Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 2506–2510, April 1990
Medical Sciences

Antioxidant activities of some tryptophan metabolites: Possible implication for inflammatory diseases

(influenza/interferon-γ/oxidative stress/indoleamine 2,3-dioxygenase/3-hydroxykynurenine)

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Communicated by Bruce N. Ames, December 29, 1989 (received for review November 17, 1989)

ABSTRACT **The antioxidant properties of tryptophan and some of its oxidative metabolites were examined by measuring how efficiently they inhibited peroxyl radical-mediated oxidation of phosphatidylcholine liposomes and B-phycoerythrin. Low micromolar concentrations of 5-hydroxytryptophan, 3-hydroxykynurenine, xanthurenic acid, or 3-hydroxyanthranilic acid, but not their corresponding nonhydroxylated metabolic precursors, scavenged peroxyl radicals with high efficiency. In particular, 3-hydroxykynurenine and 3-hydroxyanthranilic acid protected B-phycoerythrin from peroxyl radical-mediated oxidative damage more effectively than equimolar amounts of either ascorbate or Trolox (a water-soluble analog of vitamin E). Enzyme activities involved or related to oxidative tryptophan metabolism, as well as endogenous concentrations of tryptophan and its metabolites, were determined within tissues of mice suffering from acute viral pneumonia. Infection resulted in a 100-fold induction of pulmonary indoleamine 2,3-dioxygenase (EC 1.13.11.17) as reported [Yoshida, R., Urade, Y., Tokuda, M. & Hayaishi, O. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4084–4086]. This was accompanied by a 16- and 3-fold increase in the levels of lung kynurenine and 3-hydroxykynurenine, respectively. In contrast, endogenous concentrations of tryptophan and xanthurenic acid did not increase and 3-hydroxyanthranilic acid could not be detected. The activity of the superoxide anion ($O_2^{\cdot-}$)-producing enzyme xanthine oxidase increased 3.5-fold during infection while that of the $O_2^{\cdot-}$-removing superoxide dismutase decreased to 50% of control levels. These results plus the known requirement of indoleamine 2,3-dioxygenase for superoxide anion for catalytic activity suggest that viral pneumonia is accompanied by oxidative stress and that induction of indoleamine 2,3-dioxygenase may represent a local antioxidant defence against this and possibly other types of inflammatory diseases.**

It is now well recognized that reactive oxygen species produced by activated phagocytes are involved in inflammatory processes and can contribute to cell and tissue damage either directly or through activation of proteases (1). This seems likely to be of particular relevance under conditions where the activation of phagocytic cells is not controlled by the immune system, as is the case when certain paramyxoviruses and influenza viruses directly activate appropriate cells to produce reactive oxygen species (2–4). In addition to inflammation, oxygen radicals also have been implicated in the pathogenesis of cancer, atherosclerosis, and other diseases (5). Hence it is of general interest to examine how organisms protect themselves against oxidative damage.

Humans have a wide range of general antioxidant defences, including proteins [e.g., superoxide dismutase (SOD, EC 1.15.1.1), catalase (EC 1.11.1.6), and metal-binding proteins] and various small molecules (e.g., reduced glutathione and vitamins C and E) (6). Evidence is emerging that metabolic end products, such as uric acid, bilirubin, and homovanillic acid, are also important physiological antioxidants (7–9) that may exert their protective function in a local environment. For example, human albumin-bound bilirubin can protect albumin-bound fatty acids from oxidation (10), thereby acting as a site-specific antioxidant in human blood plasma (11). The antioxidant properties of 5-hydroxytryptophan (5HTrp), an oxidative metabolite of tryptophan, also has been described (12).

