Niger. J. Physiol. Sci. 32(June 2017) 21-25
www.njps.com.ng

Niger. J. Physiol. Sci.

Effect of Cortisol on Plasma Lactate Levels following Cortisol-induced Hyperglycaemia in Common African Toad, *Bufo regularis*

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Summary: Previous studies in man have shown that cortisol induces hyperglycemia through gluconeogenesis. However, the metabolic substrates involved in cortisol-induced hyperglycemia and the role of adrenergic receptors in lactate production in toads have not been well studied. This study investigated the effects of adrenergic receptor blockers in cortisol-induced hyperglycemia and blood lactate levels in the common African toad (*Bufo regularis*). Each toad was fasted and anaesthetized with sodium thiopentone given intraperitoneally (50mg/kg/i.p). The animals (control) received 0.7% amphibian saline while animals (untreated) received cortisol intravenously (50µg/kg/i.v). In pre-treatment groups, animals received propanolol (0.5 mg/kg/i.v), prazosin (0.2 mg/kg/i.v) and combination of propanolol (0.5mg/kg/i.v) and prazosin (0.2 mg/kg/i.v) respectively followed by administration of cortisol 50µg/kg/i.v. Thereafter, blood samples were collected for estimation of glucose and lactate using the modified glucose oxidase method and colorimetric method respectively. Cortisol caused significant increase in blood glucose level (($p<0.05$) and reduction in blood lactate levels. Pre-treatment with Prazosin (0.2 mg/kg/i.v) caused significant ($p<0.05$) increase in blood glucose level and significant reduction in blood lactate levels while pre-treatment with Propanolol (0.5mg/kg/i.v) abolished cortisol-induced hyperglycemia and caused increase in blood lactate levels compared with the untreated group. The combination of both blockers abolished the hyperglycemic effect of cortisol and caused increase in the blood lactate levels. The results of this study show that cortisol-induced hyperglycemia is a consequent of gluconeogenesis and mediated through the beta-adrenergic receptors. The results also show that lactate is produced and used as a gluconeogenic substrate to induce cortisol hyperglycemia in the Common African toad *bufo regularis*. The beta adrenergic receptors are involved in the use of lactate to induce cortisol hyperglycemia in the Common African toad *Bufo-regularis*.

Keywords*:* Cortisol, Hyperglycemia, Lactate, Prazosin, Propanolol, Common African toad

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Manuscript Accepted: May, 2017

INTRODUCTION

The essential energy metabolic substrate of the body cells is glucose. It enters the circulation either from endogenous sources through glycogenolysis and gluconeogenesis or from external sources via the digestive tract or intravenously (Butler and Rizza, 1989). Catecholamines and glucagon stimulate glycogenolysis and gluconeogenesis while cortisol stimulates the latter. Cortisol, glucagon and the catecholamines are called counter-regulatory hormones because they oppose the effects of insulin and act synergistically to increase hepatic glucose production. Lactate and pyruvate are from glycogenolysis and glycolysis in peripheral tissue especially muscle (Brooks, 1985). Iñigo San Millán (2014) reported lactate as a key regulator of intermediary metabolism, regulating substrate utilization. Lactate is crucial for the brain as the main fuel that neurons use and it is essential for long-term memory and could even be involved in Alzheimer's disease. It is not only an anaerobic metabolic end product, but it also signals to the animal its unfavourable situation and causes it to move to a more stable environment (Iñigo San Millán, 2014). Although, lactate is produced in all tissues, skeletal muscle, brain, red blood cells, and renal medulla are responsible for the majority of its production. In healthy individuals, there is a continuous cycle of lactate production and metabolism, which ensures that blood lactate concentrations are normally low (De Backer, 2003). Higher blood lactate concentrations occur when lactate production exceeds clearance or when clearance capacity is decreased or more frequently when both occur simultaneously (Manikis *et al*, 1995).

Cortisol, a steroid hormone, causes hyperglycemia through gluconeogenesis (Baxter, 1979; Renauld and Moon, 1980; Khani and Tayek, 2001). Previous studies in humans showed that cortisol can increase hepatic glucose production and blood glucose levels through gluconeogenesis (Khani and Tayek, 2001). Glucocorticoids enhance beta receptor mediated

responses, myocardial contractility, hepatic and muscle glucose metabolism (Davies and Lefkowtiz, 1984).

