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# BIOCHEMICAL STUDIES ON THE BLOOD AND TISSUE COMPONENTS OF THE COMMON EGYPTIAN TOAD *BUFO REGULARIS*

By

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# دراسات بيوكيميائية على مكونات دم وأنسجة الضفدع المصري بوفو ريجيولارس

سهير النجدي و منصور زهرة و الأحمدي الذهبي و محمود الصباغ

لقد تم دراسة تأثير الأنسيولين بمفرده أو الكورتيزون بمفرده أو كليهما معا ، على مستويات الجلوكوز وحامض اللاكتيك وحامض البيروفيك وعلى أنشطة إنزيمات كولين استيريز والفوسفاتيز القلوي واسبارتات أمنيو ترانسفيزيز وكذلك آلانين أمينوترا نفسيرير بالاضافة إلى تقدير كمية البروتين الكلي في الدم ومصل الدم والأنسجة في كل من ذكور وإناث الضفدع بوفو ريجيولارس وذلك في شهري يونيو وأغسطس . وقد تبين عامة أن الأنشطة الأيضية كانت أعلى في أغسطس منها في يونيو . وأدى حقن الانسيولين بالحقن البريتوني إلى حدوث هبوط في مستويات السكر في الدم ولكن تأثيره على المواد الأيضية الأخرى والانزيمات الموجودة في الدم ومصل الدم والأنسجة كانت متباينة . وأدى حقن الكورتيزون بمفرده عن طريق الحقن البريتوني إلى حدوث زيادة في مستوى الجلوكوز وحامض اللاكتيك وحامض البيروفيك في الدم وكذلك في كمية الجليكوجين في الكبد والعضلات . كما أدى حقن الكورتيزون بمفرده أيضاً إلى حدوث زيادة عامة في أنشطة كل من آلانين أمينو ترانسفيريز والفوسفاتيز القلوي وكذلك كولين استيريز في مصل الدم والكبد وأنسجة العضلات . وعند حقن الأنسولين مع الكورتيزون فانه قد فشل في معاكسة تأثيرات الكورتيزون على مستويات الجلوكوز وحامض اللاكتيك وحامض البيروفيك في الدم وذلك في شهر أغسطس ، ولكن لوحظ تباين هذه التأثيرات في شهر يونيو .

*Key Words: Bufo regularis,* Blood and tissue components, Metabolic effects of insulin and glucocorticoids.

## ABSTRACT

The effect of insulin and/or cortisone on the blood, serum and tissue levels of glucose, glycogen, pyruvic acid, lactic acid cholinesterase, alkaline phosphatase, aspartate amino transferase (AST), alanine amino-transferase (ALT) and total serum protein fractions was investigated in both males and females of *Bufo regularis* once in June and August. Metabolic activities in general were higher in August than in June. The intraperitoneal injection of insulin produced hypoglycemia but its effect on other metabolites and enzymes in blood, serum and tissues were found to be variable. The intraperitoneal injection of cortisone increased blood glucose, liver and muscle glycogen, blood lactate and blood pyruvate. It also produced a general increase in the activities of AST, ALT, alkaline phosphatase and cholinesterase in serum, liver and muscle tissues. When insulin was injected together with cortisone they failed to antagonize the effects of cortisone on blood glucose, lactic and pyruvic acids in August but its effect was found to be variable in June. The importance of these findings was discussed in relation to the findings of the other investigators.

## INTRODUCTION

Among vertebrates, the pancreatic hormones, especially insulin, are central to metabolic regulations under ordinary conditions [1]. In addition, several hormones come to play a role in times of stress or during development. The glucocorticoids make gradual adjustments during prolonged stress, fasting or starvation [2].

The glucocorticoids antagonize the uptake of glucose by muscle and fat and promote the transfer of amino acids from the hepatic gulconeogenesis; they also increase the amounts of enzymes concerned with gluconeogenesis. Glucocorticoids are 21-carbon steroids with many actions, the most important of which is to promote gluconeogenesis [3]. These patterns of hyperglycemic control are similar from fishes to mammals, although sensitivities to different hormones, drugs, and experimental manipulations vary [3,4]. They affect carbohydrate, lipid and protein metabolism. They decrease blood glucose level, have a lipogenic action, as well as a potent inhibitors of lipolysis in liver and adipose tissue and thus have an indirect anabolic effect. being a general anabolic effect on protein metabolism in that they stimulate protein synthesis and retard protein degradation [3].

Since the toad is so extensively used nowadays in the laboratory in the different physiological, pharmacological and biochemical experiments, the present investigation was performed to study the sensitivity of male and female *Bufo regularis* to injections of insulin and/or coticosteroids. The effects on cholinesterase, alkaline phosphatase, aspartate amino transferase (AST), alanine amino transferase (ALT) and total serum proteins as well as serum protein fractions have been investigated once during June and another time during August.

## MATERIAL AND METHODS

A number of 450 adult males and 450 adult females of *Bufo regularis* of average body weight 15-20 g were used in our experiments. The animals were collected from natural breeding fields and ponds surrounding Mashtal El Kaddy near Zagazig City in June and in August. The animals were kept for two days before the start of the experiment in cages containing soil from the normal environment to adapt them to the experimental conditions. The temperature was kept at 25 $^{o}$C and the relative humidity at 50%. The animals were divided into 5 groups, each of 45 males and 45 females. The animals in each group were treated as follows:

**Group I (Normals) :** The animals were kept for four days in the same laboratory conditions as the animals in the other groups to be considered as normal untreated animals.

**Group II (Controls):** Each animal was intraperitoneally injected daily with 1ml saline [0.9% NaCl, pH 7.4] for four ys.

**Group III (Insulin):** Each animals was intraperitoneally injected daily with 1 ml saline containing 1 I.U. Insulin for four days.

