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Chicken and Turkey Spermatozoal Superoxide Dismutase: A Comparative Study[1]

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ABSTRACT

Superoxide dismutase (SOD) is one of several enzymes in aerobic cells which scavenge for toxic derivatives of oxygen. These derivatives cause lipid peroxidation, a type of cellular damage that may in part explain the loss of fertilizing capability of chicken and turkey spermatozoa stored in vitro. The objective of the present research was to extract SOD from chicken and turkey erythrocytes and spermatozoa and to compare SOD activities between the two types of spermatozoa and between the spermatozoa and isogeneric erythrocytes, cells rich in SOD.

Based upon activity of purified extracts, chicken spermatozoa contained 1.48 ± 0.08, while turkey spermatozoa contained 0.32 ± 0.01 activity units/10^9 cells. Chicken and turkey erythrocytes had 2.65 ± 0.20 and 2.95 ± 0.39 units/10^9 cells, respectively. When SOD activity was expressed as density, i.e., units/mm^3 of cell volume, chicken spermatozoa had an activity density of 10.6×10^{-2} units/mm^3, 4.6 times more than turkey spermatozoa, which had 2.3×10^{-2} units/mm^3. The activity density for erythrocytes from both species was $\sim 2.0 \times 10^{-2}$ units/mm^3. On the basis of cyanide inhibition, erythrocytes contained only cupro-zinc SOD, but spermatozoa contained both cupro-zinc and mangano superoxide dismutases. The lower SOD activity in turkey spermatozoa may result in a greater susceptibility to oxygen toxicity for turkey semen stored in vitro.

INTRODUCTION

Oxygen toxicity occurs when derivatives of molecular oxygen, i.e., the superoxide anion radical, hydrogen peroxide, and the hydroxyl radical, poison cells (Fridovich, 1978). Even though the superoxide anion is reducing in nature (George, 1965), it can act indirectly as an oxidant by giving rise to the hydroxyl radical, an inducer of biomembrane peroxidation (Fong et al., 1973). Aerobic cells have a complement of enzymes which scavenge for biological oxidants (Tappel, 1978). However, not each cell type is equally endowed with these enzymes. Theoretically, two weak points exist: 1) an inadequate rate of O_2^- dismutation

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

catalyzed by superoxide dismutase (superoxide: superoxide oxidoreductase:EC 1.15.1.1; SOD) and 2) an inadequate rate of H_2O_2 reduction to water catalyzed by either a catalase or peroxidase (Fig. 1). Abu-Erreish et al., (1978) postulated that oxygen toxicity in ram spermatozoa may be mediated by H_2O_2. The spermatozoa appeared to have ample SOD but insufficient glutathione peroxidase activity. Comparable studies of enzymes which counteract oxygen toxicity have not been conducted with chicken or turkey spermatozoa, but are warranted, because these cells, especially turkey spermatozoa, rapidly lose their fertilizing capability when stored in vitro. Therefore, the purpose of the present research was to extract SOD from chicken and turkey erythrocytes and spermatozoa, and to compare SOD activities between the two types of spermatozoa and between spermatozoa and isogeneric erythrocytes, cells rich in SOD.

MATERIALS AND METHODS

Purificatoin of Erytbrocyte Superoxide Dismutase

Blood was collected from three 52-week-old

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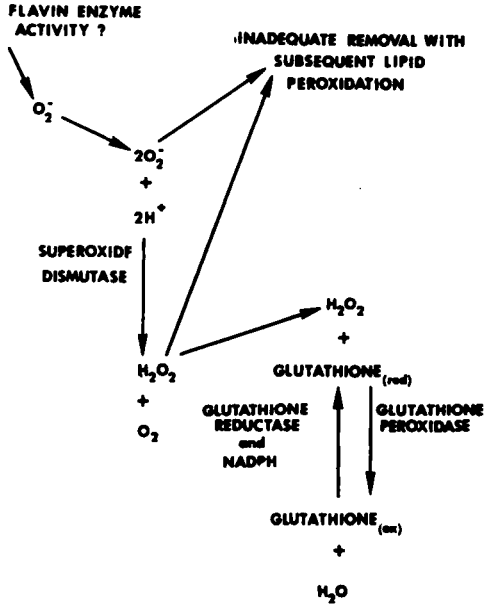