The levels of metabolism-derived antioxidants are likely to be subjected to enzymatic up- and down-regulation in response to stress by oxidants. For example, a variety of conditions that are or can be associated with oxidative stress, such as endotoxemia (13) or injection of interferons (14), induce heme oxygenase (15), the rate-limiting enzyme in the formation of bilirubin. Interferon-γ (IFN-γ) is a central cytokine of inflammatory processes with antimicrobial (e.g., antiviral) activity (16). Although the molecular mechanisms underlying these activities remain to be determined, IFN-γ is known to prime phagocytic cells for enhanced production of reactive oxygen species (17) and to induce a number of enzymes (16). Among these, indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.17) (18) is of some interest. This ubiquitous enzyme cleaves the pyrrole ring from tryptophan and other indoleamines (Fig. 1) by using superoxide anion radical ($O_2^{\cdot-}$) as cofactor and substrate in its catalytic process (19). Furthermore, its activity apparently increases more than 100-fold in the lungs of mice infected with influenza virus (20). The kynurenine (Kyn) formed by the action of IDO can be further metabolized by the Kyn pathway to various phenolic compounds (Fig. 1).

In this study we have tested how efficiently tryptophan metabolites of the Kyn pathway can scavenge peroxyl radicals. To examine the potential physiological importance of these metabolites as antioxidants, we measured their concentrations as well as the activities of relevant enzymes in tissues of mice suffering from influenza, an infection that has been proposed to be associated with oxidative stress (3, 4, 21). The results obtained show that several tryptophan metabolites are potent radical scavengers and that 3-hydroxyanthranilic acid (3HAA) and 3-hydroxykynurenine (3HKyn) can protect B-phycoerythrin (PE) from peroxyl radical-mediated oxidation for longer periods of time than equimolar concentrations of ascorbic acid and a water-soluble analog of vitamin E. Of the antioxidant active metab-

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Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; 3HAA, 3-hydroxyanthranilic acid; 3HKyn, 3-hydroxykynurenine; 5HTrp, 5-hydroxytryptophan; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-γ; Kyn, kynurenine; PtdCho, phosphatidylcholine; PE, B-phycoerythrin; SOD, superoxide dismutase; TDO, tryptophan 2,3-dioxygenase; XDH, xanthine dehydrogenase; XO, xanthine oxidase; XA, xanthurenic acid.

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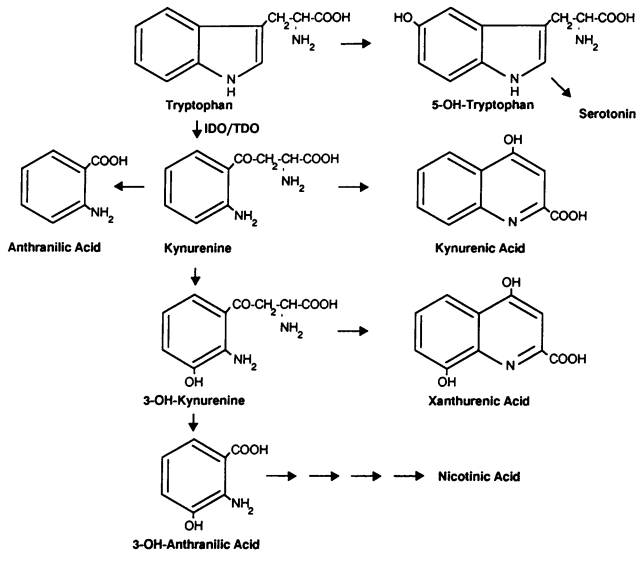

FIG. 1. Oxidative tryptophan metabolism.

olites, 3HKyn may be of particular physiological relevance as its concentration in the lung increased 3-fold during viral pneumonia.

MATERIALS AND METHODS

PE, catalase, and SOD were obtained from Calbiochem; ascorbic acid and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Aldrich; 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH) was from Polysciences; conjugated bilirubin (ditaurate·2Na) was from Porphyrin Products (Logan, UT); reduced and oxidized NAD was from Boehringer Mannheim. The decarboxylase inhibitor Benserazide [1-DL-seryl-2-(2,3,4-trihydroxybenzyl)-hydrazine] was generously given to us by F. Gey (Hoffmann–La Roche). Organic solvents (LiChrosolv; Merck) and nanopure water were used for all experiments. For the xanthine dehydrogenase/oxidase (XDH/XO, EC 1.2.3.2) assay and all experiments testing antioxidant activities of tryptophan and its metabolites, Chelex-100 (Bio-Rad)-treated transition-metal-free buffers were used. All other chemicals were from Sigma or Merck and were of the highest quality available.