**Group IV (Cortisone):** Each animal was intraperitoneally injected daily with 1 ml saline containing 1 ug of cortisone for four days.

**Group V (Insulin + Cortisone)** : Each animal was intraperitoneally injected daily with 1 ml saline containing 1 I.U. of insulin and 1 ug of cortisone for four days.

The insulin and cortisone used were of the mammalian type.

All the animals were sacrificed 24 hours after the last injection [27] and the normal untreated animals were also sacrificed at the same time. This experiment was performed twice, once on animals collected in June then repeated exactly, the same on animals collected in August.

The methods of biochemical analysis applied were as follows:

Aspartate Aminotransferase (AST) was determined by the method described by Bergmeger *et al.* [5], Alanine Aminotransferase (ALT) according to Bergmeyer *et al.* [6], Cholinesterase (C.E.) as described by Illman [7], and Alkaline phosphatase (A.P.) according to Moss *et al.* [8].

Blood glucose was determined according to Cooper and Mc Danial[9], tissue Glycogen by Fong *et al* [10], lactic and pyruvic acids by Gloster and Harris, [11]. Collogel electrophoresis of the plasma proteins was performed according to the method of Chemerton [12]. The statistical analysis of the results were done using the Students' t-test [13].

## RESULTS

The results are presented in Tables (1-7)

## DISCUSSION

The normal mean blood glucose levels presently obtained in normal and control *Bufo regularis* ranged between 59.9 ±8.8 and 60.0 ±7.0 mg/100 ml in males and between 67.8 8.4 and 76.7±7.6 in females, in June and between 54.44± 6.8 and 71.12±7.71 in males and between 66.66 9.1 and 72.22±6.0 in females in August (Table 1,2). Lower values were obtained by Abdel Daium [4] and Fouda [15], however, their values were always higher during summer than during winter months. Our samples of *Bufo regularis* were investigated during the active breeding months (June and August). Smith [16] reported higher blood sugar level at the spawning season in March, June and July and also in November. The hyperglycemia of June and July was accompanied by rapid development of the fat body. Generally, blood sugar in Amphibia was found to show higher values at the time of breeding than during non-breeding season [17].

On the other hand our liver and muscle glycogen levels

## Table (1)

**Blood glucose, pyruvic acid, lactic acid, serum C.E, A.P, AST, ALT and total protein levels in male and female *Bufo regularis Reuss* in June**

| Group | Males | | | | | | | | Females | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glucose mg/100ml | Pyruvic acid mg/100ml | Lactic acid mg/100ml | C.E. mg/ml | A.P mg/100ml | AST u/ml | ALT u/ml | Total protein g/100ml | Glucose mg/100ml | Pyruvic acid mg/100ml | Lactic acid mg/100ml | C.E. mg/ml | A.P. mg/100ml | AST u/ml | ALT u/ml | Total protein g/100 ml |
| Control mean ± S.E. | 59.9± 8.0 | 3.4± 0.3 | 27.2± 1.9 | 0.25± 0.02 | 11.1± 0.4 | 28.3± 0.25 | 41.7± 2.0 | 2.8± 0.04 | 76.7± 7.6 | 3.9± 0.6 | 36.0± 2.8 | 0.29± 0.02 | 12.8± 0.4 | 27.1± 0.3 | 42.3± 2.3 | 2.8± 0.06 |
| Normal mean ± S.E. control V.S. normal P < | 60.0± 7.0 N.S. | 2.8± 0.2 N.S. | 23.9± 3.4 N.S. | 0.27± 0.02 N.S. | 8.1± 0.3 0.001 ↓ | 26.6± 0.4 0.01 ↓ | 37.1± 1.3 N.S. | 2.6± 0.04 N.S. | 67.8± 8.4 N.S. | 3.0± 0.2 N.S. | 39.2± 3.3 N.S. | 0.25± 0.02 N.S. | 9.0± 0.3 0.001 ↓ | 56.6± 0.4 N.S. | 38.4± 1.1 N.S. | 2.7± 0.04 N.S. |
| Insulin mean ± S.E. control V.S. Insulin P < | 6.6± 3.1 0.001 ↓ | 5.8± 0.4 0.001 ↑ | 33.5± 2.8 N.S. | 0.3± 0.02 N.S. | 10.8± 0.4 N.S. | 28.0± 0.3 N.S. | 44.4± 1.6 N.S. | 2.9± 0.05 N.S. | 5.5± 2.6 0.001 ↓ | 6.5± 0.3 0.001 ↑ | 57.6± 8.8 0.05 ↑ | 0.3± 0.02 N.S. | 11.4± 0.4 0.02 ↓ | 29.1± 0.4 0.001 ↑ | 43.4± 1.5 N.S. | 2.9± 0.06 N.S. |
| Insulin + cortisone mean ± SE control V.S. Insulin + cortisone P < | 46.7± 8.3 N.S. | 5.5± 0.4 0.001 ↑ | 23.2± 1.9 N.S. | 0.35± 0.02 0.01 ↑ | 12.8± 0.4 0.01 ↑ | 29.4± 0.3 0.05 ↑ | 56.9± 1.9 0.001 ↑ | 2.8± 0.05 N.S. | 55.6± 8.8 N.S. | 6.1± 0.3 0.01 ↑ | 21.3± 1.9 0.001 ↓ | 0.4± 0.01 0.001 ↑ | 13.3± 0.3 N.S. | 29.5± 0.4 0.001 ↑ | 54.8± 2.7 0.01 ↑ | 2.8± 0.06 N.S. |
| Cortisone mean ± S.E. control V.S. cortisone P < | 423.3± 15.6 0.001 ↑ | 6.3± 0.4 0.001 ↑ | 42.8± 5.9 0.02 ↑ | 0.63± 0.02 0.001 ↑ | 18.5± 0.6 0.001 ↑ | 31.2± 0.3 0.001 ↑ | 62.9± 1.2 0.001 ↑ | 2.9± 0.04 N.S. | 423.3± 16.8 0.001 ↑ | 6.3± 0.3 0.001 ↑ | 42.8± 4.6 N.S. | 0.7± 0.02 0.001 ↑ | 19.7± 0.4 0.001 ↑ | 30.6± 0.3 0.001 ↑ | 61.4± 1.8 0.001 ↑ | 2.8± 0.04 N.S. |
| Insulin V.S. Insulin + cortisone | 0.001 | N.S. | 0.01 | N.S. | 0.01 | 0.01 | 0.001 | N.S. | 0.001 | N.S. | 0.001 | 0.001 | 0.001 | N.S. | 0.001 | N.S. |
| Insulin V.S. cortisone | 0.001 | N.S. | N.S. | 0.001 | 0.001 | 0.001 | 0.001 | N.S. | 0.001 | N.S. | N.S. | 0.001 | 0.001 | 0.01 | 0.001 | N.S. |
| Insulin + cortisone V.S. cortisone | 0.001 | N.S. | 0.01 | 0.001 | 0.001 | 0.001 | 0.02 | N.S. | 0.001 | N.S. | 0.001 | 0.001 | 0.001 | 0.05 | 0.05 | N.S. |

