OXIDATIVE METABOLISM OF CHICKEN POLYMORPHONUCLEAR LEUCOCYTES DURING PHAGOCYTOSIS

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Summary

The oxidative response to phagocytosis by chicken polymorphonuclear leucocytes was investigated as compared to guinea pig polymorphonuclear leucocytes.

The polymorphs from both species respond to phagocytosis with an increased oxygen consumption, an increased generation of O_2^- and H_2O_2, and an increased oxidation of glucose through the hexose monophosphate shunt. The rate of oxygen consumption, and generation of O_2^- and H_2O_2 by phagocytosing chicken polymorphonuclear leucocytes is considerably lower than with phagocytosing guinea pig polymorphonuclear leucocytes. By contrast, the extent of hexose monophosphate shunt stimulation in chicken polymorphs is comparable to that of guinea pig polymorphs. Evidence is presented suggesting that H_2O_2 is preferentially degraded in chicken cells through the glutathione cycle, whereas catalase and myeloperoxidase are the two main H_2O_2 degrading enzymes in guinea pig cells.

The 20,000 g fraction of the postnuclear supernatant of chicken polymorphs contains a cyanide-insensitive NADPH oxidizing activity which is stimulated during phagocytosis. Similar properties for the NADPH oxidizing activity of guinea pig polymorphs have been previously reported.

It is concluded that the metabolic burst of phagocytosing chicken polymorphonuclear leucocytes is qualitatively similar to that of guinea pig polymorphonuclear leucocytes, but the latter cells are more active in all the biochemical parameters that have been measured. The difference in the H_2O_2 degradation pathways between the two species is accounted for by the lack of myeloperoxidase and catalase in chicken polymorphs.

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Introduction

Polymorphonuclear leucocytes are known to produce hydrogen peroxide during the respiratory stimulation associated with the ingestion of particles[1-3]. The oxygen taken up by the cell is univalently reduced to superoxide anion (O_2^-) which, in turn, dismutates, with formation of hydrogen peroxide[4-6]. The fate of the hydrogen peroxide in these cells is dual, 1) it may be destroyed within the cell by enzymes such as myeloperoxidase, catalase or glutathione peroxidase, and/or 2) it may leak out of the cell. The amount of peroxide released depends at least on the rate of formation and the efficiency of the intracellular H_2O_2-destroying systems.

It has been reported that production of H_2O_2 concomitant with the increment of respiration occurring during phagocytosis is not demonstrable in chicken polymorphonuclear leucocytes[7]. On this basis, it has been suggested that these cells also fail to produce or release O_2^-[7]. This would set chicken polymorphs apart from all other types of phagocytes as far as the mechanism of metabolic activation during phagocytosis is concerned.

In the present paper the oxidative metabolism of chicken polymorphonuclear leucocytes has been reinvestigated as compared to guinea pig polymorphonuclear leucocytes which represent a classical model for the study of the phagocytosis stimulated metabolism. We conclude that the mechanism of the stimulated oxidative metabolism of polymorphs from both species is qualitatively similar including O_2^- and H_2O_2 generation.

Materials and Methods

Cell preparation

Male adult chickens (Ubard strain) were injected intraperitoneally with 70 ml of 1% sterile sodium caseinate solution. The exudate was collected 5 hours later. The exudates contaminated with red cells were discarded. The cells were collected by centrifugation at 150 g for 7 min, and were then resuspended in calcium-free Krebs-Ringer-Phosphate (KRP) buffer pH 7.4. The average differential count of the preparation used was, neutrophils 80–85%, mononuclear cells 11–20%, eosinophils 0–4%. Guinea pig leucocytes were obtained as previously described[8].

Phagocytosis

B. mycoides were used throughout the experiments. The bacteria were grown on nutrient agar, autoclaved, opsonized for 20 min at 37° with fresh homologous serum and were added to leucocyte suspension (4×10^7/ml) in a 100 : 1 ratio.