The antioxidant activities of tryptophan and its oxidative metabolites were tested by examining their inhibitory activity on peroxyl radical-mediated oxidation of either soybean phosphatidylcholine (PtdCho) liposomes (22) or PE (9, 23). The latter is an intensely colored fluorescent protein that is highly susceptible to oxidation. As oxidative damage to PE results in a loss of fluorescence, antioxidant activity of a compound is indicated by its capacity to prevent AAPH-induced decrease in fluorescence.

Outbred and pathogen-free female Swiss ICR mice (ICR-ZH, Institute for Labortierkunde, Zurich), aged 4–6 weeks, were used for all experiments. Animals were infected intranasally with 8–10 LD_{50} of influenza A/PR8/34. Control or infected mice were anesthetized, bled by subclavicular artery section, and killed by cervical dislocation. Tissues were removed, washed in ice-cold 10 mM sodium phosphate-buffered saline, dabbed dry, and homogenized immediately for subsequent analyses.

IDO activity was measured as described by Yoshida *et al.* (24), using 200 mM of unlabeled L-tryptophan and D-tryptophan as substrates for the pulmonary and hepatic enzyme, respectively. Tryptophan 2,3-dioxygenase (TDO, EC 1.13.11.11) activity was calculated by subtracting IDO activity, obtained using D-tryptophan as substrate, from total dioxygenase activity, determined using L-tryptophan as substrate. As no exogenous hematin was added to the reaction mixtures, only holoenzyme activities were measured. Tryptophan 5-monooxygenase (EC 1.14.16.4) activity was assayed as described by Hasegawa and Ichiyama (25) without preincubation of the reaction mixture with dithiothreitol (15 mM), as such preincubation did not enhance enzyme activity. Lung homogenate [1:10 (wt/vol) in 50 mM sodium phosphate buffer (pH 7.8)] was assayed for total SOD and XDH/XO activities as described (refs. 26 and 27, respectively). For the latter, the homogenization buffer was degassed with argon and supplemented with 0.5 mM phenylmethylsulfonyl fluoride and 1 mM EDTA with or without 5 mM dithiothreitol. XDH/XO activities were measured by continuously monitoring formation of uric acid and NADH at 295 nm and 340 nm, respectively.

Endogenous levels of lung tryptophan and Kyn were determined by HPLC as described (28), using fluorimetric (285/345 nm, excitation/emission wavelengths) and spectrophotometric (360 nm) detection, respectively. 5HTrp was separated on a 5-μm LC-18 column (25 × 0.46 cm with guard column; Supelco) using 100 mM sodium acetate/100 mM citrate, pH 4.1, containing 30 μM EDTA and 4% (vol/vol) acetonitrile as mobile phase at 1.2 ml/min and detected fluorimetrically (302/345 nm, excitation/emission wavelengths) (29). Special care was taken in the analyses of tissue levels of 3HKyn and 3HAA, as these compounds easily autoxidize. Lungs were removed from exsanguinated animals, stored immediately on dry ice, and homogenized in 5 vol of ice-cold 5% (vol/vol) metaphosphoric acid within 5 min. Extracts were centrifuged at 4°C and 10,000 × g for 5 min, and the supernatants were extracted with an equal volume of ethyl acetate (for 3HKyn) or were directly filtered (0.2 μm; Gelman) [for 3HAA and xanthurenic acid (XA)] and subjected to HPLC analysis. 3HKyn and 3HAA were detected electrochemically (30) and XA was detected as described (31). Recoveries of authentic standards of 3HKyn, 3HAA, and XA when added to lungs prior to homogenization were 93 ± 4% (n = 4), 75 ± 4% (n = 3), and 41 ± 4% (n = 4), respectively. Concentrations of 3HKyn, 3HAA, and XA were calculated by comparing their peak areas with those of standards (prepared freshly in 10 M HCl) and expressed on a wet-weight basis. Detection limits for both 3HKyn and 3HAA were 0.05 pmol.