N.S. = Not Significant at the 5% level

↑ = Significantly higher

↓ = Significantly lower

## Table (2)

**Blood glucose, pyruvic acid, lactic acid, and serum levels of C.E., A.P., AST, ALT and total protein levels in male and female *Bufo regularis Reuss* in August**

| Group | Males | | | | | | | | Females | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glucose mg/100ml | Pyruvic acid mg/100ml | Lactic acid mg/100ml | C.E. mg/ml | A.P mg/100ml | AST u/ml | ALT u/ml | Total protein g/100ml | Glucose mg/100ml | Pyruvic acid mg/100ml | Lactic acid mg/100ml | C.E. mg/ml | A.P. mg/100ml | AST u/ml | ALT u/ml | Total protein g/100 ml |
| Control mean ± S.E. | 71.107± 7.709 | 2.8+ 0.19 | 22.6± 1.6 | 0.26± 0.016 | 11.19± 0.28 | 25.5± 0.34 | 42.6± 2.06 | 2.75± 0.053 | 72.219± 6.013 | 3.1± 0.2 | 25.7± 1.65 | 0.34± 0.019 | 11.64± 0.32 | 26.46± 0.3 | 40.46± 1.59 | 2.75± 0.04 |
| Normal mean ± S.E. | 54.44± 6.797 | 2.3± 0.11 | 22.1± 2.2 | 0.27± 0.02 | 7.32± 0.31 | 25.7± 0.37 | 39.13± 1.4 | 2.67± 0.039 | 66.66± 9.056 | 2.6± 0.187 | 22.4± 2.02 | 0.26± 0.019 | 7.96± 0.34 | 24.7± 0.3 | 39.8± 1.6 | 2.77± 0.038 |
| Control V.S. normal P < | N.S. | 0.05 ↓ | N.S. | N.S. | 0.001 ↓ | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | 0.01 ↓ | 0.001 ↓ | 0.001 ↓ | N.S. | N.S. |
| Insulin mean ± S.E. | 23.33± 12.47 | 5.45± 2.01 | 91.35± 4.8 | 0.26± 0.015 | 10.37± 0.3 | 28.13± 0.33 | 42.6± 1.7 | 2.99± 0.047 | 6.66± 39.17 | 4.45± 0.30 | 96.6± 3.1 | 0.29± 0.016 | 12.0± 0.36 | 28.06± 0.40 | 44.06± 1.68 | 2.78± 0.057 |
| Control V.S. Insulin P < | 0.01 ↓ | N.S. | 0.001 ↑ | N.S. | N.S. | 0.001 ↑ | N.S. | 0.01 ↑ | 0.001 ↓ | 0.001 ↑ | 0.001 ↑ | N.S. | N.S. | 0.01 ↑ | N.S. | N.S. |
| Insulin + Cortisone mean ± S.E. | 13.108± 15.589 | 8.08± 0.2 | 34.79± 1.8 | 0.42± 0.018 | 12.86± 0.349 | 30.09± 0.3 | 49.2± 2.12 | 2.7± 0.051 | 173.32± 17.72 | 7.86± 0.3 | 42.8± 3.5 | 0.37± 0.029 | 13.58± 0.34 | 29.36± 0.36 | 59.33± 2.016 | 2.8± 0.065 |
| Control V.S. Insulin + Cortisone P < | 0.01 ↑ | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | 0.05 ↑ | N.S. | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | N.S. | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | N.S. |
| Cortisone mean ± S.E. | 205.55± 14.05 | 2.8± 0.2 | 34.00± 2.57 | 0.55± 0.024 | 19.06± 0.428 | 31.79± 0.26 | 63.00± 0.94 | 2.91± 0.063 | 293.32± 23.61 | 2.8± 0.3 | 30.79± 2.24 | 0.51± 0.023 | 20.0± 0.26 | 31.8± 0.23 | 65.13± 1.895 | 2.98± 0.08 |
| Control V.S. cortisone P < | 0.001 ↑ | N.S. | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | N.S. | 0.001 ↑ | N.S. | N.S. | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | 0.001 ↑ | N.S. |
| Insulin V.S. Ins. + Cort. | 0.001 | N.S. | 0.001 | 0.001 | 0.001 | 0.001 | 0.05 | 0.001 | 0.001 | 0.001 | 0.001 | 0.05 | 0.01 | 0.05 | 0.001 | N.S. |
| Insulin V.S. Cortisone | 0.001 | N.S. | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | N.S. | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | N.S. |
| Ins. + Cort. V.S. Cortisone | 0.01 | 0.001 | N.S. | 0.001 | 0.001 | 0.001 | 0.001 | 0.02 | 0.001 | 0.001 | 0.01 | 0.001 | 0.001 | 0.001 | 0.05 | N.S. |