FIG. 1. Schematic diagram depicting hypothetical superoxide anion reaction pathways in spermatozoa. The superoxide radical (O_2^-) can be generated by the univalent reduction of oxygen or the activity of several enzymes such as the flavin enzymes. The O_2^- is then dismutated, a reaction catalyzed by superoxide dismutase. The biological oxidant arising from dismutation, hydrogen peroxide, is detoxified by reduction to water, a reaction most likely catalyzed by glutathione peroxidase in sperm. Inadequate activities of the above enzymes or insufficient concentrations of glutathione and NADPH could lead to peroxidation of membrane lipids and thus irreversibly damage spermatozoa.

cockerels from brown egg strains and three 46-week-old Large White breeder tom turkeys by exsanguination using heparin (1000 units/ml, H 7005, Sigma Chemical Co., St. Louis, MO) as an anticoagulant. Erythrocyte (RBC) concentration was determined with a hemocytometer. Erythrocytes were washed, then hemolyzed, and the hemolyzates chemically extracted according to the method of Crapo et al. (1978) with the exception that they were frozen and stored at −20°C before extraction. Additional purification was accomplished by ion-exchange chromatography on 1.6 × 18 cm columns of diethylamino ethyl (DEAE) and carboxymethyl (CM) cellulose, D-8382 and C-2883 medium mesh, respectively (Sigma). The individual turkey RBC extracts were purified on both DEAE and CM-cellulose, whereas the chicken extracts were purified on CM-cellulose only. The DEAE-cellulose column was packed and equilibrated with a 2 mM potassium phosphate buffer, pH 7.8, and proteins were eluted with a linear gradient of 2 to 333 mM potassium phosphate buffer, pH 7.8 (total eluant volume = 500 ml). The CM-cellulose column was packed and equilibrated with 2 mM potassium acetate buffer, pH 5.4, and elution was achieved using a linear gradient of 2 to 500 mM potassium acetate buffer, pH 5.4 (total eluant volume = 200 ml). Eluted fractions with SOD activity were dehydrated and thus concentrated by covering filled dialysis bags (cutoff = MW 12,000) with polyethylene glycol (P2263, Sigma). The concentrated SOD from CM-cellulose-purified chicken and turkey erythrocyte extracts was dissolved in 50 mM potassium phosphate buffer, 0.1 mM EDTA, pH 7.8, for assay of SOD activity.

Purification of Spermatozoal and Seminal Plasma Superoxide Dismutase

Semen was collected by abdominal massage (Burrows and Quinn, 1937) from the brown egg strain cockerels and Large White tom turkeys, and sperm cell concentration determined with a Model B Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). Spermatozoa were separated from seminal plasma by centrifugation at 2000 × g, washed in Millonig's phosphate buffer, (pH 7.3; Millonig, 1962), recentrifuged, then suspended in deionized water and stored at −20°C. The seminal plasma was recentrifuged at 12,800 × g to remove residual spermatozoa and stored at −20°C. Sufficient spermatozoa were thawed and pooled to provide a suspension of at least 7.0×10^{11} total cells (this required ∿140 and 100 ml of whole semen from chickens and turkeys, respectively). The suspension was chilled in an ice bath, and EDTA and a trypsin inhibitor (T4385, Sigma) were added to a concentration of 0.1 mM and 7 mg/ml, respectively. The trypsin inhibitor was included to counteract acrosin, a trypsin-like enzyme, which is released during sperm cell disruption. A French pressure cell (20,000 psi) was used to lyse the spermatozoa, and the suspension of lysed cells was ultracentrifuged at 150,000 × g for 1 h. Two layers of debris sedimented, and the efficiency of cellular disruption was assessed by determining the composition of the layers by electron microscopy (Fig. 2). The top layer was predominantly flagellar microtubules with a few acrosomes, whereas the bottom layer was largely chromatin. No intact spermatozoa and only occasional mitochondria were observed, indicating that spermatozoa and their mitochondria were lysed.