Protein concentrations were determined with either a Bradford reagent (Bio-Rad kit) or the Peterson-modified method of Lowry (Sigma kit).

The unpaired student t test (one-tailed) was used to evaluate changes from infected to control groups. Significance was accepted at the $P < 0.01$ level, unless stated otherwise.

RESULTS

Exposure of multilamellar PtdCho liposomes to peroxyl radicals, generated in the aqueous phase at constant rate by the water-soluble azo-compound AAPH, resulted in immediate and linear formation of PtdCho hydroperoxide (Fig. 2). Addition of tryptophan, Kyn, kynurenic acid, or anthranilic acid at 20 μM had no significant effect on the extent of PtdCho hydroperoxide formation. In contrast, in the presence of 5HTrp, 3HKyn, XA, or 3HAA at 20 μM, oxidation of PtdCho liposomes was inhibited almost completely within the first 30–50 min, indicating that these compounds efficiently scavenge peroxyl radicals. In comparison, under these conditions, 20 μM conjugated bilirubin and 20 μM ascorbic acid or 20 μM Trolox inhibited AAPH-induced oxidation of PtdCho liposomes completely for 65 and 68 min, respectively (33). The fact that hydroxylated tryptophan metabolites, but not their metabolic precursors, possess antioxidant activity is in line with the phenolic structure and oxidizability of the former. Thus, by using cyclic voltammetry we observed that, in 100 mM sodium phosphate (pH 7.0),

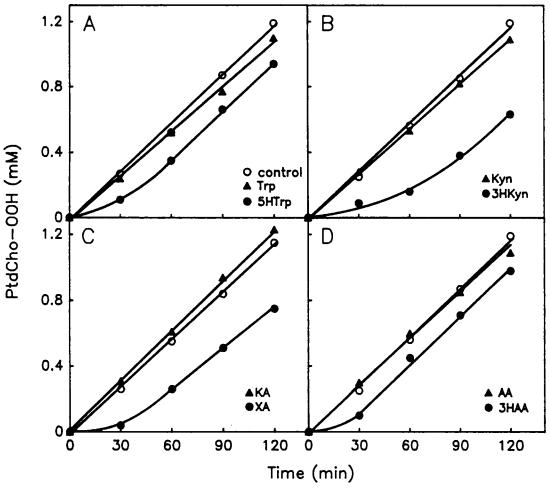

FIG. 2. Inhibition of AAPH-induced oxidation of PtdCho liposomes by tryptophan and some of its metabolites. Purified PtdCho liposomes (20 mM) were incubated at 37°C under air in sodium phosphate-buffered saline (100 mM) containing AAPH (10 mM) in the absence (open circles) or presence (solid symbols) of 20 μM tryptophan or one of its metabolites. At various time points an aliquot of the reaction mixture was removed and analyzed for PtdCho hydroperoxide (PtdCho-OOH) by HPLC as described (32). Data represent typical results obtained in two to three independent experiments. AA, anthranilic acid; KA, kynurenic acid.

