters measured.

Seasonal variation of laboratory frogs

One group of North Dakota frogs was received in September and maintained in the laboratory until the following August. Initially, in September, 6 animals were sampled for blood glucose, and 6 more were sampled for both glycogen and glucose. Simultaneously, in August, 6 freshly collected North Dakota animals and 6 laboratory maintained animals were sampled for glycogen and glucose.

TABLE 12

SEASONAL STUDIES OF NORTH DAKOTA *RANA PIPIENS*

| Body Weight (grams) | Liver Weight (grams) | Liver Glycogen (g/100 grams of liver) | Total Liver Glycogen (mg) | Muscle Glycogen (g/100g) | Blood Glucose (mg/100 cc) | Fat Body Weight (mg) | Fat Body Weight (mg/g) |
|---|---|---|---|---|---|---|---|
| A. March 4, 1969 | | | | | | | |
| 42.4 | 1.48 | 12.7 | 188 | 1.6 | 14.2 | 22.2 | 0.5 |
| 43.2 | 2.29 | 13.5 | 309 | 1.4 | 12.7 | 32.2 | 0.7 |
| 32.8 | 2.17 | 8.8 | 191 | 1.8 | 11.2 | 39.0 | 1.2 |
| 47.3 | 1.63 | 10.7 | 174 | 0.5 | 15.1 | 25.6 | 0.5 |
| 46.4 | 1.45 | 9.5 | 138 | 2.0 | 19.5 | 39.4 | 0.9 |
| 43.5 | 1.45 | 7.3 | 106 | 1.7 | 12.4 | 36.0 | 0.8 |
| Means: 45.9 | 1.75 | 10.4 | 184 | 1.5 | 14.2 | 32.4 | 0.8 |
| B. August 7, 1969 | | | | | | | |
| 34.0 | 0.99 | 5.7 | 56 | 0.8 | 16.5 | 390.6 | 11.5 |
| 26.4 | 0.69 | 4.3 | 30 | 0.8 | 21.2 | 186.0 | 7.0 |
| 42.0 | 1.09 | 3.2 | 35 | 0.7 | 20.4 | 385.0 | 9.2 |
| 33.5 | 0.65 | 9.6 | 62 | 0.6 | 23.5 | 191.8 | 6.0 |
| 31.7 | 0.57 | 4.2 | 24 | 0.4 | 30.1 | 149.4 | 4.7 |
| 30.2 | 1.08 | 10.8 | 117 | 0.8 | 12.7 | 373.8 | 12.4 |
| Means: 33.0 | 0.85 | 6.3 | 54 | 0.7 | 20.7 | 279.6 | 8.5 |
| March p=.013 | .001 | .047 | .002 | .013 | .013 | .001 | .001 |
| Oct. p= n.s. | .001 | .004 | .001 | .001 | n.s. | .009 | .004 |
| C. October 13, 1969 | | | | | | | |
| 36.2 | 1.49 | 11.1 | 165 | 1.2 | 15.3 | 210.0 | 5.5 |
| 40.0 | 1.63 | 14.3 | 233 | 1.9 | 17.5 | 110.0 | 2.8 |
| 49.3 | 1.72 | 13.5 | 232 | 1.6 | 23.5 | 85.0 | 1.7 |
| 40.5 | 1.50 | 13.4 | 201 | 1.5 | 14.7 | 126.0 | 3.1 |
| 27.0 | 1.25 | 9.4 | 118 | 1.3 | 14.7 | 96.6 | 3.5 |
| 45.5 | 1.92 | 13.3 | 255 | 1.9 | 31.2 | ---- | ---- |
| Means: 39.7 | 1.59 | 12.5 | 199 | 1.6 | 18.5 | 125.5 | 3.3 |
| March p= n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | .002 | .002 |

The September collected frogs had retained their characteristically high liver glycogen concentration after 11 months in the laboratory (Table 13). The glycogen levels were not significantly different from those present upon arrival in the fall, but were much higher ($p=.015$) than those of animals freshly collected in the summer from the same geographic area. Blood glucose levels were the same in all three groups. The data indicate that under these circumstances seasonal cyclicity in liver glycogen was halted.

Figure 6 and Table 14 give further information regarding North Dakota frogs which had been in the laboratory for at least two weeks prior to sampling and were considered to be acclimated to laboratory conditions. These animals were the control animals used in hormone experiments throughout the year. Blood glucose concentrations of all 107 animals sampled were submitted to a test of normality (Snedecor, 1956) and were found to be normally distributed about a mean of 19.3 mg% (Figure 6). The blood glucose levels exhibited seasonal fluctuation (Table 14) with a 3.9 mg% difference between fall peak and winter low levels.

## Comparison of glycogen and glucose in frogs from North Dakota and Michigan

During October of 1969 frogs from two collection sites in North Dakota and Michigan were obtained and comparisons

TABLE 13

LIVER GLYCOGEN AND BLOOD GLUCOSE OF NORTH DAKOTA FROGS MAINTAINED IN THE LABORATORY AND FRESHLY COLLECTED NORTH DAKOTA FROGS

| Laboratory Frogs | | | | Freshly Collected Frogs | |
|---|---|---|---|---|---|
| Collected and sampled in September | | Sampled 11 months later, in August | | Collected and sampled in August | |
| Liver Glycogen (g/100 g liver) | Blood Glucose (mg%) | Liver Glycogen (g/100 g liver) | Blood Glucose (mg%) | Liver Glycogen (g/100 g liver) | Blood Glucose (mg%) |
| 11.1 | 18.5 | 13.5 | 22.8 | 5.7 | 16.5 |
| 14.3 | 19.7 | 5.5 | 13.3 | 4.3 | 21.2 |
| 13.5 | 18.0 | 15.1 | 15.9 | 3.2 | 20.4 |
| 13.4 | 25.2 | 13.2 | 19.1 | 9.6 | 23.5 |
| 9.4 | 16.4 | 11.5 | 18.5 | 4.2 | 30.1 |
| 13.3 | 25.7 | | | 10.8 | 12.7 |
| | 10.3 | | | | |
| | 16.3 | | | | |
| | 22.8 | | | | |
| | 26.0 | | | | |
| | 11.7 | | | | |
| | 15.7 | | | | |
| Means: | | | | | |
| 12.5% | 18.9% | 11.7% | 17.9% | 6.3% | 20.7% |
| Sept. p = | | n.s. | n.s. | | |
| Freshly collected p = | | .015 | n.s. | | |

*p is the probability of a higher U in the Mann Whitney U test

FIGURE 6 - Distribution Histogram of Blood Glucose Levels of Frogs Maintained in the Laboratory with the Normal Distribution Superimposed (Appendix II. A.)

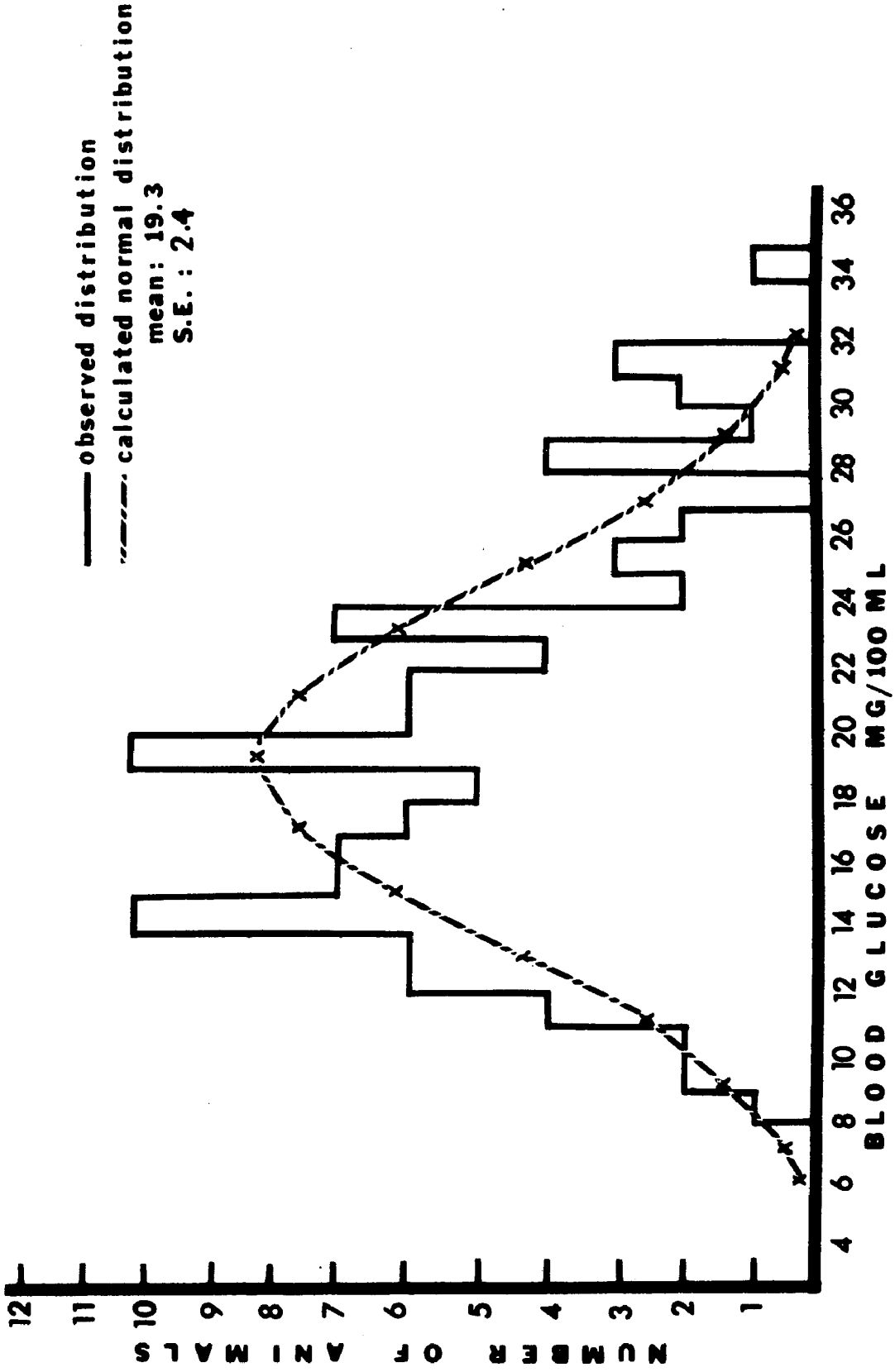

observed distribution
calculated normal distribution
mean: 19.3
S.E.: 2.4
NUMBER OF ANIMALS
1
2
3
4
5
6
7
8
9
10
11
12
BLOOD GLUCOSE MG/100 ML
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36

TABLE 14

MEAN BLOOD GLUCOSE CONCENTRATIONS FOR
NORTH DAKOTA FROGS SACRIFICED THROUGHOUT THE YEAR

| | Sept. to Jan. | Jan. to March | July to Aug. | Yearly |
|---|---|---|---|---|
| Mean Blood Glucose mg% | 19.9 | 16.0 | 19.5 | 19.3 |
| Number of Samples | 69 | 27 | 11 | 107 |
| Standard Error | 2.4 | 0.5 | 1.5 | 2.4 |
| Range | 10.8-34.5 | 8.5-24.5 | 12.7-30.1 | 8.5-34.5 |

of variation in carbohydrate reserves in the two groups made. There was no significant difference in blood glucose levels of the two groups (Table 15). The most obvious difference was that in mid to late October the frogs from Whitmore Lake had not stored the high autumn levels of either liver or muscle glycogen which was characteristic of the North Dakota animals (Table 15). The levels of liver glycogen did appear to be rising in the Whitmore Lake frogs during late October but the difference from early October was not significant. Frogs at this locality entered the water prior to hibernation the third week of November. Rapid increase in glycogen storage probably began immediately before hibernation because animals emerging from hibernation in the spring had higher mean glycogen levels than the October frogs (14.6% and 9.5%; Tables 9 and 15).

Fat body weights of North Dakota frogs were much higher than those of Whitmore Lake frogs during October (3.3 and 1.9 mg/g body weight, respectively). By the end of October fat bodies from the Whitmore Lake group weighed only 0.7 mg/g body weight as compared to an average of 3.3 mg/g for the North Dakota animals. This small fat body size was also observed at the University of Michigan Amphibian Facility for many more frogs from this locality (P. Hejmadi, personal communication).

TABLE 15

A COMPARISON OF NORTH DAKOTA AND WHITMORE LAKE
*RANA PIPIENS* DURING OCTOBER

| Body Weight (grams) | Liver Weight (grams) | Liver Glycogen (g/100 g of liver) | Total Liver Glycogen (mg) | (mg/g body wt) | Muscle Glycogen (g/100 g) | Blood Glucose (mg/100 cc) | Fat Body Weight (mg) | mg/g body wt) |
|---|---|---|---|---|---|---|---|---|
| A. North Dakota frogs October 13, 1969 | | | | | | | | |
| 36.2 | 1.49 | 11.1 | 165 | 4.5 | 1.2 | 15.3 | 210.0 | 5.5 |
| 40.0 | 1.63 | 14.3 | 233 | 4.8 | 1.9 | 17.5 | 110.0 | 2.8 |
| 49.3 | 1.72 | 13.5 | 232 | 4.7 | 1.6 | 23.5 | 85.0 | 1.7 |
| 20.5 | 1.50 | 13.4 | 201 | 4.9 | 1.5 | 14.7 | 126.0 | 3.1 |
| 27.0 | 1.25 | 9.4 | 118 | 4.3 | 1.3 | 14.7 | 96.6 | 3.6 |
| 45.5 | 1.92 | 13.3 | 255 | 5.6 | 1.9 | 31.2 | ----- | --- |
| ---- | ---- | ---- | --- | --- | --- | 12.5 | ----- | --- |
| Means: | | | | | | | | |
| 39.7 | 1.59 | 12.5 | 199 | 5.0 | 1.6 | 18.5 | 129.5 | 3.3 |
| B. Whitmore Lake frogs October 3, 1969 | | | | | | | | |
| 33.2 | 0.94 | 8.2 | 77 | 2.3 | 0.9 | 11.5 | 126.0 | 3.8 |
| 23.9 | 0.74 | 3.0 | 22 | 0.9 | 0.4 | 23.8 | 0.0 | 0.0 |
| 31.8 | 0.92 | 12.6 | 116 | 3.6 | 0.5 | 28.4 | 77.5 | 2.4 |
| 35.0 | 1.25 | 7.0 | 87 | 2.5 | 0.8 | 8.5 | 145.2 | 4.1 |
| 32.1 | 0.57 | 0.8 | 5 | 0.2 | 0.6 | 15.8 | 10.0 | 0.3 |
| 29.5 | 0.82 | 10.6 | 87 | 2.9 | 0.6 | 19.6 | 19.5 | 0.6 |
| Means: | | | | | | | | |
| 30.9 | 0.87 | 7.0 | 65.7 | 2.1 | 0.64 | 17.9 | 63.0 | 1.9 |
| North Dakota * p= | .002 | .008 | | .001 | .001 | n.s. | n.s. | n.s. |

*p=probability of higher U in Mann Whitney U test.

TABLE 15 (Continued)

| Body Weight (grams) | Liver Weight (grams) | Liver Glycogen (g/100 g of liver) | Total Liver Glycogen (mg) | Total Liver Glycogen (mg/g body wt) | Muscle Glycogen (g/100 g) | Blood Glucose (mg/100 cc) | Fat Body Weight (mg) | Fat Body Weight (mg/g body wt) |
|---|---|---|---|---|---|---|---|---|
| C. Whitmore Lake frogs October 31, 1969 | | | | | | | | |
| 33.4 | 0.70 | 8.6 | 60 | 1.8 | --- | 21.8 | 10.0 | 0.3 |
| 21.3 | 0.89 | 13.4 | 119 | 5.1 | --- | 12.3 | 17.0 | 0.8 |
| 35.8 | 1.51 | 6.5 | 98 | 2.7 | --- | 6.4 | 34.0 | 0.9 |
| 23.5 | 0.83 | 9.2 | 76 | 3.2 | --- | 13.6 | 19.0 | 0.8 |
| 26.0 | 0.91 | 7.4 | 67 | 2.6 | --- | 21.8 | 10.0 | 0.4 |
| 24.5 | 0.79 | 12.1 | 96 | 3.5 | --- | 14.2 | 26.0 | 1.1 |
| Means: | | | | | | | | |
| 27.4 | 0.94 | 9.5 | 86 | 3.1 | - | 15.1 | 19.3 | 0.7 |
| North Dakota | p=.004 | .021 | | .013 | | n.s. | .002 | .002 |

Descriptions of seasonal variation in energy reserves of Rana pipiens (Mizell, 1965) and Rana temporaria (Smith, 1954) are very similar. Both species exhibit lowest blood glucose levels in the winter, accompanied by large quantities of stored liver glycogen and, presumably, fat, as indicated by weighty fat bodies. Blood glucose levels increase during early spring and may reach peak levels during amplexus, as in the toad, Bufo bufo (Hermansen and Jorgensen, 1969). Throughout the summer blood glucose levels are regulated at higher levels than are seen during the winter. While food is abundant throughout the summer, carbohydrate is not stored maximally until late fall after most of the gonadal and fat body enlargement has taken place. Blood glucose levels are also high in the fall.

The data observed here for Rana pipiens substantiates most aspects of the pattern described by Mizell (1965). It also demonstrates the previously undescribed similarity between liver and muscle glycogen seasonal fluctuation. However, there is no consensus as to whether laboratory acclimated animals exhibit seasonal variation in glycogen and glucose levels. Bartell (1969) observed monthly differences in blood glucose levels of Ambystoma tigrinum adults and larvae stored for various periods at a uniform temperature. Bergerhoff and Hanke (1968) found seasonal blood glucose fluctuation in Rana temporaria stored in a

cold room for 3 to 4 weeks prior to use. On the other hand, Hermansen and Jorgensen (1969) observed no differences in blood glucose levels of summer collected frogs which had been kept at 20 to 22°C for days or weeks. In the present study blood glucose levels in the laboratory population of frogs, all of which had been in the laboratory for at least two weeks, were lowest in the winter but the difference observed between seasonal means was only half that seen between seasonal means of freshly collected animals. In fact the winter levels were higher and summer levels lower than those observed in freshly collected animals. Seasonal variation in liver glycogen concentration was apparently abolished in the group of animals observed over an 11 month period. One can conclude from this that while some variation is evident in the laboratory population, it is not so extensive as that occurring in the animals under natural environmental conditions.

The finding that frogs from Michigan and North Dakota differed extremely in liver and muscle glycogen levels and fat body weights has important implications for future experiments and for other amphibian physiologists. If frogs from these two different localities had been used in an experiment the variation in stored energy reserves would have been increased tremendously. Therefore, it is important to keep groups of frogs of different localities

and seasons separate or to stipulate in orders to biological suppliers that only frogs from a single locality or season are required.

## Metamorphic Changes in Carbohydrate Levels

Larvae of two species of amphibians, Rana clamitans (Frye, 1964) and Ambystoma tigrinum (Bartell, 1969) have been shown to have blood glucose levels lower than adults. The purpose of this study was to determine whether glucose levels are also lower in tadpoles of Rana pipiens, and to extend the observations to a comparison of liver glycogen levels. If glucose levels are higher in adults, adrenalin and glucagon could contribute to the increase by their hyperglycemic activity. Therefore tadpole responses to these hormones should be studied along with those of the adults.

Blood glucose and liver glycogen levels were determined on groups of tadpoles at Taylor-Kollros (1946) stages IV-VI, XII-XV, XX-XXI, and XXII-XXIII. In nature tadpoles not in metamorphic climax normally have filled guts, therefore in the laboratory they were fed ad libitum, and not fasted before study. In younger animals, stages IV-VI, blood and liver samples from two or three animals were pooled for analysis; in older animals determinations were run on samples from single individuals. Data from tadpoles was

compared with the data for summer frogs which had been in the lab for a minimum of two weeks (see Appendix II. B. for raw data). Comparisons were made between tadpoles and summer frogs because the tadpoles metamorphose in the summer and therefore coexist with summer adults in nature.

Tadpole and adult glucose levels are compared in Figure 7. During premetamorphosis, which includes stages IV through XV, glucose levels in the tadpoles were slightly but significantly lower than in either adult or late climax (stages XXII-XXIII) animals ($p=.003$ for stages IV-VI and XXII-XXIII, $p=.025$ for stages IV-VI and the adults, $p<.001$ for stages XII-XV and adults). During late metamorphic climax (stages XXII-XXIII) blood glucose levels were higher than at all earlier stages, and were not significantly different from the adult levels (statistics are in Appendix II. B. 2).

Changes in liver glycogen levels were quite striking during metamorphosis (Figure 8). A significant build-up of glycogen occurred between stages IV-VI and prometamorphic animals of stages XVIII-XIX. During early climax a dramatic fall in glycogen levels occurred from a mean of 8% fresh weight of liver in stage XVIII-XIX animals to a mean of 1.7% in stage XX-XXI animals ($p<.001$). Then, during late climax stages, XXII-XXIII, glycogen levels were not significantly different from an average summer adult level of 6.3 per cent ($p<.535$).