The supernatant was chemically extracted for SOD by the method of Weisiger and Fridovich (1973). Spermatozoal SOD was purified by ion-exchange chromatography on both DEAE and CM-cellulose and concentrated as previously outlined. The concentrated SOD from the CM-cellulose was dissolved in 50 mM potassium phosphate buffer, 0.1 mM EDTA, pH 7.8, for assay of activity.

Plasma samples were thawed and pooled to yield 80 ml of chicken and 68 ml of turkey seminal plasma. The pooled plasma was ultracentrifuged at 150,000 × g for 1 h, and supernatants were extracted for SOD by the method of Weisiger and Fridovich (1973). Following extraction, the SOD was dissolved in 50 mM potassium phosphate buffer, 0.1 mM EDTA, pH 7.8, and dialyzed (cutoff = 12,000 MW) for 24 h at 4°C against the same buffer. The extract was then assayed for SOD activity.

Assay of Superoxide Dismutase Purity and Activity

Protein concentration of purified RBC, spermatozoal, and seminal plasma SOD extracts in 50 mM

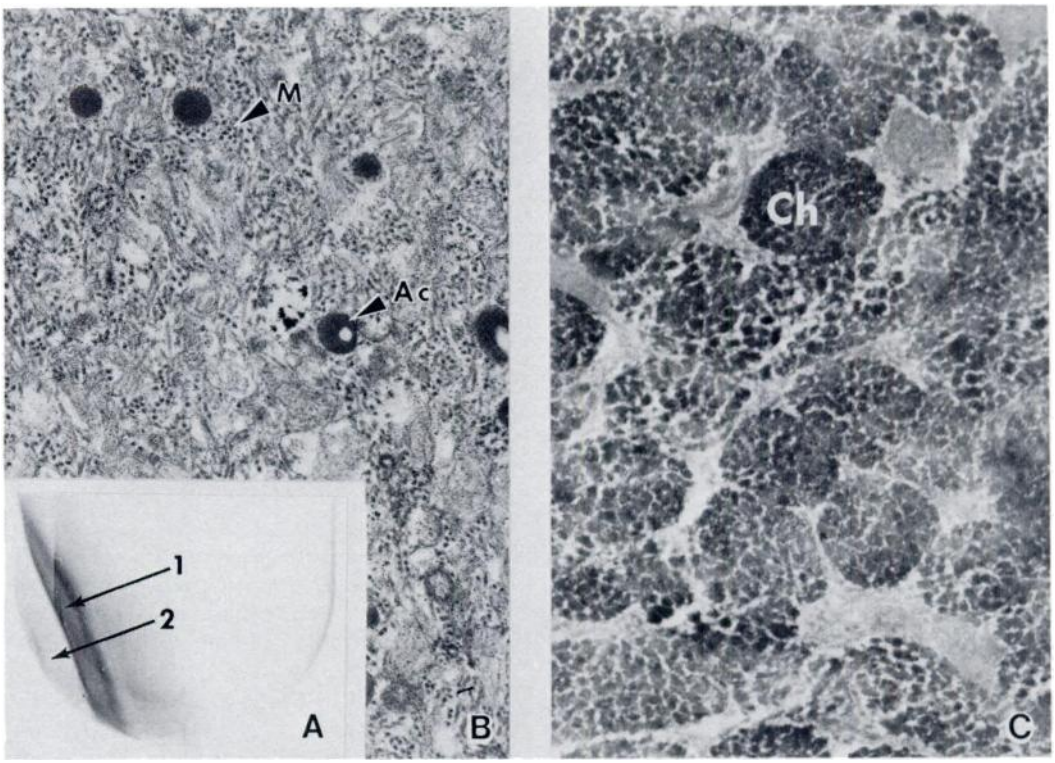

FIG. 2. Ultracentrifugation (A) of lysed turkey spermatozoa caused sedimentation of debris into an opaque brown upper layer (1) and a dense white lower layer (2). The composition of the upper layer, determined by the electron microscope (B), was primarily flagellar microtubules (M). A few intact acrosomes (Ac) were also observed. The lower layer (C) was predominantly chromatin (Ch). B, X9500; C, X39,500.

