0163-4984/99/6801-0063 $14.00

Tissue-Specific Antioxidant Profiles and Susceptibility to Lipid Peroxidation of the Newly Hatched Chick

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Received May 19, 1998; Revised July 8, 1998; Accepted July 30, 1998

ABSTRACT

The hatching process is characterized by a range of adaptive changes, and a newly hatched chick is considered as an intermediate stage between prenatal and postnatal development. The aim of the present study was to evaluate the characteristic relationships between tissue-specific fatty acid composition and antioxidant protection in newly hatched chicks. Liver, yolk sac membrane, heart, kidney, lung, and four brain regions (cerebrum, cerebellum, stem, and optic lobes) were collected. Fatty acid composition of total lipids and phosphoglycerides, α-tocopherol, lutein, ascorbic acid, reduced glutathione, and the activities of Mn- and Cu,Zn-superoxide dismutase (SOD) and Se-dependent and non-Se-glutathione peroxidase (GSH-Px), and catalase (CAT) were determined. The levels of Fe, Cu, Zn, and Mn as well as tissue susceptibility to lipid peroxidation were also studied. The tissues of the newly hatched chick showed distinctive features in fatty acid profiles, antioxidant accumulation, and susceptibility to lipid peroxidation. The brain clearly displayed the greatest susceptibility to spontaneous and Fe-stimulated lipid peroxidation, was highly unsaturated and contained very low levels of vitamin E, no detectable carotenoids, low GSH-Px, and low CAT activity. At the same

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time, the brain was characterized by high ascorbic acid concentration and comparatively high SOD activity. It was suggested that in postnatal development, antioxidant enzymes presumably play the major role in antioxidant protection of the chick tissues.

Index Entries: Chicken embryo; antioxidants; antioxidant enzymes; lipid peroxidation; fatty acids; PUFA.

INTRODUCTION

A newly hatched chick is considered as an intermediate stage between prenatal and postnatal development. The hatching process is characterized by a range of physiological, biochemical, and behavioral adaptive changes necessary for the postnatal development *(1)*. Lipid metabolism in the chick embryo is characterized by the accumulation of polyunsaturated fatty acids (PUFAs) in the lipids of the developing tissues *(2)*. Similarly α-tocopherol and carotenoid accumulation in the tissues reach their maxima at hatching *(3)*.

The hatching process is characterized by a transition from chorioallantoic to pulmonary respiration, exposure to atmospheric oxygen, and an increase of 60% in the rate of oxidative metabolism *(4)*, a situation that can lead to free-radical overproduction. The rates of free-radical formation and lipid peroxidation have been shown to be positively correlated in vitro and in vivo with O_2 tension *(5)*. On the other hand, inadequate oxygen supply limits embryo growth, whereas the chick embryo responds to a positive increase in O_2 supply by increasing its antioxidant defense *(6)*.

Major changes in lipid and antioxidant composition of the liver have been shown to occur during the early neonatal period *(2)*. These changes are primarily associated with significant alterations in levels of highly polyunsaturated lipids and antioxidant systems, in particular, α-tocopherol and carotenoid concentrations *(3)*. In general, antioxidant systems of the chick embryo include antioxidant enzymes, fat-soluble natural antioxidants such as vitamins E and A, carotenoids, and water-soluble antioxidants such as ascorbic acid and and reduced glutathione *(3,7,8)*. Nevertheless, the tissue-specific features of antioxidant distribution and regulation of antioxidant systems in newly hatched chicken and their relationships with the level of tissue lipid unsaturation have not been studied. Such information would be essential for understanding adaptive mechanisms of the newborn and to find possible ways of increasing antioxidant protection during stress conditions of hatch and as a result to increase chicken viability in postnatal development.

The aim of the present study was to evaluate the tissue-specific features in fatty acid composition and antioxidant protection in newly hatched chicks.