FIGURE 7 - Blood Glucose Levels in Metamorphosing Tadpoles and in Summer Adults (means $\pm$ standard errors of data in Appendix II. B. 1, adjacent numbers are the number of samples in each group)

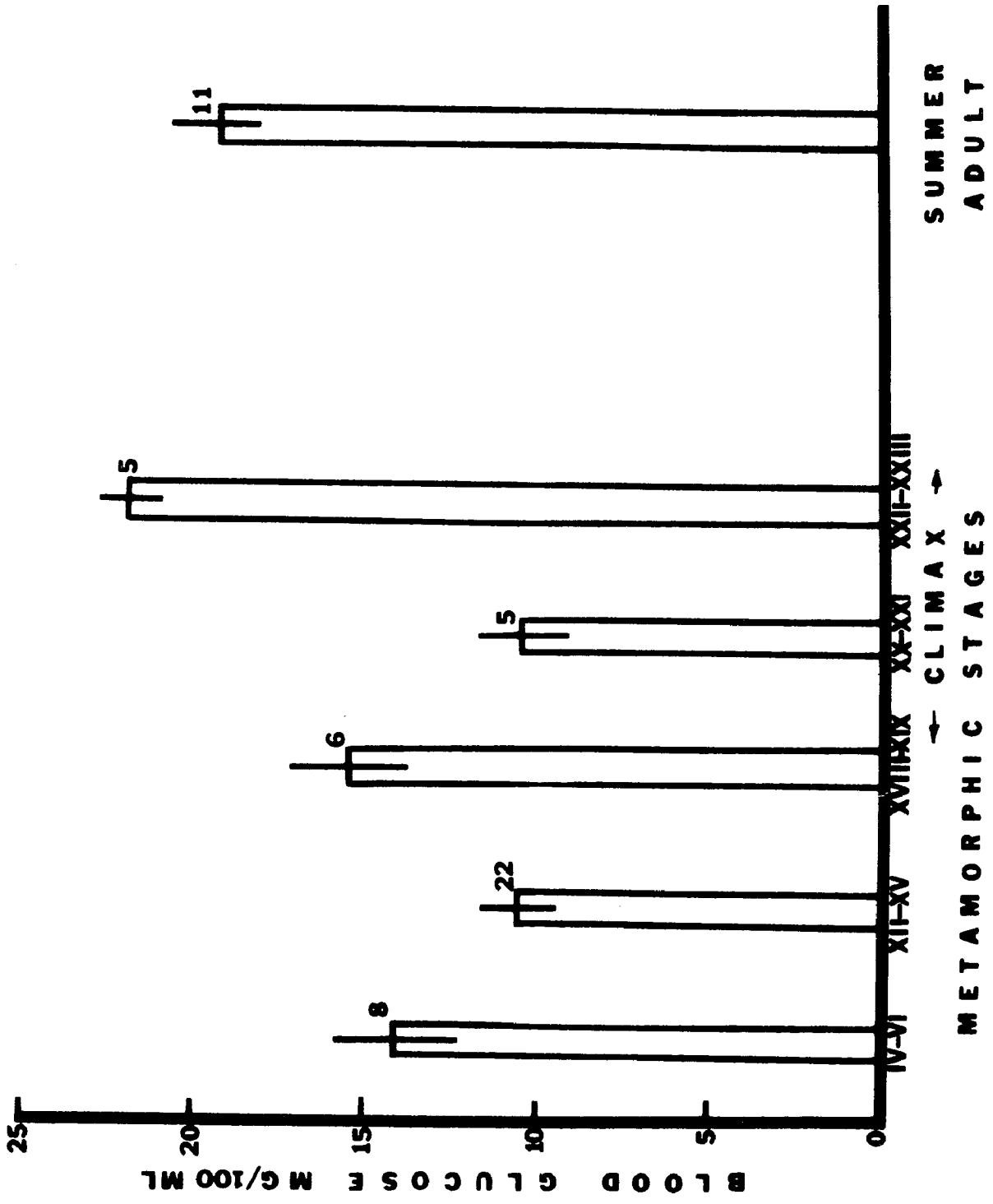
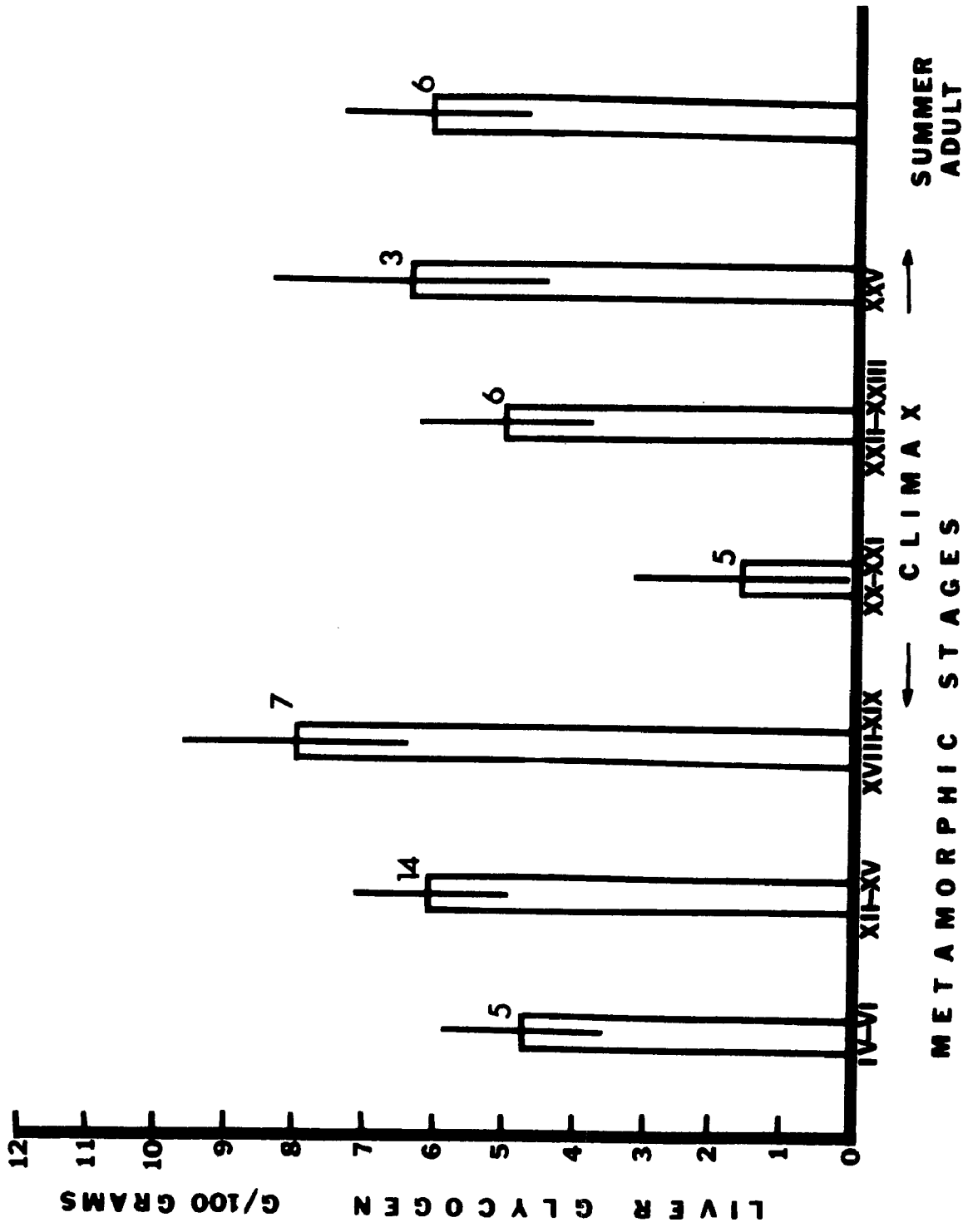

BLOOD GLUCOSE MG/100 ML
0
5
10
15
20
25
8
22
6
5
5
11
IV–VI
XII–XV
XVII–XIX
XX–XXI
XXII–XXIII
METAMORPHIC STAGES → CLIMAX →
SUMMER ADULT

FIGURE 8 - Liver Glycogen Levels in Metamorphosing Tadpoles and in Summer Adults (mean glycogen in g/100 g fresh liver weight ± standard errors of raw data in Appendix II. B. 1., numbers near top of bars are the numbers of animals in each group)


LIVER GLYCOGEN G/100 GRAMS
0
1
2
3
4
5
6
7
8
9
10
11
12
5
14
7
5
6
3
6
IV–VI
XII–XV
XVIII–XIX
XX–XXI
XXII–XXIII
XXV
METAMORPHIC STAGES
CLIMAX
SUMMER ADULT

Rana pipiens tadpole blood glucose levels were lower than adult levels (19.5 mg%) by at most 9.1 mg% at stages XX-XXI (10.4 mg%). A similar difference of 9.4 mg% between larval (18.4 mg%) and adult (27.8 mg%) Ambystoma tigrinum blood glucose levels was reported by Bartell (1969). Frye (1964) observed a greater difference of 18 mg% between tadpole (22 mg%) and adult (40 mg%) blood sugar levels in Rana clamitans.

Liver glycogen in Rana pipiens gradually increased from stages IV-VI to metamorphic climax. During climax, when the tadpoles stopped feeding, liver glycogen was utilized. Later, in climax stages XXII-XXV, glycogen levels increased to adult levels. Liver glycogen storage early in metamorphosis and its utilization during metamorphic climax have been described in Rana temporaria (Bilewicz, 1938) and in Xenopus laevis (Hanke and Leist, 1971). However, the recovery of glycogen levels has not been reported. Although the animals are still not feeding during stages XXII-XXV, both liver glycogen and blood glucose increased. These increases may have been accomodated by the shunting of materials being resorbed from the tail tissue into new carbohydrate.

## Summary of Part I

Blood glucose in Rana pipiens is regulated at a comparatively low concentration. Values obtained for the absolute concentration of sugar in the blood vary according to the techniques used in obtaining the blood and measuring the sugar in it. Enzymatic methods of glucose determination, utilizing glucose oxidase and hexokinase, give lower absolute values than those techniques which also detect other reducing substances. This is especially true when reducing techniques are coupled with tungstic acid deproteinization, as in the Folin-Malmros technique.

Although pithing frogs is a commonly used method for rapid immobilization prior to biopsy, its use is not ideal when blood glucose is to be determined as it increases variability in the glucose levels. On the other hand, excessive use of anesthetics, especially prolonged anesthesia and multiple anesthetizations, can cause severe hyperglycemia and lactacidemia.

Data reported here provide evidence that leopard frogs can maintain their blood glucose at constant levels throughout months of fasting at room temperature. However, the data also suggest that there is a correlation between dietary status and blood glucose level in that absorptive levels are significantly higher than postabsorptive. Postabsorptive levels are maintained for extended periods of

time and, apparently, at the expense of liver and muscle glycogen content only after extreme fasting. A fast of 3 to 5 days prior to experimental use was found to produce postabsorptive animals with apparently stable glycogen concentrations. Therefore, a fast of this length is a desirable preexperimental condition.

The factors discussed above cause variations about norm levels, but in the case of Rana pipiens the norm itself varies at different times of the year. Blood glucose average values are higher in summer and fall than winter while liver and muscle glycogen peak concentrations are reached in late fall with minimal levels in summer. Fat storage in the fat body occurs in late summer and fat may be utilized during winter and especially in spring.

Seasonal variation is most evident in freshly collected animals, with laboratory housing serving to moderate the seasonal influence. The extreme of this phenomenon was the cessation of seasonal changes in liver glycogen through an extended period of laboratory existence in one group of animals collected in the fall.

Rana pipiens from different collection areas may also differ in glycogen levels and fat body size at the same time of the year. Blood glucose seems less variable in this regard.

Normal levels change throughout metamorphosis. Tadpoles in premetamorphosis have significantly lower blood glucose values than adults, while the adult level is equaled in tadpoles in stages of tail resorption. Liver glycogen is stored in increasing amounts during prometamorphosis and is mobilized and then restored to summer adult levels during late metamorphic climax.

## CHAPTER III. EFFECTS OF ADRENALIN AND GLUCAGON ON CARBOHYDRATE METABOLISM

In Part I we have seen that frogs regulate their blood glucose at low levels and maintain unusually high liver and muscle glycogen levels. While frogs do have the potential to mobilize glucose fairly rapidly, for example, during exercise, their low resting glucose levels could be the result of a hormonal system balanced such that insulin, a hypoglycemic and glycogenic hormone, predominates. It is possible to postulate that hyperglycemic, glycogenolytic hormones, such as adrenalin and glucagon, are less significant in amphibian than in mammalian carbohydrate regulation. Full examination of this postulate will require complete exploration of the endocrinology of carbohydrate metabolism in amphibians, including the demonstration of the presence of and measurement of the physiological levels of the relevant hormones in amphibian tissues, determination of the conditions in which they are secreted, and examination of their effects in target tissues. The present investigation is concerned with only the latter aspect of this problem, namely measurement and comparison of the hyperglycemic and glycogenolytic activities of adrenalin and glucagon in frogs and tadpoles.

Additional significance is given to this study by the demonstration that in mammals adrenalin is a relatively impotent, perhaps non-physiological hepatic glycogenolytic agent, compared to glucagon (Ezdinli and Sokal, 1966), and that glucagon has little or no glycogenolytic activity in muscle whereas this in an important part of the physiological action of adrenalin (Ellis, 1956). In mammals there may be a dichotomy in the physiological roles of adrenalin and glucagon with only the latter hormone directly stimulating hepatic glycogenolysis. If this phenomenon is universal among vertebrates, then injected glucagon should cause liver glycogenolysis in a frog such as Rana pipiens, even though this species has few alpha cells (Seiden, 1945) and might lack the ability to secrete glucagon. If glucagon does not cause liver glycogenolysis, adrenalin might be expected to regulate glycogenolytic activity in these animals. This would mean that an evolutionary increase in specialization of these hormonal roles had taken place in some higher vertebrate group.

Blood glucose levels in tadpoles studied in Part I were even lower than comparable adult levels. It might follow that the hyperglycemic potential of adrenalin and/or glucagon is not realized prior to metamorphosis because of some unknown factors that keep the blood glucose levels low or that the hormones, themselves, do not function as hyperglycemic agents until sometime during metamorphosis. Any

increased hyperglycemic function could contribute to the increased blood glucose levels and to the maturation of the pancreatic beta cells, both of which are observed during metamorphosis.

## Responses of Intact Frogs to Adrenalin and Glucagon

The objective of this study was to establish dose-response relationships of frogs to adrenalin and glucagon as a basis for comparing the potency and the target tissue specificty of the two hormones. Responses measured were changes in blood glucose and lactate concentrations and muscle and liver glycogen concentrations. A rise in blood glucose in response to these hormones reflects hepatic glycogenolytic action, and muscle glycogenolysis and glycolysis, whereas rises in blood lactate are presumed to be a consequence primarily of muscle glycolysis (Sokal and Sarcione, 1958; Buchwald and Cori, 1931).

### Methods

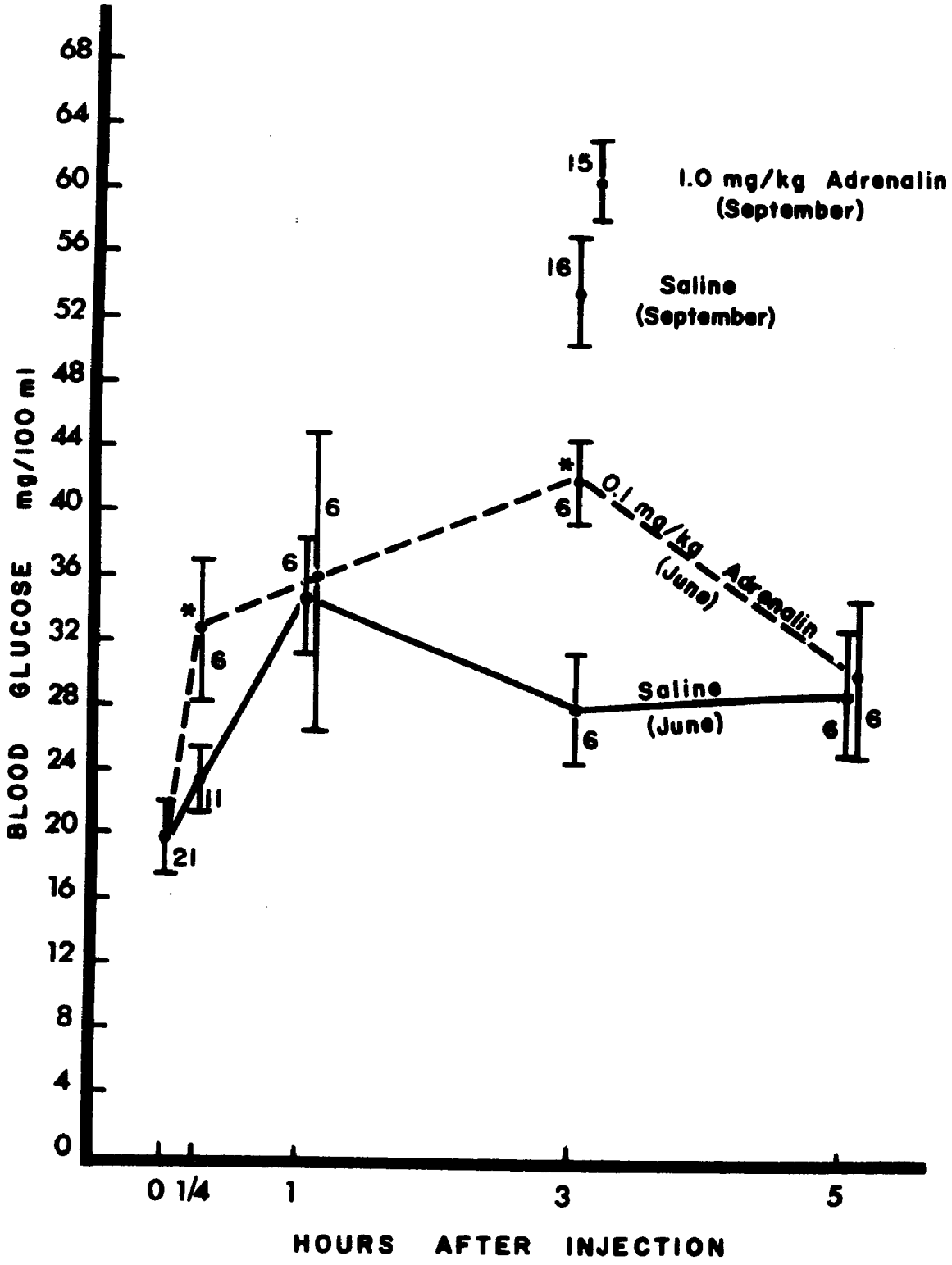
Adult male Rana pipiens weighing 30 to 80 grams, collected from the Red River Valley of North Dakota, were used in these experiments. They were obtained from the collectors during winter, summer, and fall. In addition to the general maintenance and experimental conditions described in the general Materials and Methods, those procedures and conditions which were found to be most

conducive to uniform responses in Part I were followed. Namely, frogs were handled and anesthetized minimally, were post-absorptive, and were inactive during the experiments. Where reason of analysis varied, care was taken to record the results according to the season. Although no systematic analysis of seasonal variation was intended, the results show that very significant seasonal differences in hormone sensitivity occur.

Pilot experiments were done to determine the feasibility of intravenous hormone injection. The results of experiments with intravenous injection of adrenalin in June and September are shown in Figure 9. Although hyperglycemia was seen within 15 minutes after adrenalin injection, and persisted for several hours, blood glucose also increased in placebo injected controls, presumably as a result of the initial anesthesia required for intravenous injection. Thus, the effects of the exogenous hormone at two dose levels and during 2 seasons were not clearly different from saline controls, and this approach was abandoned. All subsequent experiments were done by intraperitoneal injection of the hormones. This method is desirable because initial anesthesia is not required and it permits direct comparison of tadpole and frog responses.

Following intraperitoneal injection of the unanesthesized frogs, they were placed in moist, aerated plastic food crispers until sampling time. Sampling intervals were

FIGURE 9 - Effect of Intravenous (Femoral Vein) Adrenalin on Blood Glucose in June and September (means $\pm$ standard errors of data in Appendix III. 1 and 2, adjacent numbers are numbers of animals in each group, asterisks indicate significant difference ($p \leq .05$) from control)

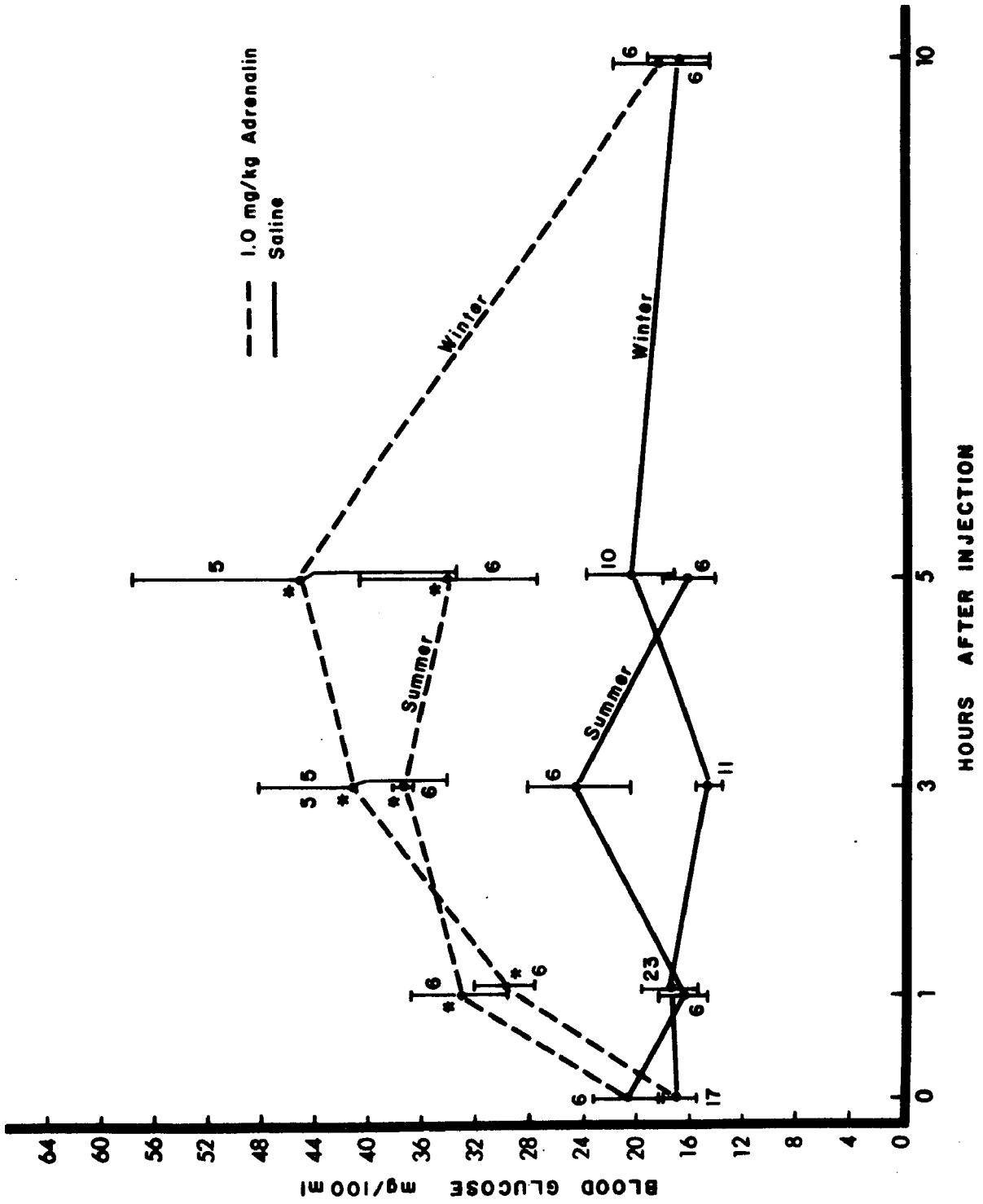

68
64
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
BLOOD GLUCOSE mg/100 ml
15
1.0 mg/kg Adrenalin
(September)
16
Saline
(September)
*
6
0.1 mg/kg Adrenalin
(June)
6
6
*
6
11
21
Saline
(June)
6
6
6
0 1/4
1
3
5
HOURS AFTER INJECTION

0, 1, 3, 5, 10, and 24 hours post-injection. Serial samples were not taken from individual animals because of the effects of anesthesia previously described. Thus, 6 frogs were used to obtain samples for each data point, and as many as 7 dozen frogs were required for one experiment comparing responses to adrenalin, glucagon, and saline injections. Hormone doses used were 1, 0.5, 0.10 and 0.05 mg/kg for adrenalin, and 2, 1, 0.10 and 0.05 mg/kg for glucagon. Tissue samples were taken by anesthetizing the animals, then taking blood, liver, and muscle samples in rapid succession. The sequence of sampling was uniform and the rate as fast as possible, so that variations in the data due to sampling procedure hopefully were minimized. The results are illustrated in figures 10 - 18. Complete data on all experiments are included in Appendix III. 4-11.

## Results

Blood glucose responses of intact frogs: Blood glucose responses to a 1 mg/kg dose of adrenalin were quite similar during summer and winter (Fig. 10). No differences in the summer and winter hormone treated or control groups were observed except at 3 hours where the summer control was higher ($p<.05$) than the winter control. Therefore, summer and winter blood glucose responses to adrenalin were treated together in subsequent dose response experiments. Responses to glucagon at a dose of 1 mg/kg were virtually

FIGURE 10 - Effects of Adrenalin on Frog Blood Glucose in Summer and Winter (means $\pm$ standard errors of data in Appendix III. 4., numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from respective seasonal control)


1.0 mg/kg Adrenalin
Saline
Winter
Summer
Winter
Summer
BLOOD GLUCOSE mg/100 ml
HOURS AFTER INJECTION

identical in summer and fall, but were sufficiently lower in winter to prohibit treatment of the seasonal dose-response data as a single curve (Figures 11 and 13).

Some dose-response relationships are illustrated in Figures 12 and 13, and data for additional doses are given in Appendix III. 5-8. Hyperglycemia was produced by hormone doses above 0.05 mg/kg. There appeared to be only slight gradations in response to dose increases above 0.10 mg/kg. The 1 mg/kg dose of adrenalin caused extreme reddening of the skin, and secretion by the skin glands. Consequently, no higher doses were attempted, and it was assumed that 1 mg/kg of adrenalin was a maximally effective dose. Glucagon responses were maximal at 1 mg/kg in summer and fall (Figure 11) but at 2 mg/kg in winter (Figure 13).

The responses to adrenalin and glucagon were very similar in pattern and magnitude of the response. Generally, hyperglycemia was apparent by one hour after injection, and lasted for at least 5 hours. Maximum glucose levels were attained at 3 hours post-injection, and by 10 hours the blood glucose values had returned to normal. The highest mean blood glucose concentration reached after either hormone, 58.7 mg%, occured after adrenalin treatment in the fall. This value was three times the initial level of 19.5 mg%.