N.S. = Not Significant at the 5% level; ↑ = Significantly high; ↓ =Significantly low

## Table (3)

**Muscular glycogen, pyruvic acid, Lactic acid, C.E., A.P., AST, ALT levels in Male and Female *Bufo regularis Reuss* in June**

| Group | Males | | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P mg/100g | AST u/mg | ALT u/mg | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P. mg/100g | AST u/mg | ALT u/mg |
| Control mean ± S.E. | 2.5± 0.45 | 6.23± 0.75 | 253.96± 21.09 | 13.5± 1.59 | 2.62± 0.175 | 8.97± 0.50 | 15.9± 1.25 | 6.12± 0.45 | 5.64± 0.70 | 229.22± 19.66 | 8.60± 0.49 | 2.02± 0.11 | 6.99± 0.23 | 12.40± 1.02 |
| Normal mean ± S.E. | 1.98± 0.189 | 6.344± 0.82 | 218.3± 25.26 | 11.99± 1.55 | 2.04± 0.13 | 8.22± 0.37 | 18.4± 0.7 | 9.64± 1.26 | 6.49± 0.80 | 337.25± 43.15 | 7.45± 1.25 | 1.50± 0.10 | 5.97± 0.35 | 13.15± 0.84 |
| Control V.S Normal P < | N.S. | N.S. | N.S. | N.S. | 0.02↓ | N.S. | N.S. | 0.02↑ | N.S. | 0.05↑ | N.S. | 0.001↓ | 0.02↓ | N.S. |
| Insulin mean ± S.E. | 7.32± 1.8 | 9.23± 0.52 | 262.09± 15.03 | 10.51± 0.9 | 3.39± 0.159 | 8.9± 0.4 | 20.09± 0.99 | 6.22± 0.48 | 6.20± 0.55 | 238.90± 20.77 | 8.17± 0.99 | 3.20± 0.23 | 8.61± 0.35 | 19.13± 0.68 |
| Control V.S. Insulin P < | 0.02↑ | 0.01↑ | N.S. | N.S. | 0.01↑ | N.S. | 0.02↑ | N.S. | N.S. | N.S. | N.S. | 0.001↑ | 0.001↑ | 0.001↑ |
| Insulin + Cortisone mean ± S.E. | 11.86± 1.24 | 7.4± 0.46 | 164.38± 16.84 | 10.58± 0.92 | 3.49± 0.164 | 9.13± 0.35 | 19.8± 0.69 | 14.25± 2.21 | 6.95± 0.40 | 164.09± 13.07 | 10.60± 0.70 | 3.45± 0.20 | 8.35± 0.51 | 17.79± 1.11 |
| Control V.S. Insulin + Cortisone P < | 0.001↑ | N.S. | 0.01↓ | N.S. | 0.01↑ | N.S. | 0.01↑ | 0.01↑ | N.S. | 0.01↓ | N.S. | 0.001↑ | 0.05↑ | 0.01↑ |
| Cortisone mean ± S.E. | 13.9± 0.86 | 8.8± 0.9 | 220.13± 17.32 | 18.6± 1.24 | 4.85± 0.2 | 8.76± 0.41 | 19.7± 0.84 | 12.96± 0.84 | 5.35± 0.39 | 189.69± 12.30 | 17.01± 0.77 | 4.97± 0.26 | 8.92± 0.53 | 20.27± 1.24 |
| Control V.S. Cortisone P < | 0.001↑ | 0.05↑ | N.S. | 0.02↑ | 0.001↑ | N.S. | 0.02↑ | 0,001↑ | N.S. | N.S. | 0.001↑ | 0.01↑ | 0.01↑ | 0.001↑ |
| Insulin V.S. Ins. + Cort. | 0.05 | 0.02 | 0.001 | N.S. | N.S. | N.S. | N.S. | 0.01 | N.S. | 0.01 | N.S. | N.S. | N.S. | N.S. |
| Insulin V.S. Cortisone | 0.01 | N.S. | N.S. | 0.001 | 0.001 | N.S. | N.S. | 0.001 | N.S. | 0.05 | 0.001 | 0.001 | N.S. | N.S. |
| Ins. + Cort. V.S. Cortisone | N.S. | N.S. | 0.05 | 0.001 | 0.001 | N.S. | N.S. | N.S. | 0.02 | N.S. | 0.001 | 0.001 | N.S. | N.S. |

N.S. = Not Significant at the 5% level; ↑ = Significantly high; ↓ =Significantly low

## Table (4)

### Muscular glycogen, pyruvic acid, Lactic acid, C.E., A.P., AST, ALT in Male and Female *Bufo regularis Reuss* in August

