

Title	Indoleamine 2,3-dioxygenase : with Special Reference to the Mechanism of Interferon Action
Author(s)	Hayaishi, Osamu
Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1985, 28(1-2), p. 39-49
Version Type	VoR
URL	https://doi.org/10.18910/82417
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

https://ir.library.osaka-u.ac.jp/

The University of Osaka

## COMMEMORATIONAL LECTURE

## INDOLEAMINE 2,3-DIOXYGENASE —WITH SPECIAL REFERENCE TO THE MECHANISM OF INTERFERON ACTION—

## OSAMU HAYAISHI

Osaka Medical College, Takatsuki, Osaka Japan

I deem it a great privilege and honour to be invited to the Golden Jubilee Celebration of the Research Institute for Microbial Diseases and to present a lecture to such a distinguished audience. Today, I have chosen to talk about "Indoleamine 2,3-dioxygenase", because, first, my life work on oxygenases began about 40 years ago when I was associated with this institute, and second, this enzyme plays an important role in the bacterial infection and interferon action.

By way of introduction, I would like to talk about first historical background of this work, namely tryptophan metabolism and inflammation and other pathological conditions. Then, I shall speak about properties and induction mechanism of indoleamine 2,3-dioxygenase, hereafter abbreviated as IDO. Finally, I shall discuss possible biological significance of this enzyme with special reference to the mechanism of interferon action.

Tryptophan, an essential amino acid, is a precursor of a number of physiologically important metabolites, such as serotonin, NAD and so forth, in mammals including humans. The major metabolic pathways of tryptophan in mammals, shown in Fig. 1, are no doubt familiar to all of you. Tryptophan is not only the precursor of kynurenine, anthranilic, kynurenic and xanthurenic acids but is also converted to many other intermediates and is ultimately metabolized to acetyl CoA and  $CO_2$ , leading to the complete oxidation of the tryptophan molecule. Alternatively, it is converted to important coenzymes, NAD and NADP and then to poly (ADP-ribose). Poly (ADP-ribose) is a homopolymer of ADPribose derived from NAD, covalently modifies various nuclear enzymes and proteins, plays a crucial role in the repair of damaged DNA, and hence is involved in oncogenesis, differentiation and mutation.

The rate limiting step that regulates the formation of these biologically important metabolites appears to be the first one, namely the oxidative cleavage of the indole ring of tryptophan yielding formylkynurenine. The enzyme responsible for this reaction was initially isolated from rat liver by Professor Y. Kotake, the first director of this institute.

Because the enzyme cleaves the pyrrole moiety of tryptophan, Kotake termed it tryptophan pyrrolase (Fig. 2). In 1957, we demonstrated that molecular oxygen was incorporated into formylkynurenine and thereafter the enzyme was renamed tryptophan 2,3-dioxygenase. It contains protoporphyrin IX as its sole prosthetic group; therefore it



FIGURE 1. Major metabolic pathways of tryptophan.



FIGURE 2. Tryptophan 2,3-dioxygenase (pyrro-lase).

is a typical heme-containing dioxygenase. A number of previous studies showed that this enzyme is solely responsible for the formation of kynurenine from tryptophan in mammals and that it is the rate limiting step of the kynurenine pathway. In support of this assumption, when a large amount of tryptophan is given to animals, or glucocorticoid is injected to stimulate gluconeogenesis from amino acids, this enzyme is induced in the



FIGURE 3. Effects of tryptophan and hydrocortisone on TPO in adrenalectomized rats.

liver as shown in Fig. 3.

When either tryptophan or hydrocortisone is given to adrenalectomized rats, the tryptophan dioxygenase activity in the liver increased about 5-fold as compared with the control; when both were given simultaneously, the increment was even larger. These results indicated that tryptophan dioxygenase plays a crucial role in the degradation of tryptophan in the liver, under physiological conditions.

On the other hand, it has long been known that various tryptophan metabolites, including kynurenine, 3-hydroxy-kynurenine, 3-hydroxy-anthranilic acid, and so forth are excreted in large amounts in the urine of patients under various pathological conditions (Table 1).

For example, in a large percentage of patients with rheumatoid arthritis, tuberculosis, various types of blood disorders including leukemia, and Hodgkin's disease, and urological disorders, excess amounts of tryptophan metabolites such as kynurenine, 3-hydroxy-

Diseases	Cases of abnormal urinary excretion/patients (%)	Abnormal urinary excretion of
Rheumatoid arthritis	9/12 (75)	Kyn
Tuberculosis	11/13 (85)	3-OH-AA
Leukemia	15/22 (68)	Kyn, 3-OH-AA
Hodgkin's disease	28/28 (100)	Kyn, 3-OH-Kyn, 3-OH-AA
Bladder tumor	44/178 (25)	Kyn, 3-OH-Kyn, 3-OH-AA
Prostate diseases	17/122 (15)	Kyn, 3-OH-Kyn, 3-OH-AA

TABLE 1. Urinary excretion of tryptophan metabolites under pathological conditions in man



FIGURE 4. Lack of correlation between TPO activity and urinary excretion of kynurenine.