MATERIALS AND METHODS

Animals

Fertilized eggs of the Ross-308 broiler-breeder strain were obtained from a commercial hatchery and incubated at 37.5°C and 60% relative humidity in a forced-draft incubator with automatic egg turning. Following hatching at 21 d, the chickens were maintained in the incubator at a reduced temperature of 30°C for 24 h with access to drinking water but with no food provision before being sacrificed to provide samples of brain, liver, heart, lung, kidney, and yolk sac membrane (YSM). The determination of ascorbic acid, glutathione content, and enzyme activities together with lipid extraction were performed on portions of fresh tissues. Determinations of α-tocopherol, carotenoids, and trace metals were performed on samples of tissues following storage at –20°C under nitrogen for up to 2 wk.

Analytical Procedures

Fatty Acid Analysis

Total lipids were extracted by standard procedures following homogenization in a suitable excess of chloroform/methanol (2:1, v/v) *(9)*. Total lipid concentrations were determined gravimetrically. Lipids were separated into their major classes by thin-layer chromatography on silica gel G using a solvent system of hexane–diethyl ether–formic acid (80:20:1 v/v). Portions of the total lipid and phospholipid were subjected to transmethylation *(10)* and the composition of the resultant fatty acid methyl esters was determined by gas–liquid chromatography using a CP9001 instrument (Chrompack, Middleburg, The Netherlands) fitted with a 30 m × 0.25 mm capillary column system (film thickness 0.25 μm; Carbowax, Alltech, Carnforth, UK). Integration of the peaks and subsequent data handling were performed using an EZ Chrom Data System (Scientific Software Inc., San Jose, CA, USA), enabling the fatty acid composition and the total amount of derived fatty acid from each sample to be quantified. The identities of the peaks were verified by comparison with the retention times of standard fatty acid methyl esters.

The tissue concentrations of fatty acyl double bonds were expressed in terms of a double-bond index (DBI) defined by

$$\text{DBI} = F[\text{p16:1} + \text{p18:1} + 2(\text{p18:2}) + 3(\text{p18:3}) + 4(\text{p20:4}) + 5(\text{p20:5}) \; 5(\text{p22:5}) + 6(\text{p22:6})]$$

where F represents mg total lipids/g tissue and p16:1 and so forth are the molar proportions of each fatty acid.

Determination of Vitamins A and E

Vitamins A and E were determined by the method of McMurray et al. *(11)*, as previously described *(3)*. In brief, the samples were saponified

with ethanolic KOH in the presence of pyrogallol, and the retinol and tocopherols were extracted from the mixture with hexane. The extract was dried under nitrogen, redissolved in methanol, and injected into a high-performance liquid chromatographic (HPLC) system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co. LTD) with a JASCO Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb type S30DS2, 3-μ C_{18} reverse-phase HPLC column, 15 cm × 4.6 mm (Phase Separations Limited, Clwyd, UK). Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.1 mL/min. Fluorescence detection of retinol involved excitation and emission wavelengths of 330 and 480 nm, respectively. The relevant wavelengths for tocopherols detection were 295 and 330 nm. Standard solutions of all-trans retinol and α-tocopherol in methanol were used for instrument calibration and tocol was used as an internal standard.

Determination of Carotenoids

Two milliliters of tissue or yolk homogenate (20% in 0.01*M* phosphate buffer, pH 7.4) was mixed with 2 mL of ethanol. Hexane (5 mL) was then added and the mixture was shaken vigorously for 5 min. The hexane phase, containing the carotenoids, was separated by centrifugation and collected. The extraction was repeated one more time with 5 mL hexane. Hexane extracts were combined and analysis of the carotenoids extracted from the tissues was performed by HPLC using a Spherisorb type S30DS2, 3-μ C_{18} reverse-phase HPLC column, 25 cm × 4.6 mm by elution with a mobile phase of acetonitrile/dichloromethane/methanol (7:2:1, v/v) *(12)*. Lutein, zeaxanthine, citranaxanthin, carotenoic acid, β-cryptoxanthin, lycopene, and α- and β-carotene were used as calibration standards.

Determination of Ascorbic Acid, Reduced Glutathione and Metals

The ascorbic acid content of the tissues was determined spectrophotometrically by the method of Omaye et al. *(13)* based on the oxidation of ascorbic acid and the reaction of the oxidation products with 2,4-dinitrophenylhydrazine to form the derivative bis-2,4-dinitrophenylhydrazone. The latter was determined by its absorbance at 520 nm. The reaction was performed in the presence of thiourea to provide a mildly reducing medium in order to prevent interference from non-ascorbic acid chromogens.