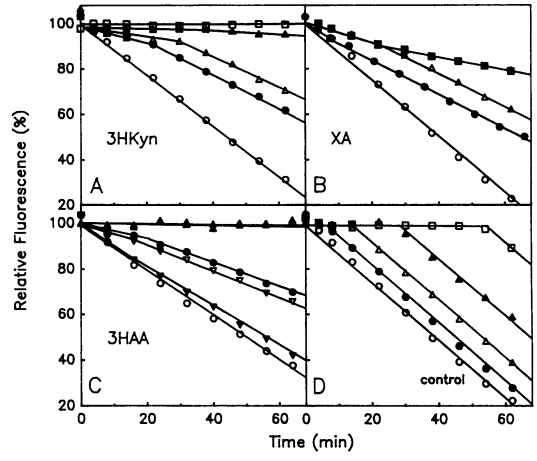

FIG. 3. Inhibition of AAPH-induced loss of fluorescence of PE by various tryptophan metabolites and known antioxidants. PE (17 nM) was incubated under air at 37°C in 75 mM sodium phosphate (pH 7.0) containing AAPH (4 mM) alone (○) or in the presence of the following compounds. (*A*) 3HKyn. (*B*) XA at 1 (●), 2 (△), 5 (▲), or 10 (□) μM. (*C*) 3HAA at 0.2 (▼), 0.5 (▽), 1 (●), 2 (△), or 5 (▲) μM. (*D*) Comparative efficiency by which Trolox at 1 (●) or 9.5 (□) μM, or ascorbate at 2 (△) or 5 (▲) μM scavenged peroxyl radicals under identical conditions. Data shown represent typical results obtained in two or more independent experiments.

the oxidizing potentials for tryptophan, Kyn, kynurenic acid, and anthranilic acid were +0.86, 1.01, and 0.85 V, respectively, and those of 5HTrp, 3HKyn, XA, and 3HAA were +0.56, 0.36, 0.61, and 0.33 V, respectively.

Although the antioxidant activity of 5HTrp, the tryptophan monooxygenase product (Fig. 1), has been noted (12), to our knowledge, those of the metabolites formed during the dioxygenase-initiated or Kyn pathway have not been described previously. Therefore, we investigated their antioxidant properties in some more detail, using the PE assay (9, 23). As shown in Fig. 3, incubation of PE with AAPH resulted in a linear decrease in fluorescence. 3HKyn at low micromolar concentrations inhibited the loss of PE fluorescence in a dose-dependent manner with total protection achieved at 5 μM and higher (Fig. 3*A*). 3HAA was even more efficient, inhibiting oxidation of PE completely at concentrations as low as 2 μM (Fig. 3*C*). XA also inhibited modification of PE, though less efficiently (Fig. 3*B*). Full protection was not observed even when tested at 10 μM, indicating that the rate of reaction of XA with peroxyl radicals is slower than that of 3HKyn or 3HAA. Interestingly, however, the antioxidant activity of XA, when used at higher concentrations, seemed to increase after an initial period of about 30 min. Partial protection of peroxyl radical-mediated oxidation of PE was also observed with conjugated bilirubin (data not shown), another known metabolic end product with antioxidant properties (33).

Under identical experimental conditions, ascorbate and Trolox, a water-soluble analog of vitamin E, completely prevented the loss of PE fluorescence for a period of time whose length was directly proportional to the initial concentration of the antioxidant. For example, 5 μM ascorbate totally prevented the loss of PE fluorescence for up to 26 min (i.e., the break point in Fig. 3*D*) after which fluorescence decreased at the same rate as in the control sample. In sharp contrast, either 5 μM 3HKyn or 5 μM 3HAA protected PE from AAPH-induced damage for periods of at least 60 min (Fig. 3 *A* and *C*). These results indicate that equimolar amounts of 3HKyn and 3HAA can scavenge more peroxyl radicals than either ascorbate or Trolox, a finding similar to that reported for biliverdin (33).

To evaluate the possible relevance of oxidative tryptophan metabolites as *in vivo* antioxidants, we used a mouse model of influenza, as such infection apparently results in a massive induction of IDO in the lung (20). Indeed, we also observed a 100-fold induction of pulmonary IDO activity in the first 6 days after infection, concomitant with a 16-fold increase in endogenous levels of Kyn, the stable break-down product of *N*-formylkynurenine formed initially by the action of IDO. The activity of IDO also increased 4-fold in the liver while hepatic TDO activity was only slightly elevated (Fig. 4*A*). In contrast to IDO, the activity of liver tryptophan 5-monooxygenase, the enzyme producing 5HTrp from tryptophan, did not increase during infection (9.3 ± 2.3, in control liver, vs. 7.1 ± 3.6 pmol per mg of protein per min at day 6).