FIGURE 11 - Effects of Glucagon on Frog Blood Glucose in Summer, Fall and Winter (means $\pm$ standard errors of data in Appendix III. 6-7, numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from respective seasonal control)

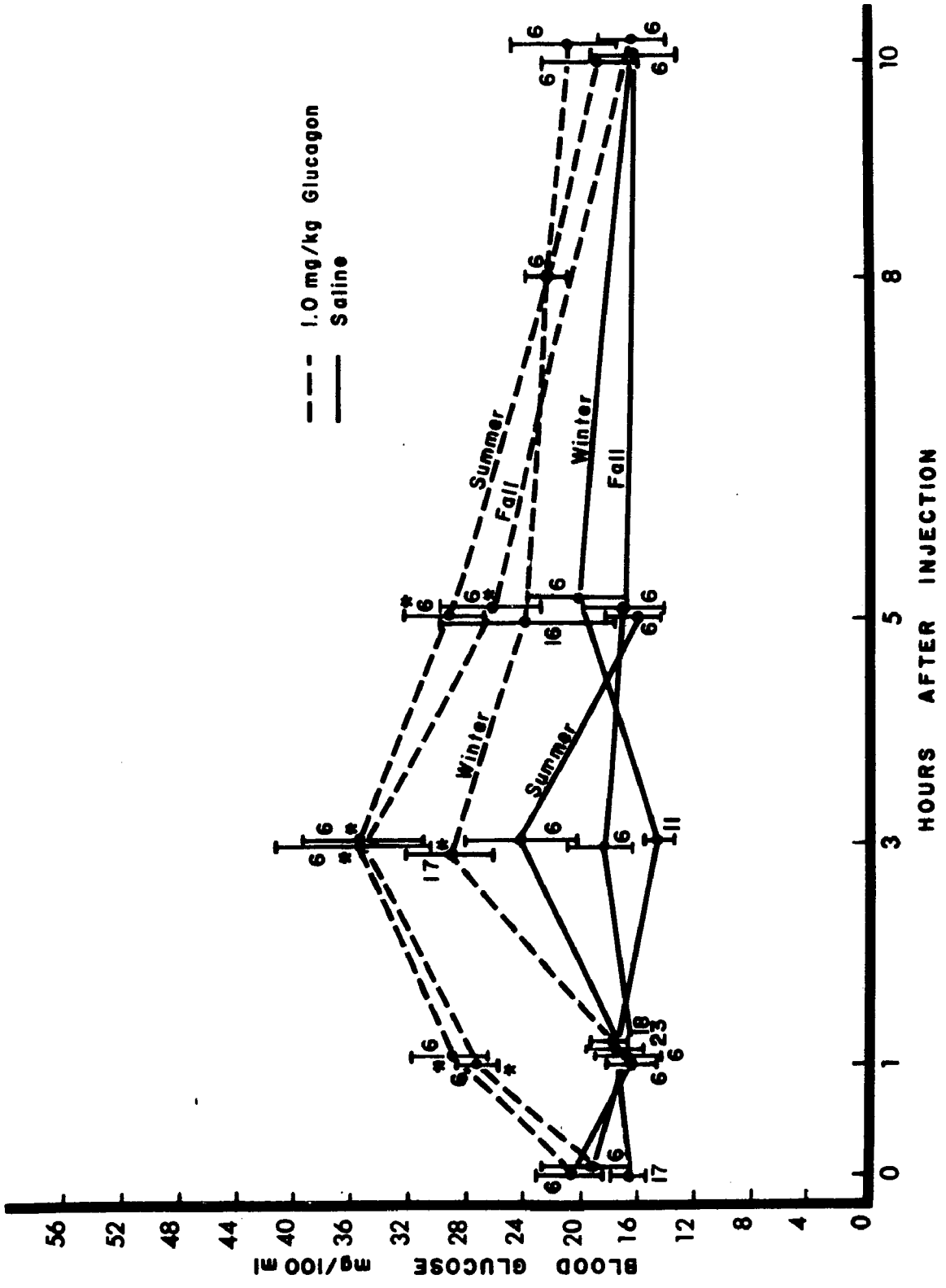

1.0 mg/kg Glucagon
Saline
Summer
Fall
Winter
Fall
Winter
Summer
BLOOD GLUCOSE mg/100 ml
HOURS AFTER INJECTION

FIGURE 12 - Effects of Different Doses of Adrenalin on Frog Blood Glucose. Data of Summer and Winter Experiments Combined (means $\pm$ standard errors of data in Appendix III. 5 and 6, numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from controls)

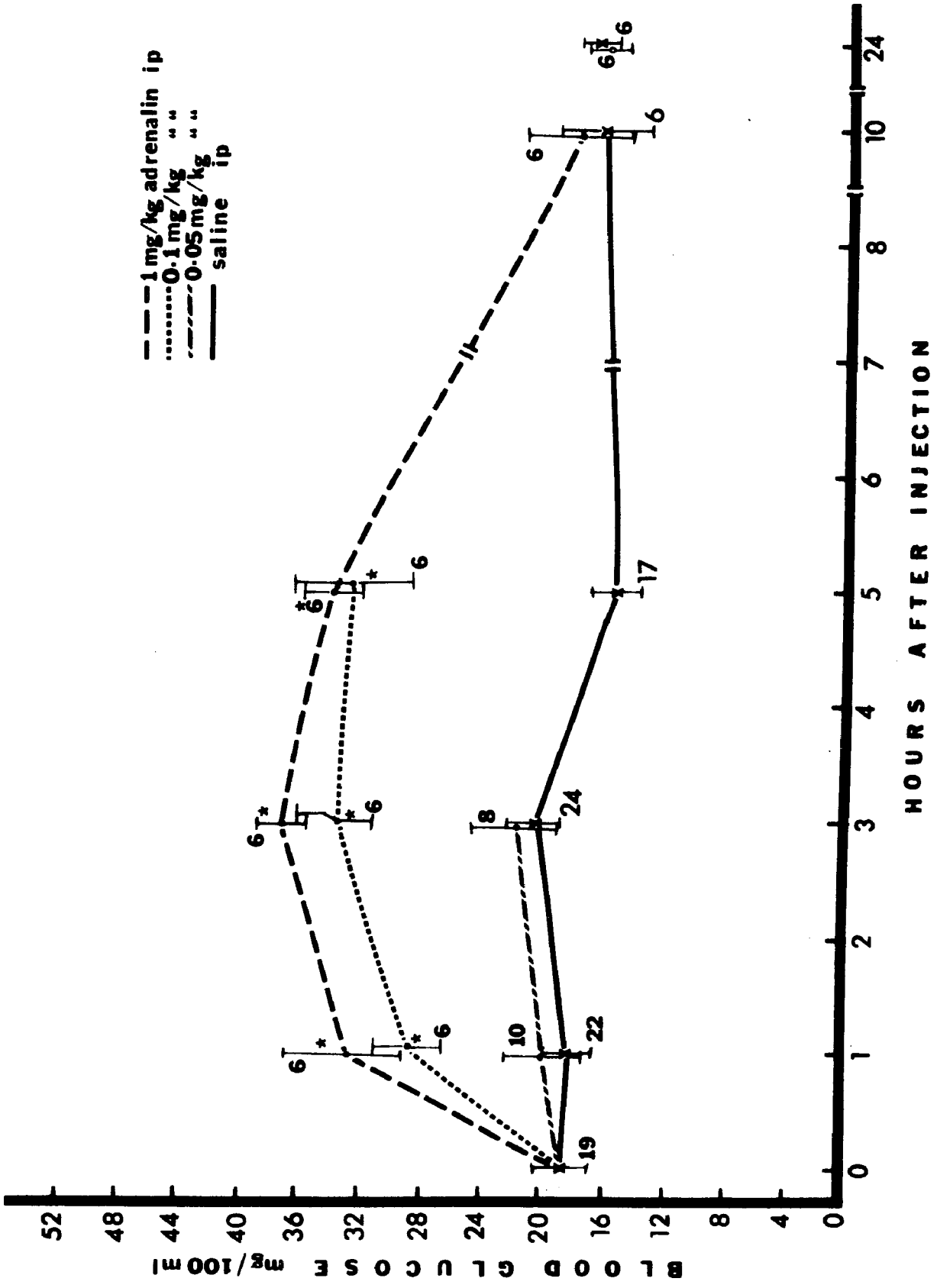
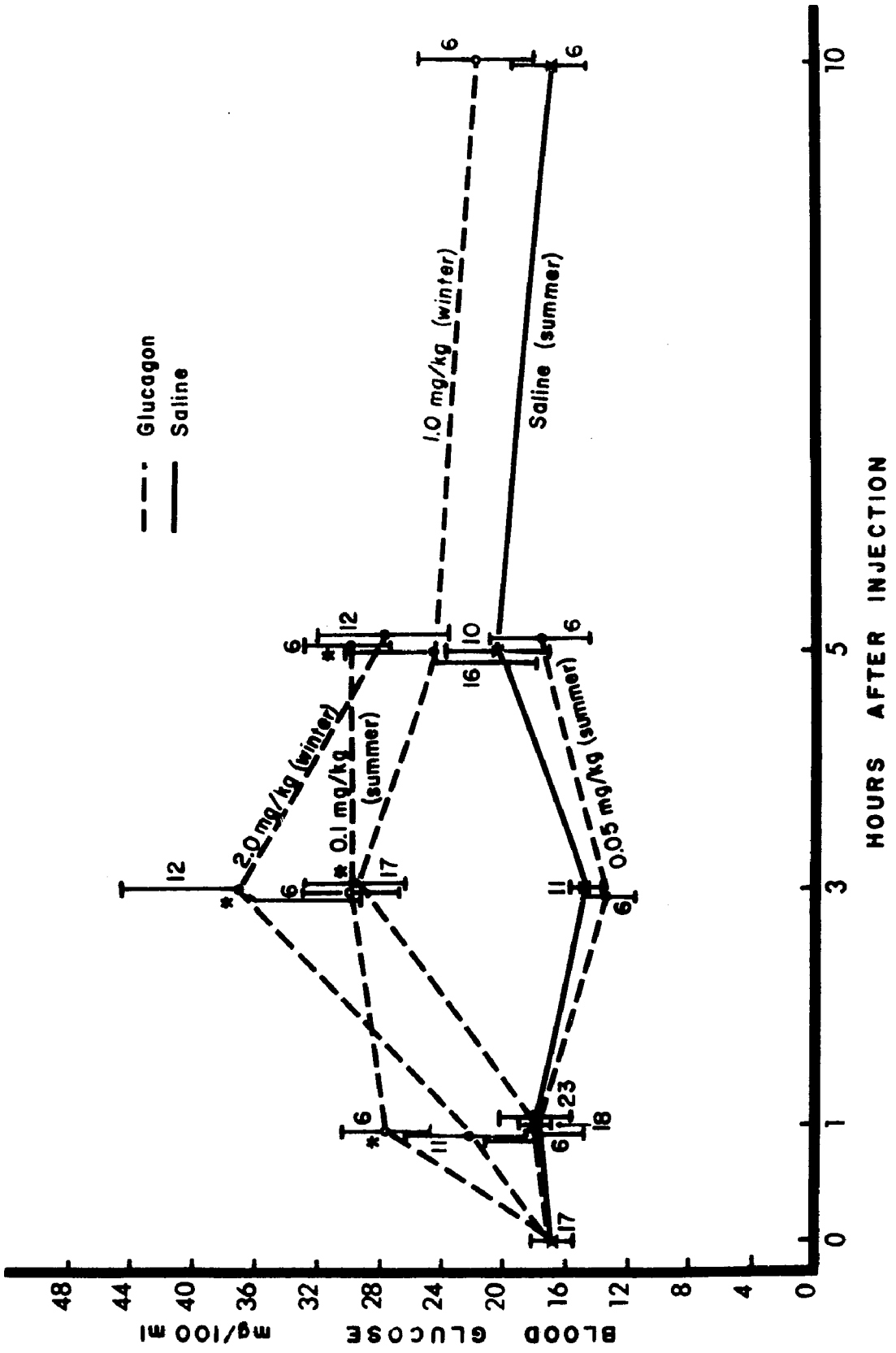

1 mg/kg adrenalin ip
0.1 mg/kg " "
0.05 mg/kg " "
saline ip
BLOOD GLUCOSE mg/100ml
HOURS AFTER INJECTION

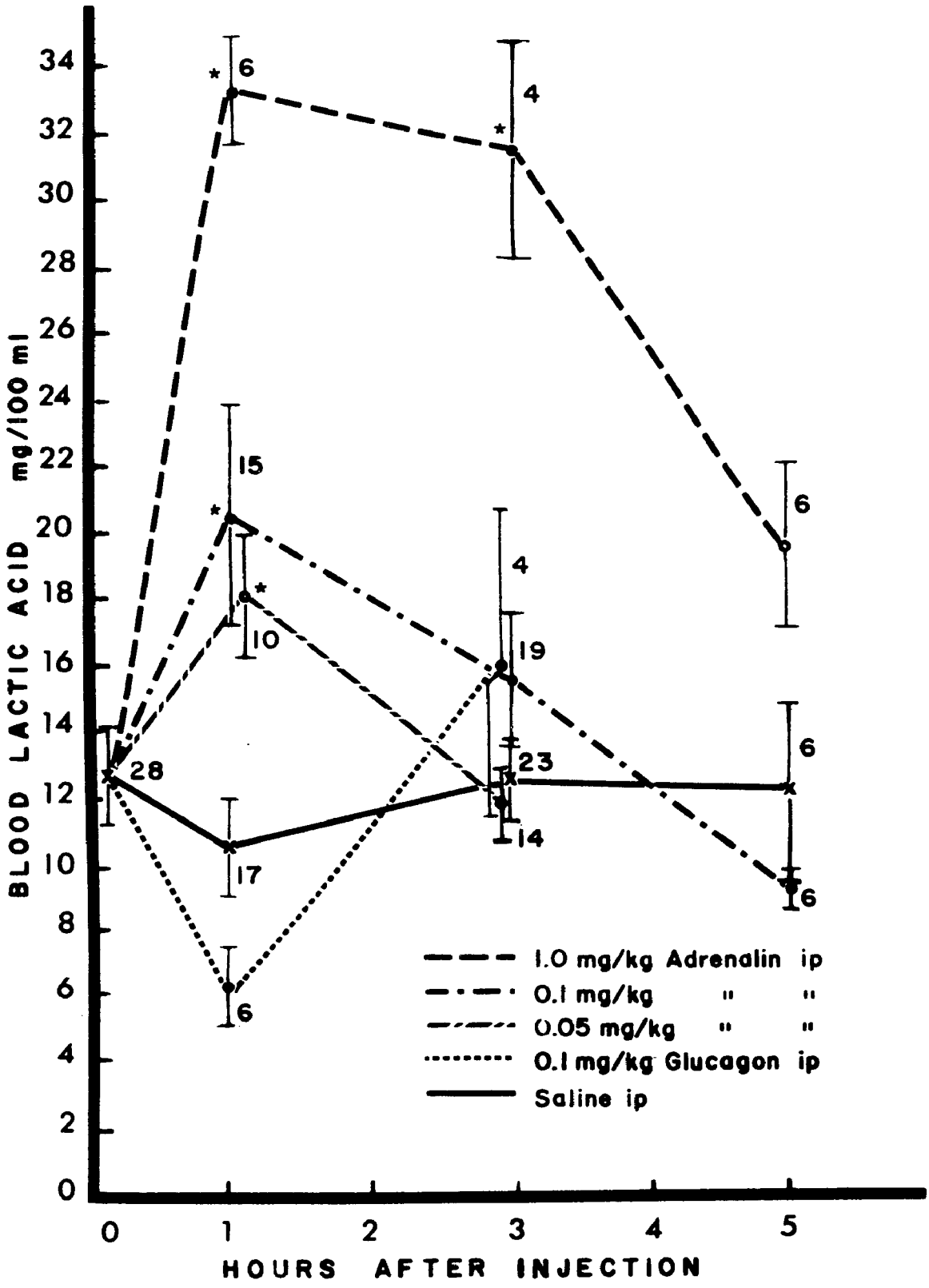
FIGURE 13 - Effects of Different Doses of Glucagon on Frog Blood Glucose (means $\pm$ standard errors of data in Appendix III. 8., numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from controls)


Glucagon
Saline
1.0 mg/kg (winter)
Saline (summer)
2.0 mg/kg (winter)
0.1 mg/kg (summer)
0.05 mg/kg (summer)
BLOOD GLUCOSE mg/100 ml
HOURS AFTER INJECTION

Blood lactate responses of intact frogs: Blood lactate was analyzed in frogs collected in summer. A graded response to different adrenalin doses was observed (Figure 14). Adrenalin at a dose of 1 mg/kg produced peak lactate concentrations at 1 hour and 3 hours after injection, but lactate levels had dropped to control levels by 5 hours. Lower doses resulted both in lower peak responses and shorter durations of response. A dose of 0.05 mg/kg of adrenalin did stimulate an increase in lactic acid in the blood but was ineffective in elevating blood glucose (Figures 14 and 12). The maximum mean level of lactate reached in the dose response studies after adrenalin treatment was 33.4 mg%, which represents an increase of approximately 154% over the initial value of 13 mg%. This per cent increase was slightly greater in magnitude than that observed for blood glucose (100% increase, Figure 12), but the response was not as prolonged. A dose of 0.1 mg/kg glucagon had no effect on blood lactate (Figure 14). The apparent decrease at 1 hour was of borderline statistical significance ($p=.052$). Only a slight and transitory response was seen at a dose of 1 mg/kg (Appendix III. 11) in September.

Muscle and liver glycogen responses of intact frogs: No changes in muscle and liver glycogen of summer frogs were seen in response to 1 mg/kg of adrenalin or glucagon

FIGURE 14 - Effects of Different Doses of Adrenalin and Glucagon on Frog Blood Lactate (means $\pm$ standard errors of data in Appendix III. 10., numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from control)


BLOOD LACTIC ACID mg/100 ml
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
HOURS AFTER INJECTION
6
4
15
10
4
19
6
28
23
17
14
6
6
1.0 mg/kg Adrenalin ip
0.1 mg/kg " "
0.05 mg/kg " "
0.1 mg/kg Glucagon ip
Saline ip

within 5 hours after injection of the hormones (Figures 15 and 16). Mean muscle glycogen levels at the various intervals measured ranged from 0.8 to 1.2 per cent. Mean liver glycogen concentrations at the same intervals ranged from 5.9 to 9.4 per cent.

Seasonal differences in responses to adrenalin and glucagon: Blood glucose responses to glucagon were slightly less in winter than in summer. The highest mean glucose concentration observed after glucagon treatment in winter was 36.9 mg% for a 2 mg/kg dose at 3 hours after injection (Figure 13). In addition, in most glucagon experiments no response was observed at 1 hour in winter. However, a 1 hour response was obtained with 0.1 mg/kg in one experiment (Appendix III. 8.C.) demonstrating that while the winter response was usually slower to develop, this was not always the case. Winter responses to glucagon were sometimes slow to develop, sometimes abbreviated, and always less than those observed in the summer and fall. However, the minimal effective dose tested (0.1 mg/kg) was effective in winter as well as in summer animals.

Striking seasonal variations in blood glucose response to adrenalin were seen (Figure 17). Several aspects of the fall response, as compared to the response of summer or winter frogs, as previously depicted in Figure 12, are notable: (1) the peak response in the fall (58.7 mg%) was

FIGURE 15 - Effects of Adrenalin and Glucagon on Frog Muscle Glycogen. (Mean ± standard errors of data in Appendix III. 9., numbers are numbers of animals in each group)

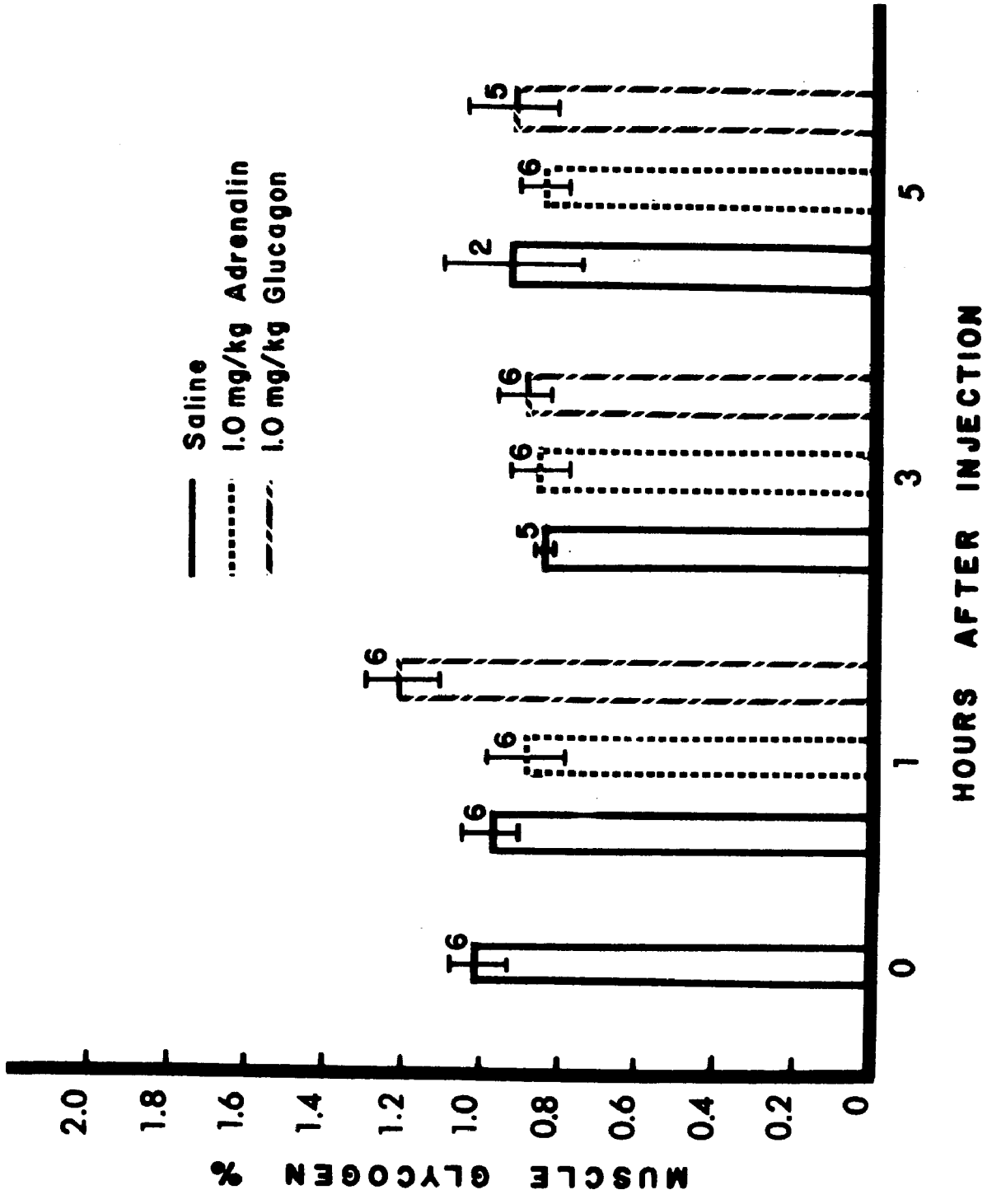

Saline
1.0 mg/kg Adrenalin
1.0 mg/kg Glucagon
MUSCLE GLYCOGEN %
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
1
3
5
HOURS AFTER INJECTION

FIGURE 16 - Effects of Adrenalin and Glucagon on Frog Liver Glycogen. Summer (means ± standard errors of data in Appendix III. 9., 6 animals per group)