Oxygen uptake

The rate of oxygen uptake by resting and phagocytosing leucocytes was measured at 37° with a Clark oxygen electrode attached to a plastic chamber as previously described[9].

Hydrogen peroxide production

The formation of H_2O_2 was assayed with three different methods, a) polarographically, b) colorimetrically, and c) fluorimetrically.

In the polarographic assay[1] the oxygen consumption by phagocytosing cells was recorded for 3–4 min in the presence or absence of 2mM cyanide. Excess catalase (from beef liver, Boehringer, Mannheim, GmbH, Germany) was then added into the electrode chamber, and a backward deflection of the recording trace was observed indicating that oxygen was released in the medium from H_2O_2.

The colorimetric method was that described by THURMAN *et al.*[10]. After recording the oxygen uptake of phagocytosing polymorphonuclear leucocytes with a Clark oxygen electrode for various time intervals, 1 ml of the mixture was rapidly drawn from the electrode chamber and trichloroacetic acid was added to it at a final concentration of 10% (w/v). After centrifugation, the clear supernatant was used for assays.

For the fluorimetric assay, the method described by ROOT and CHANCE[3] with some modifications was used. In this method the decay of scopoletin fluorescence, following its oxidation by horse radish peroxidase in the presence of H_2O_2 is measured. While oxygen uptake by phagocytosing cells was being recorded with a Clark oxygen electrode, aliquots of the mixture were drawn at various time intervals from the electrode chamber and were immediately centrifuged in an Eppendorf microcentrifuge model 3,200. The clear supernatant was used for assays. The spectrophotofluorimeter cuvette contained 3 ml of KRP, 2.5 μM scopoletin (Sigma Chemical Co, St. Louis, Mo, USA) and 0.166 μM horse radish peroxidase (Sigma Chemical Co). The volume of the samples that was added to the cuvette varied from 10 to 100 μl. A standard curve with a titrated solution of fresh H_2O_2 was prepared before each experiment.

Superoxide anion production

The assay was based on the superoxide dismutase inhibitable reduction of cytochrome c[4–6]. The assay medium was the same as for oxygen uptake measurements plus 200 μM cytochrome c (from horse heart, grade VI, Sigma Chemical Co). The experimental details have been described elsewhere[11].

$^{14}CO_2$ production from glucose

The rate of oxidation of glucose-1-^{14}C and glucose-6-^{14}C (from the Biochemical Centre, Amersham, England) was determined by incubating the leucocytes in Erlermeyer flasks equipped with a center well. The main compartment of the flask contained $1\text{-}2 \times 10^7$ leucocytes and 0.5 mM cold glucose in a final volume of

2 ml. The center well contained 0.25 ml of 20% KOH. The reaction was started by adding 0.1–0.5 μCi of labeled glucose and, when required, bacteria (ratio cells : bacteria, 1 : 100). The flasks were sealed with a rubber cap and incubated for 10 min at 37°. The reaction was stopped by injecting through the cap 1 ml of 1 N H_2SO_4. After standing for 20 min at 37° and for additional 30 min in the cold room, the content of the center well was transferred into scintillation vials containing 10 ml of Bray's scintillation medium[12]. The vials were counted in a Beckman LS 100 liquid scintillation spectrometer.

Cell homogenization and preparation of the granules

The leucocytes were incubated with or without bacteria in KRP containing 0.5 mM glucose for 5 min at 37°. After dilution with ice-cold KRP, the leucocytes were sedimented at 150 g for 10 min so that most of the bacteria remained in the supernatant. The packed cells were suspended in ice-cold 0.34 M sucrose (buffered at pH 7 with Na-bicarbonate) to a concentration of $3–4 \times 10^8$ leucocytes/ml and homogenization was performed in a Potter type homogenizer equipped with a teflon pestle driven by a motor. The homogenization was stopped when 90% of the cells were broken as judged by light microscopic examination. Usually this process did not take more than 3–4 min. The homogenate was diluted with 0.34 M sucrose and centrifuged at 250 g for 7 min to remove nuclei, cell debris and unbroken cells. The supernatant fraction was centrifuged at 20,000 g for 20 min and the sedimented granules were suspended in 0.34 M sucrose.