Of the antioxidant-active tryptophan metabolites, lung levels of 3HKyn increased 3-fold at day 3 after infection, whereas its transaminated and stable end-product XA was reduced to 25% by day 3 and returned to control values at the end of infection (Fig. 4*B*). Plasma levels of XA did not increase (data not shown), indicating that the decrease in pulmonary concentrations of this metabolite was not due to its excretion into the circulation. Despite the increase in endogenous levels of its metabolic precursor 3HKyn, we failed to detect any significant amounts of 3HAA in lungs of either control or infected animals.

As IDO uses $O_2^{-\cdot}$ as cofactor and substrate (19), it was of interest to examine whether virus infection also caused an alteration in the activities of lung enzymes involved in the production or removal of $O_2^{-\cdot}$. Total SOD activity decreased to about 50% of control levels at the late stages of infection (Fig. 4*A*) ($P < 0.03$), while that of XO was increased 3.5-fold at day 6 (Table 1). Nearly 70% of the total XO activity derived from the so-called reversible form of XO [i.e., an oxidatively modified form of XDH that can revert back to native XDH in the presence of reducing agents (34)]. The irreversible form of XO, produced by proteolytic modification (34), was also present at elevated amounts at day 6 after infection.

DISCUSSION

Small molecules such as certain vitamins and metabolites contribute to the defences that higher organisms possess

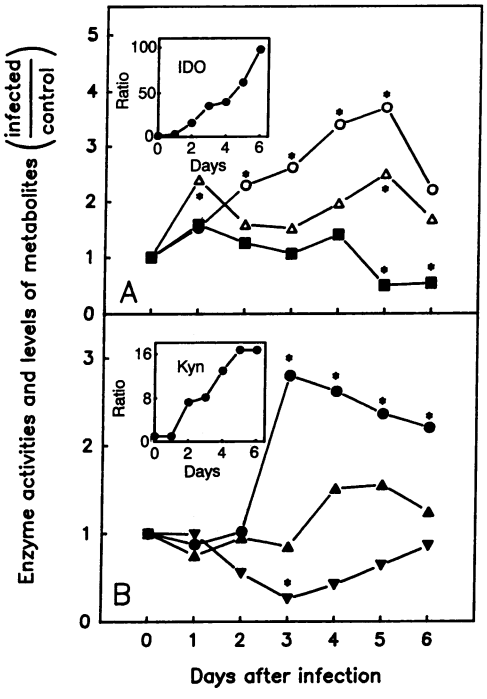

FIG. 4. Influenza A/PR8/34-induced changes in enzyme activities and endogenous levels of metabolites derived from the Kyn pathway. Mice were killed before or after infection and their lungs (solid symbols) and livers (open symbols) were homogenized and analyzed for enzyme activities (*A*) or tryptophan and its metabolites (*B*). Results are expressed as ratios of the values obtained for infected and control tissues. Control enzyme activities were 0.8 ± 0.4, 1.3 ± 0.5, and 19.7 ± 7 pmol of Kyn formed per mg of protein per min for lung IDO, liver IDO, and TDO, respectively, and 48.4 ± 14.9 units/mg of protein for SOD. Endogenous concentrations (in μM) of tryptophan, Kyn, 3HKyn, and XA in control lung were 36.7 ± 17.7, 1.3 ± 0.3, 0.1 ± 0.04, and 7.7 ± 2.6, respectively. Each point represents the results of three to six independent experiments. Asterisks indicate results significantly different from control. ○, IDO; △, TDO; ■, SOD; ●, 3HKyn; ▲, tryptophan; ▼, XA.