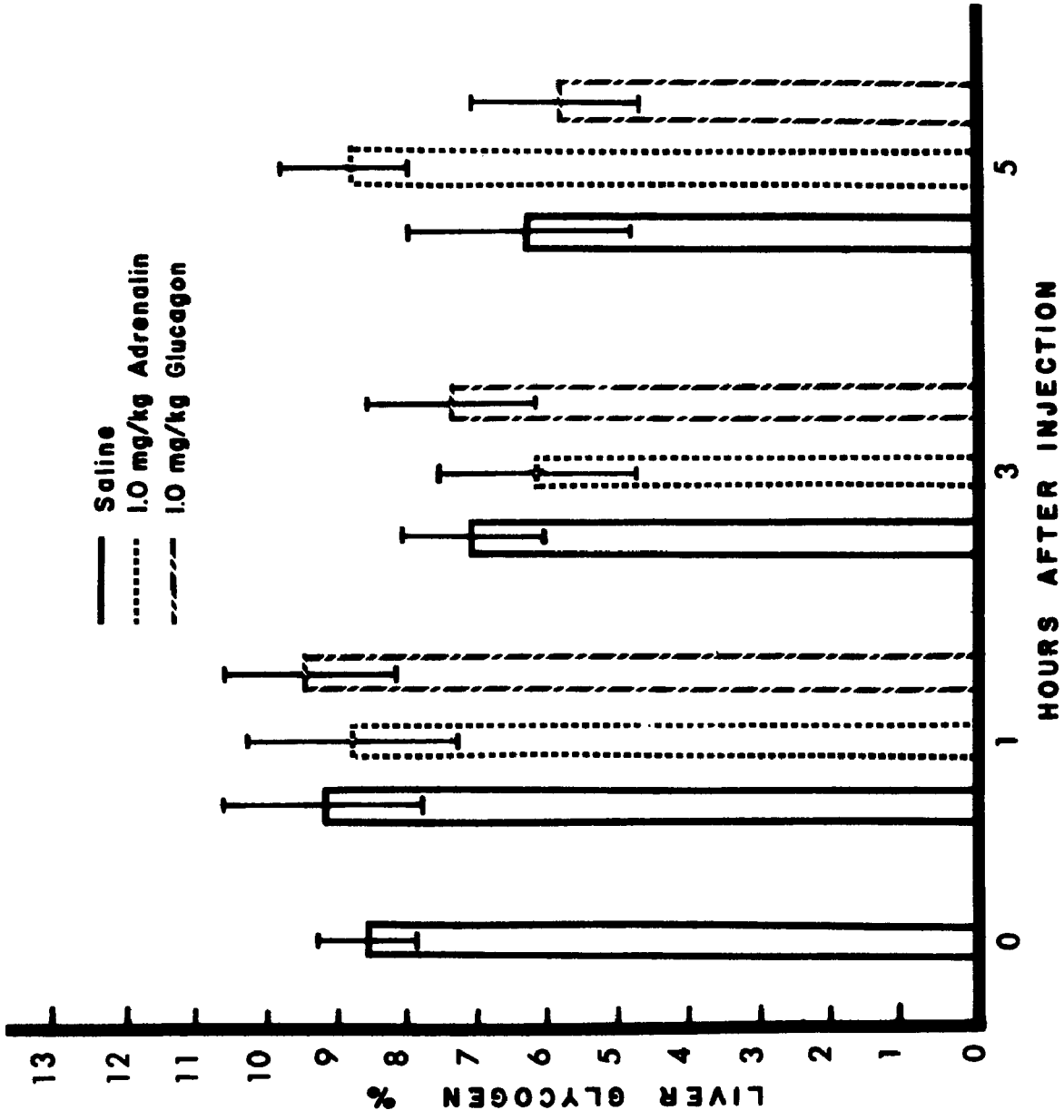
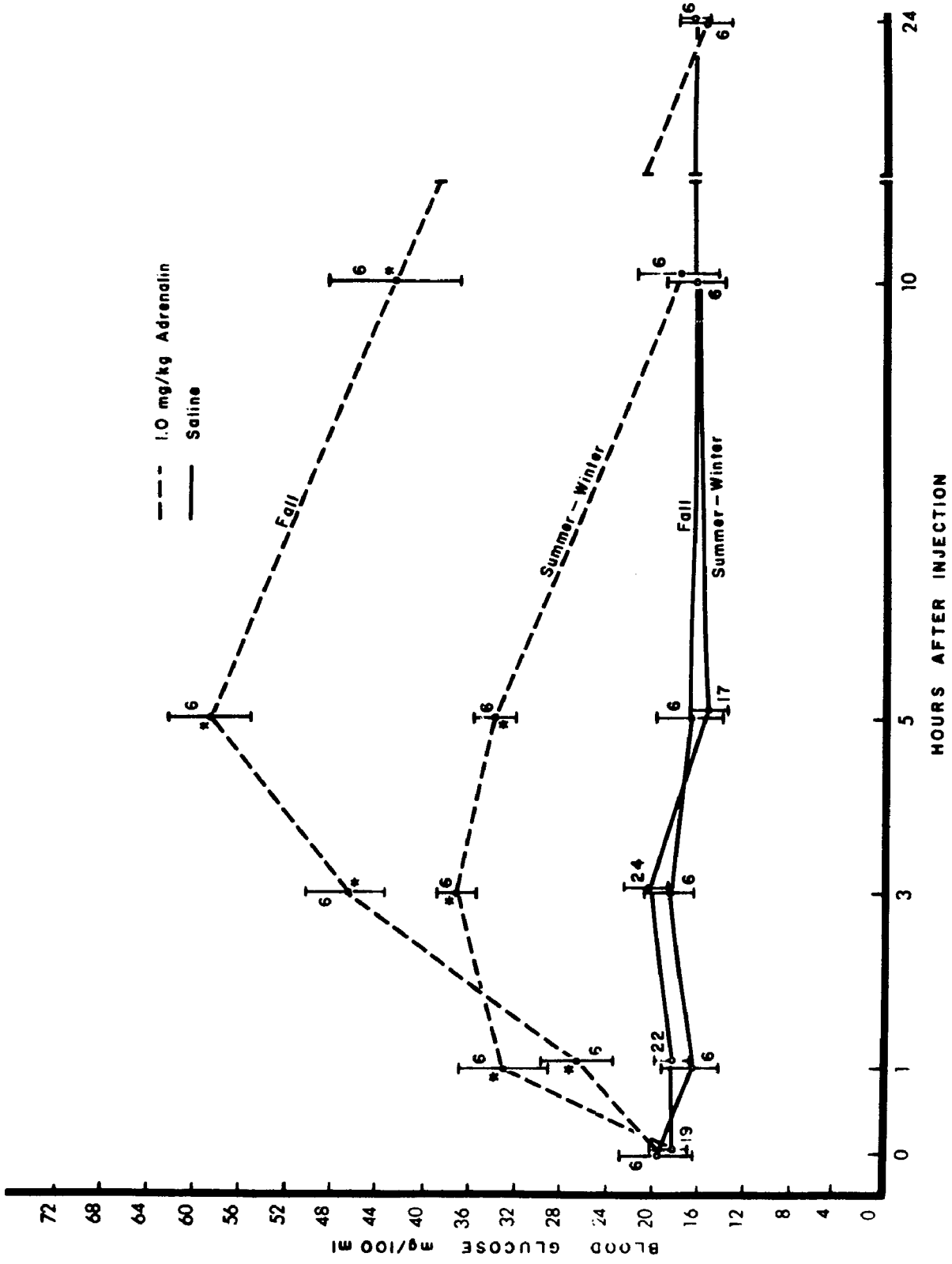

Saline
1.0 mg/kg Adrenalin
1.0 mg/kg Glucagon
LIVER GLYCOGEN %
0
1
2
3
4
5
6
7
8
9
10
11
12
13
0
1
3
5
HOURS AFTER INJECTION

FIGURE 17 - Effects of Adrenalin on Frog Blood Glucose in Fall as Compared to Summer or Winter (means $\pm$ standard errors of data in Appendix III. 5 and 6, numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from controls)


1.0 mg/kg Adrenalin
Saline
Fall
Summer–Winter
Fall
Summer–Winter
BLOOD GLUCOSE mg/100 ml
HOURS AFTER INJECTION
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
0
1
3
5
10
24

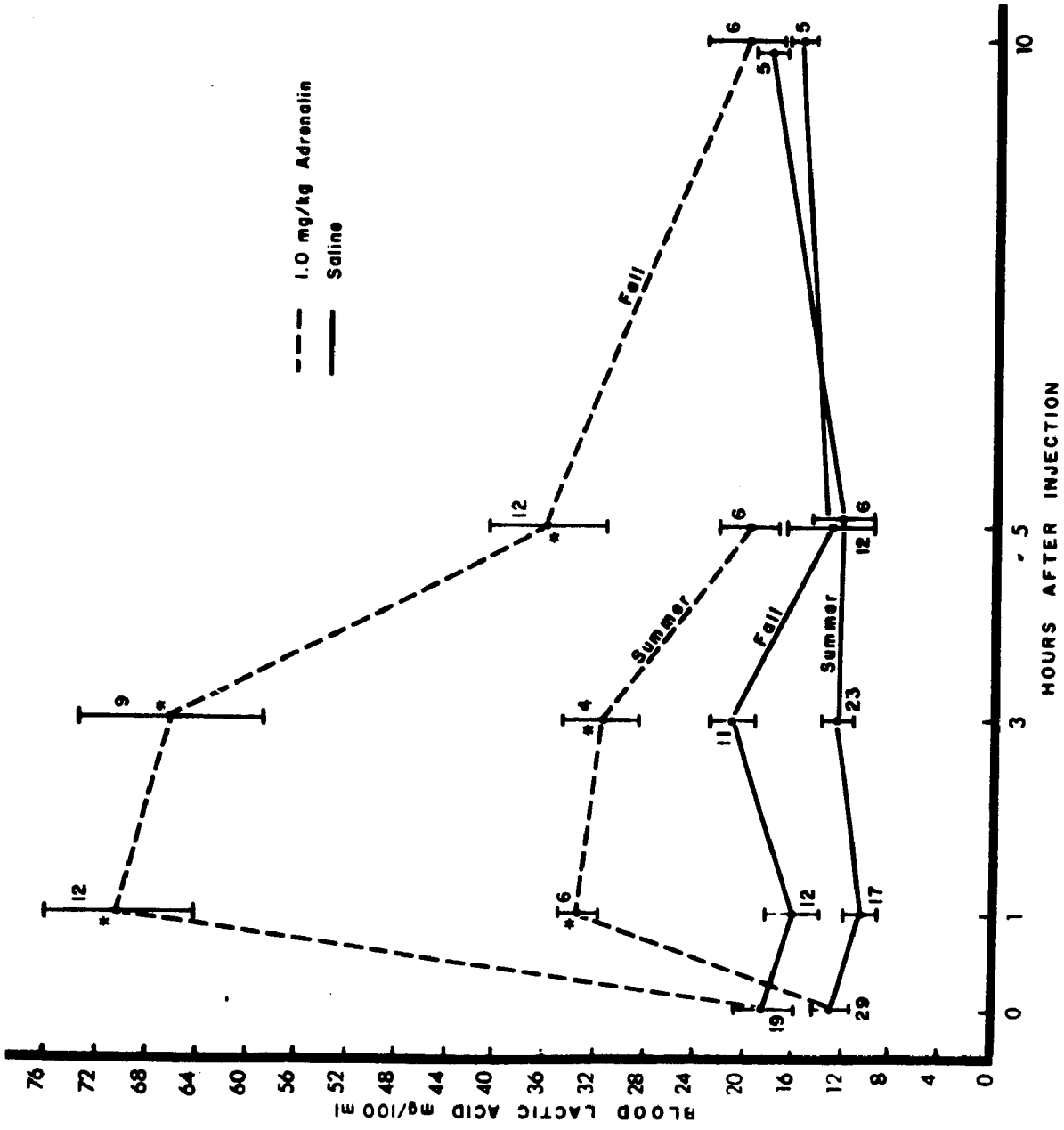
60 percent higher than the peak response in summer and winter animals (37 mg%); (2) the peak value was reached later in fall frogs; and (3) hyperglycemia persisted longer in the fall than in the other seasons.

Lactate responses to adrenalin in fall were even more exaggerated than blood glucose responses. The peak lactate level (70 mg%) was 100 per cent greater than the peak at other seasons (Figure 18). The lactacidemia persisted longer in the fall animals, being elevated at 5 hours, whereas lactate levels were not significantly different from saline controls at 5 hours in the summer animals. After glucagon treatment lactate was elevated in the fall animals 1 hour after the injection of a 1 mg/kg dose, but was no longer increased at 3 hours (Appendix III. 10) and was not seen at all after other doses.

## Discussion

Responses to Adrenalin: Although adrenalin effects, notably hyperglycemia, have been described in all groups of vertebrates, conclusions concerning the comparative sensitivity to and physiological role of adrenalin in the various groups are not reliable for several reasons. First, there is very little data, based on only a few species in the several classes of vertebrates. Second, the route of administration, which has very important effects on the characteristics of the response (Eadie, 1929) has varied,

FIGURE 18 - Effects of Adrenalin on Frog Blood Lactic Acid in Fall as Compared to Summer or Winter (mean $\pm$ standard errors of data in Appendix III. 11, numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from controls)


1.0 mg/kg Adrenalin
Saline
Fall
Summer
Fall
Summer
BLOOD LACTIC ACID mg/100 ml
HOURS AFTER INJECTION

so that responses are usually not directly comparable. Third, and most important, there is no information about the relationship between an injected dose and the amount of hormone actually reaching the target tissues, compared to endogenously secreted hormone. Although these considerations will not always be mentioned at the appropriate points, they must be kept in mind in this or any other discussion of the comparative effects of hormones.

The comparative response of the frog to adrenalin can be evaluated from the dose responses curve, and the time course, or magnitude and duration of the response. Fairly large doses of adrenalin were required to produce either hyperglycemia or lactacidemia in Rana pipiens. The lowest tested doses which caused hyperglycemia and lactacidemia were 0.10 and 0.05 mg/kg, respectively. Similar sensitivity exists in other anurans. The lowest intravascular dose of adrenalin producing a hyperglycemic response in anurans was 0.03 mg/kg in Rana catesbeiana (Wright, 1959). It is expected that larger doses would be required if the adrenalin were administered intraperitoneally. The toad, Bufo bufo, responded with hyperglycemia to a intralymphatic injection of 2 mg/kg but not 0.01 mg/kg (Hermansen and Jorgensen, 1969). A urodele, Triturus vulgarus, was more sensitive, responding with hyperglycemia to a 0.01 mg/kg intraperitoneal dose (Matei-Vladescu, 1965). In the rat the

lowest intraperitoneal dose causing hyperglycemia is between 0.01 and 0.10 mg/kg (Ellis and Anderson, 1951). Thus, there is considerable overlap in lower hyperglycemic doses of adrenalin in several amphibians and the laboratory rat. Comparable data is too scanty to make exact sensitivity comparisons for blood lactate responses in amphibians and higher vertebrates.

The hyperglycemic response of Rana pipiens to adrenalin was not clearly related to dose. Because the 0.10 mg/kg dose produced a response that was consistently lower, but which overlapped the response produced by the 1.0 mg/kg dose, a dose response may have been present. A dose of 0.075 mg/kg would have been useful in determining whether the response was a dose or a threshold type response. Blood lactate increased with dose between the doses of 0.05 mg/kg and 1.0 mg/kg (Figure 14). There is no consistent pattern among vertebrates with regard to dose relationships (Patent, 1970). Alligator blood glucose and lactate responses to adrenalin are both graded, with intramuscular doses as low as 0.01 mg/kg being effective (Stevenson, et al., 1957). Glucose increase in rat blood is graded between intraperitoneal doses of 0.10 and 0.30 mg/kg.

Responses of Rana pipiens to adrenalin are slower to develop and are more prolonged than responses of mammals. For example, in the frog peak glucose levels are not reached until 3 hours and the response lasts between 3 and 10 hours

in summer animals (Figure 10); the fall responses are even more prolonged (Figure 17). The rat given 0.10 mg/kg adrenalin intraperitoneally has a peak blood glucose response at 30 minutes and the response is complete by two hours after the injection (Ellis, 1951). The rapidity with which blood glucose increases in frog blood must, at least in part, be a function of the absorption of hormone. Significant increases are observed earlier with intravenous than with intraperitoneal injection (Figure 9). A slower hormone absorption with intraperitoneal injection could also contribute to the prolonged glucose responses in these experiments. In mammals, the subcutaneous route, which is the most slowly absorbing route, also produces blood responses of the longest durations (Eadie, 1929; Sokal and Sarcione, 1958). The prolonged amphibian response reported here is probably also due to relatively low rates of glucose utilization, reflecting the lower metabolic rates in the frog at room temperature. Other causes for the prolongation could include slower adrenalin inactivation and prolonged phosphorylase activation.

Blood lactate was dramatically elevated in the Rana pipiens response to adrenalin. While developing more rapidly and of shorter duration than the hyperglycemic reaction, the lactacidemia was still slow and long lasting (Figure 14). There were two notable differences between

blood glucose and lactate responses. First, the amount of lactate increase was observed to be related clearly to the hormone dose, and, second, lower adrenalin doses (0.05 mg/kg) than are required to produce a rise in glucose produce a rise in blood lactate.

There is very little comparable data on lactate responses of other amphibians or other lower vertebrates. Lactate increased in the whole frog (Rana pipiens) after adrenalin (Buchwald and Cori, 1931). The alligator, while exhibiting a slowly developing hyperglycemia, responded rapidly, within 1 hour, with a 70 mg% increase in lactic acid (Frye and Farrar, unpublished data); this response was similar to the response of Rana pipiens in fall.

The slow and prolonged responses of Rana pipiens to adrenalin observed in this study appear to be characteristic of other amphibians and many other poikilotherms. In the dogfish shark blood glucose levels rise slowly, reaching a peak at 2 hours and remain elevated for the 48 hour duration of the experiment following a 0.5 mg/kg intramuscular adrenalin injection (Patent, 1970). Alligator responses are also of long duration lasting up to 70 hours after a 1 mg/kg intramuscular injection (Stevenson et al., 1957). Goldfish responses resemble those of Rana pipiens in duration (Young and Chavin, 1965).

Two differences between amphibian and mammalian blood glucose and lactate responses exist: the poikilothermic responses are slower and more prolonged, and they are initiated from different basal levels. These factors make comparison of the magnitude of responses difficult. Another parameter of the response is the total change in glucose above control levels, as measured by the area under the hyperglycemic or lactacidemic curve. The total hyperglycemia in the summer-winter frog after adrenalin (Figure 12) is 67% of the total hyperglycemia in rats given the same dose (Ellis, 1956). Exact comparisons can not be made in the case of lactate because the most closely comparable data on adrenalin induced lactacidemia in mammals is based on subcutaneous injections (Bloom and Russell, 1955). If total lactacidemia of frogs and rats given 0.20 mg/kg intraperitoneally and subcutaneously is compared, the response of the frog can be estimated to be about 80 per cent of the rat response. However, if, as expected, the response of the rat to the same dose would be reduced if it were given intraperitoneally, the response of the two species might be roughly equivalent. Lactate responses as large as those observed in fall frogs are seldom seen in comparable mammalian experiments.

Although hyperglycemia and lactacidemia were induced by adrenalin in frogs, liver or muscle glycogen depletion

was not observed in this study. However, this observation is not inconsistent with a primary effect of adrenalin upon glycogenolysis since glycogenolysis of only 1 mg of liver glycogen is required to produce an instantaneous increase in frog blood glucose of 15 mg%. Loss of 1 mg of glycogen from the liver is not detectable with the methods employed. In addition differences in the amount of liver glycogen between frogs contribute to the difficulty in demonstrating statistically significant changes in glycogen content. In spite of these factors, however, the magnitude of the changes in tissue glycogen induced by adrenalin in frogs is much smaller than in mammals. For example, rat muscle glycogen decreased 40 per cent from initial concentrations after a subcutaneous adrenalin dose of 0.30 mg/kg (Bloom and Russell, 1955) whereas a three fold higher dose caused no glycogen depletion in the frog. Changes of the magnitude seen in mammals would have been readily detectable in this assay. It is probable that the lack of a significant drop in tissue glycogen is due to a combination of factors, including the slow, prolonged nature of the response, and the rapid replacement of the glycogen by gluconeogenesis and glycogenesis.

Responses to Glucagon: Intraperitoneal injection of glucagon in doses as low as 0.10 mg/kg caused near doubling of Rana pipiens blood glucose levels. Some other anurans

are known to respond to glucagon, although the doses which have been used are sometimes very large. For example, the toad, Bufo arenarum, showed an increase in blood glucose from 19 mg% to 64 mg% within 1 hour after subcutaneous injection of a 5 mg/kg dose (Penhos and Lavintman, 1964). The bullfrog has also been shown to respond to glucagon (Wright, 1959). In urodeles a complete lack of hyperglycemic response has been reported in the salamander, Taricha torosa (Wurster and Miller, 1960), although this is not a completely reliable report, since the experimental design consisted of daily glucagon injections, followed by analysis of a single blood sample, which could have missed a relatively transient response. Moreover, in the same species Miller (1960) states that a mild rise in blood glucose is observed after an intracardiac injection of glucagon at a comparatively low dose of 0.01 mg/kg. It is not yet clear whether glucagon resembles adrenalin in promoting glycogenolysis in other lower vertebrates. Glucagon has no effect in the ratfish (Patent, 1970), and causes only a slight hyperglycemic response in the skate (Grant, 1964) and the dogfish (Patent, 1970).

As in the case of adrenalin the response of Rana pipiens to glucagon was slow to develop and of long duration. Virtually identical responses were elicited by the same mg/kg doses of adrenalin and glucagon during summer, but

glucagon induced responses were slightly less in winter than summer responses to either adrenalin or glucagon. Other vertebrates, such as the goldfish and alligator, which have prolonged adrenalin responses also exhibit prolonged glucagon hyperglycemia (Young and Chavin, 1965; Stevenson et al., 1957).

Comparisons between the responses of mammals and Rana pipiens show that: (1) because of the longer duration of response in the frog, the total glucagon hyperglycemia in response to an equivalent dose can be equal in the two groups; frogs injected intraperitoneally with 0.10 mg/kg glucagon in summer exhibited at least 110 per cent of the total change in blood glucose observed for non fasted mice given 0.10 mg/kg subcutaneously (Staub et al., 1955); (2) doses required in mammals for a significant hyperglycemic response are on the order of 1 to 10 µg/kg, given subcutaneously, and in frogs are in the order of 50 to 100 µg/kg given intraperitoneally.

As in the case of adrenalin, glucagon did not cause glycogen depletion in the liver of Rana pipiens. The explanations for this are probably the same as those discussed in connection with adrenalin.

The consistent failure of glucagon to increase blood lactate was expected, and is presumed to indicate that; as with mammals (Sutherland, 1951), muscle glycogenolysis and lactate generation are not stimulated by this hormone.

Seasonal differences in responses to adrenalin and glucagon: During the fall adrenalin induced hyperglycemia and lactacidemia were more than doubled as compared to winter and summer responses to the same dose (Figure 17 and 18). Hyperglycemia and lactacidemia in response to other manipulations, such as anesthesia and handling, were also enhanced in the fall. No difference in glucagon induced hyperglycemia of fall and summer animals was observed.

There are many possible explanations for the fall increase in adrenalin sensitivity. (1) Perhaps the simplest explanation would be a slower rate of adrenalin inactivation in the fall. That a dose of 0.10 mg/kg gives an almost equal effect to that observed for a 1.0 mg/kg dose argues against this explanation. Smith (1954) used slower inactivation as an explanation for the observed seasonal hyperglycemic excitability in Rana temporaria. A related explanation might be that adrenalin absorption rate from the injection site varies with season.

(2) The correlation between high glycogen levels in fall and increased adrenalin effectiveness might suggest a causal relationship between glycogen concentration and the response to adrenalin. But if glycogen concentration were the only cause of the enhanced response adrenalin should have been equally effective in winter when glycogen levels are still high and glucagon should have been more effective

in fall as well. At muscle glycogen concentrations below 0.4% frog gastrocnemius phosphorylase a activity was positively correlated with the glycogen concentration and was increased proportionally in the presence of adrenalin (Danforth, 1965). But at higher glycogen levels the enzyme activity was independent of glycogen concentration. If this holds true in liver, it seems unlikely that the increase in glycogen *per se* from summer to fall levels would provide the basis for enhanced adrenalin effect.