Determination of NAD(P)H oxidase activity

The rate of NADH and NADPH oxidation was measured by determining the rate of O_2 consumption with a Clark type oxygen electrode as previously described[8,13]. Enzymatically reduced NADH and NADPH (grade II) were purchased from Boehringer, Mannheim, GmbH, Germany.

Results

Oxygen uptake, O_2^- and H_2O_2 generation

The results of simultaneous determinations are shown in Table 1. Chicken leucocytes responded to phagocytosis with an increased oxygen consumption as do the polymorphonuclear leucocytes of all the other species so far

Table 1
Oxygen uptake and generation of O_2^- and H_2O_2 by chicken and guinea pig polymorphonuclear leucocytes.

		nmoles/2 min/2×10^7 cells				
		O_2		H_2O_2		O_2^-
		$-NaN_3$	$+NaN_3$	$-NaN_3$	$+NaN_3$	$-NaN_3$
Chicken	Resting cells	13.9 ± 1.2 (5)	12.7 ± 1.5 (4)	—	—	— (5)
	Phagocytosing cells	33.7 ± 1.6 (4)	27.03 ± 1.1 (6)	3.33 (2)[a] 3.12 (1)[b] 3.26 ± 0.22 (3)[c]	5.03 ± 0.47 (4)[a] 5.87 (2)[b] 5.31 ± 0.33 (6)[c]	9 ± 0.73 (6)
Guinea pig	Resting cells	18.7 ± 3.2 (3)	16.2 ± 2.8 (3)	undetectable	3.8 ± 2.8 (3)	5.4 ± 4.1 (5)
	Phagocytosing cells	91.9 ± 4.1 (5)	119.5 ± 7.0 (5)	2.8 (2)	106.4 ± 2.3 (5)	77.1 ± 7.8 (6)

The basic assay medium was KRP pH 7.4 containing 0.5 mM glucose. For O_2^- assay, 200 μM ferricytochrome c and when required 35 μg/ml of superoxide dismutase was added to the medium. 10^7 guinea pig cells/ml and $2–4 \times 10^7$ chicken cells/ml were used in each assay. Heat killed and serum opsonized B. mycoides was the phagocytosable particle (ratio cell : bacteria, 1 : 100). Volume 2 ml. Temperature 37°. $NaN_3 = 2$ mM. Results are given as mean ± SEM. The number of experiments is shown in parenthesis.

[a] fluorimetric method
[b] colorimetric method
[c] mean of a and b

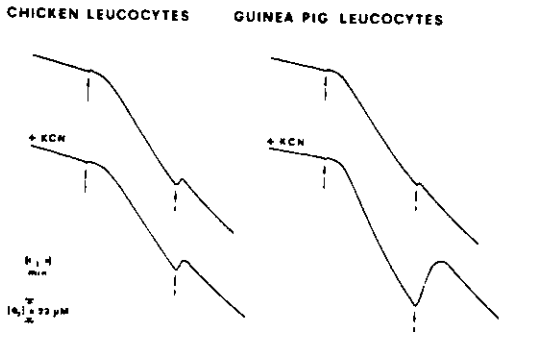

Fig. 1. Polarographic assay of oxygen uptake and H_2O_2 generation by chicken and guinea pig polymorphonuclear leucocytes. The basic assay medium was Krebs-Ringer-Phosphate pH 7.4 containing 0.5 mM glucose. Volume 2 ml. Temperature 37°. 9.5×10^7 chicken cells and 2.5×10^7 guinea pig cells were used in each assay. Heat-killed B. mycoides opsonized with homologous serum was added at the solid arrows (ratio cells:bacteria, 1:100). Excess catalase was added at the broken arrows.

tested. The respiratory increment in chicken leucocytes was considerably lower than that observed in guinea pig cells. The oxygen uptake by phagocytosing chicken leucocytes was not significantly affected by azide, whereas guinea pig leucocytes showed a higher oxygen consumption in the presence of this inhibitor than in its absence.