against oxidative damage (6–12, 33). One important observation of the present study is that some of the oxidative tryptophan metabolites of the Kyn pathway are also powerful antioxidants. To assess their antioxidant capacities we measured how efficiently they inhibited peroxyl radical-induced damage to either soybean PtdCho or PE. A comparison of the results obtained with these two methods revealed quantitative but not qualitative differences (see Figs. 2 and 3). For example, while XA was at least as efficient as 3HKyn and 3HAA in preventing AAPH-induced formation of PtdCho hydroperoxide, it was much less efficient in preventing AAPH-induced loss of PE fluorescence. These differences are likely due to variations in reaction rates of peroxyl radicals with either PE or PtdCho. From reported reaction rate constants (23, 35), it can be estimated that PE reacts about 40 times faster with peroxyl radicals than PtdCho. Therefore, under experimental conditions where radical generation is rate-limiting, the PE fluorescence-based assay is a more sensitive test for rapidly reacting peroxyl radical scavengers than the PtdCho oxidation assay. More importantly, our results further show that 3HKyn and 3HAA, like vitamin C, Trolox, and reduced pyridine nucleotides (9), belong to the class of small molecules that react very rapidly with peroxyl radicals and hence are potentially important biological antioxidants.

Table 1. XDH/XO activities in lung of influenza-infected mice

Activity	Activity		*P* value
	Control	Day 6	
Total	0.51 ± 0.21	1.81 ± 0.62	<0.006
XDH	0.30 ± 0.29	0.24 ± 0.44	N.S.
XO_{rev}	0.15 ± 0.15	1.33 ± 0.74	<0.017
XO_{irrev}	0.08 ± 0.03	0.39 ± 0.23	<0.031

Activities are expressed as nmol of uric acid or nmol of NADH formed per mg of protein per min. XO_{rev} was calculated by subtracting XO_{irrev} (determined in the absence and presence of NADH and dithiothreitol, respectively) from total XO activity (27). Total activity (XDH/XO) was determined separately from total XO activity, explaining the minor discrepancies between the values given for total activity and the corresponding sums of activities of XDH, XO_{rev}, and XO_{irrev}. XO_{rev}, reversible form of XO; XO_{irrev}, irreversible form of XO; N.S., not significantly different. Results are expressed as mean ± SD; for control, $n = 3$; for day 6, $n = 6$.

Our study did not address the mechanism of antioxidant activity of tryptophan metabolites. However, the fact that all phenolic metabolites but not their nonhydroxylated metabolic precursors showed antioxidant activities points toward the importance of the phenolic moiety as the active entity. More surprisingly, among the phenolic tryptophan metabolites equimolar concentrations of 3HKyn and 3HAA were more efficient in protecting PE from peroxyl radical-mediated damage than either ascorbic acid or Trolox. The results indicate that, unlike these latter antioxidants, each molecule of 3HKyn and 3HAA can scavenge more than two peroxyl radicals. Preliminary experiments in our laboratory suggest that this high antioxidant efficiency may be the result of dimerization of 3HKyn and 3HAA upon reaction with free radicals, to products that also have high peroxyl radical-scavenging activity. Interestingly, dimerization of phenoxyl radicals has been reported to occur in the cases of homovanillic acid (36) and tyrosine (37).

As organisms in general respond to oxidative stress through adaptation of their antioxidant defence mechanisms, it was of interest to examine whether such stress can also lead to an alteration in oxidative tryptophan metabolism and, possibly, endogenous levels of antioxidatively active metabolites. We used a mouse model of influenza infection, as this disease is known to be associated with a dramatic increase in lung IDO activity (20) and has been suggested to be associated with oxidative stress (3, 4, 21). Our results provide indirect evidence for the latter, as shown by the infection-induced 50% depression of SOD activity (Fig. 4) and 3.5-fold increase in activity of the $O_2^{-\cdot}$ producing enzyme XO (Table 1; see also ref. 21). Nearly 70% of the latter activity arose from the reversible form of XO that is produced from XDH through oxidative modification (34). Furthermore, we have observed that influenza infection is accompanied by increased *in vivo* production of H_2O_2 within the lung (G. D. Buffinton, E.P., and R.S., unpublished data), directly supporting the hypothesis that oxidative stress occurs during viral pneumonia.