potassium phosphate buffer, 0.1 mM EDTA, pH 7.8, was determined using the Bio-Rad protein assay (Bio-Rad, Richmond, CA). To assess purity, aliquots of RBC and spermatozoal extracts containing 20–30 μg protein were subjected to electrophoresis in polyacrylamide gels (Cyanogum 41, Fisher Scientific; 4% acrylamide concentrating gels, pH 6.7; and 9% resolving gels, pH 8.9) polymerized with ammonium persulfate. Electrophoresis was conducted at 4 mA/gel at 5°C with bromphenol blue (B0126, Sigma) as a tracking dye. Gels were then either stained for protein with Coomassie G 250 (B1131, Sigma) according to the method of Blakesly and Boezi (1977), or stained for SOD activity with nitro blue tetrazolium (N6876, Sigma) according to Beauchamp and Fridovich (1971), with one modification: the concentration of nitroblue tetrazolium was 5 mM.

Superoxide dismutase activity and inhibition of activity with 1 mM KCN was quantitated according to the method of Crapo et al. (1978) with a Gilford Model 250 spectrophotometer (Gilford Instrument, Oberlin, OH). Absorbance of the reaction mixture at 550 nm increased at a rate of 0.020 absorbance units/min as ferricytochrome c, the O_2^- detector, was reduced to ferrocytochrome c by O_2^- generated by xanthine and xanthine oxidase. Addition of SOD decreased the rate of ferrocytochrome C reduction, a unit of activity being defined as the amount of SOD required to effect a 50% decrease in the Δ absorbance/min.

RESULTS

Erythrocyte Superoxide Dismutase

In preliminary experiments, extracts from pooled blood were used to establish chromatography conditions for purification of chicken and turkey RBC SOD. The enzymes from both species had low affinity for DEAE and high affinity for CM-cellulose. Electrophoresis of the CM-cellulose-purified SOD showed protein bands that varied in relative mobility and activity between batches. This observation was attributed to possible genetic variation between birds, and all subsequent work was conducted with blood obtained from individuals.

Turkey SOD extracts were purified by ion-exchange chromatography on DEAE and then CM-cellulose, and representative chroma-

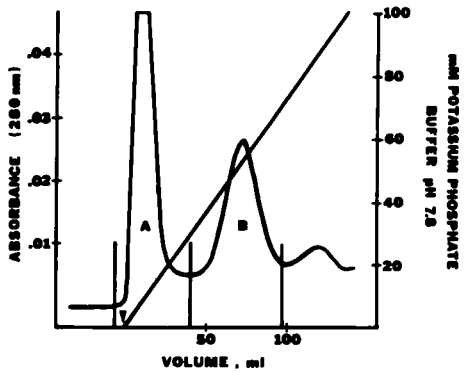

FIG. 3. Chromatography of turkey erythrocyte superoxide dismutase eluted from a 1.6 X 18 cm column of DEAE-cellulose. Chromatography was conducted at 22°C with a flow rate of 1 ml/min. The potassium phosphate gradient was initiated (arrow) when the nonabsorbed protein began to emerge from the column. The peaks which contained SOD activity are demarcated by vertical lines. In this example, peak A contained 5.80 mg of protein, had a specific activity of 518 units/mg, and a total of 3004 units. Peak B contained 2.96 mg of protein, had a specific activity of 1266 units/mg, and a total of 3747 units. This elution pattern was consistent for chromatography of turkey RBC SOD on DEAE-cellulose.

tograms are shown in Figs. 3 and 4. Chicken RBC extracts were purified on CM-cellulose only, as the combined effects of the dimensions of the DEAE-cellulose column and the smaller amount of enzyme obtainable from individual chickens resulted in excessive dilution. The elution pattern of chicken RBC SOD from the CM-cellulose column was similar to that for the turkey depicted in Fig. 4. In both cases ~95% of the SOD activity of the CM-cellulose elutate was contained in two overlapping peaks (Fig. 4, A) which were highly resolved from all others. The remaining 5% of the SOD activity in the CM-cellulose eluate was contained in a trailing peak represented by peak B in Fig. 4.

Electrophoresis of protein (Fig. 5) from concentrated eluates corresponding to peak A in Fig. 4 showed that the protein of both chicken and turkey extracts was predominantly SOD.