It has been shown in a previous study that beta adrenergic receptors played dominant role in cortisol-induced hyperglycemia in toads (Isehunwa *et al*, 2013). Previous studies in humans (Khani and Tayek, 2001) and reptiles (Renauld and Moon, 1980) have shown the effect of cortisol on lactate production but there is little or no information on the effect of cortisol on lactate production in the toads. The present study investigated the effect of cortisol on plasma levels of lactate in cortisol-induced hyperglycemia and the possible involvement of adrenergic receptors in lactate production. The study also tried to relate the role of lactate production in the hyperglycemia induced by cortisol in the common African toad *Bufo regularis*.

MATERIALS AND METHODS

Experimental Design

One hundred adult toads (*bufo regularis*) of both sexes weighing between 65-110g were used for the study. The toads obtained from the banks of slow-moving streams, around ponds and wet bushes were randomly picked as found during the night search. Hence, selection of the animal is unbiased. Each animal was fasted 24 h and anaesthesized with sodium thiopentone 50mg/kg body weight given intraperitoneally. The animal was secured on its back on a dissecting board. The truncus arteriosus was dissected free from surrounding connective tissue and used for blood collection. The anterior abdominal vein was cannulated for drug injection. Each toad was heparinised (170 units/0.1 ml) and allowed 30 mins stabilization. After stabilization period, basal blood collection (0 min) was made from the truncus arteriosus. The animals were randomly divided into five groups (1-V) of 20 toads per group. Toads in group I (control) received intravenous (i.v) injection of 0.65% amphibian saline while toads in group II (untreated) received cortisol (50µg/kg i.v).Toads in groups III, IV and V were pre-treated with propranolol (0.5mg/kg i.v), prazosin (0.2mg/kg i.v) or combination prazosin (0.2mg/kg i.v) and propranolol (0.5mg/kg i.v) respectively, 30 mins thereafter cortisol (50µg/kg i.v) is injected. In each animal, 0.05 ml per sample was drawn directly from the truncus arteriousus for glucose determination. Blood samples were collected at time interval of 0, 30, 60, and 90 mins, post-injection. Each drug injection was in a total volume between 0.1 and 0.12 ml given intravenously through the anterior abdominal vein cannula. Blood glucose was determined immediately using modified glucose oxidase method of Trinder (1969). Because of the small size of the toad, animals were sampled only once in each experiment and then sacrificed.

Determination of Blood Lactate Levels.

Blood lactate was determined using the colorimetric method by Taylor (1996). 1ml of blood was added to 2ml of perchloric acid to deproteinize it. The mixture was centrifuged for five minutes. 0.5ml of the top clear liquid was taken into test-tubes and the samples were run in duplicates. 3ml of concentrated H_2SO_4 was added and the mixture was shaken thoroughly. The samples were incubated for 10minutes and later cooled to room temperature using water bath. 50µL of $CuSO_4$ reagent and 100µL of PP reagent were added simultaneously and the mixture is shaken thoroughly. The tubes were left at room temperature for at least 30minutes and then read absorbance at 570nm. The values of lactate were obtained with simple proportionality. The proportionality equation is obtained from the standard curve plotted.

Statistical analysis

All values given are mean ± S.E.M of the variables measured. Values between two groups were compared using students' t -test while One-way analysis of variance (ANOVA) was used to compare mean values in multiple groups.

RESULTS

Effects of 0.7% amphibian saline and cortisol on blood glucose and lactate levels.

Injection of 0.7% amphibian saline had no effect on blood glucose as well as lactate levels. However, the mean fasting blood glucose and lactate levels in toad, *bufo regularis*, were 42.6 ± 5.1 mg/dl and 38.1 ± 2.0 mg/dl respectively (Figures 1 and 2). Injection of cortisol (50µg/kg) caused significant increase in blood glucose level from a mean basal value 58.8 ± 9.2 to maximum 138.8 ±8.1 mg/dl 90 min post injection (figure1). Pretreatment with prazosin prevented the increase in blood glucose levels caused by cortisol injection while propranolol pretreatment abolished the increase in glucose levels caused by cortisol injection. The combination of both blockers completely abolished cortisol-induced hyperglycemia in the toad compared with the untreated (cortisol) toads.

Effect of 0.7% amphibian saline and cortisol (50µg/kg) on blood lactate levels.

Amphibian saline had no effect on blood lactate levels. Cortisol injection (50µg/kg) caused reduction in blood lactate levels 60min and 90min compared with control post-injection period (figure 2).