In view of the known requirement for and utilization of $O_2^{-\cdot}$ by IDO and its lower K_m for $O_2^{-\cdot}$ when compared with SOD (38), IDO has been suggested to represent an antioxidant defence (39–41). Removal of $O_2^{-\cdot}$ by IDO is likely to be of particular physiological importance during viral pneumonia, a condition where pulmonary IDO activity is increased 100-fold (Fig. 4; ref. 20), $O_2^{-\cdot}$ is produced at elevated rates, and endogenous SOD activity is decreased substantially. Indeed, endogenous levels of lung Kyn increased 16-fold during infection, implying (19) that, at least in this organ, IDO actually removed increasing amounts of $O_2^{-\cdot}$ as pneumonia progressed. Furthermore, and more importantly, our results indicate that the antioxidant activity of IDO may act at yet another level. Upon consumption of the potential prooxidant $O_2^{-\cdot}$, the dioxygenase initiates the formation of certain tryptophan metabolites (e.g., 3HKyn) that are very powerful antioxidants. Conversion of a potential prooxidant into anti-

oxidants has been proposed as the mechanism of antioxidant defence provided by heme oxygenase (15). Such mechanism is in sharp contrast to the action of SOD, which converts $O_2^{-\cdot}$ into an oxidant, i.e., H_2O_2.

It remains to be established whether the decrease in endogenous concentrations of XA and 3HKyn during the first and last 3 days of infection, respectively (Fig. 4), reflects actual antioxidant protection provided by these two metabolites. In the light of the great number of competing cellular antioxidants and the small concentrations of XA and 3HKyn measured in the lungs of infected mice, such antioxidant action may actually seem rather unlikely. Also, competition experiments showed that when tested in a homogeneous system and at equimolar concentrations, both 3HKyn and 3HAA failed to prevent AAPH-mediated oxidation of ascorbic acid (data not shown), indicating that the reaction rates of these tryptophan metabolites with peroxyl radicals are smaller than that of ascorbate. However, during infection the concentrations of ascorbic acid and reduced glutathione present in lung homogenates decreased by 35 and 45% to 0.8 ± 0.1 and 0.7 ± 0.1 mM, respectively (T. Hennet, E.P., and R.S., unpublished data). Furthermore, it is important to note that these results represent concentrations of antioxidants present in lung homogenates. They do not take into consideration possible variations in the levels of antioxidants as a result of differences in cell types and/or compartmentalization and hence cannot exclude the possibility of much higher local concentrations of antioxidant active tryptophan metabolites. Indeed, histological examination revealed that influenzal pneumonia was characterized by areas of intense inflammation adjacent to areas of essentially unchanged architecture (M. Adé-Damilano, E.P., and R.S., unpublished data). Moreover, in endotoxin-treated mice, IDO activity was found to be localized exclusively in lung interstitial cells (42), a site where resident and infiltrated macrophages are present during viral pneumonia. These cells may induce oxidative stress upon appropriate stimulation. Activated macrophages show increased IDO activity (43) and produce various tryptophan metabolites of the Kyn pathway (44).

The physiological function of IDO is not known at present. In addition to its potential function as antioxidant, induction of IDO has been proposed to provide the host cell with protection against a number of different microorganisms (18, 45) and tumors (46, 47) through depletion of intracellular tryptophan, to produce cytotoxic metabolites (44), and to regulate the concentrations of certain neurotransmitters and immunomodulators (20). IFN-γ seems to be the principle *in vivo* inducer of IDO (48). As a key mediator of inflammation, this lymphokine is known to prime macrophages for enhanced production of reactive oxygen species (17). The results presented here are consistent with the hypothesis that IFN-γ-mediated induction of IDO may represent a local antioxidant defence against pneumonia and other inflammatory diseases acting at two levels—i.e., removing $O_2^{-\cdot}$ and thereby producing potent antioxidants.

We thank Dr. B. Kräutler (Federal Institute of Technology, Zürich) for the help with cyclic voltammetry measurements, the Theodor Kocher Institute, Berne for the use of the fluorimeter, T. Hennet for the preparation of the virus suspension, and T. Hennet and the members of our research group for stimulating discussions and cooperation in supplying animal tissues. We also thank Drs. C. L. Geczy and N. H. Hunt for carefully reading the manuscript. This work was supported by Grant 31-9221.87 from the Swiss National Science Foundation to E.P. and R.S.

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