Based upon the total SOD activity of the CM-cellulose eluates, the units of SOD activity per 10^9 RBCs and per unit volume of RBCs were calculated and found comparable between chickens and turkeys (Table 1). Mean units/10^9 RBCs from cockerels and toms were 2.65 ± 0.20 and 2.95 ± 0.39, respectively. These values along with data from the preliminary work indicate a range of 2–4 units/10^9 RBCs for chickens and turkeys. The density of SOD activity in cockerel RBCs was calculated to be 2.1 x 10^{-2} and 1.9 x 10^{-2} units/mm^3 (Table 1) for tom RBCs. Therefore, it is concluded that the SOD activities of chicken and turkey RBCs are similar and are attributable to the cupro-zinc form of the enzyme on the basis of inhibition with 1 mM KCN.

Spermatozoal and Seminal Plasma Superoxide Dismutase

The purification of spermatozoal SOD required DEAE and CM-cellulose chromatography. Neither chicken nor turkey spermatozoal SOD adsorbed to DEAE-cellulose. Adsorption did occur on CM-cellulose, and representative chromatograms for the elution of chicken and turkey spermatozoal SOD are shown in Fig. 6A and B, respectively. After eluates corresponding to the SOD-containing peaks in Fig. 6 were concentrated to equivalent volumes, average total protein was 1.29 ± 0.28 mg for chickens and 0.29 ± 0.06 mg for turkeys (Table 2). The specific activity averaged 1038 ± 142 and 934 ± 175 units/mg protein for chickens and turkeys, respectively (Table 2).

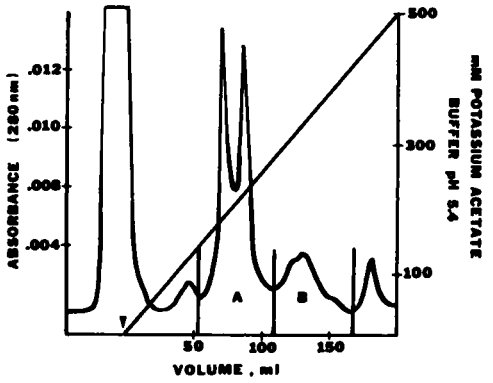

FIG. 4. Chromatography of turkey erythrocyte superoxide dismutase eluted from a 1.6 X 18 cm column of CM-cellulose. Chromatography was conducted at 22°C with a flow rate of 1 ml/min. The potassium acetate gradient was initiated (arrow) after all nonadsorbed protein had been eluted from the column. The peaks which contained SOD activity are demarcated by vertical lines. In this example, peak A contained 1.28 mg of protein, had a specific activity of 2232 units/mg, and a total of 2857 units. Peak B contained 0.22 mg of protein, had a specific activity of 454 units/mg, and a total of 100 units. This elution pattern was observed for the chromatography of both chicken and turkey RBC SOD on CM-cellulose.

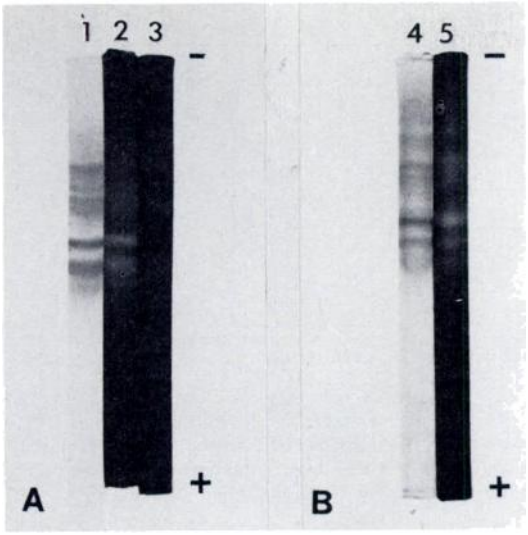

FIG. 5. Polyacrylamide gels showing erythrocyte (RBC) superoxide dismutase (SOD) bands spearated by electrophoresis.

A) Gels 1 and 2 contained 20 μg of DEAE and CM-cellulose purified turkey RBC SOD. Gel 1 was stained with Coomassie G 250 for protein and gel 2 with nitro blue tetrazolium (NBT) for SOD activity. Gel 3 contained 30 μg BSA and served as a control for the specificity of the NBT stain. At least seven protein bands were NBT positive for SOD activity.