| Group | Males | | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P mg/100g | AST u/mg | ALT u/mg | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P. mg/100g | AST u/mg | ALT u/mg |
| Control mean ± S.E. | 1.88± 0.14 | 7.40± 1.05 | 190.82± 12.95 | 9.60± 0.79 | 2.27± 0.10 | 7.24± 0.47 | 13.47± 0.70 | 6.69± 0.33 | 6.80± 0.57 | 154.84± 7.11 | 7.47± 0.63 | 1.88± 0.50 | 6.43± 0.39 | 11.65± 1.06 |
| Normal mean ± S.E. | 2.80± 0.34 | 5.78± 0.60 | 127.07± 6.32 | 11.49± 0.77 | 1.95± 0.10 | 7.66± 0.28 | 16.17± 0.86 | 4.30± 0.53 | 6.67± 1.27 | 206.15± 16.97 | 11.40± 1.10 | 1.90± 0.10 | 7.34± 0.46 | 15.82 1.22 |
| Control V.S Normal P < | 0.05↑ | N.S. | 0.001↑ | N.S. | 0.05↓ | N.S. | 0.05↑ | 0.02↑ | N.S. | 0.01↑ | 0.01↑ | N.S. | N.S. | 0.02↑ |
| Insulin mean ± S.E. | 2.07± 0.16 | 6.40± 0.50 | 264.60± 21.70 | 12.25± 0.80 | 3.09± 0.19 | 7.40± 0.36 | 17.73± 1.01 | 2.78± 0.48 | 5.87± 0.58 | 338.02± 14.61 | 14.46± 10.90 | 3.16± 0.16 | 7.60± 0.42 | 18.24± 1.30 |
| Control V.S. Insulin P < | N.S. | N.S. | 0.01↑ | 0.05↑ | 0.001↑ | N.S. | 0.01↑ | N.S. | N.S. | 0.001↑ | N.S. | 0.001↑ | N.S. | 0.001↑ |
| Insulin + Cortisone mean ± S.E. | 8.95± 0.76 | 14.80± 0.89 | 265.70± 23.85 | 15.78± 1.40 | 4.03± 0.29 | 8.75± 0.50 | 20.53± 1.08 | 10.90± 0.50 | 13.35± 0.90 | 204.28± 13.16 | 15.70± 0.98 | 3.66± 0.20 | 7.30± 0.53 | 18.89± 0.98 |
| Control V.S. Insulin + Cortisone P < | 0.001↑ | 0.001↑ | 0.02↑ | 0.001↑ | 0.001↑ | 0.05↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.01↑ | 0.001↑ | 0.001↑ | N.S. | 0.001↑ |
| Cortisone mean ± S.E. | 9.67± 0.48 | 11.50± 0.98 | 206.66± 12.70 | 20.67± 1.16 | 5.70± 0.33 | 10.47± 0.59 | 24.14± 1.24 | 5.87± 0.61 | 12.57± 0.89 | 226.48± 5.89 | 18.44± 0.91 | 5.35± 0.27 | 9.69± 0.43 | 22.56± 1.18 |
| Control V.S. Cortisone P < | 0.001↑ | 0.01↑ | N.S. | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ |
| Insulin V.S. Ins. + Cort. | 0.001 | 0.001 | N.S. | 0.05 | 0.01 | 0.05 | N.S. | 0.001 | 0.001 | N.S. | N.S. | N.S. | N.S. | N.S. |
| Insulin V.S. Cortisone | 0.001 | 0.001 | 0.005 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | N.S. | 0.001 | 0.01 | 0.05 |
| Ins. + Cort. V.S. Cortisone | N.S. | 0.02 | 0.05 | 0.02 | 0.001 | 0.05 | 0.05 | 0.001 | N.S. | N.S. | 0.05 | 0.001 | 0.01 | 0.05 |

N.S. = Not Significant at the 5% level; ↑ = Significantly high; ↓ =Significantly low

## Table (5)

### Hepatic glycogen, pyruvic acid, Lactic acid, C.E., A.P., AST, ALT in Male and Female *Bufo regularis Reuss* in June

| Group | Males | | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P mg/100g | AST u/mg | ALT u/mg | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P. mg/100g | AST u/mg | ALT u/mg |
| mean ± S.E. Control | 4.96± 0.4 | 15.03± 1.08 | 64.85± 9.468 | 5.9± 0.68 | 5.23± 0.44 | 6.5± 0.5 | 10.36± 1.04 | 4.28± 0.35 | 14.68± 1.53 | 38.61± 3.759 | 6.99± 0.88 | 6.04± 0.54 | 7.27± 0.58 | 11.52± 1.12 |
| Normal mean ± S.E. | 3.4± 0.9 | 18.3± 3.00 | 114.97± 21.33 | 5.25± 0.78 | 7.75± 0.622 | 4.2± 0.35 | 6.05± 0.26 | 3.3± 0.5 | 15.29± 2.52 | 71.12± 12.94 | 4.5± 0.86 | 8.28± 0.64 | 4.74± 0.45 | 6.09± 0.54 |
| Control V.S Normal P < | N.S. | N.S. | 0.05↑ | N.S. | 0.01↑ | 0.001↓ | 0.001↓ | N.S. | N.S. | 0.05↑ | 0.05↓ | 0.02↑ | 0.01↓ | 0.001↓ |
| Insulin mean ± S.E. | 2.4± 0.23 | 19.968± 2.198 | 95.5± 14.88 | 6.3± 0.6 | 9.388± 0.595 | 7.67± 0.45 | 13.1± 0.98 | 4.13± 0.78 | 12.84± 1.24 | 64.73± 5.65 | 5.96± 0.64 | 9.53± 0.711 | 7.36± 0.44 | 14.2± 0.75 |
| Control V.S. Insulin P < | 0.001↓ | N.S. | N.S. | N.S. | 0.001↑ | N.S. | N.S. | N.S. | N.S. | 0.001↑ | N.S. | 0.001↑ | N.S. | 0.05↑ |
| Insulin + Cortisone mean ± S.E. | 7.5± 0.76 | 12.7± 1.5 | 102.88± 13.059 | 8.95± 0.72 | 11.52± 0.569 | 9.13± 0.47 | 15.48± 0.936 | 4.2± 0.47 | 12.55± 0.95 | 103.62± 21.88 | 8.98± 0.86 | 12.36± 0.58 | 9.58± 0.44 | 16.8± 0.89 |
| Control V.S. Insulin + Cortisone P < | 0.01↑ | N.S. | 0.05↑ | 0.01↑ | 0.001↑ | 0.01↑ | 0.01↑ | N.S. | N.S. | 0.01↑ | N.S. | 0.001↑ | 0.01↑ | 0.01↑ |
| Cortisone mean ± S.E. | 5.2± 0.57 | 13.466± 0.94 | 98.07± 12.85 | 12.96± 1.2 | 14.74± 0.917 | 8.0± 0.45 | 14.5± 0.75 | 10.089± 1.249 | 7.036± 0.47 | 68.23± 2.9 | 17.4± 1.3 | 23.74± 1.86 | 10.26± 0.59 | 18.012± 1.028 |
| Control V.S. Cortisone P < | N.S. | N.S. | 0.05↑ | 0.001↑ | 0.001↑ | 0.05↑ | 0.01↑ | 0.001↑ | 0.001↓ | 0.001↑ | 0.001↑ | 0.001↑ | 0.01↑ | 0.001↑ |
| Insulin V.S. Ins. + Cort. | 0.001 | 0.02 | N.S. | 0.01 | 0.02 | N.S. | N.S. | N.S. | N.S. | N.S. | 0.02 | 0.01 | 0.01 | N.S. |
| Insulin V.S. Cortisone | 0.001 | 0.02 | N.S. | 0.001 | 0.001 | N.S. | N.S. | 0.001 | 0.001 | N.S. | 0.001 | 0.001 | 0.001 | 0.01 |
| Ins. + Cort. V.S. Cortisone | 0.05 | N.S. | N.S. | 0.01 | 0.01 | N.S. | N.S. | 0.001 | 0.001 | N.S. | 0.001 | 0.001 | N.S. | N.S. |