Effects of cortisol injection on blood lactate levels during prazosin and propranolol pretreatments

Injection of cortisol 50µg/kg caused significant reduction in blood lactate levels 60min and 90min post-injection period. Pretreatment of the toads with propranolol caused increase in blood lactate levels 60min and 90min post-injection time compared with the cortisol (untreated) toads while pretreatment with

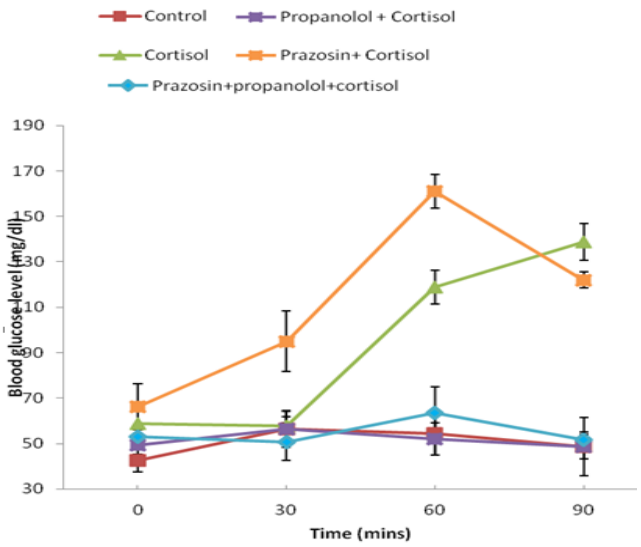

Figure 1. Effects of 0.7% amphibian saline, cortisol (50 µg/kg) in untreated toads and in prazosin treated (0.2 mg/kg), propranolol treated (0.5 mg/kg), and combination of prazosin (0.2 mg/kg) and propranolol (0.5 mg/kg) on glucose levels of the toads. The points are mean ± S.E.M. of five determinations.

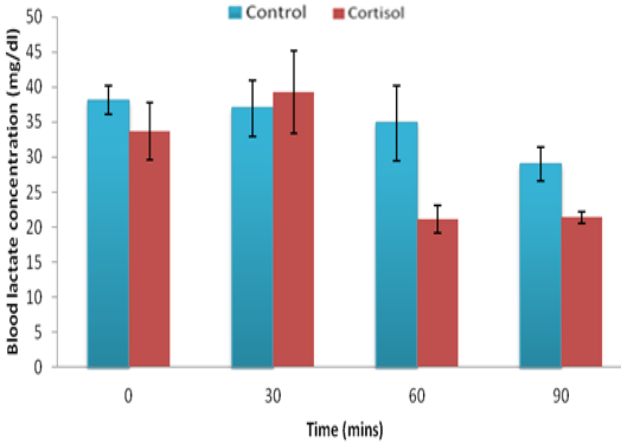

Figure 2. Effect of 0.7% amphibian saline and cortisol (50µg/kg) on blood lactate levels in the toads.

prazosin caused significant reduction in blood lactate levels 30min, 60min, and 90min post-injection period compared with untreated (cortisol group) (figure 3). However, combination of prazosin and propranolol blockers caused increase in blood lactate levels 60min and 90min post-injection compared with untreated (cortisol group) (Figure 3).

DISCUSSION

The hyperglycemic effect of cortisol observed in the present study *Bufo regularis* is consistent with previous studies in frogs (Hanke, 1974, 1978; Broughton and DeRoos, 1984), and in toads (Isehunwa *et al*, 2013). Cortisol causes hyperglycemia through gluconeogenesis (Baxter, 1979; Renauld and Moon, 1980; Gurwitz *et al*, 1994; Khani and Tayek, 2001). In the fasting state, gluconeogenesis maintains blood glucose levels. Krebs (1963) defined gluconeogenesis as the formation of carbohydrate from any non-carbohydrate precursors. Cortisol being a gluconeogenic hormone may cause rise in glucose levels through the use of gluconeogenic substrates such as lactate, amino acids and fatty acids.

The mean fasting blood lactate levels of 23-39 mg/dl observed in the present study is similar to that reported in Rana species (Hutchison and Turney, 1975, Farrar & Frye 1979a, MbangKollo and DeRoos, 1983, Goldman 1988, Peterson and Gleeson, 2007, San Millian, 2014). Blood lactate levels in the Common African toad *bufo regularis* has not been reported. The observation of the present study in which cortisol injection caused significant rise in glucose levels and concomitant reduction in blood lactate levels at 60 min & 90 min post-injection period seems to suggest that lactate could be a possible substrate involved in cortisol- hyperglycemia.