B) 30 μg of CM-cellulose purified chicken RBC SOD stained as for gels 1 and 2 in (A). At least seven protein bands contained SOD activity.

Yields and purification factors for chicken and turkey spermatozoal SOD could not be accurately determined due to strong interference with the spectrophotometric assay prior to CM-cellulose chromatography. However, electrophoresis of protein from peaks containing SOD activity indicated that the enzyme had been substantially purified (Fig. 7). Chicken sperm extracts produced four bands which were positive for SOD activity (Fig. 7A), and the turkey extract had at least three bands (Fig. 7B). A possible fourth band of turkey sperm SOD may have been obscured by unexplained diffuse staining at the top of the gel.

Total units of SOD activity averaged 1365 ± 370 and 253 ± 5 for chicken and turkey sperm extracts, respectively (Table 2). Units of SOD activity/10^9 spermatozoa averaged 1.48 ± 0.08 for chicken spermatozoa but only 0.32 ± 0.01 for turkey spermatozoa (Table 2). Both of these values are considerably lower than the corresponding RBC values (Table 1). However, this is to be expected as the spermatozoa are appreciably smaller than RBCs, e.g., the mean corpuscular volume calculated for turkey RBCs was approximately 157 μm^3, whereas the volume of a turkey spermatozoon was estimated to be 14 μm^3. Therefore, a better comparison can be made on the basis of SOD activity density, i.e., units/mm^3. The density of spermatozoal activity was calculated to be 10.6 x 10^{-2} units/mm^3 for chickens and 2.3 x 10^{-2} units/mm^3 for turkeys (Table 2). Thus, when comparing activities per unit volume between spermatozoa and RBCs, chicken spermatozoa had ∿500% of the activity of chicken RBCs. Turkey spermatozoa, however, had approximately the same activity per unit volume as either turkey or chicken RBCs but only 22% as much SOD activity as chicken spermatozoa.

In preliminary experiments, unsuccessful attempts were made to purify a cupro-zinc SOD after mild lysis of spermatozoa. Therefore, the mitochondria were assumed to be the primary source of sperm SOD. Mitochondria were lysed with a French pressure cell (Fig. 2), and sperm SOD was purified by the technique of Weisiger and Fridovich (1973), originally developed for the purification of mangano SOD from chicken liver mitochondria. However, on the basis of inhibition with 1 mM KCN, the spermatozoal extracts from chickens and turkeys appeared to contain cupro-zinc SOD. Turkey spermatozoal SOD activity was 72% inhibited by 1 mM KCN, but chicken spermatozoal SOD only 34%, indicating a greater percentage of mangano SOD in the chicken extract. Unfortunately, no differentiation could be made between the mangano and cupro-zinc enzymes by the NBT stain, and insufficient amounts of sperm SOD were obtained for further purification and metal analysis.

Superoxide dismutase activity was also

TABLE 1. Activity of chicken and turkey erythrocyte superoxide dismutase extracts after carboxymethyl cellulose chromatography.

Source	Units/10^9 erythrocytes	Units/mm^3
Chicken (3)[a]	2.65 ± 0.20[b]	2.1 X 10^{-2}
Turkey (3)	2.65 ± 0.39	1.9 X 10^{-2}

[a]Denotes the number of individual bird replicates.

[b]Values reported as mean ± SEM.