FIGURE 5. Indoleamine 2,3-dioxygenase.

kynurenine and so forth were excreted in the urine. However, under these conditions, no one has been able to demonstrate the induction or elevation of the tryptophan dioxygenase activity in the liver. Actually all attempts to show correlations between the TPO activity and the urinary excretion of tryptophan metabolites have been uniformly unsuccessful and this phenomenon has long remained a mystery.

Fig. 4. shows "the lack of correlation between TPO activity and urinary excretion of kynurenine" in patients with bladder cancer, according to Galiani and coworkers. The vertical axis represents TPO activity determined with surgical needle biopsy liver specimens. Horizontal axis kynurenine in the urine. These findings indicated that abnormal increases in kynurenine in the urine are not due to increased activity of tryptophan oxygenase in the liver. Therefore, the excretion of large amounts of kynurenine and its metabolites could not be explained on the basis of excess production of kynurenine in

 TABLE 2.
 Tryptophan 2,3-dioxygenase (TPO)
 and indoleamine 2,3-dioxygenase (IDO)

	Source	Substrate	Oxygen
TPO	Liver	L-Tryptophan	$O_2$
IDO	Brain	L-&D-Tryptophan	l
	Lung	5-Hydroxy-L&D- tryptophan	$O_2\overline{\cdot}$
	Stomach	Tryptamine	&
	Intestine Epididymis	Serotonin	$O_2$
	etc.	etc.	

the liver.

We, then, asked ourselves the following questions. What is the mechanism of tryptophan degradation under these various pathological conditions? What is the biological significance of this phenomenon? Is tryptophan dioxygenase actually the rate-limiting enzyme in the major metabolic pathway of tryptophan in mammals?

In 1963 we found from rabbit intestine another enzyme that also catalyzes the formation of kynurenine from tryptophan. In contrast to tryptophan dioxygenase, this enzyme exhibits broad substrate specificity and acts upon various indoleamine derivatives, including tryptophan. Therefore it was termed indoleamine 2,3-dioxygenase, abbreviated as IDO (Fig. 5). As shown here, indoleamine dioxygenase utilizes molecular oxygen as well as superoxide anion, a univalently reduced molecular oxygen for activity. Distribution and properties of these two enzymes are shown in Table 2.

As can be seen here, tryptophan dioxygenase, abbreviated as TPO, is found only in the liver, and acts only on tryptophan. In contrast, indoleamine dioxygenase is widely distributed in various tissues and organs, except the liver. It has a broad substrate specificity, and acts upon not only tryptophan but also 5-hydroxytryptophan, tryptamine, serotonin, etc, although tryptophan is a far better substrate in terms of Km and Vmax. Tryptophan dioxygenase utilizes molecular oxygen, but indo-

TABLE 3. Molecular and catalytic properties

	Indoleamine Dioxygenase	Tryptophan Dioxygenase
M.W.	42,000	167,000
Subunits	1	4
Carbohydrate	4.8%	
Protoheme IX	1	2
Turnover number	120	420

learnine dioxygenase requires and utilizes superoxide anion and molecular oxygen for activity. The molecular properties of the purified enzyme preparations of these two oxygenases are also different, as shown in Table 3.

Although both indoleamine dioxygenase and tryptophan dioxygenase are hemoproteins, their molecular properties are clearly different with regard to the molecular weight, subunit structure, carbohydrate and heme contents and the turnover number. They are also different in terms of immunological properties. Thus, there are two entirely different enzymes both catalyzing essentially idential reactions, namely the formation of formylkynurenine from tryptophan. However, unlike tryptophan dioxygenase, indolearnine dioxygenase is not induced by either tryptophan or glucocorticoid. What is then the physiological function of indoleamine dioxygenase?

One of the most striking features of this enzyme is the fact that the purified enzyme is by itself totally inactive, even in the presence of oxygen, and requires the superoxide anion. Evidence for the participation of the superoxide anion in the indoleamine dioxygenase catalyzed reaction has already been published in detail and is briefly summarized as follows.