(3) It is possible that changes in metabolic control which lead to glycogen accumulation also cause an increase in the amount and/or activity of adrenalin sensitive phosphorylase, but this is made unlikely by arguments against high glycogen concentrations being the cause.

(4) Another possibility is that glycogen, when deposited rapidly, as it may be in the fall, is structurally different and, perhaps, more accessible to the phosphorylase. Baic (personal communication) has tentative evidence that the ultrastructural form of glycogen in frog liver is different in fall and spring.

(5) Finally, there is the possibility that the change in adrenalin sensitivity, as demonstrated in an *in vivo* assay, is due not to a real change in the adrenalin responsive mechanism in liver and muscle, but to a decrease in the rate of removal of glucose and lactate from blood by

other mechanisms. The fact that there is no unusual autumnal effect of glucagon suggests that this is not the basis for the elevated hyperglycemic response in fall.

The biological significance of the accumulation of glycogen in fall and the increase in glycogen mobilization in response to adrenalin is unknown. Glycogen accumulation can be interpreted as storage of energy reserves for use during winter hibernation or spring breeding activity, but the susceptibility of this glycogen to mobilization by adrenalin seems to be contradictory to this interpretation. Possibly these phenomena are complimentary components of a mechanism which makes increased amounts of energy available during fall migration. Until more is known about the energy requirements of migration, the merits of this suggestion cannot be judged.

## Responses of Intact Tadpoles to Adrenalin and Glucagon

Hormones which cause an increase in blood glucose, such as adrenalin and glucagon, could contribute to the increase in blood glucose level and increased beta cell function which are observed during metamorphosis (Part I; Frye, 1964, 1965; Bartell, 1969). Since it is not known when in development sensitivity to adrenalin and glucagon appears, the objective of these experiments was to determine whether tadpoles respond to adrenalin and glucagon in a manner similar to that seen in frogs.

## Methods

The methods are the same as those described in Part I. Hormones were injected intraperitoneally just as in the frog studies, and blood and tissue samples were taken at the intervals indicated in the figures.

## Results

Blood glucose was elevated by both adrenalin and glucagon at a dose of 1 mg/kg (Figure 19). In both cases the response was apparent at 1 hour (the earliest interval at which glucose was measured) and persisted for between 3 and 5 hours.

Blood lactate was increased over the 4 hour saline control level by adrenalin (1 mg/kg) at 5 hours (Figure 20). Lactate values of saline injected controls were elevated significantly over initial values at 1 hour after injection, and the absence of a significant adrenalin effect is probably due to this factor rather than to a slow response to adrenalin.

Liver glycogen depletion was observed in tadpoles receiving glucagon (1 mg/kg) but not adrenalin (Figure 21). Glycogen levels were lower at 3 hours (1.5%) and 5 hours (2.7%) than the saline control tadpoles at 4 hours (5.3%).

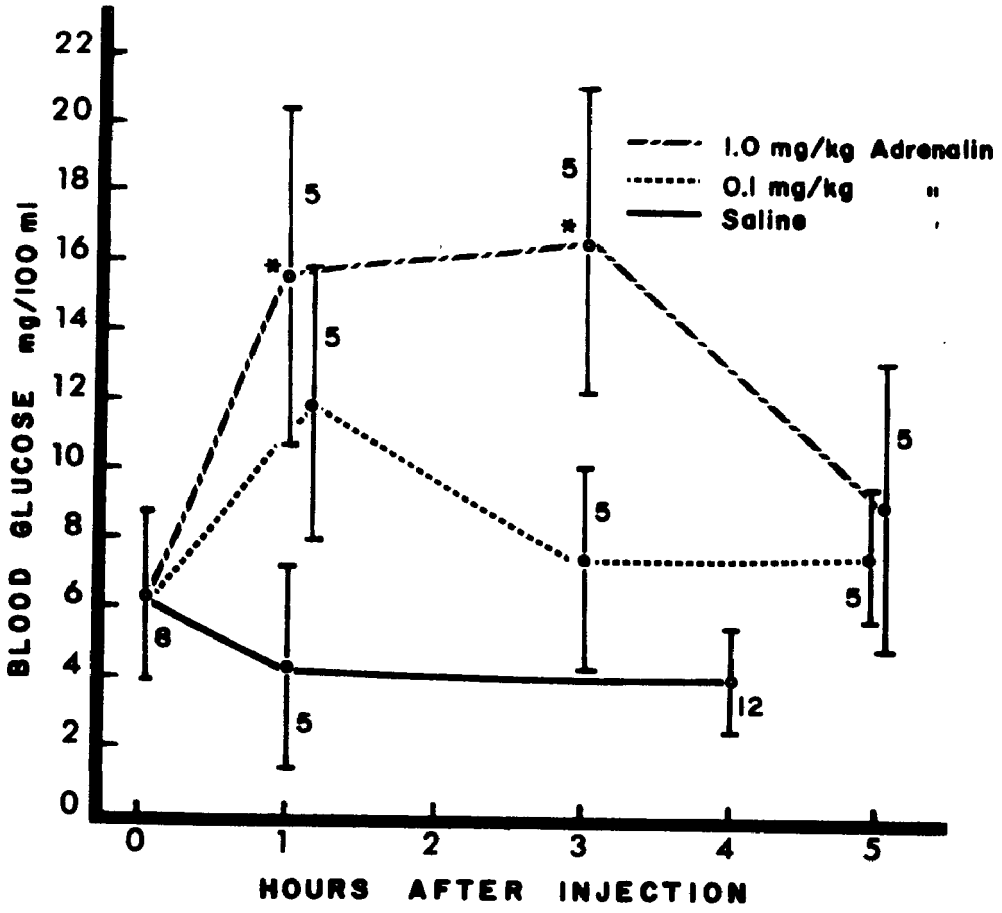
## Discussion

Tadpoles responded to adrenalin and glucagon with hyperglycemia. Lactacidemia was not clearly demonstrated

FIGURE 19 - A. Effect of Adrenalin on Tadpole Blood Glucose (mean $\pm$ standard errors of data in Appendix III. 12)

B. Effect of Glucagon on Tadpole Blood Glucose (mean $\pm$ standard errors of data in Appendix III. 12)

(numbers are numbers of tadpoles in each group, asterisks indicate significant ($p \leq .05$) difference from controls)


1.0 mg/kg Adrenalin
0.1 mg/kg "
Saline "
BLOOD GLUCOSE mg/100 ml
HOURS AFTER INJECTION

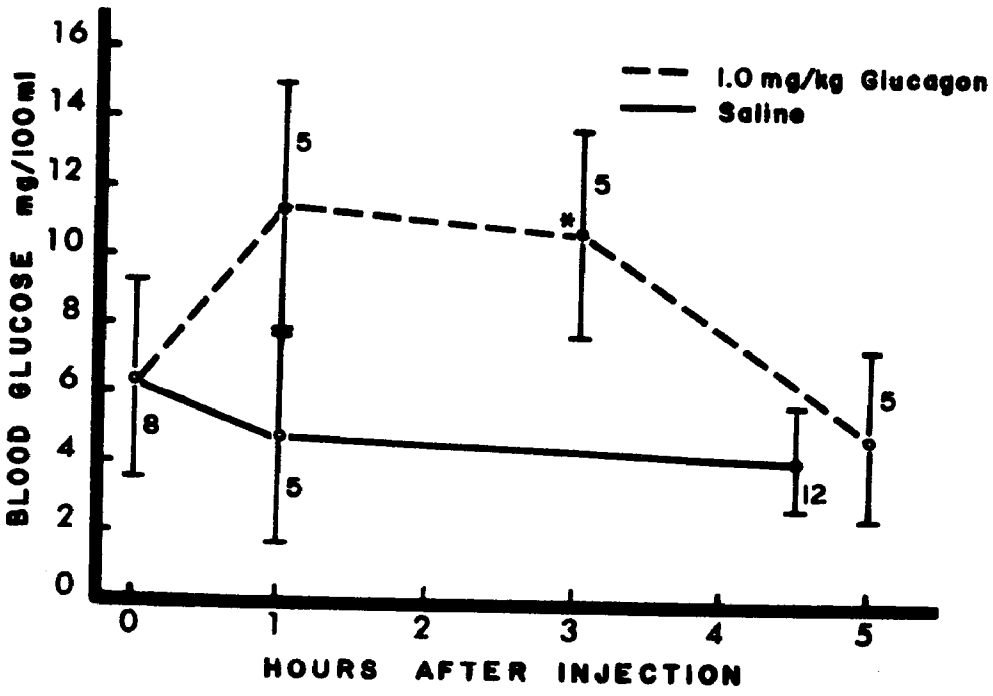

1.0 mg/kg Glucagon
Saline
BLOOD GLUCOSE mg/100ml
HOURS AFTER INJECTION

FIGURE 20 - Effect of Adrenalin and Glucagon on Tadpole Blood Lactate (means $\pm$ standard errors of data in Appendix III. 13, numbers are numbers of animals in each group, asterisks indicate significant ($p \leq .05$) difference from saline controls)

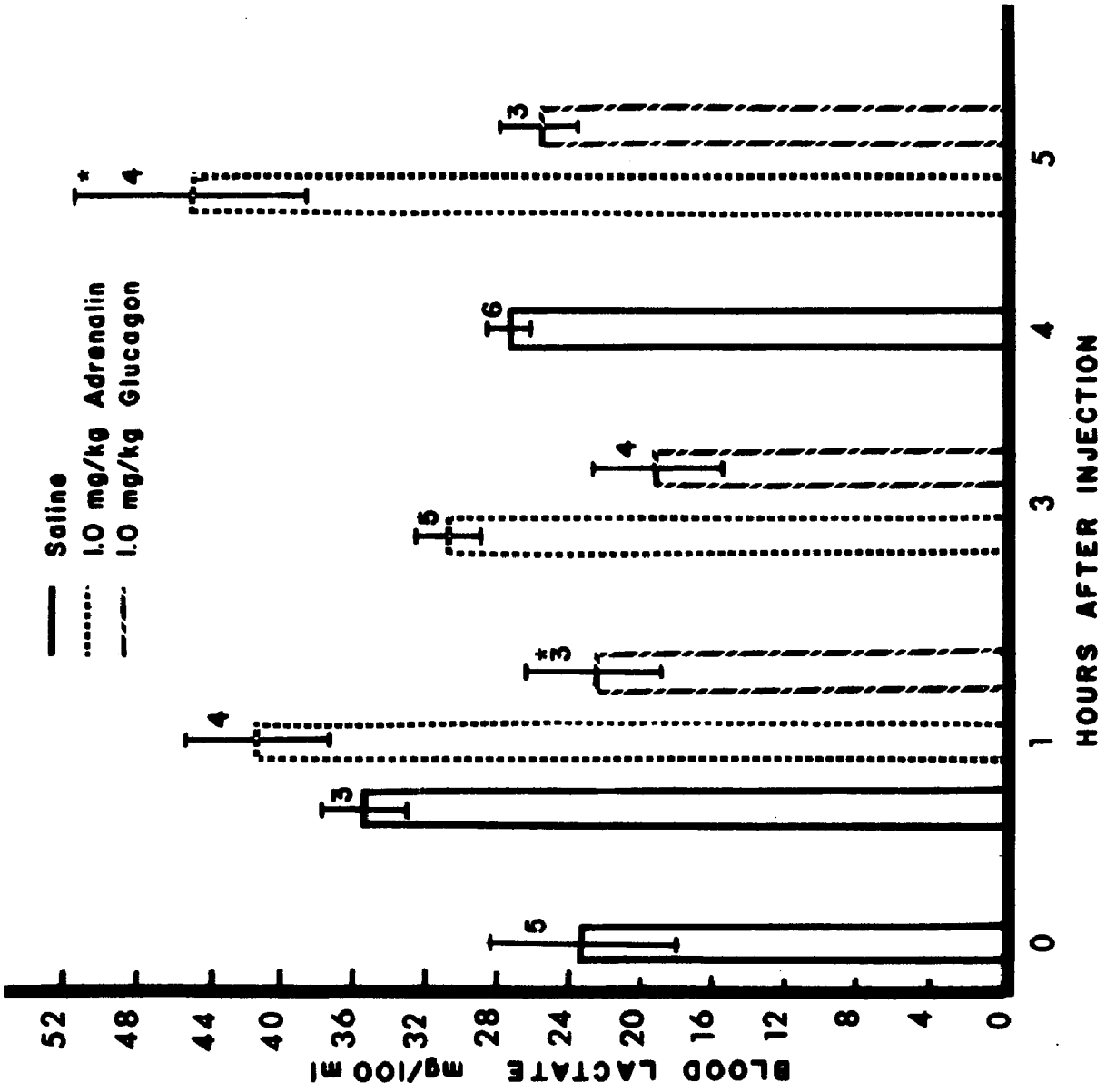
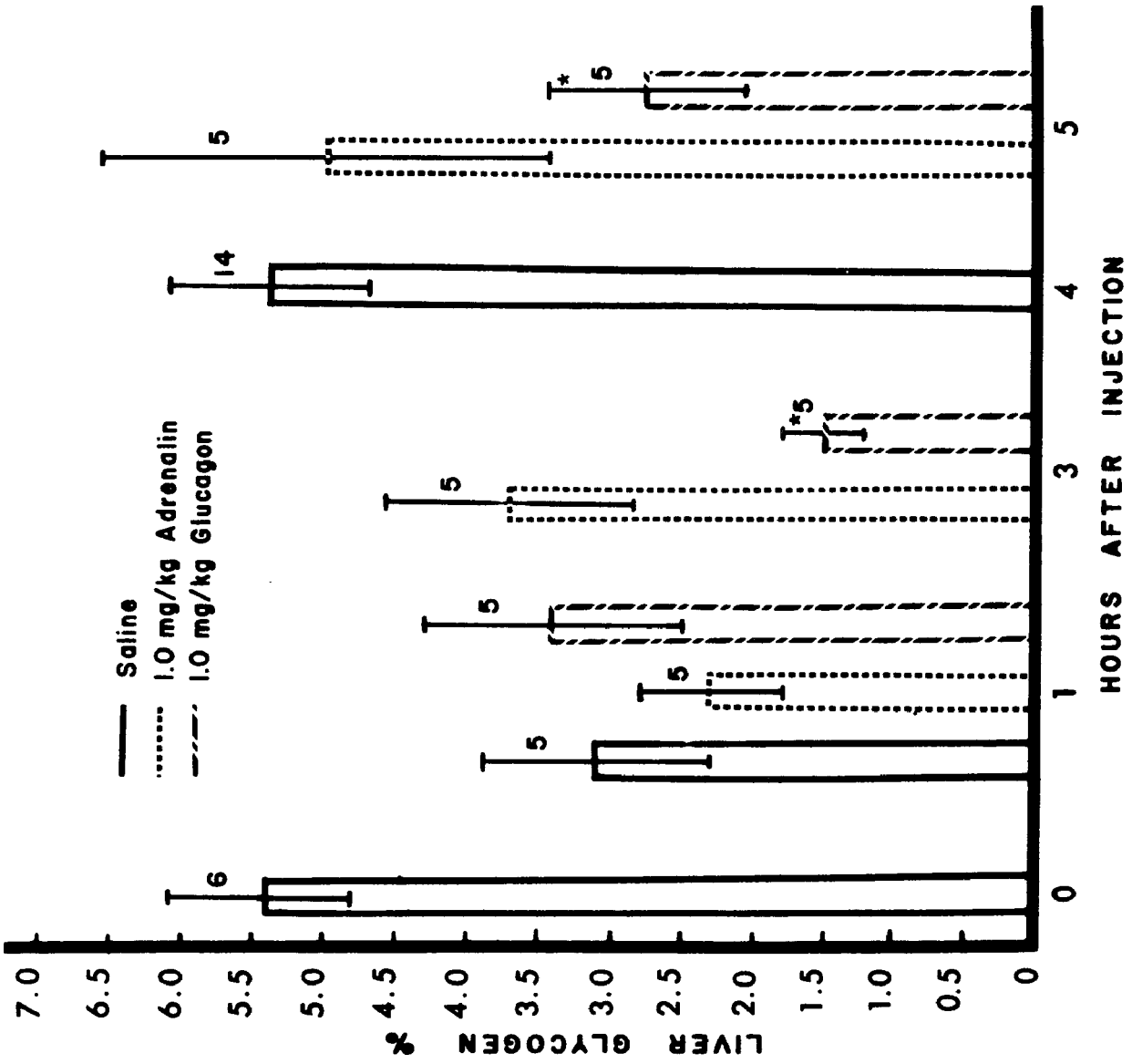

Saline
1.0 mg/kg Adrenalin
1.0 mg/kg Glucagon
BLOOD LACTATE mg/100 ml
HOURS AFTER INJECTION
0
4
8
12
16
20
24
28
32
36
40
44
48
52
0
1
3
4
5
*4
I6
4
I5
*I3
4
I3
5

FIGURE 21 - Effect of Adrenalin and Glucagon on Tadpole Liver Glycogen (means ± standard errors of data in Appendix III. 14, numbers are numbers of animals in each group, asterisks indicate significant difference from controls)


Saline
1.0 mg/kg Adrenalin
1.0 mg/kg Glucagon
LIVER GLYCOGEN %
HOURS AFTER INJECTION
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1
3
4
5
6
14
*5

but saline control lactate levels were frequently elevated. At 5 hours after adrenalin lactate levels were significantly elevated above the 4 hour saline controls. Liver glycogen decrease was observed after glucagon but not after adrenalin. This may indicate that in the tadpole glucagon hyperglycemia is due either to greater glycogen mobilization or to slower replacement of glycogen than in the frog liver in which glycogen depletion was not observed. The lack of detectable glycogen depletion in the adrenalin treated tadpoles could be due to concomitant lactate gluconeogenesis and glycogenesis, but there also could be absolute differences in tadpole liver sensitivity to the two hormones.

The significance of responses to adrenalin and glucagon in tadpoles is that potentially important roles for these hormones during metamorphosis are indicated. Chromaffin cells first appear in the developing Rana pipiens adrenal when hind limb buds make their first appearance (Stenger and Charripper, 1946). Therefore, adrenalin could be secreted throughout most of larval development. But there is no direct evidence on this point. It is not known whether alpha cells or glucagon are present in tadpoles.

The role of adrenalin and glucagon in producing blood glucose increase as a factor contributing to development of adult blood glucose levels and of beta cell function has been previously suggested. These hormones could also

mediate the cataclysmic liver glycogen depletion associated with the cessation of feeding during metamorphic climax (Part I, Figure 8).

## Effects of Adrenalin and Glucagon on Glucose Release from Liver Slices

In mammals both adrenalin and glucagon have the ability to stimulate hepatic glycogenolysis by activation of phosphorylase via the increased production of cyclic adenosine monophosphate. Failure to observe glycogen depletion in the liver of Rana pipiens which received adrenalin or glucagon injection raises the possibility that the hyperglycemia caused by adrenalin and glucagon might, in part, be due to other effects of the two hormones. For example, adrenalin hyperglycemia could be due to gluconeogenesis from lactate. Similarly, if lipolysis is an important effect of these hormones, the accumulation of fatty acids and ketones could inhibit glucose utilization, through preferential lipid oxidation and consequent restraint of the phosphofructokinase reaction (Newsholme et al., 1962), and thus promote hyperglycemia. The experiments with isolated liver slices to be described in this section were done to determine more directly whether the addition of adrenalin and/or glucagon causes hepatic glycogenolysis.

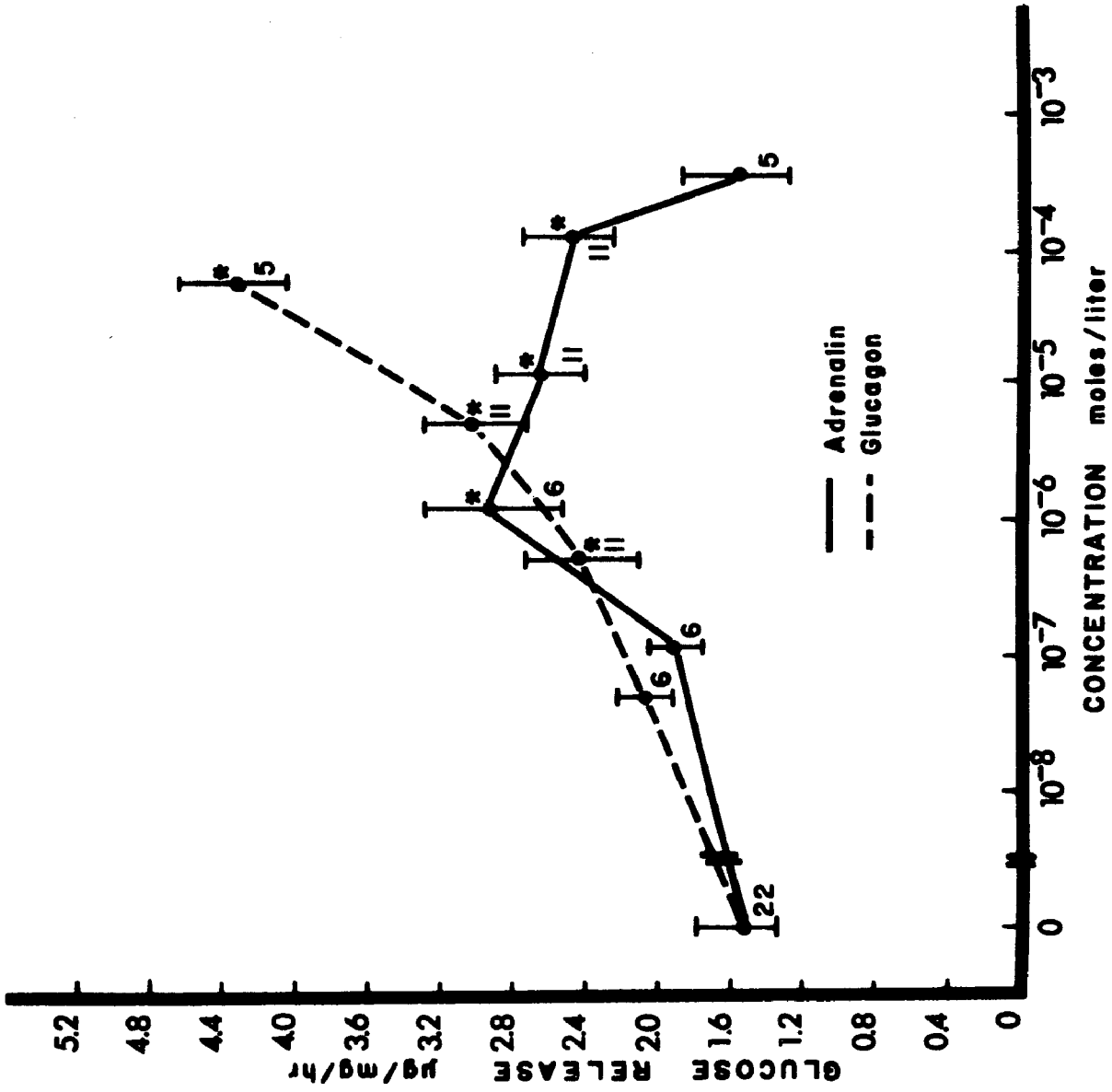
## Methods

The basic procedures have been described in the Materials and Methods. Several initial experiments were done to determine the best incubation conditions to produce maximal response to hormones added to the medium. A comparison was made between adrenalin effects incubated in phosphate saline buffer at pH 7.4, and in amphibian Ringers at pH 7.8. In addition, several different temperatures and incubation times were compared for their effect on glucose release in response to adrenalin. Results of these experiments (Appendix IV.1 and 2) indicated that incubation at 25°C for 1 hour in amphibian Ringers was optimal for studying hormone effects.