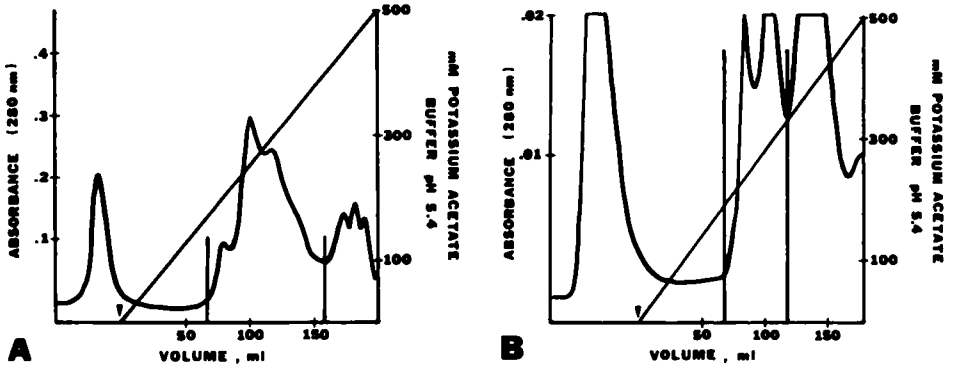

FIG. 6. Chromatograms depicting elution of (A) chicken and (B) turkey spermatozoal superoxide dismutase from a 1.6 × 18 cm column of CM-cellulose. Chromatography was performed at 22°C with a flow rate of 1 ml/min. The potassium acetate gradients were initiated (arrows) after the nonadsorbed protein had been eluted. Superoxide dismutase activity was found in the peaks demarcated by vertical lines. Total protein of these peaks averaged 1.29 ± 0.28 (n = 2) and 0.29 ± 0.06 mg (n = 2) and specific activity averaged 1038 ± 142 and 934 ± 175 units/mg for chickens and turkeys, respectively.

detected in seminal plasma. Chicken seminal plasma had an activity of 2.25 units/ml and turkey seminal plasma 2.05 units/ml. Table 3 shows the distribution of SOD activity in semen. In either species, 59% or more of the total activity was found to come from the cell fraction which comprised 20% or less of total semen volume.

DISCUSSION

Several similarities were shown between the superoxide dismutases of chicken, turkey, and mammalian erythrocytes (Crapo et al., 1978). The techniques used for purification and activity determination of mammalian SOD were suitable for the characterization of chicken and turkey SOD. Similarly, chicken and turkey RBC SOD, like mammalian, appears to be a cupro-zinc enzyme and is stable to freezing. Units of activity/10^9 cells were comparable between avian and mammalian RBCs, as our data suggest a range of 2–4 units/10^9 RBCs and Abu-Erreish et al. (1978) reported 3.3 units/10^9 ram RBCs. However, this is not to suggest the density of SOD activity is comparable, as avian RBCs are larger than mammalian (Sturkie and Griminger, 1976).

Unlike mammalian RBC SOD (ovine or bovine), electrophoresis of chicken and turkey RBC SOD revealed numerous bands having SOD activity. Weisiger and Fridovich (1973) reported multiple forms of chicken liver cytosol cupro-zinc SOD but were uncertain whether they were true isozymes or were a common protein derivatized by post-translational changes. Therefore, the bands shown in Fig. 5 should not necessarily be considered isozymes even though they may exist.

Chicken and turkey spermatozoa, like ram

TABLE 2. Total protein and activity of chicken and turkey sperm superoxide dismutase extracts after carboxymethyl cellulose chromatography.

Source	Total protein in purified extract (mg)	Total units	Specific activity (units/mg protein)	Units/10^9 spermatozoa	Units/mm^3
Chicken (2)[a]	1.29 ± 0.28[b]	1365 ± 370	1038 ± 142	1.48 ± 0.08	10.6×10^{-2}
Turkey (2)	0.29 ± 0.06	258 ± 5	934 ± 175	0.32 ± 0.01	2.3×10^{-2}

[a] Denotes replicates of pooled samples with equivalent sperm cell number.

[b] Values reported as mean ± SEM.

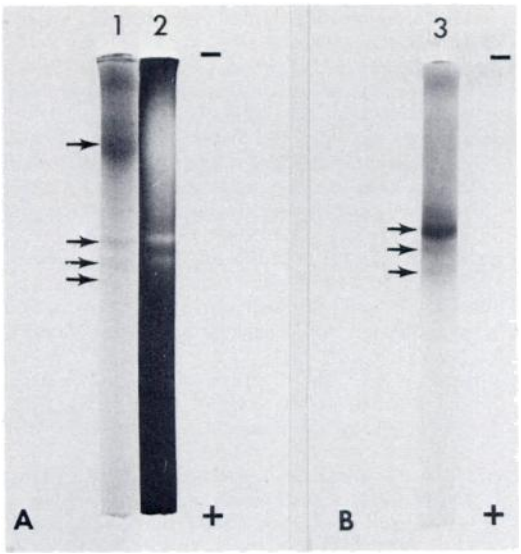

FIG. 7. Polyacrylamide gels showing spermatozoal superoxide dismutase (SOD) bands separated by electrophoresis.