Reduced glutathione was determined by the method of Griffith *(14)* via the determination of total glutathione following enzymatic recycling with glutathione reductase; oxidized glutathione was determined in the presence of 2-vinylpyridine and reduced glutathione was calculated as the difference between total and oxidized glutathione.

Selenium concentrations were determined using hydride generation atomic absorption spectrometry as described by Wilson et al. *(15)*. In the

method, the organic matter in the sample is destroyed by the action of the nitric/perchloric/sulfuric acids. The selenium in the residue is dissolved in 3*M* hydrochloric acid and any selinate converted to selenite by gentle heating. The method employed a hydride generator (P.S. Analytical LTD, Orpington, Kent, UK, Model 10.004), a fluorescence detector (P.S. Analytical LTD., Model 10.033), an autosampler (P.S. Analytical LTD, Model 20.099), and a computer with Avalon Software. Concentrations of the other metals (Zn, Cu, Mn, and Fe) were determined by atomic absorption spectrophotometry as described by Wilson et al. *(16)*.

Assay of Antioxidant Enzyme Activities

Tissue samples were washed in potassium phosphate buffer (10 m*M*, pH 7.4) at 4°C, homogenized in nine volumes of the same buffer, and supplemented with 30 m*M* KCl, as described by Wilson et al. *(16)*. The protein content of an aliquot of the homogenate was determined using a Bio-Rad dye-binding kit system (Bio-Rad Laboratories GmbH, Munich, Germany). The remainder of the homogenate was centrifuged (3500*g*, for 30 min at 4°C) and the enzyme activities of the supernatant were determined. Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were determined spectrophotometrically using kit systems (Randox Ltd., Crumlin, Nothern Ireland). GSH-Px activity was measured by a coupled reaction with excess glutathione reductase and monitoring the NADPH oxidation at 340 nm. Total GSH-Px activity was determined using cumene hydroperoxide as a substrate and the activity of Se-dependent GSH-Px was measured with H_2O_2 as a substrate *(17)*. Units of glutathione peroxidase activity were expressed as micromole of NADPH oxidized per minute.

Total SOD activity was determined employing xanthine and xanthine oxidase to generate superoxide radicals and their reaction with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a Red Formazan dye. A unit of SOD activity was defined as the quantity of SOD required to produce 50% inhibition of the rate of this reaction. To determine Mn–SOD, the measurement was performed in the presence of 5 m*M* sodium cyanide *(18)*. The activities of the enzymes are expressed as units per milligram supernatant protein.

Determination of Lipid Peroxidation

Lipid peroxidation was estimated by spectrophotometric determination of thiobarbituric acid reacting substances (TBARS) as previously described *(3)*. Tissue homogenates (10% w/v), in sodium phosphate buffer, 10 m*M*, pH 7.4, containing 1.15% (w/v) KCl were prepared. Homogenates were incubated at 37°C for 60 min in the presence or absence of 0.1 m*M* $FeSO_4$ under an air atmosphere with gentle shaking. At the end of the incubation, butylated hydroxytoluene was added to a concentration of 0.01% (v/v). TBARS assays were performed according to the method of Ohkawa et al. *(19)*. 1,1,3,3-Tetramethoxypropane was

used as a standard for TBARS determination. Results were expressed as microgram malondialdehyde (MDA) per gram fresh tissue.

Chemicals

Fatty acid methyl ester standards, 2,4-dinitrophenylhydrazine,lutein were obtained from Sigma (Poole, UK); α-tocopherol, δ-tocopherol, and β-carotene were obtained from Fluka (Gillingham, Dorset, UK); zeaxanthin from Apin (Abingdon, UK), and tocol, γ-tocopherol, lycopene, and β-cryptoxanthin were a gift from Hoffman–La Roche (Basel, Switzerland).

Statistical Analysis

All results are presented as mean ± SEM of five replicate measurements from each tissue and each biochemical parameter indicated. Statistical analysis was performed by the Student's *t*-test using one-way analysis of variance (ANOVA).