Figure 1 shows the recorded tracings of the polarographic assay of oxygen uptake and H_2O_2 generation. The presence of H_2O_2 in the medium is indicated by the backward deflection of the trace after catalase addition. Hydrogen peroxide could be detected in the medium of phagocytosing chicken leucocytes both in the presence and in the absence of cyanide, whereas no H_2O_2 was detectable with guinea pig cells in absence of the inhibitor.

For a better quantitation of hydrogen peroxide production, the scopoletin method and the thiocyanate method were used. Even with these two procedures appreciable amounts of H_2O_2 could be measured in the incubation medium of phagocytosing chicken leucocytes, with or without azide. The amount of H_2O_2 measured with phagocytosing chicken cells accounted for 19.6% of the oxygen consumed in the presence of azide. With phagocytosing guinea pig cells the amount of H_2O_2 that accumulated in the medium in the absence of azide was comparable to that observed with chicken cells under the same experimental conditions. However, if the cells were allowed to phagocytose in the presence of azide the amount of H_2O_2 was several times higher than in absence of the inhibitor and was also markedly higher than that found in the medium of phagocytosing chicken leucocytes. The H_2O_2 measured with phagocytosing guinea pig cells accounted for about 89% of the oxygen taken up in the presence of azide.

A small but definite amount of superoxide anion was measured in the incubation medium of chicken phagocytosing leucocytes, whereas no superoxide anion could be detected in the medium of resting cells. The amount of superoxide anion released by phagocytosing chicken leucocytes was considerably lower than that released by phagocytosing guinea pig leucocytes.

Oxidation of glucose-1-^{14}C and glucose-6-^{14}C

Table 2 shows the effect of phagocytosis on the $^{14}CO_2$ production from glucose-1-^{14}C by chicken and guinea pig leucocytes. Chicken polymorphonuclear leucocytes responded to phagocytosis with an increased production of $^{14}CO_2$ from glucose-1-^{14}C. The production of

Table 2

Effect of bacteria or H_2O_2 addition on the $^{14}CO_2$ production from glucose-1-^{14}C by chicken and guinea pig polymorphonuclear leucocytes.

	cpm/10 min/10^7 cells		
	—	+H_2O_2	+bacteria
Chicken leucocytes	1,684 ± 218 (4)	30,670 ± 1,214 (4)	31,603 ± 3,913 (4)
Guinea pig leucocytes	1,902 ± 43 (4)	9,260 ± 446 (4)	46,928 ± 2,917 (4)

The experimental details are described under methods. The specific activity of glucose was 7.4×10^5 cpm/μmole. H_2O_2 was 0.5 mM. The results are given as mean ± SEM of the number of experiments indicated in parenthesis.

$^{14}CO_2$ from glucose-6-^{14}C was markedly lower than that from glucose-1-^{14}C and was not appreciably stimulated by addition of particles (not shown). The preferential stimulation of $^{14}CO_2$ production from glucose-1-^{14}C indicates that an increased activity of the hexose monophosphate shunt takes place in phagocytosing chicken leucocytes. Similar results were obtained with guinea pig cells in keeping with those published in previous papers[14,15].

The data of Table 2 also show that the effect of the addition of H_2O_2 to resting cells was markedly different in the two cell types. The extent of the hexose monophosphate shunt stimulation in chicken cells was comparable to that induced by phagocytosis, whereas in guinea pig cells the stimulation induced by H_2O_2 was markedly lower than that induced by phagocytosis.