## Results

In the absence of added adrenalin or glucagon frog liver slices released glucose at a rate of 1.6 µg/mg/hour (Figure 22). Adrenalin stimulated glucose release at concentrations between $1.4 \times 10^{-6}$ and $1.4 \times 10^{-4}$ M; those concentrations below and above these levels were ineffective. Glucagon stimulated glucose release at concentrations as low as $7.2 \times 10^{-7}$ M. Glucose release rates increased with increasing hormone concentrations up to and including $7.2 \times 10^{-5}$ M. The rate of glucose release was increased from 1.6 to 3 µg/mg/hour, an 88 per cent increase above initial levels, with adrenalin. The highest dose of glucagon caused

FIGURE 22 - Effect of Adrenalin and Glucagon on Glucose Release from Frog Liver Slices (means ± standard errors of data in Appendix IV. 3, numbers are the numbers of flasks in each group, asterisks indicate significant difference from initial)


GLUCOSE RELEASE µg/mg/hr
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
0
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
10⁻³
CONCENTRATION moles/liter
Adrenalin
Glucagon
5
11
6
22

release of 4.4 µg/mg/hour.

In the initial experiments with tadpole liver slices, neither adrenalin nor glucagon caused any increase in glucose release over control values (Table 16 A and 17 A). However, the rate of glucose release in all sets of slices, including controls as well as hormone treated slices, was high (2.6 µg/mg/hour) compared to the rate of release from control slices of frog liver. When the tadpoles were moderately cooled by placing them in cold water for 2-3 minutes prior to liver removal, the rate of release in control slices when incubated at 25°C was reduced. Therefore, on the assumption that the apparent lack of a hormone effect in this experiment was due to the high control rates rather than to real hormone insensitivity, the experiment was repeated on animals which were cooled prior to spinal section and removal of the liver. A significant effect of adrenalin was seen at all concentrations from $1.4 \times 10^{-6}$ to $1.4 \times 10^{-4}$ M (Table 16 B and 16 C). The rates of glucose release obtained after hormone treatment of liver slices from cooled animals are similar to the initial values observed in liver slices from uncooled animals, suggesting that the latter animals are maximally stimulated.

Glucagon caused significant increases in glucose release from liver slices of cooled tadpoles at concentrations between $7.2 \times 10^{-5}$ and $7.2 \times 10^{-8}$ M, though the

TABLE 16

EFFECT OF ADRENALIN ON GLUCOSE RELEASE FROM TADPOLE LIVER SLICES

| Adrenalin Conc. | Moles/L: | $5.7 \times 10^{-4}$ | $1.4 \times 10^{-4}$ | $1.4 \times 10^{-5}$ | $1.4 \times 10^{-6}$ | $1.4 \times 10^{-7}$ | 0.0 |
|---|---|---|---|---|---|---|---|
| | µg/ml: | 100 | 25 | 2.5 | 0.25 | 0.025 | 0.0 |
| A. Room temperature animals | | | | | | | |
| Mean glucose release (µg/mg/hr) | | -- | 2.60 | 3.02 | 2.04 | 2.02 | 2.61 |
| Standard Error | | -- | 0.35 | 0.24 | 0.18 | 0.24 | 0.16 |
| Number of flasks | | -- | 5 | 5 | 5 | 5 | 20 |
| p* | | -- | >.05 | >.05 | >.05 | >.05 | |
| B. Cooled animals | | | | | | | |
| Mean glucose release (µg/mg/hr) | | 2.64 | 2.98 | 2.87 | 2.73 | -- | 2.08 |
| Standard Error | | 0.24 | 0.33 | 0.29 | 0.28 | -- | 0.10 |
| Number of flasks | | 5 | 5 | 5 | 5 | -- | 10 |
| p | | <.025 | <.01 | <.01 | <.025 | -- | |
| C. Animals cooled to immobility | | | | | | | |
| Mean glucose release (µg/mg/hr) | | -- | -- | -- | 2.31 | 1.91 | 1.10 |
| Standard Error | | -- | -- | -- | 0.26 | 0.28 | 0.14 |
| Number of flasks | | -- | -- | -- | 4 | 4 | 4 |
| p | | -- | -- | -- | <.01 | >.05 | |

*p is probability of a higher t in Student's t test.

TABLE 17

EFFECT OF GLUCAGON ON GLUCOSE RELEASE FROM TADPOLE LIVER SLICES

| Glucagon Conc. | Moles/L: | $7.2 \times 10^{-5}$ | $7.2 \times 10^{-6}$ | $7.2 \times 10^{-7}$ | $7.2 \times 10^{-8}$ | $7.2 \times 10^{-9}$ | 0.0 |
|---|---|---|---|---|---|---|---|
| | µg/ml: | 250 | 25 | 2.5 | 0.25 | 0.025 | 0.0 |
| A. Room temperature animals | | | | | | | |
| Mean glucose release (µg/mg/hr) | | -- | 3.30 | 2.36 | 2.58 | 2.10 | 2.61 |
| Standard Error | | -- | 0.50 | 0.26 | 0.29 | 0.40 | 0.16 |
| Number of flasks | | -- | 5 | 5 | 5 | 5 | 20 |
| p* | | -- | >.05 | >.05 | >.05 | >.05 | |
| B. Cooled animals | | | | | | | |
| Mean glucose release (µg/mg/hr) | | 11.41 | 3.09 | 2.85 | 2.53 | -- | 2.08 |
| Standard Error | | 1.31 | 0.18 | 0.54 | 0.22 | -- | 0.10 |
| Number of flasks | | 4 | 5 | 5 | 5 | -- | 10 |
| p | | <.001 | <.001 | >.05 | =.05 | -- | |
| C. Animals cooled to immobility | | | | | | | |
| Mean glucose release (µg/mg/hr) | | -- | -- | -- | 1.58 | 1.20 | 1.10 |
| Standard Error | | -- | -- | -- | 0.21 | 0.37 | 0.14 |
| Number of flasks | | -- | -- | -- | 4 | 5 | 4 |
| p | | -- | -- | -- | >.05 | >.05 | |

*p is probability of a higher t in Student's t test.

response at this lower concentration was of borderline significance (p=.05) in one experiment and not significant in the other (Table 17 B and 17 C). Large release rates (11.4 µg/mg/hour) were found at the highest glucagon dose. As with frogs, a significant difference was observed in the adrenalin and the glucagon effects, in that the highest hormone concentrations of adrenalin were ineffective, whereas all concentrations of glucagon above the minimum level were stimulatory.

In addition to the effect on glucose release, adrenalin appeared to cause constriction of blood vessels in liver slices of both frogs and tadpoles. This was shown by the fact that as the dose of adrenalin was increased the amount of blood appearing in the medium also increased. A good dose response curve to adrenalin might have been obtained by measurement of the hemoglobin concentration in the incubation medium. Glucagon did not have this effect.

## Discussion

Isolated frog liver slices respond similarly to adrenalin and glucagon in dose requirements and in glucose release rates at the lower concentrations. At the lowest effective concentration for each hormone ($7.2 \times 10^{-7}$ M glucagon and $14 \times 10^{-7}$ M adrenalin) similar glucose release rates of 2.44 and 2.95 µg/mg/hour, respectively, were observed.

Adrenalin and glucagon responses were dissimilar at higher dose levels in the frog liver slices. Adrenalin was increasingly less effective above the $1.4 \times 10^{-6}$ M level. This correlated with the appearance of blood in the incubation medium. A plausible explanation for these observations may be that the hormone caused enough constriction of the blood vessels to effectively hinder its own penetration of the inner tissue mass while causing blood to be extruded from the vessels. In contrast glucagon showed continually increasing effects at high doses.

All the properties of adrenalin and glucagon dose response relationships observed in frog liver slices were also seen in tadpole liver slices. This includes even the peculiar responses at high doses. Two possible differences are observed: tadpole slices may be slightly more sensitive to glucagon than were the frog slices, and tadpole slices may have a greater potential for increasing glucose output in response to large glucagon doses.

Glucose release rates of frog liver slices were 5 to 10 times lower than rates which have been reported for mammalian slices. Rana pipiens slices averaged 1.5 µg/mg/hour at 25°C, whereas rabbit slices release 14 to 28 µg/mg/hour at 37°C (Tyberghein et al., 1956) or 9 to 12 µg/mg/hour (Sutherland and Cori, 1951). The 12 degree temperature difference must contribute significantly to the difference

in glucose release rates. However, comparisons of release rates obtained under the optimal incubation conditions for each species are meaningful and of physiological significance in understanding the carbohydrate regulation of each animal. For example, frog blood glucose levels correlate with the lower glucose output of the frog liver slices in vitro.

Frog and mammal slice responses to adrenalin are virtually identical in per cent increase and in minimal sensitivity. Rana pipiens liver slice release rates increased after adrenalin by at most 88 per cent after doses as low as 0.25 µg/ml ($1.4 \times 10^{-6}$ M). Rabbit slices also exhibit maximal increases of 88 per cent and doses as low as 0.25 µg/ml were effective (Sutherland, 1950). Comparisons of the slice responses to glucagon cannot be made because neither maximal responses or minimal effective doses were determined exactly.

In conclusion adrenalin and glucagon were effective in stimulating glucose release in frog and tadpole liver slices. The increase in glucose released from the hormone treated slices is presumed to be due to increased glycogenolysis. Adrenalin and glucagon, therefore, have the potential to stimulate liver glycogenolysis in intact frogs and tadpoles.

## SUMMARY OF PART II

Adrenalin dose response studies conducted in intact frogs during summer and winter indicated that hyperglycemic responses did not clearly increase with dose increases above 0.10 mg/kg. Further experiments are needed to determine whether a threshold phenomenon is to be expected. In the dose response experiments blood glucose was, at most, doubled, and the hyperglycemic responses developed slowly and were prolonged. Blood lactate increase was definitely graded between doses of 0.05 and 1.0 mg/kg. Blood lactate was increased by 154%; the lactacidemia was slow to develop and was prolonged. Liver and muscle glycogen stores were not depleted. In the fall adrenalin caused greater hyperglycemia and lactacidemia than occurred in summer and winter: blood glucose levels were 60% higher, blood lactate levels were 100% higher, and both responses lasted longer.

Glucagon dose response studies in intact frogs revealed a hyperglycemic response similar in magnitude and duration, at the same mg/kg dose levels but at greatly dissimilar molar dosages, to the adrenalin dose responses. There was no consistent glucagon effect on blood lactate. Glucagon did not cause liver and muscle glycogen depletion. Fall responses to glucagon were identical to the summer responses. The only seasonal aspect of the glucagon response was a slightly decreased hyperglycemia in winter.

Frog adrenalin and glucagon hyperglycemic responses were similar in magnitude and duration at equal mg/kg dose levels during summer. In the fall adrenalin was more potent than glucagon at any of the seasons studied. Adrenalin was a potent stimulator of lactacidemia but glucagon was not. Neither hormone caused liver or muscle glycogen depletion.

Doses of adrenalin which caused hyperglycemia in frogs were not unlike those required in comparable experiments with other anurans and the laboratory rat. Both lactacidemic and hyperglycemic responses in the frog developed more slowly and were more prolonged than in the mammalian responses. As a consequence, total increase in glucose and lactate were similar in comparable frog and mammalian experiments. No glycogen depletion was observed in the frog after adrenalin, but glycogen loss comparable to that observed in mammals would have been detectable.

Glucagon doses effective in stimulating frog hyperglycemia were much larger than required for a stimulatory effect in mammals. However, total increase in blood glucose after glucagon was equal to the two because of the prolonged frog response. Glycogen loss equivalent to that seen after glucagon treatment in mammals was not observed, but would have been detectable.

Tadpoles developed hyperglycemia and lactacidemia after injection of a 1 mg/kg dose of adrenalin, but no glycogen depletion was observed. Glucagon (1 mg/kg) caused both hyperglycemia and glycogen depletion, but no lactacidemia. The only definite difference seen in frog and tadpole responses to either hormone was that glycogen depletion observed in the tadpole liver was not seen in frog liver.

Adrenalin concentrations between $1.4 \times 10^{-6}$ M and $1.4 \times 10^{-4}$ M were effective in stimulating glucose release from frog liver slices. Both lower and higher doses were ineffective. Glucose release rates were increased by at most 88% after incubation with adrenalin. This degree of increase was very similar to the increase observed in mammalian liver slices treated with adrenalin.

Glucagon concentrations from $7.2 \times 10^{-7}$ M up to, and including $7.2 \times 10^{-5}$ M elevated glucose release rates of frog liver slices. Even at the highest glucagon concentration no evidence of decreased effectiveness was observed.

Adrenalin and glucagon stimulated glucose release rates only in tadpoles that had been cooled prior to liver removal. Adrenalin was effective in cooled animals at concentrations of $1.4 \times 10^{-6}$ M to $1.4 \times 10^{-4}$ M. Higher doses were ineffective. Glucagon concentrations between $7.2 \times 10^{-8}$ M and $7.2 \times 10^{-5}$ M were stimulatory, but no evidence of decreased effectiveness with increase in hormone concentration was observed.

The only difference observed in frog and tadpole liver slice responses to adrenalin and glucagon was a slightly greater glucagon sensitivity in tadpole liver.

# CHAPTER IV. CONCLUSIONS

This investigation conducted in frogs and tadpoles has had two major purposes: (1) to determine the normal levels of blood glucose and lactate and liver and muxcle glycogen in relatively unperturbed animals under various natural and artificial conditions; and (2) to characterize and compare the effects of adrenalin and glucagon upon carbohydrate and lactate levels in blood and carbohydrate levels in liver and muscle.

Observations directed toward the first purpose have demonstrated that Rana pipiens, as is also the case for some other amphibians, regulates blood glucose at remarkably low levels, and maintains both liver and muscle glycogen at high levels as compared to mammals. In addition, this work has shown that there are appreciable differences from season to season in blood and tissue levels of carbohydrate and lactate, and that the blood levels of glucose are significantly lower during the larval phase of the life cycle than after metamorphosis. The basis of these features of carbohydrate metabolism in the frog is not presently known, either as regards to the metabolic and regulatory mechanisms which underlie them, or as regards to their significance.

I have postulated that the metabolic and regulatory mechanisms may relate to a particular balance of effects of several hormones affecting carbohydrate metabolism in the frog as compared to mammals, and discussed the very limited data which exists relative to this point (page 2). In addition to the probable role of hormonal activity, the characteristic carbohydrate levels in the frog undoubtedly must relate to some distinctive features of the pathways of intermediary metabolism. For example, compared to mammals, (1) there may be differences in the relative activities of key enzymes which control glycogenesis, glycogenolysis, glycolysis and fermentation, and gluconeogenesis; (2) unusual allosteric effects of substrates or products upon these enzymes may exist, which could affect absolute concentrations of various carbohydrates and intermediary products; (3) differences in reaction rates or equilibra may exist, due, for example, to the conditions of pH, hydration, and so on in the intracellular reaction environment, which could affect the concentrations of various carbohydrates and metabolites; and (4) possible differences may exist in availability of substrates to liver and/or muscle. There is no comparative evidence with which to evaluate these possibilities, but they suggest the probable complexity of mechanisms that may account for the findings made in this study.

The significance of the characteristic regulatory levels of carbohydrates in amphibians can likewise only be speculated upon. In mammals, the relatively high blood glucose levels are presumed to relate to the function of maintaining a constant, rapidly available supply of this important substrate to tissues, which they metabolize at a relatively high rate. This is especially important in the case of nervous tissue, in which glucose is an essential substrate. Thus, the low blood glucose levels of amphibians could have several meanings. (1) It is possible that nervous tissue of the frog does not depend upon blood glucose with no observable behavioral or neurological disturbances (Wright, 1959; Bartell, 1969; Copeland and deRoos, 1972). Perhaps amphibian nervous tissue can utilize non carbohydrate substrates, such as fatty acids. (2) It is possible that the demand for sugar is filled by some other circulating carbohydrate and that low blood glucose levels are only a consequence of its presence. Non reducing sugars such as fructose have not been ruled out as potential energy sources. This study has shown that glucose is the most prominent reducing sugar in blood, and that other reducing sugars are unlikely to be present in sufficient concentrations to replace glucose as the major energetic substrate.

One of the principal reasons for characterizing the normal levels of glucose, glycogen, and lactate in this study was to establish baselines from which to evaluate the

effects of hormones. Several significant observations were made. (1) Different methods of glucose assay can give significantly different estimates of glucose concentration. This is probably a major factor in the great variability in normal blood glucose levels which has been reported in the literature. (2) Anesthesia can cause hyperglycemia and hyperlactacidemia, especially when the anesthetic is applied repeatedly, or for a long period of time. Care must be exercised to standardize methods of anesthetizing the animals during experimental work. (3) Activity or food consumption prior to blood sampling may cause abnormally high glucose or lactate levels. Frogs must be fasted for a standard interval, and kept as undisturbed as possible when used for studies on glucose and lactate regulation. (4) Seasonal and other factors operative in frogs supplied from different collection areas by commercial dealers may contribute to the overall variability in carbohydrate and lactate values in a given species of frog. (5) Finally, significant variation in glucose and glycogen occurs during development, and therefore, experimental animals should be uniform with respect to developmental stage.

Observations directed toward the purpose of characterizing and comparing the effects of adrenalin and glucagon on carbohydrate levels of Rana pipiens have demonstrated that both hormones cause hyperglycemia and that adrenalin

causes hyperlactacidemia in both frogs and tadpoles. Some evidence was obtained in the present study that stimulation of liver glycogenolysis is one mechanism by which both adrenalin and glucagon produce hyperglycemia. The increased glucose release from liver slices incubated with the hormones is indicative of an increase in glycogenolysis. In intact frogs glucagon and adrenalin injections each cause hyperglycemia but no liver glycogen depletion is detectable. This is not inconsistent with hormone induced glycogenolysis since minimal glycogen losses could account for the observed hyperglycemia. Other mechanisms of increasing blood glucose levels such as gluconeogenesis from lactate and amino acids and, possibly, decreased glucose utilization are activated by these hormones in mammals (Ellis, 1951; Foa, 1968) and may also be activated in Rana pipiens. Stimulation of muscle glycogenolysis and glycolysis is the presumed mechanism by which adrenalin causes lactacidemia.

The significance of adrenalin and glucagon responses in Rana pipiens can only be discussed speculatively. Neither hormone appears to predominate over the other in carbohydrate regulation. Rather, each hormone probably has areas of regulation in which it dominates. For example, adrenalin is more directly active in muscle carbohydrate regulation than is glucagon. Glucagon, in only one experiment, caused only transient hyperlactacidemia. Muscle

tissue is more responsive to adrenalin than is liver: lowest doses caused lactacidemia, but not hyperglycemia and dose response curves were obtained in lactate studies but not clearly in glucose studies. Glucagon, on the other hand, probably acts primarily on the liver, and frog liver may be more sensitive to glucagon than to adrenalin. Evidence for this is scanty, however, and based on the fact that hyperglycemia results after the injection of much lower amounts of glucagon than adrenalin when calculated on a mole/kg basis. Liver slice experiments failed to reveal any difference in liver sensitivity to adrenalin and glucagon.

The exact physiologic roles of adrenalin and glucagon in frog carbohydrate regulation are not known, although these hormones could be active in any situation where extra glucose is required for energy. Adrenalin would be most effective in situations involving extended periods of anaerobic conditions such as in prolonged activity or in a diving escape response. Both adrenalin and glucagon could help maintain blood glucose levels at the expense of liver glycogen during a long term fast, but it is doubtful that fasts lasting for several months are ever encountered by frogs in nature. Both hormones could also contribute to maintenance of blood glucose and liver glycogen levels in fasts of shorter duration, if they have the capacity to stimulate fatty acid oxidation and/or gluconeogenesis, as

they do in mammals (Ellis, 1951; Foa, 1968).

This work has also shown that appreciable seasonal differences exist in blood glucose and lactate responses to adrenalin. The locus of the enhanced adrenalin effect in fall is probably muscle because glucagon hyperglycemia is not concomitantly increased. Theoretically several mechanisms could underlie this seasonal enhancement of adrenalin responses. These include: (1) slower rates of adrenalin inactivation in the fall; (2) more efficient adrenalin absorption form the injection site during the fall; (3) seasonal sensitivity of adrenalin receptor sites in muscle; and (4) seasonal amplification of adrenalin induced muscle phosphorylase activation. The significance of the increased adrenalin effectiveness in autumn may lie in some, as yet, undetermined role of adrenalin in frog migration or other prehibernatory activity.

The significance of the responses of tadpoles to adrenalin and glucagon may lie in the potential to increase blood glucose. This, in turn, could help initiate beta cell function, insulin secretion, and ultimately, provide for the system of carbohydrate regulation found in adult frogs. Equally important may be the potential of each hormone to stimulate liver glycogenolysis in tadpoles. During metamorphic climax tadpoles stop feeding, mobilize their stored glycogen (this study; Hanke and Leist, 1971)

and presumably utilize glucose released until feeding can be resumed. Both adrenalin and glucagon could initiate this process.