RESULTS

Polyunsaturated Fatty Acids in the Tissues

The distribution of long-chain PUFAs within the phospholipids of the tissues are shown in Table 1. In accordance with the proportions of total PUFAs in the phospholipids, the tissues can be placed in the following descending order: liver > kidney = muscle > heart > brain > YSM > lung. However, based on the DBI, the tissues can be placed in the following descending order: YSM > liver > brain > kidney > muscle = heart > lung. Nevertheless, expressed on a per gram lipid basis, the brain contains the highest concentration of double bonds and YSM the lowest.

Fat-Soluble and Water-Soluble Antioxidants in the Tissues

Concentrations of vitamins A and E and lutein + zeaxanthin within the tissues are shown in Table 2. The tissues were characterized by widely differing and distinctive levels of fat-soluble antioxidants. Thus, the liver and YSM displayed, by far, the highest levels of the fat-soluble antioxidants and the brain the lowest levels of such metabolites. In the brain, the level of vitamin E was some 100 times lower when compared to its concentration in the liver. A similar difference was found in the level of vitamin A, whereas carotenoids were undetectable in the brain.

Apart from the relatively high level of vitamin A within the kidney, vitamins A and E and carotenoid concentrations in the other tissues were all of a similar concentration and also very low compared to that of the liver.

Table 1
Main PUFAs in the Phospholipids of the Tissues of a Newly Hatched Chick (wt%)

Tissue	18:2n-6	20:4n-6	22:6n-3	DBI**
Liver	12.33± 0.37[a]	21.51± 1.38[a]	10.12± 1.30[a]	161.88
Brain	1.54± 0.05[b]	9.51± 0.23[b]	16.17± 0.28[b]	69.87
Kidney	11.84± 0.49[ac]	19.90 ± 0.77[ac]	6.25± 0.22[c]	62.16
Heart	9.77± 0.50[cd]	21.84± 0.27[ac]	3.26± 0.27[d]	52.16
Lung	8.48± 0.59[cd]	10.08± 0.39[b]	3.32± 0.14[d]	36.12
Thigh Muscle	15.03± 0.55[e]	15.25± 0.25[d]	8.06± 0.63[e]	53.59
YSM	14.89± 0.73[ae]	8.65± 0.58[b]	2.86± 0.13[d]	290.97

Note: Values are mean ± SEM (n = 5). Numbers with different superscripts are significantly different with respect to the column.
**DBI is the double-bond index of total lipids.

The liver and kidney also contained high concentrations of GSH. The ratio of reduced glutathione (GSH)/oxidized glutathione (GSSG) (not shown) in all the tissues was high varying from 9.1 (YSM) up to 12.4 (liver). The liver, kidney, and lung were characterized by high levels of ascorbic acid.

Antioxidant Enzymes in the Tissues

Table 3 shows the activities of SOD, GSH-Px, and catalase (CAT) in the tissues. The tissues displayed a considerable variation in the Mn–SOD activity, with the heart having the highest value and lung the lowest. By contrast, the lung was characterized by high Cu,Zn–SOD activity; in the heart, activity of Cu,Zn–SOD was comparable to the other tissues. Based on the total SOD activity, the tissues could be placed in the following descending order: heart > muscle > YSM > kidney > lung > liver.

As can be seen, the tissues differed markedly in the GSH-Px activities. In all the tissues, Se-dependent GSH-Px was the main enzymic form, comprising from 65% (lung) up to 90% (heart) of the total enzyme

Table 2
Antioxidant Distribution in the Tissues
of a Newly Hatched Chick

Tissue	Vitamin E, μg/g tissue	Vitamin A, μg/g tissue	Lutein+ Zeaxanthin, μg/g tissue	Ascorbic acid, μg/g tissue	Glutathione, nM/mg protein
Liver	678.16± 44.2 [a]	10.34± 1.11[a]	30.76± 2.76[a]	150.95± 12.44[a]	43.31± 3.76[a]
Brain	6.62± 0.44 [b]	0.11± 0.013[b]	nd	839.14± 55.12[b]	40.19± 3.88[ab]
Kidney	19.48± 1.23[c]	1.26± 0.13[c]	2.33± 0.31[bc]	130.60± 10.33[a]	54.71± 4.23[a]
Heart	24.25± 1.77[c]	0.35± 0.021[d]	3.32± 0.36[b]	59.01± 4.66[c]	32.94± 4.34[b]
Lung	21.03± 1.55[c]	<0.05	2.65± 0.16[bc]	124.63± 11.62[a]	23.57± 2.11[bc]
Thigh Muscle	14.80± 1.36[d]	0.14± 0.022[b]	1.95± 0.17[cd]	57.92± 6.33[c]	21.26± 1.60[cd]
YSM	220.65± 17.66[e]	3.04± 0.041[e]	18.55± 1.33[e]	nd	4.19± 1.10[e]