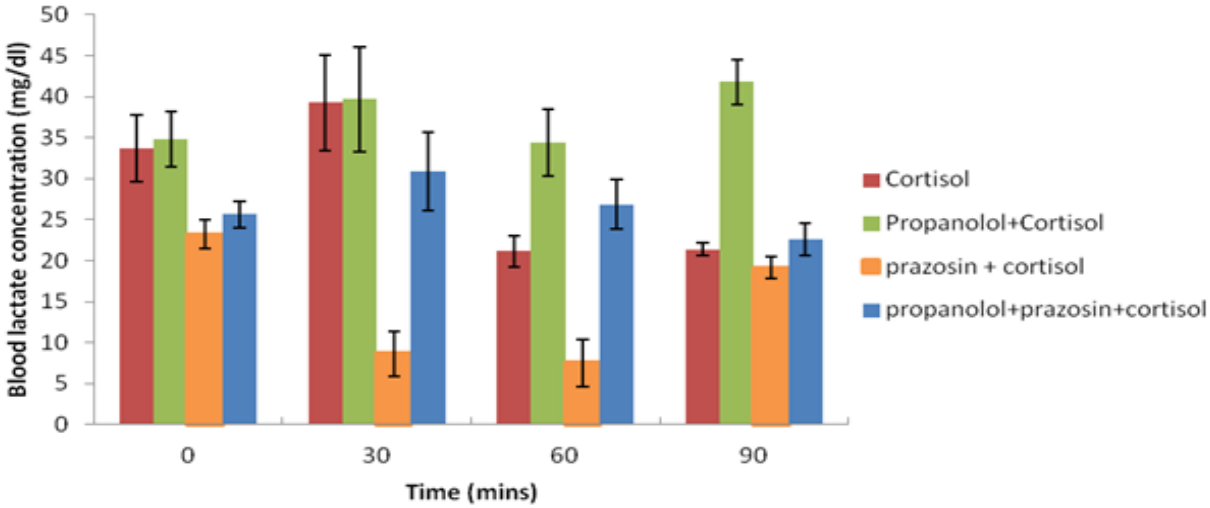

Figure 3: Comparison of effects of cortisol (50 µg/kg) in untreated (cortisol) toads and in prazosin treated (0.2 mg/kg), propranolol treated (0.5 mg/kg), and combination of prazosin (0.2 mg/kg) and propranolol (0.5 mg/kg) on blood lactate levels in the toads. The points are mean ± S.E.M. of five determinations.

It is interesting to note that the period of cortisol-induced hyperglycemia correlated with the period of reduction in blood lactate levels following cortisol injection. In the fasting state, gluconeogenesis contributes significantly to blood glucose levels (Rui, 2014). Cortisol is a gluconeogenic hormone and may cause rise in glucose levels through the use of gluconeogenic substrates such as alanine, lactate and fatty acids (Berneis *et al*, 1997; Djurhuus *et al*, 2002). The decrease in lactate levels observed in the present study following cortisol injection is possibly due to the fact that lactate was used partly for glucose production when glycogen store was depleted. Jacobs (1981) reported that a diet low in carbohydrate may lead to depletion of glycogen stores and ultimately lead to decrease in lactate levels. Therefore, gluconeogenesis from blood lactate may be partly responsible for the observed increase in blood glucose in the present study. This is consistent with the study in humans (Khani and Tayek, 2001) that cortisol caused gluconeogenesis. Previous studies also confirmed the use of lactate as a substrate for glucose formation in the frogs (Goldman, 1988; Peterson and Gleeson, 2007). However, the results of the present study contrast the findings of Hanke (1978) in frogs and AlNagdy *et al* (1995) in Common Egyptain toad. The latter reported increase in lactate level after cortisol injection.