# APPENDIX I. ANESTHETIC EFFECTS ON BLOOD GLUCOSE AND LACTATE IN NORTH DAKOTA *RANA PIPIENS*

Appendix I. A. Handling and Anesthetic Effects on Blood Glucose in North Dakota *Rana pipiens*

| Animal # | Sample and/or Anesthetic Times: Zero time | Mean ±S.E. | One hour | Mean ±S.E. | Three hour | Mean ±S.E. |
|---|---|---|---|---|---|---|
| 1. Effect of multiple anesthetic applications (0.5% Finquel) with blood samples taken at all time intervals. | | | | | | |
| 1 | 18.3 | | 39.6 | | 53.8 | |
| 2 | 13.6 | | 20.5 | | 38.0 | |
| 3 | 14.2 | | 38.0 | | 40.3 | |
| 4 | 22.3 | | 62.6 | | 69.0 | |
| 5 | 12.5 | | 23.0 | | 37.5 | |
| | | 15.9 ± 1.8 | | 37.8 ± 2.3 | | 54.6 ± 8.5 |
| 2. Effect of multiple anesthetic application with blood withdrawn only at three hours. (0.5% Finquel) | | | | | | |
| 10 | --- | | --- | | 34.5 | |
| 11 | --- | | --- | | 37.5 | |
| 12 | --- | | --- | | 30.0 | |
| 13 | --- | | --- | | 31.0 | |
| 14 | --- | | --- | | 83.5 | |
| 15 | --- | | --- | | 28.7 | 40.8 ± 8.7 |
| 3. Effect of continuous anesthetic application for 3 hours: Initial Finquel concentration 0.5%, Maintained for 3 hours in 0.06% Finquel | | | | | | |
| 1 | 18.3 | | --- | | --- | |
| 2 | 13.6 | | --- | | --- | |
| 3 | 14.2 | | --- | | --- | |
| 4 | 22.3 | | --- | | --- | |
| 5 | 12.5 | | --- | | --- | |
| | | 15.9 ± 1.8 | | | | |
| 6 | --- | | --- | | 48.0 | |
| 7 | --- | | --- | | 31.8 | |
| 8 | --- | | --- | | 23.0 | |
| 9 | --- | | --- | | 74.0 | 44.2 ±15.2 |
| 4. Anesthesia without handling. Blood samples at all intervals. (0.5% Finquel) | | | | | | |
| 16 | 11.0 | | 32.5 | | 51.0 | |
| 17 | 15.8 | | 45.0 | | 65.7 | |
| 18 | 21.5 | | 33.6 | | 70.8 | |
| 19 | 17.0 | | 30.0 | | 45.0 | |
| 20 | 24.2 | | 40.6 | | 69.0 | |

Appendix I. A. 4. continued

| Animal # | Sample and/or Anesthetic Times: Zero time | Mean ±S.E. | One hour | Mean ±S.E. | Three hour | Mean ±S.E. |
|---|---|---|---|---|---|---|
| 21 | 17.0 | 17.6 ± 1.8 | 43.3 | 37.5 ± 2.3 | 71.2 | 61.9 ± 4.2 |
| Blood samples at 3 hours only: | | | | | | |
| 22 | --- | | --- | | 55.3 | |
| 23 | --- | | --- | | 68.0 | |
| 24 | --- | | --- | | 38.5 | |
| 25 | --- | | --- | | 56.6 | |
| 26 | --- | | --- | | 43.3 | 52.3 ± 5.2 |
| 5. Anesthetic Dose Studies Sept. 1967 | | | | | | |
| 0.5% Finquel see parts 2. and 4. | | | | | | |
| 0.25% Finquel: | | | | | | |
| 27 | --- | | --- | | 47.0 | |
| 28 | --- | | --- | | 52.8 | |
| 29 | --- | | --- | | 54.2 | |
| 30 | --- | | --- | | 60.0 | |
| 31 | --- | | --- | | 48.0 | |
| 32 | --- | | --- | | --- | 51.6 ± 2.0 |
| 0.125% Finquel: | | | | | | |
| 33 | --- | | --- | | 54.2 | |
| 34 | --- | | --- | | 43.3 | |
| 35 | --- | | --- | | 70.8 | |
| 36 | --- | | --- | | 57.0 | |
| 37 | --- | | --- | | 60.6 | |
| | | | | | | 57.1 ± 4.2 |
| 6. Finquel (0.5%) and dihydroergotamine October 1967 | | | | | | |
| Saline controls: | | | | | | |
| 38 | 21.9 | | --- | | --- | |
| 39 | 18.6 | | --- | | --- | |
| 40 | 21.9 | | --- | | --- | |
| 41 | 23.0 | | --- | | --- | |
| 42 | 14.6 | | --- | | --- | |
| 43 | 25.8 | | --- | | --- | |
| | | 22.4 ± 1.7 | | | | |
| 44 | --- | | --- | | 70.6 | |
| 45 | --- | | --- | | 57.0 | |
| 46 | --- | | --- | | 58.8 | |
| 47 | --- | | --- | | 71.0 | |
| 48 | --- | | --- | | 66.0 | |
| | | | | | | 53.9 ± 2.9 |

Appendix I. A. 6. continued

| Animal # | Sample and/or Anesthetic Times: Zero time | Mean ±S.E. | One hour | Mean ±S.E. | Three hour | Mean ±S.E. |
|---|---|---|---|---|---|---|
| | Dihydroergotamine 1 mg/Kg: | | | | | |
| 49 | --- | | --- | | 76.3 | |
| 50 | --- | | --- | | 41.6 | |
| 51 | --- | | --- | | --- | |
| 52 | --- | | --- | | 45.5 | |
| 53 | --- | | --- | | 70.2 | |
| | | | | | | 50.9 ± 4.2 |
| | Dihydroergotamine 10 mg/Kg: | | | | | |
| 54 | --- | | --- | | 85.0 | |
| 55 | --- | | --- | | 70.6 | |
| 56 | --- | | --- | | 33.8 | |
| 57 | --- | | --- | | 60.3 | |
| 58 | --- | | --- | | 55.8 | 61.1 ± 4.2 |
| | Dihydroergotamine 25 mg/Kg: | | | | | |
| 59 | --- | | --- | | 44.8 | |
| 60 | --- | | --- | | 67.0 | |
| 61 | --- | | --- | | 56.0 | 55.9 |
| 7. | Finquel (0.5%) and Hexamethonium | | | | February 1969 | |
| | Saline controls: | | | | | |
| 62 | 14.8 | | --- | | --- | |
| 63 | 17.3 | | --- | | --- | |
| 64 | 14.0 | | --- | | --- | |
| 65 | 18.4 | | --- | | --- | |
| 66 | 13.0 | 15.5 ± 1.0 | --- | | --- | |
| 67 | --- | | 16.4 | | --- | |
| 68 | --- | | 26.6 | | --- | |
| 69 | --- | | 21.5 | | --- | |
| 70 | --- | | 20.7 | | --- | |
| 71 | --- | | 21.5 | | --- | |
| 72 | --- | | 27.5 | 22.4 ± 1.7 | --- | |
| | Hexamethonium 50 mg/Kg injected at zero time: | | | | | |
| 73 | --- | | 31.0 | | --- | |
| 74 | --- | | 29.0 | | --- | |
| 75 | --- | | 19.8 | | --- | |
| 76 | --- | | 28.4 | | --- | |
| 77 | --- | | 22.8 | 26.2 ± 2.1 | --- | |
| | Hexamethonium 25 mg/Kg injected at zero time: | | | | | |
| 78 | --- | | 27.0 | | --- | |
| 79 | --- | | 23.9 | | --- | |
| 80 | --- | | 33.4 | | --- | |
| 81 | --- | | 27.5 | | --- | |
| 82 | --- | | 20.7 | 26.5 ± 2.1 | --- | |

Appendix I. A. 7. continued

| Animal # | Sample and/or Anesthetic Times: Zero time | Mean ±S.E. | One hour | Mean ±S.E. | Three hour | Mean ±S.E. |
|---|---|---|---|---|---|---|
| Saline controls: | | | | | | |
| 83 | 27.3 | | 25.3 | | 37.5 | |
| 84 | 16.8 | | 19.2 | | 33.8 | |
| 85 | 22.3 | | 30.7 | | 34.8 | |
| 86 | 11.8 | 19.6 | 13.7 | 20.2 | 17.5 | 30.8 |
| Hexamethonium 100 mg/Kg injected at zero time: | | | | | | |
| 87 | 12.5 | | 10.0 | | 18.6 | |
| 88 | 20.5 | | 20.5 | | 55.5 | |
| 89 | 27.3 | | 28.8 | | 37.5 | |
| 90 | 21.0 | | 33.0 | | 37.5 | |
| 91 | 15.5 | 19.4 | 30.0 | 25.1 | 58.0 | 42.4 ± 9.2 |
| 8. Multiple anesthetization with 0.5% Finquel and Equithesin | | | | | | |
| Finquel: | | | | | | |
| 92 | --- | | --- | | 72.0 | |
| 93 | --- | | --- | | 28.0 | |
| 94 | --- | | --- | | 44.5 | |
| 95 | --- | | --- | | 52.0 | |
| 96 | --- | | --- | | 66.0 | |
| 97 | --- | | --- | | 69.0 | 55.3 ± 3.9 |
| Equithesin: | | | | | | |
| 98 | --- | | --- | | 48.0 | |
| 99 | --- | | --- | | 34.0 | |
| 100 | --- | | --- | | 66.0 | |
| 101 | --- | | --- | | --- | |
| 102 | --- | | --- | | 50.0 | |
| 103 | --- | | --- | | --- | 49.5 ± 6.4 |

Appendix I. B. Handling and Anesthetic Effects on Blood Lactate in North Dakota *Rana pipiens*

| Animal # | Sample and/or Anesthetic Times: Zero time | Mean ±S.E. | One hour | Mean ±S.E. | Three hour | Mean ±S.E. |
|---|---|---|---|---|---|---|
| 1. Effect of multiple anesthetization September 1967 | | | | | | |
| 1 | 15.0 | | --- | | --- | |
| 2 | 24.2 | | --- | | --- | |
| 3 | 12.8 | | --- | | --- | |
| 4 | 25.6 | | --- | | --- | |
| 5 | 20.0 | 19.5 ± 2.5 | --- | | --- | |
| 6 | --- | | --- | | 44.0 | |
| 7 | --- | | --- | | 76.1 | |
| 8 | --- | | --- | | 26.8 | |
| 9 | --- | | --- | | 46.4 | |
| 10 | --- | | --- | | 53.3 | 49.3 ± 8.0 |
| 2. Effect of multiple anesthetization and dihydroergotamine | | | | | | |
| 1 mg/Kg dihydroergotamine injected at zero time: | | | | | | |
| 11 | --- | | --- | | 76.3 | |
| 12 | --- | | --- | | 41.6 | |
| 13 | --- | | --- | | 45.5 | |
| 14 | --- | | --- | | 70.2 | 58.4 ± 8.7 |
| 10 mg/Kg dihydroergotamine injected at zero time: | | | | | | |
| 15 | --- | | --- | | 92.8 | |
| 16 | --- | | --- | | 60.2 | |
| 17 | --- | | --- | | 48.9 | |
| 18 | --- | | --- | | 76.7 | |
| 19 | --- | | --- | | 60.3 | 67.8 ± 7.6 |
| 3. Effect of multiple anesthetization and hexamethonium | | | | | | |
| February 1968 | | | | | | |
| Saline controls: | | | | | | |
| 1 | 27.2 | | 18.8 | | 64.0 | |
| 2 | 20.8 | | 27.6 | | 42.4 | |
| 3 | 30.4 | | 19.4 | | 72.0 | |
| 4 | 19.2 | | 27.9 | | 76.0 | |
| 5 | 33.6 | 26.2 | 27.0 | 22.3 | 44.0 | 60.5 |
| Hexamethonium 100 mg/Kg injection at zero time | | | | | | |
| 6 | 18.4 | | --- | | --- | |
| 7 | 46.4 | | 68.8 | | 93.0 | |
| 8 | 20.9 | | 34.4 | | 32.0 | |
| 9 | 14.4 | 25.3 | 36.2 | 46.4 | 38.4 | 54.4 |

# APPENDIX II. COMPARISONS OF ADULT AND TADPOLE CARBOHYDRATE LEVELS

Appendix II. A. Mean blood glucose concentrations of North Dakota frogs maintained in the laboratory and used as zero time control animals through out the year Sept. 1968-October 1969

| Animal # | Sacrifice date | Blood Glucose mg% | Animal # | Sacrifice Date | Blood Glucose mg% |
|---|---|---|---|---|---|
| 1 | Sept. 3, | 28.2 | 41 | Nov. 22, | 20.5 |
| 2 | 1968 | 28.8 | 42 | 1968 | 14.0 |
| 3 | " | 23.0 | 43 | " | 15.7 |
| 4 | " | 20.2 | 44 | " | 14.5 |
| 5 | " | 28.8 | 45 | " | 19.5 |
| 6 | " | 19.0 | 46 | Nov. 29 | 14.4 |
| 7 | " | 28.8 | 47 | " | 18.3 |
| 8 | " | 25.8 | 48 | " | 11.2 |
| 9 | " | 23.0 | 49 | " | 17.7 |
| 10 | " | 31.0 | 50 | " | 19.4 |
| 11 | " | 23.0 | 51 | " | 31.4 |
| 12 | " | 34.5 | 52 | Dec. 5 | 12.9 |
| 13 | Sept. 11 | 20.7 | 53 | " | 12.3 |
| 14 | " | 20.0 | 54 | " | 10.8 |
| 15 | " | 19.0 | 55 | " | 13.4 |
| 16 | " | 30.5 | 56 | " | 14.4 |
| 17 | " | 14.5 | 57 | Dec. 10 | 25.0 |
| 18 | " | 22.5 | 58 | " | 23.5 |
| 19 | " | 15.0 | 59 | " | 19.8 |
| 20 | " | 29.0 | 60 | " | 15.0 |
| 21 | " | 17.0 | 61 | " | 21.4 |
| 22 | " | 24.2 | 62 | " | 26.6 |
| 23 | Oct. 15 | 21.9 | 63 | Jan. 6, | 9.7 |
| 24 | " | 16.6 | 64 | 1969 | 14.7 |
| 25 | " | 21.9 | 65 | " | 11.3 |
| 26 | " | 23.0 | 66 | " | 13.0 |
| 27 | " | 14.6 | 67 | " | 13.3 |
| 28 | " | 25.8 | 68 | Jan. 29 | 16.2 |
| 29 | Oct. 28 | 17.9 | 69 | " | 18.3 |
| 30 | " | 13.0 | 70 | " | 12.2 |
| 31 | " | 17.9 | 71 | " | 17.2 |
| 32 | " | 19.5 | 72 | " | 16.2 |
| 33 | " | 22.3 | 73 | " | 15.2 |
| 34 | " | 19.5 | 74 | Feb. 27 | 24.5 |
| 35 | Nov. 12 | 10.3 | 75 | " | 13.0 |
| 36 | " | 16.3 | 76 | " | 20.3 |
| 37 | " | 22.8 | 77 | " | 15.3 |
| 38 | " | 26.0 | 78 | " | 19.8 |
| 39 | " | 11.7 | 79 | " | 8.5 |
| 40 | Nov. 22 | 16.8 | 80 | March 4 | 14.2 |

Appendix II. A. continued

| Animal # | Sacrifice Date | Blood Glucose mg% | Animal # | Sacrifice Date | Blood Glucose mg% |
|---|---|---|---|---|---|
| 81 | March 4 | 12.7 | 94 | July 8 | 19.1 |
| 82 | " | 11.2 | 95 | " | 18.5 |
| 83 | " | 15.1 | 96 | Aug. 21 | 16.5 |
| 84 | " | 19.5 | 97 | " | 21.7 |
| 85 | " | 12.4 | 98 | " | 20.4 |
| 86 | March 12 | 16.0 | 99 | " | 23.5 |
| 87 | " | 9.6 | 100 | " | 30.1 |
| 88 | " | 21.5 | 101 | " | 12.7 |
| 89 | " | 19.8 | 102 | Oct. 13 | 15.3 |
| 90 | " | 21.8 | 103 | " | 17.5 |
| 91 | July 8, | 22.8 | 104 | " | 23.5 |
| 92 | " | 13.3 | 105 | " | 14.7 |
| 93 | " | 15.9 | 106 | " | 14.7 |
| | | | 107 | " | 31.2 |

Appendix II. B. 1. Blood glucose and liver glycogen concentrations of tadpoles of different metamorphic stages

| Animal # | Body Weight (mg or g) | Stage | Blood Glucose (mg %) | Mean Glucose (mg %) | Liver Glycogen (g/100 g liver) | Mean (g %) |
|---|---|---|---|---|---|---|
| 1 | 592 mg | IV-VI | 19.5 | | 6.9 | |
| 2 | 873 | " | | | | |
| 3 | 1005 | " | 15.3 | | 4.1 | |
| 4 | 625 | " | | | | |
| 5 | 755 | " | 21.4 | | 4.4 | |
| 6 | 627 | " | | | | |
| 7 | 709 | " | 16.6 | | 3.6 | |
| 8 | 654 | " | | | | |
| 9 | 658 | " | 13.0 | | 4.3 | |
| 10 | 766 | " | | | | |
| 11 | - | " | 9.5 | | - | |
| 12 | - | " | | | | |
| 13 | - | " | 9.9 | | - | |
| 14 | - | " | | | | |
| 15 | - | " | 7.2 | | - | |
| 16 | - | " | | 14.1 ± 1.7 | | 4.6 ± 1.8 |
| 17 | 3.0 g | XII-XV | 11.3 | | 8.2 | |
| 18 | 2.8 | " | 9.2 | | 6.7 | |
| 19 | 3.3 | " | 10.8 | | 5.5 | |
| 20 | 2.9 | " | 11.8 | | 5.1 | |
| 21 | 2.3 | " | 13.4 | | 5.0 | |
| 22 | 2.8 | " | 7.3 | | 6.4 | |
| 23 | 1.6 | " | 14.6 | | 6.8 | |
| 24 | 2.5 | " | 7.3 | | 5.7 | |
| 25 | 1.8 | " | 19.7 | | 5.5 | |
| 26 | 2.1 | " | 8.9 | | 7.3 | |
| 27 | 2.6 | " | 1.7 | | - | |
| 28 | 3.8 | " | 0.8 | | - | |
| 29 | 3.7 | " | 7.2 | | - | |
| 30 | 4.3 | " | 8.3 | | - | |
| 31 | 2.6 | " | 4.8 | | - | |
| 32 | - | " | 8.3 | | - | |
| 33 | - | " | 14.0 | | - | |
| 34 | - | " | 15.4 | | 6.1 | |
| 35 | - | " | 12.0 | | 5.7 | |
| 36 | - | " | 13.0 | | 6.2 | |
| 37 | - | " | 15.4 | | 6.9 | |
| 38 | - | " | 17.4 | | - | |

Appendix II. B. 1. continued

| Animal # | Body Weight (mg or g) | Stage | Blood Glucose (mg %) | Mean Glucose (mg %) | Liver Glycogen (g/100 g liver) | Mean (g%) |
|---|---|---|---|---|---|---|
| 39 | 4.4 g | XVIII-XIX | 12.0 | | 5.9 | |
| 40 | 4.1 | " | 8.3 | | 7.6 | |
| 41 | - | " | 16.2 | | 9.5 | |
| 42 | 2.8 | " | 19.0 | | 5.9 | |
| 43 | 2.2 | " | 20.6 | | 11.6 | |
| 44 | 4.4 | " | 16.3 | | 7.6 | |
| 45 | 3.3 | " | - | 15.4 ± 1.7 | 8.2 | 8.0 ± 1.7 |
| 46 | 2.5 | XX-XXI | 15.0 | | 0.9 | |
| 47 | 2.6 | " | 13.7 | | 2.5 | |
| 48 | 1.8 | " | 10.4 | | 3.2 | |
| 49 | 1.8 | " | 5.5 | | 0.1 | |
| 50 | 2.9 | " | 7.3 | | 2.1 | |
| | | | | 10.4 ± 1.3 | | 1.7 ± 1.5 |
| 51 | 1.6 | XXII- | 20.0 | | 5.4 | |
| 52 | 1.8 | XXIII | 19.8 | | 4.2 | |
| 53 | 2.9 | " | - | | 4.6 | |
| 54 | 2.5 | " | 24.5 | | 4.8 | |
| 55 | 2.4 | " | 21.5 | | 6.6 | |
| 56 | 2.6 | " | 24.1 | 22.0 ± 0.8 | 5.3 | 5.1 ± 1.3 |
| 57 | 2.3 | XXV | - | | 7.8 | |
| 58 | 1.9 | " | - | | 5.1 | |
| 59 | 2.0 | " | - | | 6.5 | 6.5 ± 2.1 |
| 1 | - | Summer | 22.8 | | 5.9 | |
| 2 | - | Adult | 13.3 | | 4.3 | |
| 3 | - | " | 15.9 | | 3.2 | |
| 4 | - | " | 19.1 | | 9.6 | |
| 5 | - | " | 18.5 | | 4.2 | |
| 6 | - | " | 16.5 | | 10.7 | 6.3 ± 1.2 |
| 7 | - | " | 21.7 | | | |
| 8 | - | " | 20.4 | | | |
| 9 | - | " | 23.5 | | | |
| 10 | - | " | 30.1 | | | |
| 11 | - | " | 12.7 | 19.5 ± 1.5 | | |

Appendix II. B. 2. Statistics of comparisons among metamorphic stages

a. Comparisons of blood glucose levels among tadpole stages

(Mann-Whitney U)

| Stages: | IV-VI | XII-XV | XVIII-XIX | XX-XXI | XXII-XXIII |
|---|---|---|---|---|---|
| IV-VI | - | <.001 | .377 | - | .003 |
| XII-XV | <.001 | - | <.025 | - | - |
| XVIII-XIX | .377 | <.025 | - | =.041 | =.009 |
| XX-XXI | - | - | =.041 | - | =.004 |
| XXII-XXIII | .003 | - | =.009 | =.004 | |

b. Comparisons of blood glucose levels of tadpoles and adults

(Student's T Test)

| Stages: | Summer Adult | Winter Adult |
|---|---|---|
| IV-VI | =.025 | >0.5 |
| XII-XV | <.001 | < .01 |
| XVIII-XIX | >.05 | > .4 |
| XX-XXI | <.005 | < .001 |
| XXII-XXIII | >.05 | < .05 |

c. Comparisons of liver glycogen levels of tadpoles and adults

(Mann-Whitney U)

| Stages: | IV-VI | XII-XV | XVIII-XIX | XX-XXI | XXII-XXIII | XXV |
|---|---|---|---|---|---|---|
| IV-VI | - | >.05 | =.005 | - | - | - |
| XII-XV | >.05 | - | >.05 | - | - | - |
| XVIII-XIX | .005 | >.05 | - | <.001 | - | - |
| XX-XXI | - | - | <.001 | - | =.004 | - |
| XXII-XXIII | - | - | - | =.004 | - | =.131 |
| XXV | - | - | - | - | =.131 | - |
| Adults | .214 | >.05 | .114 | .002 | >.535 | >.05 |

# APPENDIX III. RESPONSES OF ADULTS AND TADPOLES TO ADRENALIN AND GLUCAGON

Appendix III. 1. Blood glucose responses to intravenous adrenalin (June, 1968)

| Treatment | n | Blood glucose mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 Time | 21 | 19.9 | 2.1 | 11.0-36.2 | |
| 15 min | 11 | 23.4 | 2.2 | 17.0-43.7 | |
| 1 hour | 6 | 34.8 | 3.4 | 22.6-49.7 | |
| 3 hours | 6 | 28.1 | 3.5 | 18.2-49.7 | |
| 5 hours | 6 | 29.3 | 3.7 | 19.1-44.0 | |
| 24 hours | 6 | 14.5 | 2.4 | 10.5-20.5 | |
| Adrenalin: | | | | | |
| 0.10 mg/kg (0.1 mg/kg) | | | | | |
| 15 min | 10 | 32.8 | 4.2 | 23.0-50.7 | .021 |
| 1 hour | 6 | 35.9 | 9.3 | 11.0-75.3 | .531 |
| 3 hours | 6 | 42.1 | 2.3 | 35.2-48.3 | .032 |
| 5 hours | 6 | 30.2 | 4.6 | 20.3-48.0 | .469 |
| 24 hours | 6 | 19.1 | 2.4 | 12.5-23.7 | .066 |

Appendix III. 2. Blood glucose responses to intravenous adrenalin (September, 1968)

| | | | | | |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 10 | 20.1 | 0.9 | 16.0-25.5 | |
| 3 hours | 15 | 53.9 | 3.3 | 36.3-77.0 | |
| 24 hours | 5 | 11.3 | 1.3 | 9.0-15.0 | |
| Adrenalin: | | | | | |
| (1 mg/Kg) | | | | | |
| 3 hours | 16 | 60.5 | 2.8 | 35.7-74.3 | >.05 |
| 24 hours | 6 | 17.4 | 1.4 | 12.5-23.7 | .350 |

Appendix III. 3. Blood lactate responses to intravenous adrenalin (June and August, 1968)

| Treatment | n | Blood lactate mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| | | June | | | |
| Controls: | | | | | |
| 0 time | 21 | 21.6 | 3.3 | 3.9-48.7 | |
| 15 min | 6 | 18.6 | 6.7 | 4.3-49.0 | |
| 1 hour | 6 | 37.9 | 7.9 | 20.5-52.0 | |
| 3 hour | 6 | 19.3 | 4.5 | 8.2-37.8 | |
| 5 hour | 6 | 28.3 | 6.7 | 13.0-56.0 | |
| 24 hour | 6 | 44.4 | 13.2 | 18.0-100.0 | |
| Adrenalin: (0.10 mg/Kg) | | | | | |
| 15 min | 6 | 13.8 | 3.3 | 7.0-28.0 | .531 |
| 1 hour | 6 | 31.0 | 11.2 | 18.5-39.0 | .381 |
| 3 hour | 6 | 20.3 | 2.7 | 12.8-30.5 | .350 |
| 5 hour | 6 | 20.8 | 3.8 | 12.5-39.0 | .242 |
| 24 hour | 5 | 42.8 | 9.3 | 24.3-77.0 | .331 |
| | | August | | | |
| Controls: | | | | | |
| 0 time | 15 | 19.0 | 2.1 | 0.5-31.2 | |
| 3 hour | 15 | 42.7 | 4.6 | 9.0-60.7 | |
| 24 hour | 5 | 28.0 | 6.1 | 9.1-41.3 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 3 hour | 16 | 60.0 | 5.1 | 28.2-97.0 | <.025 |
| 24 hour | 6 | 63.0 | 25.0 | 13.6-141.0 | .123 |

