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TISSUE GLYCOGEN AND LACTATE HANDLING BY THE DEVELOPING DOMESTIC FOWL

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Abstract—1. The levels of glycogen and lactate in liver, intestine, yolk sac membrane and leg and breast muscle of domestic fowl from day 10 of "in ovo" development to day 5 after hatching compared with adults have been measured and compared with the circulating concentrations in blood of glucose and lactate.

2. Glycogen stores in most tissues increased before hatching to attain a minimum around the eclosion and then increased to adult values in muscle and liver.

3. Lactate maintained its plasma concentrations with higher effectiveness than plasma glucose, which increased steadily up to adult levels from hatching.

4. The study of tissue vs plasma lactate concentration ratios suggests a general activation of lactate metabolism from hatching, coinciding with the ingestion of carbohydrate-based food.

5. Both muscles studied, as well as intestine, seem to be net lactate producers; blood cells can speculatively be considered as lactate users and liver maintains its concentration of lactate very close to that of plasma, suggesting a fast utilization of this material as well as liver being the main site for control of circulating lactate.

INTRODUCTION

The importance of lactate in adult avian metabolism is considerable, as is the main energetic substrate that the chicken intestine exports to the liver (Riesenfeld *et al.*, 1982). This lactate is used as energetic or gluconeogenic substrate in the bird liver (Söling *et al.*, 1973; Dickson and Langslow, 1978; Brady *et al.*, 1979; Watford *et al.*, 1981; Sugano *et al.*, 1982; Hers and Hue, 1983) in addition to being the end product of white (glycolytic) muscle anaerobic utilization of glucose.

The actual tissue levels of lactate can provide a clue as to the relative existence of gradients between the tissue and plasma, and hence can help understand the release/uptake of this important metabolite to/from the bloodstream.

Glycemy in adult hens is maintained at high levels when considered in comparison with mammals (Pearce, 1971; 1977), related to the predominance of glucagon over insulin in birds (Prosser and Brown, 1968), and has the immediate consequence of more limited glucose uptake by most avian tissues, such as blood cells, which hardly use glucose as a source of energy (Barron and Harrop, 1928; Schweigger, 1962; Shields *et al.*, 1964; Bell and Culbert, 1968; Bell, 1971; Rosa *et al.*, 1983; Pons *et al.*, 1986). However, liver glycogen is sensitive to hormone mobilization (Freeman and Manning, 1971) and helps maintain circulating glucose homeostasis.

In the period of hatching, the bird embryo changes greatly (especially in the mature precocious bird, like the domestic fowl) from a mainly lipidic (yolk) based energetic metabolism to the more carbohydrate and low quality protein based (chicken chow) energetic metabolism that characterizes free life.

It has been intended here to correlate the levels of glycogen in the main reserve tissues of the developing chick with the tissue levels of lactate as well as with circulating concentrations of glucose and lactate so as to gain additional information about the ontogenesis of lactate production and utilization in the developing bird.

MATERIALS AND METHODS

Recently laid fertilized chicken eggs (*Gallus domesticus*, White Leghorn strain) were kept in a temperature and humidity controlled automatically turning incubator. Randomly selected eggs were taken on days 6, 10, 13, 15, 18, 19 and 20 of incubation and dissected. The yolk sac membrane was singled out and gently cleaned of its adhering yolk with 9.5 g/l NaCl. Small intestine (cleaned of its contents with saline solution), liver, leg and breast muscle were also immediately obtained as above.