N.S. = Not Significant at the 5% level; ↑ = Significantly high; ↓ =Significantly low

## Table (6)

### Hepatic glycogen, pyruvic acid, Lactic acid, C.E., A.P., AST, ALT in Male and Female *Bufo regularis Reuss* in August

| Group | Males | | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P mg/100g | AST u/mg | ALT u/mg | Glycogen g/100g | Pyruvic acid mg/100g | Lactic acid mg/100g | C.E. mg/g | A.P. mg/100g | AST u/mg | ALT u/mg |
| Control mean ± S.E. | 4.8 ±0.44 | 8.07 ±0.59 | 96.8 ±8.91 | 6.84 ±0.65 | 9.25 ±0.54 | 7.4 ±0.44 | 11.85 ±0.66 | 4.17 ±0.35 | 10.8 ±0.69 | 68.22 ±4.97 | 6.53 ±0.71 | 8.09 ±0.51 | 6.42±0.36 | 10.52 ±0.72 |
| Normal mean ± S.E. | 3.17 ±0.37 | 7.3 ±0.64 | 116.6 ±11.03 | 8.94 ±0.77 | 9.45 ±0.79 | 7.68 ±0.52 | 11.68 ±0.8 | 3.3 ±0.41 | 7.42 ±0.44 | 119.41 ±7.48 | 7.84 ±0.59 | 9.07 ±0.53 | 7.09±0.4 | 9.57 ±0.76 |
| Control V.S Normal P < | 0.01↓ | N.S. | N.S | N.S. | N.S. | N.S. | N.S. | N.S. | 0.001↑ | 0.001↑ | N.S. | N.S. | N.S. | N.S. |
| Insulin mean ± S.E. | 5.9 ±0.49 | 19.19 ±0.85 | 159.113±7.79 | 11.73 ±0.89 | 9.4 ±0.42 | 7.14 ±0.3 | 13.28 ±0.7 | 6.04 ±0.55 | 17.55 ±0.5 | 101.24±8.44 | 111.56 ±0.88 | 10.23 ±0.54 | 7.77±0.4 | 15.84 ±0.86 |
| Control V.S. Insulin P < | N.S. | 0.001↑ | 0.001↑ | 0.001↑ | N.S. | N.S. | N.S. | 0.01↑ | 0.001↑ | 0.01↑ | 0.001↑ | N.S. | 0.002↑ | 0.001↑ |
| Insulin + Cortisone mean ± S.E. | 6.49 ± 0.33 | 24.99 ± 0.95 | 415.55 ± 34.914 | 12.35 ± 1.02 | 10.74 ± 0.59 | 8.16 ± 0.42 | 14.5 ± 0.79 | 6.4 ± 0.43 | 22.9 ± 1.66 | 37.01 ± 16.06 | 13.18 ± 0.97 | 11.8 ± 0.51 | 9.3 ± 0.48 | 15.9 ± 1.11 |
| Control V.S. Insulin + Cortisone P < | 0.01↑ | 0.001↑ | 0.001↑ | 0.001↑ | N.S. | N.S. | 0.02↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.01↑ | 0.001↑ | 0.001↑ |
| Cortisone mean ± S.E. | 16.89 ± 1.013 | 23.75 ± 1.14 | 512.18 ± 40.69 | 12.43 ± 1.199 | 19.89 ± 1.00 | 9.05 ± 0.45 | 16.92 ± 0.96 | 11.59 ± 0.6 | 18.38 ± 1.076 | 403.04 ± 21.35 | 4.14 ± 1.23 | 22.7 ± 1.24 | 9.88 ± 0.5 | 18.03 ± 0.88 |
| Control V.S. Cortisone P < | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.02↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ | 0.001↑ |
| Insulin V.S. Ins. + Cort. | N.S. | 0.001 | 0.001 | N.S. | N.S. | N.S. | N.S. | N.S. | 0.01 | 0.001 | N.S. | N.S. | 0.02 | N.S. |
| Insulin V.S. Cortisone | 0.001 | 0.01 | 0.001 | N.S. | 0.001 | 0.01 | 0.01 | 0.001 | N.S. | 0.001 | N.S. | 0.001 | 0.01 | N.S. |
| Ins. + Cort. V.S. Cortisone | 0.001 | N.S. | N.S. | N.S. | 0.001 | N.S. | N.S. | 0.001 | 0.05 | N.S. | N.S. | 0.001 | N.S. | N.S. |