Note: Values are mean ± SEM (n = 5). Numbers with different superscripts are significantly different with respect to the column.

activity. The liver and kidney displayed the highest total GSH-Px activity and the muscle the lowest.

As in the case of GSH-Px, catalase activity was also maximal in the liver and kidney.

Metals in the Tissues

Tissue levels of Se, Zn, Cu, and Fe are shown in Fig. 1. The liver contained the highest concentrations of Zn, Cu, and Mn (data not shown). The kidney was also characterized by comparatively high levels of the metals. The liver and kidney also contained high concentrations of Se. Comparison between the tissues (excluding YSM) revealed a highly significant positive correlation between the concentration of Se and the activity of Se-dependent GSH-Px (r = 0.95, $p < 0.01$). There was also a positive correlation (r = 0.93, $p < 0.05$) between Fe content and catalase activity in all the tissues other than YSM.

Antioxidant Profile of the Brain

The antioxidant profile of the brain is shown in Table 4. The cerebellum had some distinctive features in antioxidant concentration compared

Table 3
Antioxidant Enzyme Activities in the Tissues of a Newly Hatched Chick

Tissue	Mn-SOD, U/mg protein	Cu-Zn-SOD, U/mg protein	Se- GSH-Px, mU/mg protein	Non-Se-GSH-Px, mU/mg protein	Catalase, U/mg protein
Liver	3.805± 0.23[a]	1.459± 0.16[a]	177.03± 11.42[a]	114.61± 9.22[a]	35.78± 2.14[a]
Kidney	2.978± 0.17[b]	3.154± 0.29[b]	159.82± 12.43[a]	58.62± 6.23[b]	29.541± 1.66[a]
Heart	5.789± 0.24[c]	2.734± 0.14[b]	99.02± 9.88[b]	11.63± 1.33[c]	5.753± 0.33[b]
Lung	0.086± 0.011[d]	5.785± 0.46[c]	99.83± 14.21[b]	53.01± 4.66[b]	5.966± 0.52[b]
Thigh Muscle	1.062± 0.12[d]	6.070± 0.44[c]	45.84± 4.77[c]	12.64± 0.12[c]	3.185± 0.23[c]
YSM	0.124± 0.01[e]	6.970± 0.37[c]	102.61± 9.77[b]	37.65± 4.22[d]	15.181± 2.120[d]

Note: Values are mean ± SEM (n = 5). Numbers with different superscripts are significantly different with respect to the column.