Several eggs were allowed to hatch and the resulting chicks were sacrificed at days 0, 1, 2, 3 or 5 through decapitation. The chicks were maintained in a warm and humid (25 ± 1°C, 75–90% relative humidity) environment and were fed chicken standard purina pellets and tap water. They were compared with adult hens weighing 4.5 ± 0.2 kg from the same stock. All chicks and hens were sacrificed at 08.00–10.00 hr and their neck wound blood was received into dry heparinized beakers and used for plasma separation (at 4°C) and hematocrit estimation (Ferrando *et al.*, 1981). Tissue samples were frozen in liquid nitrogen. Aliquots of these were weighed and immediately placed in 2 volumes of 30% KOH, digested for 10 min, and used for glycogen purification (Good *et al.*, 1933) and chemical measurement

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Table 1. Blood and tissue lactate levels and blood glucose of developing chicks (*Gallus domesticus*)

Parameter and tissue	Incubation (days) 10	15	18	20	0	Free life (days) 1	2	3	5	Adult	ANOVA "time" F (df)
Lactate											
Blood		2.55 ± 0.60	2.05 ± 0.19	2.43 ± 0.48	2.60 ± 0.20	2.53 ± 0.40	3.22 ± 0.86	4.70 ± 1.00	4.49 ± 0.56	3.41 ± 0.74	1.39 (6,23)ns
Plasma		3.60 ± 1.10	2.74 ± 0.68	3.28 ± 0.41	3.03 ± 0.18	2.19 ± 0.42	2.81 ± 0.31	4.35 ± 0.90	4.67 ± 0.90	3.38 ± 0.82	1.95 (6,26)ns
Glucose											
blood		3.6 ± 0.2*	4.8 ± 0.2*	5.5 ± 0.6*	6.6 ± 0.2*	8.8 ± 0.5	8.1 ± 0.8	10.5 ± 0.3	12.4 ± 1.9	10.1 ± 1.2	8.84 (6,31)†
Plasma		6.2 ± 0.1*	7.4 ± 0.1*	9.9 ± 1.4*	10.0 ± 0.3*	10.5 ± 0.3*	10.4 ± 0.3*	15.2 ± 0.5	14.2 ± 1.5	18.5 ± 1.9	15.95 (6,24)†
Lactate											
liver	1.39 ± 0.14*	1.74 ± 0.15*	2.10 ± 0.10*	1.62 ± 0.12*	2.03 ± 0.19*	2.16 ± 0.14*	4.11 ± 0.66	3.91 ± 0.39	4.32 ± 0.80	3.58 ± 0.56	6.74 (7,35)†
Intestine	5.73 ± 1.10*	2.18 ± 0.09*	3.33 ± 0.29*	6.29 ± 0.41*	10.2 ± 0.2*	15.1 ± 1.7	15.6 ± 1.6	14.4 ± 1.1	16.2 ± 1.0	17.4 ± 1.7	11.3 (7,32)†
Yolk sac membrane	1.64 ± 0.31	0.98 ± 0.20	1.47 ± 0.11	2.22 ± 0.39	2.21 ± 0.10	3.11 ± 0.42	4.39 ± 0.43	5.30 ± 0.47	6.72 ± 0.29	—	13.6 (7,32)†
Leg muscle	3.60 ± 0.21*	4.32 ± 0.39*	17.7 ± 1.4	30.0 ± 2.1*	30.7 ± 0.8*	34.1 ± 2.7*	30.6 ± 0.2*	32.6 ± 1.0*	34.7 ± 1.5*	20.2 ± 3.3	38.51 (7,35)†
Breast muscle	4.90 ± 0.89*	4.21 ± 0.32*	12.3 ± 1.1*	21.4 ± 0.4*	20.7 ± 1.5*	18.3 ± 0-8*	22.4 ± 0.8*	28.1 ± 3.4*	28.5 ± 1.0*	52.9 ± 6.0	9.66 (7,32)†

All values for blood or plasma are expressed in mMoles per liter and for tissues in mMoles per kg of fresh tissue.
All values are the mean ± S.E.M. of 68 different animals.
Statistical significance of the differences versus the adult values: * = $P < 0.05$; statistical significance of all values with respect to "time" (ANOVA): † = $P \leqslant 0.05$; ns = $P > 0.05$.

with an anthrone method (Pons *et al.*, 1981), using pure glucose as standard.