Oxidation of NADPH and NADH by chicken and guinea pig polymorphonuclear leucocyte granules

Table 3 shows that granules isolated from resting chicken polymorphonuclear leucocytes were able to oxidize both NADH and NADPH. The rate of oxidation of both coenzymes was stimulated in granules isolated from phagocytosing leucocytes. NADPH was oxidized at a higher rate than NADH by both granules from resting cells and those from phagocytosing cells. Cyanide almost totally inhibited the oxidation of NADPH by granules isolated from resting leucocytes, but had only a minor effect on the NADPH oxidizing activity of granules isolated from phagocytosing cells.

In Table 3 a typical experiment on the properties of the NADPH and NADH oxidase activities of granules isolated from resting and phagocytosing guinea pig polymorphonuclear leucocytes is also shown. The results are in agreement with those published in previous papers[8,16], and are qualitatively similar to those obtained with chicken cells. A quantitative difference however was evident between the two cell types, that is that the NADPH and NADH oxidase activities of guinea pig cells were markedly higher than those of chicken cells.

Figure 2 shows that the oxidation of NADPH by chicken leucocyte granules was coupled with H_2O_2 production, the peroxide generated being stoichiometric to the oxygen consumed.

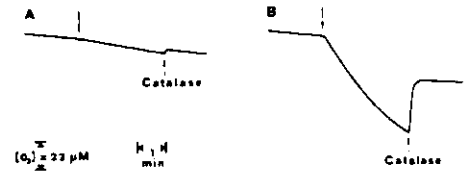

Fig. 2. NADPH oxidase activity and H_2O_2 production by granules isolated from resting (A) and phagocytosing (B) chicken polymorphonuclear leucocytes. Assay conditions are described in the legend to Table 3. Granules isolated from 4×10^7 leucocytes were added at the arrows.

Table 3
NADPH and NADH oxidase activity of granules from chicken and guinea pig polymorphonuclear leucocytes

		n atoms oxygen/min/granules from 10^8 cells			
		Granules from PMN at rest		Granules from PMN under phagocytosis	
		−KCN	+KCN	−KCN	+KCN
Chicken	NADPH	10.5 ± 10.34 (3)	2.0 (2)	254.78 ± 69.3 (3)	169.1 ± 34.5 (3)
	NADH	2.5 (2)	undetectable	7.87 ± 1.71 (3)	undetectable
Guinea pig	NADPH	1,826 (1)	365 (1)	6,250 (1)	5,580 (1)
	NADH	375 (1)	90 (1)	990 (1)	653 (1)

Assay medium: 65 mM phosphate buffer pH 5.5, 170 mM sucrose, 1 mM NADPH or 2 mM NADH, 0.5 mM $MnCl_2$ and 2 mM KCN where indicated. Temperature 37 °C. The reaction was started by adding the granule suspension. Results are given as mean ± SEM. In parenthesis the number of experiments is indicated.

Discussion

The comparative study of the phagocytosis-associated metabolic burst in chicken and guinea pig polymorphonuclear leucocytes, presented in this paper, reveals that several qualitative similarities exist between the two cell types. These include an increased oxygen consumption, an increase of the hexose monophosphate shunt, an increased generation of superoxide anion and hydrogen peroxide, and an activation of the cyanide-insensitive NADPH oxidase of the granules which is coupled with an increased hydrogen peroxide production.

Some differences have been also observed between the two types of cell. A major one is that all the biochemical activities that have been recorded, with the exception of the phagocytosis stimulated activity of the hexose monophosphate shunt, are definitely lower in chicken cells than in guinea pig cells. Other differences will emerge more clearly from the following discussion.