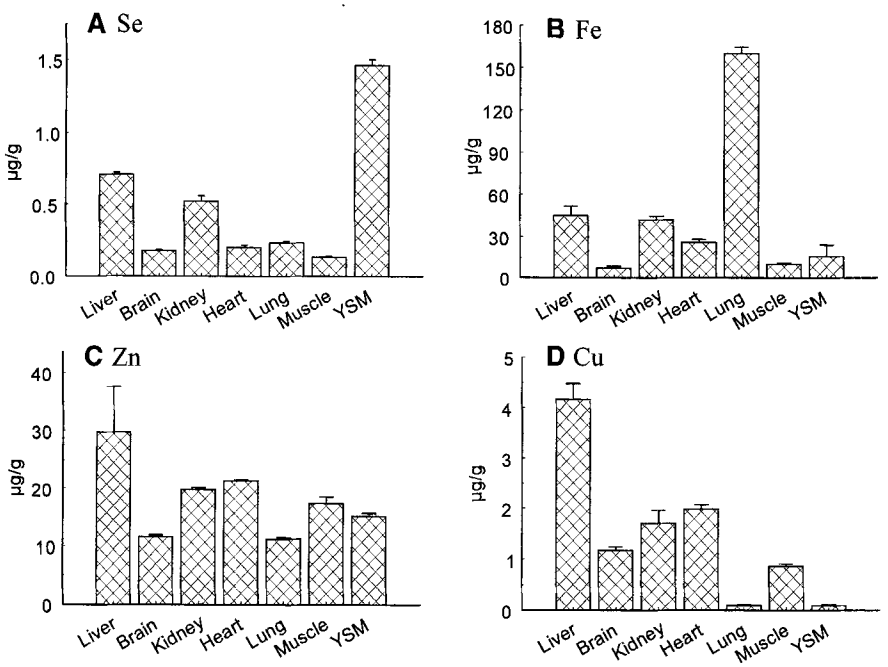

Fig. 1. Metal distribution in the tissues of a newly hatched chick: (a) selenium; (b) iron; (c) zinc; (d) copper. Values are the means (n = 5) with the SEM indicated by the error bars.

Table 4
Antioxidant Composition of the Brain of a Newly Hatched Chick

Antioxidants	Cerebrum	Cerebellum	Brain stem	Optic lobes
α-tocopherol, μg/g tissue	5.22± 0.32	7.12± 0.29**	6.12± 0.38	5.66± 0.41
Ascorbic acid, μg/g tissue	889.4± 41.2	711.3± 51.2**	747.72± 61.4	850.53± 66.4
Glutathione, nM/mg protein	41.12± 3.22	38.12± 2.88	40.13± 4.12	39.56± 3.22
Mn-SOD, U/mg protein	3.66± 0.29	2.837± 0.12*	3.91± 0.29	3.02± 0.21
Cu-Zn-SOD, U/mg protein	6.910± 0.51	6.985± 0.39	7.52± 0.60	8.03± 0.62
Se-dependent GSH-Px, mU/mg protein	29.83± 1.77	28.61± 2.33	33.14± 3.99	32.6± 2.44
Se-independent GSH-Px, mU/mg protein	6.22± 0.42	6.60± 0.46	5.57± 0.52	7.31± 0.82
Catalase, U/ mg protein	1.923± 0.11	2.365± 0.12*	1.966± 0.14	1.822± 0.15

Note: Significance compared to cerebrum. $^{**}p < 0.01$; $^{*}p < 0.05$; $n = 5$.

to the other brain regions; in particular, the cerebrum. The cerebellum was characterized by significantly increased α-tocopherol concentration and catalase activity but decreased ascorbic acid level and Mn–SOD activity compared to that in the cerebrum. In general, the brain was characterized by a low level of vitamin E and low GSH-Px and catalase activity, but a very high level of ascorbic acid and high SOD activity. In general, the brain was characterized by comparatively low levels of Se (0.187 ± 0.01) and moderate levels of Zn (11.67 ± 0.23) and Cu (1.18 ± 0.06).

The Susceptibility to Lipid Peroxidation of the Tissues

Table 5 shows the accumulation of MDA by tissue homogenates incubated in the absence or presence of Fe. Susceptibility to lipid peroxidation as indicated by MDA production differed widely between the tissues. The brain was clearly the most susceptible to lipid peroxidation and the liver the least. In the brain, MDA accumulation as a result of spontaneous lipid peroxidation exceeded levels within the lung, muscle, kidney, liver, and YSM by 3.84, 4.24, 5,00, 5.08, and 8.02 times, respectively. Similarly, Fe-stimulated peroxidation in the brain was also very much higher than that for the other tissues. Such differences were exhibited when calculated in terms of either absolute weight of tissue or unit weight of lipid within the tissues.