Substrates preference for cellular metabolism varies between species and individual tissues of vertebrates. Previous studies in humans (Consoli et al, 1990) and amphibians and reptiles (Gleeson, 1991) showed that the skeletal muscle is the major source of plasma alanine and lactate in post absorptive state and following activity respectively. Peterson and Gleeson (2009) also showed that lactate is a preferred substrate in white glycolytic muscles of frogs at rest. Like other vertebrates, lactate is a product of muscle metabolism in amphibians, present in circulation and in greater amounts during activity. (Bennett and Licht, 1974, Hutchison and Turney, 1975, Philips and Hird, 1977, MbangKollo and DeRoos, 1983). Further studies on the level of lactate in the liver, muscle and other gluconeogenic tissues like kidney and key enzymes involved in gluconeogenesis will help our understanding of gluconeogenesis in the toad.

The results of the present study in which pretreatment with propranolol abolished cortisol-induced hyperglycemia and at the same time caused increase in blood lactate levels compared with cortisol group 60min and 90min post-injection period seems to confirm that the beta adrenergic receptors are involved and play dominant role in cortisol-induced hyperglycemia. This observation is in agreement with our previous study (Isehunwa et al, 2013). The findings of the present study in which pretreatment with propranolol caused increase in blood lactate levels compared with untreated (cortisol) group seems to suggest that the mechanism of cortisol-induced hyperglycemia through the use of lactate is mediated by the beta-adrenergic receptors. Hence, blockade of beta adrenergic receptors caused abolition of cortisol hyperglycemia and resulted in increased blood lactate levels same time 60 and 90min post-injection period. The above findings thus suggest that lactate was used probably as a gluconeogenic substrate and that the beta receptors were involved in the use of lactate as a gluconeogenic substrate to induce cortisol hyperglycemia in the toad. The pathway through which lactate is converted to glucose is well-known (Katz and Tayek, 1998)

However, pretreatment with prazosin caused significant reduction in blood lactate levels and rise in cortisol-induced hyperglycemia 60min and 90min post-injection period. The findings probably show that the alpha adrenergic receptors may not play any significant role in cortisol-induced hyperglycemia and in the use of lactate as a gluconeogenic substrate in the toad. This latter observation may explain why prazosin pretreatment did not prevent the cortisol-induced hyperglycemia and caused reduction in blood lactate levels during the same period. The blockade of alpha receptors may have resulted in un-opposed activity of beta receptors causing a rise in blood glucose levels following cortisol injection.

Following pretreatment with combined α and β blockers, the rise in blood glucose levels was abolished and there was increase in blood lactate levels suggesting the non-involvement of α adrenergic receptors in the use of lactate for glucose production.

In conclusion, the results of the present study showed that lactate level decreased significantly in response to cortisol injection suggesting that lactate could have served as a gluconeogenic substrate in toads. The results also showed that beta-adrenergic receptors are involved in the use of lactate as a gluconeogenic substrate to induce cortisol hyperglycemia in the Common African toad *bufo regularis*. Further studies on the level of lactate in the liver, muscle and other gluconeogenic tissues like kidney and key enzymes involved in gluconeogenesis will help our understanding of gluconeogenesis in the toad.

REFERENCES

Al-Nagdy, S.A; Zahara, M.H; Alzahaby, A. and Elsabbagh, M.E. (1995). Biochemical studies on blood tissue component of the common African toad *Bufo regularis.* Qatar Univ.Sci.J.15 (1), 37-49.

Baxter, J. D (1979). Glucocorticoid hormone action in: Pharmacology of adrenal cortical hormones edited by Gill, GN, oxford, *pergamon press*, 67-121.

Bennett, A.F. and Licht, P. (1974). Anaerobic metabolism during activity in amphibians. Comp. Biochem. Physiol. 48A, 319-327.

Brooks, G.A. (1985). Anaerobic threshold. Review of the concept and directions for future research. Med. Sci. Sports Exerc. 17(2), 22-34

Broughton R.E, DeRoos R, (1984): Temporal effects of infused corticosterone and aldosterone on plasma glucose levels in the American Bullfrog (*Rana castebiana)* Gen. Comp. Endocrinol., 35: 205-215

Butler, P.C and Rizza, R.A. (1989). Regulation of carbohydrate mechanism and response to hypoglycemia. Endocrinol. Metabol. Clin. North America.

Berneis, K; Ninnis, R; Girard, J; Frey, B.M; Keller, U. (1997). Effects of insulin-like growth factor 1 combined with growth hormone on glucocorticoid-induced whole-body protein catabolism in man. J.Clin.Endocrinol.Metab 82:2528-2534.

Consoli, A; Nurjhan, N; Reilly,J.J; Bier, D.M. and Gerich, J.E.(1990).Contribution of liver and skeletal muscle to alanine and lactate metabolism in humans. Am. J.Physiol.259, 677-684.