A) 30 μg of DEAE and CM-cellulose purified chicken spermatozoal SOD stained with Coomassie G 250 (gel 1) for protein and nitro blue tetrazolium (NBT; gel 2) for SOD activity. Three bands with SOD activity are visible, one of which is diffuse (upper arrow). A fourth faint band was also observed (lower arrow).

B) 30 μg of DEAE and CM-cellulose-purified turkey spermatozoal SOD stained with Coomassie G 250 for protein. NBT staining showed that the region occupied by three bands (arrows) and the diffusely stained region at the top of the gel were positive for SOD activity.

(Abu-Erreish et al., 1978) and bovine spermatozoa (Magnes and Li, 1980), were found to contain SOD. The ovine spermatozoal SOD was assumed to be a cupro-zinc enzyme on the basis of inhibition with 1 mM KCN, but avian spermatozoa, like bovine, appeared to have both cupro-zinc and mangano SOD, as their SOD activity was only partially inhibited by 1 mM KCN.

Chicken spermatozoa apparently are endowed with an appreciably greater SOD activity than are turkey spermatozoa. On the basis of both units/10^9 cells and units/mm^3, chicken spermatozoa had an average of 4.6 times more SOD activity than did turkey spermatozoa. This difference in SOD activity between the two types of spermatozoa is interesting in light of accumulating data which suggest that chicken and turkey spermatozoa are metabolically dissimilar. Sexton (1974) reported that chicken spermatozoa have a higher endogenous respiratory rate than do turkey spermatozoa, and McIndoe and Mitchell (1978) isolated only lactate dehydrogenase (LDH) 1 from chicken spermatozoa, but LDH 1 through LDH 5 from turkey spermatozoa. Thus, while morphologically similar, the spermatozoa may differ markedly in certain aspects of cell physiology.

Superoxide dismutase activity of semen was predominantly found in the cell fraction (Table 3). The SOD activity of seminal plasma may arise from damaged spermatozoa or possibly from apocrine secretions of epithelial cells lining the ducts of the male reproductive tract, a common occurrence (Hess et al., 1976).

Superoxide anion can be generated by a number of enzyme systems within cells, and there is increasing evidence that obligatory aerobes must constantly utilize superoxide dismutase to counteract superoxide radicals generated via univalent reduction of oxygen (Fridovich, 1978). Dismutation of O_2^- generates hydrogen peroxide which in itself is deleterious to cells, necessitating its conversion to water by a peroxidase or to water and oxygen by catalase.

We have found that atmospheric O_2 pressure is ∿120–130 mm Hg greater than the partial pressure of O_2 in freshly ejaculated turkey semen. This implies that storing turkey semen under atmospheric conditions could lead to O_2^- toxicity due to inadequate SOD activity. The same mechanism may be apropos for chicken

TABLE 3. Distribution of superoxide dismutase in chicken and turkey semen.

Source	% Packed cell volume	Total units/ml of semen	% Total activity from spermatozoa	% Total semen activity from seminal plasma
Chicken	10	13.45	76.7	23.3
Turkey	20	4.02	59.2	40.8

semen, which also has low O_2 tension, despite the greater SOD activity. Additional weak points could be inadequate glutathione peroxidase or reductase activity or lack of available glutathione, which apparently comprise the system for eliminating H_2O_2 in sperm (Li, 1975; Abu-Erreish et al., 1978). The coenzyme for glutathione reductase is NADPH, which is produced by the hexose monophosphate shunt, a pathway probably not utilized by chicken or turkey spermatozoa (Sexton, 1974). Accumulation of O_2^- and H_2O_2 can generate a very potent oxidant, $OH\cdot$, which can peroxidize membrane lipids (Fong et al., 1973). Lipid peroxidation has been shown to occur in fowl spermatozoa stored in vitro (Fujihara and Howarth, 1978). The role of the O_2^-, H_2O_2 system in the genesis of lipid peroxidation remains to be determined, but it is feasible that this mechanism could account for the rapid loss of sperm cell viability in vitro, especially for the turkey.

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