Table 5
MDA Formation by Tissue Homogenates as a Result of Lipid Peroxidation

Tissue	Spontaneous LP, μg/g tissue/ hour	Spontaneous LP, μg/mg lipid/ hour	Fe-stimulated LP, μg/g tissue/ hour	Fe-stimulated LP, μg/mg lipid/ hour
Liver	11.26^{a}	0.10^{a}	32.11^{a}	0.29^{a}
Brain	57.22^{b}	1.46^{b}	177.4^{b}	4.53^{b}
Kidney	11.44^{a}	0.27^{c}	79.27^{c}	1.87^{cd}
Heart	9.86^{a}	0.26^{c}	43.92^{d}	1.15^{e}
Lung	14.89^{a}	0.49^{d}	42.43^{d}	1.39^{e}
Thigh Muscle	13.49^{a}	0.30^{c}	100.86^{e}	2.25^{ed}
YSM	7.13^{c}	0.019^{e}	27.11^{a}	0.073^{f}

Note: Values are mean ± SEM (n = 5). Numbers with different superscripts are significantly different with respect to the column.

DISCUSSION

The tissues of the newly hatched chick showed distinctive features in antioxidant profile and susceptibility to lipid peroxidation. The susceptibility of tissues to peroxidation depends on a number of factors, including, primarily, the content of PUFA, levels of natural antioxidants (vitamins A, E, and C and carotenoids), activities of antioxidant enzymes (SOD, GSH-Px, and CAT), their cofactors (Se, Zn, and Mn), and content and availability of prooxidant cations (Fe and Cu).

The importance of an antioxidant/prooxidant balance in the development of the chick embryo has been shown previously (3). In this respect, the hatching process can be considered as a high-stress condition to which the newly hatched chicken has to accommodate. Thus, effective antioxidant protection may be vital for posthatch viability and subsequent productive and reproductive performances.

The brain clearly displayed the greatest susceptibility to spontaneous and Fe-stimulated lipid peroxidation. This is in agreement with previous observations of the chick embryo (3). High levels of lipid unsaturation and comparatively low antioxidant protection make the brain vulnerable to free-radical attack; this is of particular importance in the chick with respect to the development of encephalomalacia, which is associated with an antioxidant system compromised through the deficiency of vitamin E (20). In such conditions, the cerebellum was shown to display particular oxidative stress. Decreased levels of ascorbic acid and Mn-SOD may also play a crucial role in the development of encephalomalacia. Packer and Landvik (21) suggested that in biological systems, vitamin E can be recycled from its oxidized form, ascorbic acid being one of the possible antioxidants involved in such a recycling (22). In the embryonic brain, it has been suggested that antioxidant defense is afforded by high levels of ascorbic acid to effect the recycling of the low concentrations of vitamin E in order to maintain physiological requirements (3).

The present data show the marked differences that exist in the fatty acid composition among the different tissues of the newly hatched chicks. The ratio of the tissue levels of vitamin E (per unit weight of lipids, ng/mg) to DBI for the tissues are as follows: liver, 37.4; brain, 2.4; kidney, 7.4; heart, 12.1; lung, 19.1; muscle, 6.2; YSM, 2.1. The ratio of vitamin E to DBI is a good indicator of tissue susceptibility to lipid peroxidation. With the sole exception of the YSM, a negative correlation ($r = -0.78$) existed for the tissues between this ratio and MDA accumulation following Fe stimulation.

The liver is the main site of natural antioxidant accumulation and metabolism. Vitamin E and carotenoid accumulation in the liver reached a maximum at hatching (3) and is accompanied by high activities of GSH-Px and CAT. Thus, the susceptibility of the liver lipids to peroxidation in the newly hatched chick and immediately following hatching is low. The high levels of endogenous antioxidants within the liver can clearly serve as a major adaptive mechanism for the protection of the tissue during the oxidative stress experienced at hatching. However, by the 10th day after hatching, the vitamin E level in the liver has decreased dramatically (23). Carotenoids can also be considered as a group of natural antioxidants of some importance for avian embryo development (3). Lutein and zeaxanthin are characterized by a high antioxidant activity (24) and are the main carotenoids accumulated in high concentration

in the liver but much lower in other tissues. In general, carotenoid accumulation in the chicken embryo occurs in a similar manner to that of vitamin E, reaching a maximum level at hatching *(3)*. Within the liver of the newly hatched chick, it has been shown that the massive accumulation of lipids occurs through the formation of droplets within the cytosol *(25)*, thus providing, in turn, a suitable intracellular milieu for the storage of large amounts of lipid-soluble vitamins.