N.S. = Not Significant at the 5% level; ↑ = Significantly high; ↓ =Significantly low

## Table (7)

### Serum protein factions of *Bufo regularis Reuss* (percentage)

| Protein Fractions | Control 1 | Control 2 | Normal | Insulin | Ins. + Cortisone | Cortisone | Insulin V.S. Insulin + Cortisone P < | Insulin V.S. Cortisone P < | Insulin + Cortisone V.S. Cortisone P< |
|---|---|---|---|---|---|---|---|---|---|
| (1)<br>Mean ± S.E.<br>P < V.S. Controls | 30.7±1.744 | 30.24±1.74 | 27.64±1.69<br>N.S. | 30.87±2.74<br>N.S. | 28.0.6±0.69<br>N.S. | 29.84±1.24<br>N.S. | N.S. | N.S. | N.S. |
| (2)<br>Mean ± S.E.<br>P < V.S. Controls | 44.76±2.32 | 39.114±3.37 | 45.99±0.7<br>N.S. | 51.76±1.46<br>0.01↑ | 53.5±1.52<br>0.01↑ | 51.4±1.014<br>0.01↑ | N.S. | N.S. | N.S. |
| (3)<br>Mean ± S.E.<br>P < V.S. Controls | 15.23±1.3 | 14.17±3.31 | 12.92±0.67<br>N.S. | 10.07±2.09<br>N.S. | 11.85±1.23<br>N.S. | 11.56±0.65<br>N.S. | N.S. | N.S. | N.S. |
| (4)<br>Mean ± S.E.<br>P < V.S. Controls | 6.3±1.8 | 10.7±3.6 | 3.35±0.296<br>N.S. | 1.694±0.46<br>0.02↑ | = 0 =<br>0.001↓ | = 0 =<br>0.001↓ | 0.01 | 0.01 | N.S. |
| (5)<br>Mean ± S.E.<br>P < V.S. Controls | 3.012±1.7 | 2.32±0.92 | 9.42±0.848<br>0.01↓ | 5.57±1.38<br>0.05↓ | 5.94±2.49<br>N.S. | 7.17±1.43<br>N.S. | N.S. | N.S. | N.S. |

N.S. = Not Significant at the 5% level; ↑ = Significantly high; ↓ =Significantly low

in *Bufo regularis* ranged, in liver, between 3.4±0.9 - 4.96±0.4 in males and 3.3±0.5 - 4.28±0.35 g/100gm in females, in June and between 3.17±0.37 and 4.8±0.44 in males and between 3.3±0.41 and 4.17±0.35 in famales in August, and in muscle it ranged between 1.88 0.14 and 2.8 0.34 in males and between 4.3±0.53 and 6.69±0.33 in females in August, which are similar to the values reported by Fouda [15] in July but his values were much lower in December. However, the values obtained by Abdel Daium [14] were lower both in July and February.

Presently, no sex differences were found in levels of blood glucose or liver glycogen either in June or in August (Table 1, 2, 5, 6). On the other hand, in case of muscle glycogen in June, the male values were found to be significantly higher than the female values and the opposite was found to be true in August (Tables 3,4).

Byrne and White [18] recorded measurable differences in energy interconversions associated with reproduction as well as with seasonal variations. Accordingly, the presently observed differences in muscle glycogen between males and females could be related to differences between the two sexes regarding breeding. This observation has been noted by some other workers [19, 20, 21, 22].

The total serum protein value in the normal untreated samples of *Bufo regularis* studied presently ranged between 2.6±0.04 and 2.8±0.04 g/100 ml with no sex differences. No significant differences were also found between the values of total serum proteins obtained in June and those obtained in August, except in the case of males injected with insulin, where proteins increased ($p<0.01$) (Table 1).

In Amphibia, total serum protein values ranged between 1.97±0.12 and 4.8 mg% as have been previously reported [15,23]. Fouda [15] also reported no sex or seasonal differences in the total serum protein values. Accordingly in the present study the electrophoretic studies of serum performed on samples of animals composed of equal number of males and females and also the results obtained in June and those obtained in August are presented together.

Five electrophoretic serum protein fractions were obtained and are presented in table 7, where, generally, in Amphibia between 5 and 9 electrophoretic serum protein fractions have been reported [24,25].

Liver AST and ALT activities were always found presently to be higher in August than in June both in male and female animals (Tables 5, 6). In August the temperature was between 30-39°C and in June it was between 28-34°C. Pasanen and Koskela [26,27] observed higher AST activities in Amphibia in summer. They considered this as indicating high rate of protein synthesis. They also stated that the presence of high mineral concentrations in various organs during the summer supports the notion of high protein synthesis. Pasanen [28] pointed out that his increased activity of AST and ALT in summer seems to be endogenous since it continues even though the frogs are in continuous darkness and without food. With few exceptions, liver and muscle cholinesterase activities were also found to be higher in August than in June. However, this was not found to be true regarding alkaline phosphatase activities (Tables 3,4,5,6).

Carbohydrate metabolism is highly governed by the endocrine system. Hanke and Neumann [29] indicated that there are three systems regulating carbohydrate metabolism in Amphibia, the hormones of the pancreatic islets, the corticosteroids, and the adrenocorticotrophic hormones and the system of the thyroid hormones and the thyroid stimulating hormones. They stated that the islet tissues with both types of endocrine pancreatic cells (a and 5) are present in Amphibia. The intraperitoneal injection of 1 I.U. of Insulin daily for 4 days lowered significantly the blood glucose levels in all samples of *Bufo regularis* studied presently after 24 hours of the last injection (Tables 1, 2). However, the results obtained for the effect of insulin on liver and muscle glycogen as well as for blood, liver and muscle pyruvic and lactic acid values were found to be variable (Tables 1, 2, 3, 4, 5, 6).