Blood samples were diluted with 10 volumes of distilled water, sonicated (30 s at 100 W) and deproteinized with 0.66 M (final concentration) perchloric acid within less than 2 min after sacrifice of the animal; they were then neutralized with $KOH/KHCO_3$, the clear supernatants being used for glucose (Hugget and Nixon, 1957) and lactate (Gutmann and Wahlefeld, 1974) enzymatic measurements. Frozen tissue samples were homogenized in 10 volumes of chilled distilled water with a Tenbroeck homogenizer. Plasma samples as well as tissue homogenates (in 10 volumes of distilled water) were deproteinized with the same perchloric acid procedure, the clear supernatants being used for lactate estimations by the same method used for whole blood.

Statistical comparison between the means was achieved by using the Student's t-test. A simple one-way analysis of variance method (ANOVA) was also used to test the significance of variation of data versus time. A P value of 0.05 has been taken as limit of statistical significance for all tests. The limit $F^{0.05}$ values used have been: $F^{0.05}_{8,60} = 2.10$; $F^{0.05}_{6,23} = 2.53$; $F^{0.05}_{6,30} = 2.42$.

RESULTS

In Table 1 the blood and plasma glucose and lactate concentrations are presented together with the tissue lactate levels measured in developing and adult domestic fowl. The blood lactate concentrations were maintained at a considerable uniformity during the period studied, as no significant differences were detected with respect to adults. Even lower variations were found for plasma lactate values. The fraction of blood lactate carried by blood cells (calculated from both plasma and blood values and hematocrit) increased from practically nil values on day 10 of incubation to a 34.9% in adults (1, 2, 20.5, 26.3, 28.5, 19.2%, respectively for days 18, 20, 0, 1, 2, 3 and 5). Blood glucose concentrations increased steadily during the incubation period, the concentrations not being statistically different from those of adults from day 1 onwards. Plasma glucose also increased to the very high adult levels more uniformly, the differences vs adults being non significant from day 2 onwards. The fraction of glucose carried in the blood cells was nil in all groups except for a mean of 10% in 1-day-old chicks.

Liver lactate maintained its values during incubation, increasing from day 1 to 2 to adult levels. Intestine lactate decreased transiently on days 15–18 from the high day 10 levels and increased again around hatching, attaining the adult levels on day 1. The lactate concentration in the vitelline sac membrane increased slowly to the highest values in 5-day-old chicks.

Leg and breast muscle contained the same low lactate concentration up to day 15 of incubation; then, despite both muscles raising their lactate, the pace of the leg muscle was higher up to day 20 when both stabilized, but leg muscle had significantly higher lactate levels throughout all the period studied (except in adults, when the breast muscle attained much higher levels).

In Fig. 1 the glycogen content of yolk sac membrane, liver and muscles of developing domestic fowl are presented. Yolk sac membrane glycogen stores were considerable just before hatching, decreasing steadily from that moment to the practically nil