Davies, A.O. and Lefkowtiz, R. J. (1984). Regulation of beta-adrenergic receptors by steroid hormones. Ann. Rev. Physiol. 46, 119-30.

De Backer, D. (2003): Lactic acidosis. *Intensive Care Med*, 29:699-702.

Djurhuus, C.B;Gravholt, C.H; Nielsens, S; Mengel, A; Christiansen, J.S;Schmitz,O.E;Moller, N.(2002). Effects of cortisol on lipolysis and regional intestinal glycerol levels in humans. Am. J. Physiol. Endocrinol. Meta. 283: E172-E177.

Farrar, E.S. and Frye, B.E. (1979a). Factors affecting normal carbohydrate levels in *Rana pipiens*. Gen. Comp. Endo. 39, 358-371.

Goldman, S.S. (1988). Gluconeogenesis in amphibian retina, lactate is preferred to glutamate as gluconeogenic precursor. Biochem. J. 254:359-365.

Gurwitz, J.H, Bohn RL, Glynn RJ, Monane M, Mogun H, Avorn J (1994). Glucocorticoids and the risk for initiation of hypoglycemic therapy. Arch. Intern. Med 154: 97-101

Hanke, W. (1974). Endocrinology of amphibia. In "chemical zoology" (M.Florkin and B.T.Scheer, eds), 9, 123-159.Academic Press, New York.

Hanke, W. (1978) The adrenal certex of amphebia. In "General, Comparative and clinical Endocrinology of the adrenal certex". (I. Chester Jones and I.W. Hendersion, eds) Academic press, New York, 2, 419.

Hutchison, V. H. and Turney, L.D. (1975) Glucose and lactate concentrations during activity in the leopard frog (*Rana pipiens). J. comp. physiol.* 99, 287-295.

Iñigo San Millán, (2014): What is Lactate and Lactate Threshold? http://home.trainingpeaks.com/blog/article/what-is-lactate-and-lactate Retrieved on 8/4/2015.

Isehunwa, G. O., Olaniyan, O. T. and Alada, A. R. A. (2013). The role of alpha and beta-adrenergic receptors in cortisol-induced hyperglycaemia in the common African toad (*Bufo regularis*). Afri. J. Biotech. vol.12 (36), pp. 5554-5558

Jacobs, L. (1981). Lactate concentrations after short maximal exercise at various glycogen levels. Acta Physiol. Scand. 111, 465-467.

Katz, J.and Tayek, J. (1998). Gluconeogenesis and Cori cycle in 12, 20, and 40-h fasted humans. Am. J.Physiol.275, E476-E484.

Khani, S and Tayek, J. A. (2001). Cortisol increases gluconeogenesis in humans. Its role in the metabolic syndrome, Clin. Sci. (Lond) 101, 739-747.

Krebs, H.A. (1963). Advan. Enzyme. Regul. 1, 385-400.

MbangKollo, D and DeRoos, R. (1983). Comparative effects of epinephrine, norepinephrine, and a gentle handling stress on plasma lactate, glucose and hematocrit levels in the American Bullfrog (*Rana catesbeiana*).

Peterson, A.M. and Gleeson, T.T. (2007). Characterization of circannual patterns of metabolic recovery from activity in Rana catesbeiana at 15^0C. J. Exp. Biol. 210, 1786-1797.

Peterson, A.M. and Gleeson, T.T. (2009). Skeletal muscle substrate utilization is altered by acute and acclamatory temperature in the American bullfrog *(Lithobates catesbeiana*). J. Exptal. Biol. 212, 2378-2385.

Philips, J. W. and Hird, F. J. R. (1977). Gluconeogenesis in vertebrate livers. Comp. Biochem. Physiol. 57B, 127-131.

Renaud, J. M. and Moon, T. W. (1980). Characterization of gluconeogenesis in hepatocytes reptiles, *Amer Zool*, 13, 67-69.

Rui, L (2014). Energy metabolism in the liver. Compr. Physiol. 4(1) :177-197.

Taylor, A.C. (1996). A Simple Colorimetric Assay for Muramic Acid and Lactic Acid. Appl. Biochem. Biotech. 56: 49-58.

Trinder, E. (1969). Determination of blood glucose using 4 amino phenazone as oxygen acceptor, *J. Chem. Pathol*, 22, 246.