This result is in agreement with the idea of Han and Neuman, [29] that in Amphibia insulin sometimes affects liver and muscle glycogen and sometimes it does not. We also add that the effect is sometimes an increase and sometimes is a decrease depending on the whole metabolism of the animal as Hank and Neuman stated. Variable effects of insulin on the liver and muscle glycogen in Amphibia has been also noted [30].

Insulin affects carbohydrate metabolism in a number of ways including increased entry of glucose into cells, increased utilization of glucose,, by various tissues and decreased production of glucose (gluconegenesis) by the liver. The net action of all the above effects of insulin is to decrease the blood glucose level. In this action insulin stands alone against an array of hormones that attempt to counteract its effect. This with no doubt represents one of the organism's most important defence mechanisms since prolonged hyperglycemia poses a potentially lethal threat to the brain and must be avoided [34].

The hypoglycemic action of insulin has been demonstrated in all vertebrate groups from the cyclostomes to the primates [32, 33], however, in contrast to mammals, the hypoglycemic effect in Amphibia may be severe without producing convulsions or causing death [34].

In general, non mammalian species require relatively larger doses of insulin to lower the blood sugar, while they respond more slowly and show less severe general reactions. The effects of injected insulin vary greatly even among mammals, probably, because of differences in dietary habits, herbivorous species are less dependent on insulin than are carnivorous species [2]. Normal blood sugar

levels in frogs and toads are generally lower than one finds in other vertebrate animals [16, 35] and they do have easily available glycogen reservoirs which can be quickly mobilized into blood glucose on demand (36). This explains the variable effects of the injected insulin observed on liver and muscle glycogen tissue and blood pyruvic and lactic acids that represents different metabolic steps in the quick exchange between tissue and blood carbohydrates. In this respect, Murray et Al [31] stated that metabolic regulation cannot be discussed in the context of a single hormone or metabolite.

Regulation is a complex process in which the flux through a given pathway is the result of the interplay of a number of hormones and metabolites. Accordingly in the present investigation the effect of cortisone administration either alone or together with insulin has been also studied. The intraperitoneal injection of 1 ug. of cortisone daily for four days increased blood glucose and liver and muscle glycogen significantly (Tables 1, 2, 3, 4, 5 6). Hanke [37] indicated that since it was found in Amphibia that ACTH, corticosterone and aldosterone gave quantitatively similar results on carbohydrate metabolism, firm distinctions between glucocorticoids and mineralocorticoids cannot be made and the use of these terms in Amphibia may in part be inappropriate.

Accordingly in the present study the we have compared the results that have been obtained using cortisone with those reported by other investigators using other types of corticoseroids. Beaumount [38] reported that induction of hepatic glycogen accumulation was due to treatment of larval Amphibians with ACTH or cortisol. Similarly, Hanke and Leist [17] found that body fluid glucose and liver and tissue glycogen increased in Amphibian larvae during premetamorphosis and at metamorphic climax and of juveniles after ACTH, corticosterone or cortisol administration, aldosterone was not as effective as other corticoids. In the present study cortisone injection increased the levels of blood lactate and blood pyruvate (Table 1, 2]. Hanke [37] reported similar findings in the level of body fluid lactate of early larval stages in Amphibia.

Generally, with few exceptions, cortisone administration increased significantly AST, ALT, Alkaline phosphatase and cholinesterase levels in serum, liver and muscle tissues of all samples *of Bufo regularis* studied presently (Tables 1, 2, 3, 4, 5, 6). Similar findings regarding AST and ALT has been noted by Hanke [22]. Murray et al. [31] indicated that glucocorticoids increase glucose production in the liver by different mechanisms. They can increase the delivery of amino acids (the gluconeogenic substance) from peripheral tissue; an effect that can partly explain the increase of AST and ALT activities due to cortisone administration. Glucocorticoids also increase the rate of gluconeogenesis by increasing the amount (and activity) of several key enzymes and "permitting" other key metabolic reactions to operate at maximal rates and promote protein metabolism which might thus explain the increased levels and activities of all metabolites and enzymes investigated, namely blood lactate and pyruvate, AST, ALT, A.P., C.E. activities in blood, liver and muscle tissues.

In the present study when insulin was injected together with cortisone it failed to antagonize the effects of cortisone on blood glucose, lactic and pyruvic acids in August. However, the effect was found to be variable in June (Tables 1, 2). Varley et al. [39] discussed the interactions insulin and the glucocorticoids. They stated that the stress of hypoglycemia induced by insulin administration stimulates the hypothalamus to release corticotrophin releasing hormones (CRH) which causes a marked increase in plasma adrenocorticotrophic hormone (ACTH) that increases plasma cortisol level. In this respect Hanke and Pehlemann [40] and Janssens [41] indicated that in Amphibia, the effects induced by Islet hormones or corticosteroids partially depend on some other parameters including season and diet. In this respect Gorbman et al [42] stated that it is difficult to rationalize the pattern of glucocorticoid action on intermediary metabolism unless we place it in the context of adaptation to stress. One of the primary stimuli of ACTH secretion is physiological or traumatic stress. Typical stresses can be starvation, disease or other harmful states during which feeding and the nutritional status of the organism are seriously compromised.

This also recalls the notation to Murray et al [31] that glucocorticoids have anabolic effects at physiological levels, but can be catabolic in certain conditions and at higher than physiological levels.

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