First, the enzyme requires and utilizes the superoxide anion for activity. The superoxide anion can be supplied as a relatively stable potassium salt,  $KO_2$  or is generated either chemically or enzymatically. The superoxide is required for the initiation of the reaction as well as to maintain the steady



FIGURE 6. Possible reaction sequence.

state of the reaction. Second, the reaction is inhibited by scavengers for the superoxide anion, such as superoxide dismutase, tyron and so forth, but scavengers for singlet oxygen or hydroxy radicals are totally without effect, even at high concentrations. Third, the superoxide anion labeled with heavy oxygen isotope, oxygen-18, is incorporated into the product of the reaction. However, when the reaction is carried out in the presence of heavy oxygen-containing air, oxygen-18 is also incorporated into the product of the reaction, to some extent. These results indicate that IDO is a unique oxygenase and requires both molecular oxygen and the superoxide anion for activity. An explanation for this dual requirement is given in the reaction scheme shown in Fig. 6.

This scheme illustrates a possible reaction sequence based on the recent kinetic experiments in our laboratory. It is obviously an oversimplification but may be useful as a point of orientation for discussing the reaction mechanism. Let us start with the native ferric enzyme. First, it binds with the substrate which is probably a very fast process; then the ES complex reacts with superoxide to form a ternary complex of Fe<sup>+3</sup>·O<sub>2</sub><sup>-</sup>·Try which is identical with the ferrous.oxygen. substrate complex. This charge transfer complex is also produced by the binding of molecular oxygen with the ferrous form of enzyme and substrate. This unstable binary complex can be produced experimentally by photolysis of Fe<sup>2+</sup> · Try · CO complex. Once the ternary

complex is produced, it decomposes to yield the reaction product, generating a ferrous form of enzyme. The first-order rate constant for this reaction is estimated to be  $2.0 \text{ s}^{-1}$ . Thus, during the steady state of the reaction cycle, K<sub>4</sub> is the rate-determining step and is in good agreement with the turn-over number of the enzyme. During the catalytic cycle, however, the enzyme is slowly oxidized with the rate constant of 0.03/sec to the ferric form which, in absence of the superoxide anion, remains inactive. In other words, one in every 60 or 70 cycles, the enzyme undergoes autooxidation to become a ferric form of enzyme, which then binds with tryptophan and the superoxide anion. In fact, when supply of the superoxide anion is cut off during the steady state of the reaction, the reaction continues for several seconds until all the enzymes are converted to the inactive ferric form. Thus the enzyme utilizes both molecular oxygen and the superoxide anion. But in order to initiate the reaction and maintain the steady state of the reaction, the superoxide anion is absolutely essential, and the supply of O<sub>2</sub><sup>-</sup> is the rate-determining step in the overall process.

Now, should this enzyme utilize the superoxide anion in vitro, the possibility still remains that these observations are an in vitro artifact and that the enzyme utilizes only molecular oxygen in vivo. To carry the point further, one may ask: Does this enzyme actually exist and function in vivo, because the superoxide anion is most unstable and the steady state concentration in the cell is presumably very low. In an attempt to answer these questions, mucosal cells from rabbit intestine were isolated and the intracellular dioxygenase activity of dispersed cell suspensions of enterocytes was determined as a model of in vivo experiments (Fig. 7).

Because superoxide dismutase, SOD, is active in enterocytes, it is reasonable to assume that the supply of superoxide anion is low and must be one of the rate-limiting factors of the intracellular IDO activity. Now



FIGURE 7. Intracellular indoleamine 2,3-dioxygenase activity and  $O_2^-$ .



FIGURE 8. Effects of inosine and allopurinol on IDO activity.

how can one regulate the level of  $O_2^{-}$  in the cell. In order to approach this problem we did the following experiments. A number of enzyme systems may generate  $O_2^{-}$  in the enterocytes but since xanthine oxidase generates  $O_2^{-}$  in vitro and is abundant in the intestine, the effect of inosine, a substrate of xanthine ixidase, on the intracellular dioxygenase activity was examined. Alternatively diethyldithiocarbamate (DDC), a copper chelator and a potent in vivo inhibitor of super-oxide dismutase, was used to inhibit the intracellular dismutase activity. Both of these reagents should increase the concentration of the superoxide anion inside the enterocytes



FIGURE 9. Effect of DDC on IDO activity.

and thereby stimulate the endogenous IDO activity in the cell. The results of such experiments are presented in the next two figures.

The time course of the reaction is shown on the left of Fig. 8. The addition of inosine increased the dioxygenase activity nearly 5 fold. The dose response curve is shown on the right. The amount of inosine required for the maximum stimulation was in the order of 0.1 mM and this effect was abolished by the addition of allopurinol, a specific inhibitor of xanthine oxidase. Neither inosine nor allopurinol had any effect on the purified IDO in vitro. These results indicate that the superoxide anion generated by the xanthine oxidase system is utilized by the indoleamine dioxygenase, and that it is one of the rate determining factors in vivo.