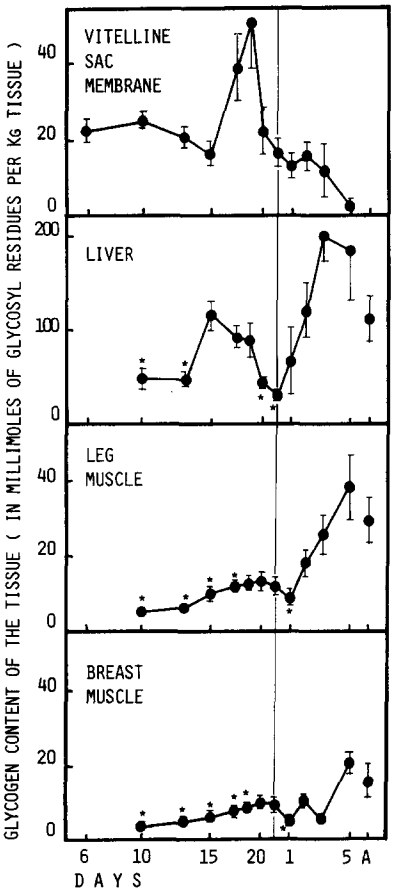

Fig. 1. Glycogen concentrations expressed as millimoles of glycosyl residues per kg of fresh tissue in the tissues of developing domestic fowl compared with the adult (A). Each dot represents the mean ± SEM of 6–8 different animals. The vertical line corresponds to the day in which the eclosion took place (day 0). The asterisk (*) represents that the group indicated is significantly different ($P < 0.05$) from that of adults. In all from parameters studied the variation with respect to time was statistically significant (ANOVA) ($P < 0.05$), with F values of 3.95 [11, 65], 6.30 [11, 77], 6.47 [11, 82] and 3.52 [11, 80] respectively (degrees of freedom between brackets) for yolk sac membrane, liver, leg muscle and breast muscle.

glycogen levels of day 5. Liver glycogen also showed a transient maximum before hatching, with very low stores on days 20 and 0, which were promptly recovered up to days 3–5 and then decreasing to the adult levels. Leg and breast muscles showed the same (low levels) pattern up to day 1, when they attained a mimimum. Thence, while breast muscle increased its levels to the discreet adult concentrations, leg muscle glycogen stores increased much more to attain the significantly higher (than those of breast muscle) adult glycogen levels practically after hatching.

DISCUSSION

The availability of glucose in the avian embryo is very limited (Freeman and Vince, 1974), as all circulating glucose must come from the very low glucidic reserves of the egg (Freeman and Vince, 1974; Roca *et al.*, 1982; 1984) or synthetized through gluconeogenesis from glycerol or precursor amino acids (Kilsheimer *et al.*, 1960; Ballard and Oliver, 1963; Yarnell *et al.*, 1966; Willier, 1968), and thus the availability of glucose from this moment on increases in a parallel way to the dwindling of fat reserves (Romanoff, 1967; Freeman and Vince, 1974) and increasing but limited amino acid utilization (Needham, 1931; Rupe and Farmer, 1955). Plasma glucose actually began to increase significantly from some time after hatching, when the animal has initiated its feeding with chicken chow, mostly carbohydrate. The lack of glucose in the blood cells agrees with the lack of utilization of this substrate by circulating avian cells (Schweigger, 1962; Shields *et al.*, 1964; Bell and Culbert, 1968; Bell, 1971; Rosa *et al.*, 1983). The transient presence of glucose in blood cells on day 1 can be a consequence of the dramatic changes that follow both access to unlimited air oxygen and carbohydrate-based food before the alterations in glycogen and glucose–lactate metabolism that occur at hatching (Pearce, 1971; 1977; Freeman and Vince, 1974) give pace to the final adult glucose homeostasis.

The maintenance of lactate levels in the developing chick plasma is higher than that of glucose in the period studied, as the standard deviation of the computed means of Table 1 for this parameter is only a 22% of the mean, while that of glucose is 32%. The lactate level maintenance in blood thus seems to be of higher homeostatic importance than that of the main inter-organal staple: glucose. Despite glucose not being used for erythrocyte metabolism, the presence of lactate in the blood is considerable. One can thus speculate on the possibility of this metabolite being a substrate for its energetic metabolism, especially after hatching, when the intestine provides a continuous stream of this acid from the glycolytic breakup of glucose (Riesenfeld *et al.*, 1982). In the presence of lactate dehydrogenase (Rosa *et al.*, 1983) and alanine transaminase, the lactate blood cell utilization can provide a source for alanine formation and output, collecting the 2-amino nitrogen of amino acid catabolism, very important in the chicken erythrocytes (Schweigger, 1962; Bell, 1971; Pons *et al.*, 1986). The liver and muscle glycogen content pattern found here agree in general terms with the data previously described in the literature (Thommes and Firling, 1964; Freeman, 1965; 1969; Daugeras, 1968; Rinaudo *et al.*, 1969; Freeman and Manning, 1971; Freeman and Vince, 1974) as did those of the yolk sac membrane (Thommes and Just, 1964; Thommes and Aglinskas, 1966; Thommes *et al.*, 1968). This is in agreement with the very low levels found in the peri-hatching period, which are recovered in the following days up to adulthood. However, the estechiometry of lactate formation and glucose and glycogen disappearance is not in full agreement with a pure carbohydrate origin of this lactate (Arese *et al.*, 1967) as probably some amino acid hydrocarbon skeletons also find their way into lactate (Arese *et al.*, 1967). The maintenance of circulating lactate levels is the consequence of its production by glycolytic (lactogenic?) tissues and the uptake by the liver for gluconeogenesis (Söling *et al.*, 1973; Dickson and Langslow, 1978; Brady *et al.*, 1979; Watford *et al.*, 1981; Hers and Hue, 1983), or, also, uptake by other