Appendix III. 4. Frog blood glucose responses to adrenalin (1 mg/Kg) in summer as compared to winter

| Treatment | n | Blood glucose mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| | | A. August | | | |
| Controls: | | | | | |
| 0 time | 6 | 20.7 | 2.4 | 12.7-30.1 | |
| 1 hour | 6 | 16.5 | 1.7 | 11.2-24.0 | |
| 3 hour | 6 | 24.3 | 3.9 | 15.5-40.0 | |
| 5 hour | 6 | 16.1 | 2.1 | 11.7-24.0 | |
| Adrenalin: | | | | | |
| 1 hour | 6 | 33.1 | 3.7 | 19.4-46.5 | .002 |
| 3 hour | 6 | 37.2 | 0.6 | 36.2-39.0 | .032 |
| 5 hour | 6 | 34.0 | 6.7 | 9.5-59.6 | <.05 |
| | | B. November-March | | | |
| Controls: | | | | | |
| 0 time | 17 | 16.8 | 1.2 | 9.5-24.5 | |
| 1 hour | 23 | 17.6 | 2.2 | 8.4-29.0 | |
| 3 hour | 11 | 14.5 | 1.1 | 9.2-21.0 | |
| 5 hour | 10 | 20.3 | 3.5 | 11.6-35.8 | |
| 10 hour | 6 | 16.7 | 2.4 | 9.5-25.0 | |
| Adrenalin: | | | | | |
| 1 hour | 6 | 29.8 | 2.3 | 22.4-35.8 | <.001 |
| 3 hour | 6 | 41.2 | 7.0 | 18.2-55.5 | <.001 |
| 5 hour | 5 | 45.6 | 12.2 | 20.5-84.0 | <.025 |
| 10 hour | 6 | 18.0 | 3.7 | 11.4-33.7 | >.05 |

Appendix III. 5. Frog blood glucose responses to adrenalin in summer and winter combined. Dose response data.

| Treatment | n | Blood glucose mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 19 | 18.7 | 1.7 | 12.5-30.1 | |
| 1 hour | 22 | 18.3 | 1.5 | 11.2-37.5 | |
| 3 hour | 24 | 20.7 | 1.6 | 12.2-40.0 | |
| 5 hour | 17 | 15.4 | 1.6 | 8.4-25.2 | |
| 10 hour | 6 | 16.3 | 3.1 | 9.7-24.7 | |
| 24 hour | 6 | 16.8 | 1.1 | 14.0-20.5 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 33.1 | 3.8 | 19.4-46.5 | .001 |
| 3 hour | 6 | 37.3 | 1.5 | 35.3-39.0 | .001 |
| 5 hour | 6 | 34.0 | 1.9 | 9.5-59.6 | .005 |
| 10 hour | 6 | 18.0 | 3.7 | 11.7-33.7 | .531 |
| 24 hour | 6 | 15.9 | 1.3 | 13.0-27.2 | .066 |
| (0.1 mg/Kg) | | | | | |
| 1 hour | 6 | 28.8 | 2.2 | 27.0-39.5 | .005 |
| 3 hour | 6 | 33.8 | 2.4 | 28.2-33.0 | .001 |
| 5 hour | 6 | 32.6 | 4.0 | 18.0-46.5 | .001 |
| (0.05 mg/Kg) | | | | | |
| 1 hour | 10 | 19.9 | 2.3 | 13.3-31.4 | .400 |
| 3 hour | 8 | 21.9 | 2.7 | 12.2-27.8 | .500 |
| (0.5 mg/Kg not presented in a Figure) | | | | | |
| 1 hour | 6 | 25.5 | 2.4 | 19.4-28.8 | .025 |
| 3 hour | 6 | 35.4 | 4.8 | 9.0-47.0 | .001 |
| 5 hour | 6 | 45.7 | 3.2 | 39.0-55.8 | .001 |

Appendix III. 6. Frog blood glucose responses to adrenalin and glucagon September

| Treatment | n | Blood glucose mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 6 | 19.5 | 3.3 | 14.7-31.2 | |
| 1 hour | 6 | 16.7 | 2.5 | 14.7-21.9 | |
| 3 hour | 6 | 18.6 | 2.3 | 13.7-21.9 | |
| 5 hour | 6 | 17.0 | 2.9 | 11.5-25.2 | |
| 10 hour | 6 | 16.3 | 3.1 | 9.7-24.7 | |
| 24 hour | 6 | 16.8 | 1.1 | 14.0-29.5 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 26.7 | 3.1 | 18.5-34.0 | .004 |
| 3 hour | 6 | 46.7 | 3.5 | 38.3-58.6 | .001 |
| 5 hour | 6 | 58.7 | 3.5 | 49.7-72.0 | .001 |
| 10 hour | 6 | 42.7 | 5.7 | 25.7-74.5 | .001 |
| 24 hour | 6 | 15.9 | 2.3 | 13.0-27.2 | .066 |
| Glucagon: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 29.1 | 2.8 | 23.0-34.0 | .001 |
| 3 hour | 6 | 35.9 | 5.4 | 24.0-70.2 | .001 |
| 5 hour | 6 | 26.3 | 3.5 | 18.1-36.3 | .013 |
| 10 hour | 6 | 19.5 | 3.4 | 13.7-27.5 | .165 |
| 24 hour | 6 | 14.2 | 1.2 | 10.3-17.5 | .120 |
| (.05 mg/Kg) | | | | | |
| 1 hour | 6 | 17.6 | 3.2 | 8.5-24.8 | >.500 |
| 3 hour | 6 | 12.9 | 1.8 | 9.0-14.7 | <.001 |
| 5 hour | 6 | 17.5 | 3.3 | 9.0-28.7 | >.100 |

**Appendix III. 7.** Frog blood glucose responses to glucagon (1 mg/Kg) in summer as compared to winter

| Treatment | n | Blood glucose mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| | | A. August | | | |
| Controls: | | | | | |
| 0 time | 6 | 20.7 | 2.4 | 12.7-30.1 | |
| 1 hour | 6 | 16.5 | 1.7 | 11.2-24.0 | |
| 3 hour | 6 | 24.3 | 3.9 | 15.5-40.0 | |
| 5 hour | 6 | 16.1 | 2.1 | 11.7-24.0 | |
| Glucagon: | | | | | |
| 1 hour | 6 | 27.2 | 1.3 | 22.5-31.0 | .001 |
| 3 hour | 6 | 35.3 | 4.3 | 22.4-46.5 | .008 |
| 5 hour | 6 | 29.7 | 2.8 | 22.2-41.8 | .002 |
| 8 hour | 6 | 22.4 | 1.4 | 18.1-26.8 | - |
| | | B. November-March | | | |
| Controls: | | | | | |
| 0 time | 17 | 16.8 | 1.2 | 9.5-24.5 | |
| 1 hour | 23 | 17.6 | 2.2 | 8.4-29.0 | |
| 3 hour | 11 | 14.5 | 1.1 | 9.2-21.0 | |
| 5 hour | 10 | 20.3 | 3.5 | 11.6-35.8 | |
| 10 hour | 6 | 16.7 | 2.4 | 9.5-25.0 | |
| Glucagon: | | | | | |
| 1 hour | 18 | 17.9 | 1.1 | 8.5-24.5 | .681 |
| 3 hour | 17 | 29.3 | 3.3 | 14.6-65.8 | <.005 |
| 5 hour | 16 | 24.0 | 6.1 | 6.0-44.0 | .40 |
| 10 hour | 6 | 21.4 | 3.7 | 13.5-38.6 | >.05 |

Appendix III. 8. Frog blood glucose responses to glucagon. Dose response data. (Control data presented under the appropriate season).

| Treatment | n | Blood glucose mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| | | A. Two mg/Kg glucagon Winter | | | |
| 1 hour | 11 | 22.2 | 4.0 | 4.2-37.0 | .84 |
| 3 hour | 12 | 36.9 | 7.8 | 9.9-59.8 | <.01 |
| 5 hour | 12 | 27.7 | 4.1 | 13.6-74.0 | .2 |
| | | B. One mg/Kg see Appendix III. 6-7 | | | |
| | | C. 0.10 mg/Kg Winter | | | |
| 1 hour | 6 | 40.3 | 2.2 | 22.0-47.6 | <.001 |
| 3 hour | 6 | 17.0 | 1.8 | 10.2-23.0 | >.05 |
| | | D. 0.10 mg/Kg Summer | | | |
| 1 hour | 6 | 27.3 | 2.9 | 14.5-31.9 | .032 |
| 3 hour | 6 | 29.5 | 3.1 | 19.7-40.0 | .155 |
| 5 hour | 6 | 29.9 | 2.8 | 19.4-39.4 | .004 |
| | | E. 0.05 mg/Kg Fall | | | |
| 1 hour | 6 | 17.6 | 3.2 | 8.5-24.8 | >.50 |
| 3 hour | 6 | 12.9 | 1.8 | 9.0-14.7 | <.001 |
| 5 hour | 6 | 17.5 | 3.3 | 9.0-28.7 | >.100 |

Appendix III. 9. Frog liver and muscle glycogen responses to a adrenalin and glucagon. August.

| Treatment | n | Mean Muscle glycogen (per cent) | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 6 | 0.99 | 0.07 | 0.74-1.18 | |
| 1 hour | 6 | 0.96 | 0.07 | 0.80-1.24 | |
| 3 hour | 5 | 0.83 | 0.01 | 0.76-0.93 | |
| 5 hour | 2 | 0.92 | - | 0.76-1.09 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 0.88 | 0.10 | 0.62-1.24 | .242 |
| 3 hour | 6 | 0.85 | 0.07 | 0.61-1.06 | >.535 |
| 5 hour | 6 | 0.84 | 0.06 | 0.62-1.02 | |
| Glucagon: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 1.20 | 0.10 | 0.87-1.55 | .047 |
| 3 hour | 6 | 0.89 | 0.07 | 0.74-1.15 | >.465 |
| 5 hour | 5 | 0.92 | 0.12 | 0.54-1.13 | |
| | | Mean Liver glycogen (per cent) | | | |
| Controls: | | | | | |
| 0 time | 6 | 8.6 | 0.7 | 6.6-11.2 | |
| 1 hour | 6 | 9.2 | 1.4 | 5.4-13.4 | |
| 3 hour | 6 | 7.1 | 1.0 | 4.4-11.0 | |
| 5 hour | 6 | 6.4 | 1.6 | 1.8-12.2 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 8.8 | 1.5 | 4.9-15.5 | .409 |
| 3 hour | 6 | 6.2 | 1.4 | 1.7-10.4 | .350 |
| 5 hour | 6 | 8.9 | 0.9 | 5.8-12.4 | .294 |
| Glucagon: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 9.5 | 1.1 | 5.8-12.9 | .242 |
| 3 hour | 6 | 7.4 | 1.2 | 3.6-10.8 | .469 |
| 5 hour | 6 | 5.9 | 1.2 | 1.7-9.2 | .469 |

Appendix III. 10. Blood lactate response to adrenalin and glucagon (August)

| Treatment | n | Blood lactate mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 29 | 13.0 | 1.3 | 2.3-28.8 | |
| 1 hour | 17 | 10.6 | 1.4 | 0.0-21.0 | |
| 3 hour | 23 | 12.6 | 1.2 | 0.0-20.0 | |
| 5 hour | 6 | 12.3 | 2.6 | 6.4-26.3 | |
| 10 hour | 5 | 18.4 | 1.3 | 13.2-21.0 | |
| Adrenalin: | | | | | |
| (1 mg/Kg) | | | | | |
| 1 hour | 6 | 33.4 | 1.5 | 21.4-47.0 | <.001 |
| 3 hour | 4 | 31.6 | 3.2 | 5.0-44.0 | .05 |
| 5 hour | 6 | 19.7 | 2.4 | 9.3-25.9 | .120 |
| (0.5 mg/Kg) | | | | | |
| 1 hour | 6 | 24.9 | 3.0 | 15.0-35.7 | <.001 |
| 3 hour | 6 | 14.1 | 4.2 | 0.0-30.0 | >.05 |
| 5 hour | 6 | 21.1 | 2.9 | 12.8-30.0 | .350 |
| (0.1 mg/Kg) | | | | | |
| 1 hour | 15 | 20.5 | 3.4 | 0.0-40.5 | <.01 |
| 3 hour | 19 | 15.7 | 1.9 | 4.0-36.8 | >.05 |
| 5 hour | 6 | 9.3 | 9.5 | 5.5-13.6 | >.531 |
| (0.05 mg/Kg) | | | | | |
| 1 hour | 10 | 18.2 | 1.8 | 10.8-29.6 | <.01 |
| 3 hour | 14 | 11.9 | 1.1 | 6.0-21.2 | >.05 |
| Glucagon: | | | | | |
| (0.10 mg/Kg) | | | | | |
| 1 hour | 6 | 6.4 | 1.2 | 3.5-10.1 | .052 |
| 3 hour | 4 | 15.9 | 4.7 | 6.2-28.0 | >.05 |

Appendix III. 11. Blood lactate response to adrenalin and glucagon (September)

| Treatment | n | Blood lactate mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 19 | 18.3 | 2.5 | 4.4-25.6 | |
| 1 hour | 12 | 16.0 | 2.2 | 5.2-29.2 | |
| 3 hour | 11 | 21.0 | 1.9 | 6.8-39.6 | |
| 5 hour | 12 | 13.3 | 3.6 | 1.8-50.4 | |
| 10 hour | 5 | 16.0 | 0.8 | 11.4-22.8 | |
| 24 hour | 5 | 15.1 | 0.9 | 12.4-17.6 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 12 | 70.4 | 6.2 | 35.6-105.6 | <.001 |
| 3 hour | 9 | 66.1 | 7.5 | 41.5-104.6 | <.001 |
| 5 hour | 12 | 36.1 | 4.8 | 17.6-72.0 | <.001 |
| 10 hour | 6 | 20.3 | 3.5 | 11.4-32.0 | .214 |
| 24 hour | 6 | 4.9 | 0.4 | 1.6- 8.0 | .002 |
| Glucagon: (1 mg/Kg) | | | | | |
| 1 hour | 6 | 35.3 | 5.1 | 21.5-62.5 | <.001 |
| 3 hour | 6 | 18.7 | 1.4 | 12.3-23.1 | >.05 |
| 5 hour | 6 | 10.5 | 4.3 | 1.3-28.8 | >.05 |
| 24 hour | 6 | 9.1 | 1.1 | 5.6-12.8 | .004 |

Appendix III. 12. Tadpole blood glucose response to adrenalin and glucagon

| Treatment | n | Blood glucose mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 8 | 6.1 | 2.8 | 0.0-16.2 | |
| 1 hour | 5 | 4.4 | 2.9 | 0.6-11.2 | |
| 4 hour | 12 | 4.0 | 1.5 | 0.0- 8.3 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 5 | 15.5 | 4.8 | 3.3-33.1 | .048 |
| 3 hour | 5 | 16.6 | 4.5 | 4.3-29.2 | <.025 |
| 5 hour | 5 | 9.0 | 4.1 | 0.0-29.5 | >.05 |
| (0.1 mg/Kg) | | | | | |
| 1 hour | 5 | 11.9 | 3.9 | 5.6-23.0 | .075 |
| 3 hour | 5 | 7.3 | 2.9 | 0.8-11.1 | >.05 |
| 5 hour | 5 | 7.6 | 1.9 | 6.4-10.5 | >.05 |
| Glucagon: (1 mg/Kg) | | | | | |
| 1 hour | 5 | 11.1 | 3.6 | 0.8-16.4 | .075 |
| 3 hour | 5 | 10.4 | 2.9 | 6.4-16.6 | <.02 |
| 5 hour | 5 | 4.8 | 2.4 | 0.8- 9.2 | >.05 |
| (0.1 mg/Kg) | | | | | |
| 3 hour | 5 | 6.0 | 2.5 | 1.1- 8.9 | >.05 |
| 5 hour | 5 | 13.0 | 2.8 | 8.5-18.5 | <.01 |

Appendix III. 13. Tadpole blood lactate responses to adrenalin and glucagon.

| Treatment | n | Blood lactate mg% Mean | Standard Error | Range | p |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 5 | 23.3 | 5.2 | 8.8-38.4 | |
| 1 hour | 3 | 35.6 | - | 33.7-37.8 | |
| 4 hour | 6 | 27.3 | 1.4 | 15.3-43.2 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 4 | 41.2 | 4.0 | 23.8-78.6 | .429 |
| 3 hour | 5 | 30.7 | 2.0 | 11.8-46.8 | .396 |
| 5 hour | 5 | 44.9 | 5.8 | 28.5-60.8 | .033 |
| Glucagon: (1 mg/Kg) | | | | | |
| 1 hour | 3 | 22.4 | 4.0 | 13.8-28.3 | .050 |
| 3 hour | 4 | 19.3 | 2.8 | 14.3-27.2 | .238 |
| 5 hour | 3 | 25.6 | - | 24.9-26.4 | .548 |

Appendix III. 14. Tadpole liver glycogen responses to adrenalin and glucagon.

| | | Mean liver glycogen (per cent) | | | |
|---|---|---|---|---|---|
| Controls: | | | | | |
| 0 time | 6 | 5.4 | 0.7 | 3.2- 7.1 | |
| 1 hour | 5 | 3.1 | 0.8 | 0.6- 4.7 | |
| 4 hour | 14 | 5.4 | 0.7 | 1.4-11.9 | |
| Adrenalin: (1 mg/Kg) | | | | | |
| 1 hour | 5 | 2.3 | 0.5 | 1.0- 4.2 | .210 |
| 3 hour | 5 | 3.7 | 0.9 | 0.4- 6.0 | >.05 |
| 5 hour | 5 | 5.0 | 1.6 | 0.2-10.3 | >.05 |
| (0.1 mg/Kg) | | | | | |
| 1 hour | 5 | 3.7 | 0.5 | 2.2- 4.5 | .50 |
| 3 hour | 5 | 3.7 | 0.5 | 2.5- 5.2 | >.05 |
| 5 hour | 5 | 6.5 | 0.5 | 4.9- 7.7 | >.05 |
| Glucagon: (1 mg/Kg) | | | | | |
| 1 hour | 5 | 3.4 | 0.9 | 1.9- 6.9 | .421 |
| 3 hour | 5 | 1.5 | 0.3 | 0.7- 2.4 | <.01 |
| 5 hour | 5 | 2.8 | 0.8 | 1.0- 5.3 | <.05>.025 |
| (0.1 mg/Kg) | | | | | |
| 3 hour | 5 | 4.4 | 1.0 | 1.6- 7.5 | >.05 |
| 5 hour | 5 | 6.1 | 0.7 | 4.3- 8.3 | >.05 |

Appendix III. 15. Adrenalin effects on blood glucose and lactate in the fed laboratory rat.

A. Blood glucose

| Rat # | Treatment | Blood glucose mg%: Initial | 15 min | 30 min | 1 hr | 1-1/2 hr | 2 hr | 3 hr |
|---|---|---|---|---|---|---|---|---|
| 1 | Saline | 119 | 153 | 146 | - | 146 | 125 | 114 |
| 2 | Saline | 121 | 138 | 132 | 132 | 123 | 119 | 116 |
| 3 | Adrenalin (0.1 mg/Kg) | 116 | 147 | 164 | 144 | 131 | 119 | 106 |
| 4 | Adrenalin (0.1 mg/Kg) | 101 | 124 | 196 | 146 | 117 | 102 | 96 |

B. Blood lactate

| | | Blood lactate mg%: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | Saline | 17.3 | 26.5 | 9.5 | - | 12.5 | 12.7 | 14.5 |
| 2 | Saline | 15.0 | 9.6 | 12.0 | 8.8 | - | 10.0 | 8.8 |
| 3 | Adrenalin (0.1 mg/Kg) | 19.0 | 17.0 | 16.5 | 20.0 | 21.5 | 19.0 | 13.7 |
| 4 | Adrenalin (0.1 mg/Kg) | 19.2 | 15.0 | 16.7 | 14.0 | 23.7 | 9.5 | 14.2 |

# APPENDIX IV. RESPONSES OF FROG LIVER SLICES TO ADRENALIN AND GLUCAGON

Appendix IV. 1. Adrenalin stimulation of glucose release from frog liver slices. Phosphate-chloride buffer (pH 7.4) compared with amphibian Ringers (pH 7.8) at 25 C.

| Incubation medium | Time of Incubation | Number Flasks | Mean Glucose Release (µg/mg/hr±S.E.) | Probability of higher t |
|---|---|---|---|---|
| Ringers | 1/2 hr | 5 | 0.68 ± 0.23 | p .05 |
| Adrenalin* Ringers | 1/2 hr | 5 | 1.13 ± 0.42 | p > .05 |
| Ringers | 1 hr | 5 | 1.68 ± 0.38 | |
| Adrenalin Ringers | 1 hr | 5 | 2.69 ± 0.20 | p < .05 |
| Phos-Cl Buffer | 1/2 hr | 5 | 1.57 ± 0.46 | |
| Adrenalin Buffer | 1/2 hr | 5 | 2.09 ± 0.34 | p > .05 |
| Phos-Cl Buffer | 1 hr | 5 | 3.29 ± 0.48 | |
| Adrenalin Buffer | 1 hr | 5 | 3.95 ± 0.64 | p > .05 |

*Adrenalin concentration = 2.5 µg/ml.

Appendix IV. 2. Adrenalin (2.5 µg/ml) stimulation of glucose release from frog liver slices at different temperatures and incubation times. (Ringers pH 7.8)

| Temperature °C | Incubation time hrs | Glucose release g/mg/time Mean ± S.E. | Number Flasks | Probability of higher t |
|---|---|---|---|---|
| 18° Control | 1/2 hr | 1.96 ± 0.21 | 5 | p > .05 |
| 18° Adrenalin | 1/2 hr | 1.95 ± 0.31 | 5 | |
| 18° Control | 1 hr | 4.20 ± 0.17 | 5 | p > .05 |
| 18° Adrenalin | 1 hr | 4.80 ± 0.46 | 5 | |
| 22° Control | 1/4 hr | 2.48 ± 0.24 | 5 | p > .05 |
| 22° Adrenalin | 1/2 hr | 2.79 ± 0.21 | 5 | |
| 22° Control | 1 hr | 3.62 ± 0.20 | 5 | p < .05 |
| 22° Adrenalin | 1 hr | 4.77 ± 0.42 | 5 | |
| 25° Control | 1/2 hr | 2.05 ± 0.13 | 6 | p > .05 |
| 25° Adrenalin | 1/2 hr | 2.03 ± 0.10 | 6 | |
| 25° Control | 1 hr | 3.54 ± 0.20 | 6 | p < .005 |
| 25° Adrenalin | 1 hr | 5.47 ± 0.38 | 6 | |
| 28° Control | 1/2 hr | 2.83 ± 0.37 | 6 | p > .05 |
| 28° Adrenalin | 1/2 hr | 3.07 ± 0.43 | 6 | |
| 28° Control | 1 hr | 5.29 ± 0.80 | 4 | p > .05 |
| 28° Adrenalin | 1 hr | 3.85 ± 0.60 | 4 | |

Appendix IV. 3. Adrenalin and glucagon effects on glucose release from frog liver slices incubated for 1 hour at 25° C in Ringers (pH 7.8).

A. Glucagon

| Glucagon Dose: | µg/ml: | 250 | 25 | 2.5 | .25 | .025 | 0 |
|---|---|---|---|---|---|---|---|
| | Moles/L: | $7.2 \times 10^{-5}$ | $7.2 \times 10^{-6}$ | $7.2 \times 10^{-7}$ | $7.2 \times 10^{-8}$ | $7.2 \times 10^{-9}$ | 0 |
| Mean glucose release | (µg/mg/hr) | 4.37 | 3.04 | 2.44 | 2.08 | 1.92 | 1.56 |
| Number of flasks | | 5 | 11 | 11 | 6 | 6 | 22 |
| Standard Error | | 0.29 | 0.29 | 0.33 | 0.13 | 0.17 | 0.23 |
| p | | <.001 | <.001 | <.05 | >.05 | >.05 | |

B. Adrenalin

| Adrenalin Dose: | µg/ml: | 100 | 25 | 2.5 | .25 | .025 | 0 |
|---|---|---|---|---|---|---|---|
| | Moles/L: | $5.7 \times 10^{-4}$ | $1.4 \times 10^{-4}$ | $1.4 \times 10^{-5}$ | $1.4 \times 10^{-6}$ | $1.4 \times 10^{-7}$ | 0 |
| Mean glucose release | (µg/mg/hr) | 1.57 | 2.52 | 2.66 | 2.95 | 1.92 | 1.56 |
| Number of flasks | | 5 | 11 | 11 | 6 | 6 | 22 |
| Standard Error | | 0.30 | 0.25 | 0.25 | 2.39 | 0.13 | 0.23 |
| p* | | >.05 | <.025 | <.001 | <.001 | >.05 | |

*p probability of a higher t.

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