An increased respiration and hexose monophosphate shunt activity in phagocytosing chicken leucocytes has been already reported by PENNIAL and SPITZNAGEL[7]. These authors, however, failed to demonstrate generation of H_2O_2, and concluded that the inability of chicken leucocytes to produce H_2O_2 "sets these cells apart from the polymorphonuclear leucocytes of all other species". The primary contribution of the present paper is that we were able to demonstrate generation and extracellular release of both superoxide anion and hydrogen peroxide in phagocytosing chicken leucocytes. Three different techniques were used to measure H_2O_2 production, including the sensitive scopoletin method, and the results matched very well. The failure of PENNIAL and SPITZNAGEL to demonstrate H_2O_2 using the technique of formate oxidation may be explained by the absence of catalase in chicken polymorphonuclear leucocytes[17,18]. This technique, in fact, relies on the peroxidase like activity of catalase which converts formate into CO_2 concomitantly with the phagocytic production of H_2O_2[19]. Formate oxidation has been reported to be stimulated by addition of exogenous catalase at least in phagocytosing human leucocytes[20,21]. PENNIAL and SPITZNAGEL could not demonstrate formate oxidation by phagocytosing chicken leucocytes, even in the presence of exogenous catalase. This result cannot be obviously accounted for by catalase deficiency in chicken cells. Since the formate oxidation technique reveals only a small fraction of the peroxide produced[2,20], it might be that the failure of PENNIAL and SPITZNAGEL to show formate oxidation by phagocytosing chicken leucocytes in the presence of exogenous catalase is attributable to the low sensitivity of the method employed. The authors failed to show H_2O_2 production even with the scopoletin method which, by contrast, we have employed with positive results. In our experiments a modification of the method as originally described by ROOT and CHANCE[3] has been used. In the original method the oxidation of scopoletin in the presence of horse radish peroxidase is measured concomitantly with phagocytosis. Operatively, the cuvette of the spectrofluorimeter is filled with both the reagents for the scopoletin reaction and leucocytes, and then the reaction is started by addition of the phagocytic particles. The rate of decay of scopoletin fluorescence is recorded and the linear part of the tracing is used for calculation of the initial rate of H_2O_2 production. The number of human cells used for each assay in the method described by ROOT and CHANCE is 2.5×10^6/ml. In preliminary experiments, we have shown that this cell concentration was too low for an evaluation of H_2O_2 produced by chicken leucocytes. On the basis of this fact a concentration of 5×10^7 leucocytes/ml was used and the suspensions were allowed to phagocytose for variable periods of time. The medium was then quickly separated from the cells and aliquots of variable size were used for the scopoletin assay of H_2O_2. This procedure offers two advantages, 1) a large number of cells can be used, and 2) the peroxide accumulated for the desired period of time can be measured. With this technique, accumulation of H_2O_2 in the medium of phagocytosing chicken leucocytes was detected both in the presence and in the absence of azide. With phagocytosing guinea pig leucocytes, only a small amount of H_2O_2 accumulated in the medium without the inhibitor, but it was about forty times higher when the inhibitor was present. This difference might be explained by the presence in guinea pig leucocytes of two hydrogen peroxide destroying enzymes such as catalase and myeloperoxidase, which are both

sensitive to azide, and by the lack of these enzymes in chicken leucocytes[17,18,22,23]. In fact we have recently shown that in phagocytosing guinea pig cells about 90% of the H_2O_2 produced is degraded by catalase and myeloperoxidase[11].

The peroxide released from phagocytosing chicken leucocytes in the presence of azide accounted for about 19.6% of the respiratory burst, whereas it amounted to about 89% of the oxygen consumed in guinea pig cells. This suggests either that H_2O_2 is not the only product of oxygen reduction in chicken cells or that H_2O_2 is predominantly disposed of by azide-insensitive intracelluar H_2O_2-utilizing pathways. The latter possibility seems to be the real one. In chicken leucocytes, which lack both myeloperoxidase and catalase, the main H_2O_2-utilizing pathway is the glutathione cycle. Two enzymes, glutathione peroxidase and glutathione reductase, are involved in this pathway according to the following equations:

$$H_2O_2 + 2GSH \xrightarrow{\text{Glutathione peroxidase}} 2H_2O + GSSG \quad (1)$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} 2GSH + NADP^+ \quad (2)$$