Table 2. Lactate tissue vs plasma concentration ratios during the development of the chick (*Gallus domesticus*)

Tissue	Incubation days			Free life days					
	15	18	20	0	1	2	3	5	Adults
Blood cells	0.00	0.03	0.05	0.63	1.33	1.43	1.12	0.82	0.87
Liver	0.48	0.64	0.49	0.67	0.99	1.46	0.90	0.93	1.00
Intestine	0.61	0.80	1.92	3.37	6.89	5.55	3.31	3.47	4.86
Yolk sac membrane	0.28	0.36	0.68	0.72	1.42	1.56	1.22	1.44	—
Leg muscle	1.20	1.58	9.15	10.10	15.57	10.89	7.49	7.43	5.64
Breast muscle	1.17	1.54	6.52	6.83	8.36	7.97	6.46	6.10	14.78

The values given have been calculated from the means of the data presented in Table 1.

tissues for energetic utilization of lactate. In Table 2 the ratios of tissue concentration (in mmol/kg of tissue) vs that of plasma are presented. Obviously both entities are not directly comparable, as the tissue contains also variable amounts of blood and the proportion of solids is much higher than in plasma, but nevertheless the ratios are comparable between themselves throughout the developmental period studied. Liver lactate is initially low, up to hatching, because the availability of lactate (and glucose) in the confined egg is limited (Freeman and Vince, 1974), and so is the gluconeogenic conversion of lactate into glucose (Freeman and Vince, 1974). After hatching, the ratio closely approaches unity, indicating that there is a fast equilibrium between circulating and liver lactate (and one can suppose, lactate utilization). This further suggests that liver is the main site of circulating lactate concentration control through its uptake and metabolization. The pattern and actual levels of lactate in the liver are comparable to those of Rinaudo *et al.* (1976).

The intestine can be defined as an important lactate producer (Riesenfeld *et al.*, 1982) both in the adult and even in the 20-day embryo, as a preparation for its role in the adult fowl; the most dramatic changes in lactate concentration ratios in intestine actually take place around hatching.

Yolk sac membrane lactate handling is low during incubation, reaching neutral values close to hatching and increasing a little afterwards, in agreement with the role of this organ as substrate retriever and its uptake of glucose (Willier, 1968) and probably lactate when the availability of the former is high, as in post-hatching free life.

Both muscles showed the same lactate concentration ratio pattern. The higher concentrations (and ratios) of leg muscle agree with its higher metabolic activity; no metabolic differentiation can be observed in both muscles up to hatching except for a coincident higher lactate production and rates from day 20 onwards. This lack of differentiation into oxidative or glycolytic muscles actually takes place after hatching (Bacou and Vigneron, 1976; García *et al.*, 1985) and is better seen in the adult ratios, which invert to those of day 1, as the less active breast muscle is converted into white glycolytic muscle (Lebherz *et al.*, 1982) in the adult.

The general pattern of lactate production by the developing bird agrees with a low key lactate/glucose metabolism during incubation which develops from day 20 to day 1 after hatching into a burst of lactate production and glycogen disappearance, followed by higher glucose availability from the diet which coincides with increased liver lactate handling capabilities and high production by intestine and muscle. It can be speculated that other tissues, such as blood cells, can retrieve lactate (not generated from glucose) and use it as metabolic substrate.

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