The importance of the heart–vascular system for the posthatch development determines the strategy of antioxidant defense. The present data show that the heart was characterized by moderate levels of both fat- and water-soluble antioxidants. Most significantly, the activity of Mn–SOD in the heart was much higher than that in the other tissues. With the considerable increase in the activity of the heart mitochondria associated with the hatching process, leakage of electrons from the electron-transport chain has been suggested to be the main source of superoxide radicals in this tissue *(26)*. SOD forms the first line of defense against free-radical damage, thus, the high activity of the mitochondrial SOD is possibly of vital importance to the heart at the critical period of hatching.

The importance of antioxidant/prooxidant balance in the lung is exemplified by its involvement in the development of pulmonary hypertension syndrome in the chicken *(27)*. The lung lipids were far less unsaturated than the other tissues presently investigated and displayed the lowest DBI. However, the lung also displayed a very high concentration of Fe; the enhanced susceptibility of the lung to spontaneous lipid peroxidation compared to other tissues may be associated with such high concentration of Fe. Nevertheless, it is clear that the lung possesses considerable protection against peroxidation through its content of Cu,Zn–SOD, Se–GSH-Px and non-Se–GSH-Px.

As in the case of the brain, the high levels of PUFAs in the muscle made the tissue vulnerable to lipid peroxidation, especially in the presence of catalytic amounts of Fe. With only low levels of fat-soluble antioxidants, comparatively low levels of Se, reduced glutathione, and ascorbic acid, the muscle presumably relies upon its high levels of Cu,Zn–SOD for antioxidant protection. In the case of the chick embryo, there is evidence that conditions under which vitamin E concentrations are compromised levels of Cu,Zn–SOD are unable to afford adequate protection, with the result that exsudative diathesis with muscular degeneration can be observed at hatching *(28)*.

A highly significant correlation between the Se level and the activity of Se–GSH-Px was found in the majority of the tissues ($p < 0.01$). A carryover effect of Se from the maternal diet via the chicks has been shown *(28)* with an associated increase in the activity of GSH-Px and reduction in exudative diathesis. It has been suggested that the effect of Se on the activity of GSH-Px is achieved through pretranslational

mechanisms, including Se–GSH-Px gene expression and cytosolic mRNA stabilization *(29)*.

Although Zn, Cu, and Mn are necessary for the activity of SOD, the present data failed to demonstrate any positive correlation between cation concentration and enzyme activity. These data are in agreement with previous observations *(16)* and suggest that the availability of metal cofactors is not the limiting step in the expression of SOD in chick tissues. By contrast, with exception of lung and YSM, there was a significant positive correlation between the catalase activity and the level of its cofactor Fe in the tissues (r = +0.93). This is consistent with the hypothesis that expression of catalase activity in certain fetal tissues depends on the availability of Fe *(16)*. The absence of such a correlation in the YSM probably reflects the role of this organ in absorption and transport of trace metals during embryogenesis; that is, the level of Fe as well as other metals in the YSM are not functional but reflect processes of their absorption from the residual yolk and transport to other tissues.

Thus, the main findings of the present work are tissue-specific distinctive features in association with the level of polyunsaturated fatty acid, antioxidant enzyme activity, natural antioxidant accumulation, and the susceptibility to lipid peroxidation.

In a previous investigation *(30)*, it was shown that different tissues of chick embryo displayed distinct developmental strategies with regard to the acquisition of antioxidant capacity, and it was suggested that during embryogenesis, natural antioxidants (vitamins A, E, and C and carotenoids) play a crucial role in antioxidant defense of the embryo tissues against lipid peroxidation.

From the present data, it may be concluded that in postnatal development, when oxygen concentrations in the tissues are higher, metabolic activity and superoxide radical production are increased and tocopherol and carotenoid concentrations are decreased; required protection is afforded through the major antioxidant enzymes SOD, GSH-Px, and CAT. To prove this hypothesis, continuation of the research is necessary during chick postnatal development.

ACKNOWLEDGMENTS

The authors are grateful to the Scottish Office Agriculture and Fisheries department for financial support, to the Royal Society for the visiting fellowship to Peter F. Surai and to Hoffman–La Roche, Basel, Switzerland for the generous gift of β-cryptoxanthin, lycopene, γ-tocopherol, and tocol.

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