Chicken leucocytes have been reported to contain both glutathione peroxidase and glutathione reductase activities[7,17]. The $NADP^+$ generated by the glutathione cycle is reduced again by the first two enzymes of the hexose monophosphate shunt, i.e. glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, with concomitant production of CO_2 from glucose carbon one. We have shown in this paper that exogenously added H_2O_2 stimulates the hexose monophosphate shunt activity of chicken cells much more than that of guinea pig cells. This supports the hypothesis that the glutathione cycle is the preferential pathway for H_2O_2 utilization in chicken cells. Assuming that all the oxygen consumed by phagocytosing chicken leucocytes is converted to H_2O_2, one would expect a rate of H_2O_2 production of 27.03 nmol/2 min/2×10^7 cells in the presence of azide. The amount of H_2O_2 measured was, however, 5.31 nmol/2 min/2×10^7 cells in the presence of azide. This suggests that 22 nmol H_2O_2/2 min/2×10^7 cells (80% of the total H_2O_2 produced) are degraded by the azide insensitive glutathione cycle in these cells. On the contrary, the H_2O_2 recovered in the medium of phagocytosing guinea pig leucocytes in the presence of azide accounts for about 89% of the oxygen consumed. This suggests that 89% of the H_2O_2 produced by guinea pig cells is destroyed by catalase and myeloperoxidase, and only 11% by other azide insensitive pathways, possibly the glutathione cycle.

The enzymatic basis of the stimulated respiration in phagocytosing leucocytes, either polymorphs or macrophages, is still a debated issue[16,24,25]. We have proposed in previous papers that the activation during phagocytosis of a NADPH oxidizing activity, which is bound to granules and is relatively insensitive to inhibition by cyanide, accounts for the increased respiration and H_2O_2 generation in either types of phagocyte[13,16,24,26,27].

This paper shows that chicken leucocytes were able to oxidize NADPH or NADH. The rate of NADPH oxidation was higher than that of NADH oxidation. Additionally, we have found that the 20,000 g fraction isolated from the homogenate of phagocytosing chicken cells had a higher NADPH and NADH oxidizing activity than the corresponding fraction isolated from resting cells, which was relatively insensitive to inhibition by cyanide. The final product of the granule-catalyzed oxidation of NADPH was found to be hydrogen peroxide.

The NADPH oxidizing activity of phagocytosing chicken leucocytes closely resembles, with respect to its biochemical properties, that of guinea pig leucocytes and meets all the requirements needed for an enzymatic activity to be regarded as the basis for the respiratory burst. It appears, therefore, that even in chicken leucocytes the phagocytosis-stimulated respiration is sustained by an activation of a granule bound NADPH oxidizing activity, as it has been demonstrated to be the case with polymorphonuclear or mononuclear leucocytes from several species, including guinea pig. A difference, however, exists between the respiratory burst of chicken leucocytes and that of guinea pig leucocytes. The respiration of the former cells is not appreciably affected by azide, whereas that of the latter cell type is 30% higher in the presence of azide than in its absence. This difference can be explained on the

basis of the lack of catalase in chicken leucocytes[17,18] and the efficiency of this enzyme in catalyzing the breakdown of H_2O_2 in guinea pig leucocytes[11]. In fact in the absence of azide the H_2O_2 formed by phagocytosing guinea pig leucocytes is degraded by catalase as follows, $H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$. We have calculated that about 46% of the total H_2O_2 formed is degraded by this mechanism[11]. In the presence of azide, which inhibits catalase, H_2O_2 is not degraded by a catalase mechanism and a higher oxygen uptake is measured as compared to that observed in absence of the inhibitor. Thus, this apparent stimulation by azide of the oxygen consumption cannot be observed in chicken leucocytes due to the absence of catalase.

In conclusion, chicken leucocytes, although their oxidative metabolism is less active than that of guinea pig leucocytes, seem to follow a pattern of oxidative response to phagocytosis similar to that of leucocytes from other species. The differences that have been observed are mainly a consequence of the deficiency in chicken cells of the two H_2O_2 degrading enzymes catalase and myeloperoxidase.

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