In the same manner, the intracellular indoleamine dioxygenase activity in the dispersed enterocytes was markedly enhanced by the addition of 5 mM DDC, an inhibitor of superoxide dismutase (Fig. 9). Diethyldithiocarbamate, however, had no activating effect on the purified indoleamine dioxygenase, in vitro. These results indicate that DDC probably inhibits superoxide dismutase, increases the intracellualr concentration of superoxide anion, and stimulates the apparent indole-



FIGURE 10. Phagocytosis and active oxygen.

amine dioxygenase activity, again indicating that  ${\rm O_2}^-$  is the rate-limiting factor, in the intact cells.

So far, I have talked about the role of superoxide in the indoleamine dioxygenase catalyzed reaction. Now let us look at the physiological function of the superoxide anion, in vivo.

The superoxide anion is an active form of oxygen and is produced in leukocytes when bacteria are ingested by leukocytes as shown in Fig. 10. During phagocytosis, NADPH oxidase on the membrane of phagosomes is activated and molecular oxygen is reduced by one electron to produce superoxide anion. The superoxide anion alone, or together with H<sub>2</sub>O<sub>2</sub> which is generated from the superoxide, kills the ingested bacteria. Therefore the superoxide anion is implicated in the defense mechanism against bacterial infection. Indoleamines are also implicated in the inflammatory processes. And as I told you at the beginning, tryptophan is also degraded in inflammatory and other pathological conditions. So, how do you put all these pieces of information together to elucidate the biological function of this enzyme? As the lung is an aerobic organ and probably generates a fair amount of superoxide anion, we decided to subject mice to viral infection to cause viral pneumonia and determined the indoleamine dioxygenase activity in the lung.

When we exposed mice to influenza virus, the virus replication in the lung began within



FIGURE 11. Induction of IDO by virus infection.

24 hours after the infection, reached a peak by the 3rd day and persisted until the 9th day, causing viral pneumonia. Thereafter, the virus was rapidly eliminated from the lung and by the 14th day almost completely disappeared (Fig. 11). The indoleamine dioxygenase activity in the lung increased almost linearly from the 5th day after infection. More than 120-fold increase was observed on the 11th day after which the activity gradually decreased to normal values over a twoweek period. These results indicate that, with viral infection, the indoleamine dioxygenase activity increases tremendously and the break-down of tryptophan and other indoleamine derivatives in the lung is accelerated to a large extent. Since this experimental approach is somewhat complicated and is difficult to quantify, we then used bacterial endotoxin instead of influenza virus in order to simplify the experimental conditions.

Bacterial endotoxin or lipopolysaccharide (LPS) of gram negative bacteria is an inflammatory agent and induces a nonspecific immune response. It is a large molecular compound consisting of 3 different portions, namely lipid A, core polysaccharide and o antigen specific polysaccharide. When LPS, the lipopolysaccharide fraction of E, coli, was given intraperitoneally to mice, again we observed a remarkable increase in the indoleamine dioxygenase activity with a concomitant increase in the plasma kynurenine level.



FIGURE 12. Plasma kynurenine level and IDO activity in lung after LPS treatment.

As can be seen in the lower panel of Fig. 12, induction of indoleamine dioxygenase in mouse lung was observed almost immediately after the intraperitoneal injection of LPS. The specific activity of IDO in the high speed supernatant of the lung homogenate increased almost immediately and linearly for about 24 hours, after about 48 hours gradually decreased and reached a normal value within seven days. A single injection of about  $20\gamma/$ mouse of LPS was sufficient to induce 30-50 fold, sometimes a 100-fold increase in this enzyme activity. As shown in the upper panel, plasma kynurenine levels also increased and gradually decreased. The time course of the increase and decrease in plasma kynurenine level is essentially identical to that of IDO activity in the lung.

Fig. 13 shows "the plasma kynurenine level and tryptophan dioxygenase activity in the liver after LPS treatment." Under these conditions, tryptophan dioxygenase activity in the liver decreased to less than 50% of the



FIGURE 13. Plasma kynurenine level and TPO activity in liver after LPS treatment.

normal activity, thus there is no correlation to the plasma kynurenine level. These results clearly indicate that the induction of indoleamine dioxygenase is related to the increase in the plasma kynurenine level and that tryptophan is probably degraded in the lung and other organs in the presence of elevated levels of IDO. Such a remarkable increase in enzyme activity is rather unusual in the mammalian system. So we investigated its mechanism. The increase in the enzyme activity under these conditions appears to be due to the net synthesis of enzyme protein rather than to the removal of inhibitors, formation of activators or other factors, because the concomitant administration of an inhibitor of RNA or protein synthesis such as Actinomycin D or cycloheximide abolished the increase in the enzyme activity produced by LPS. Immunological analyses using antibody against IDO are in line with this conclusion, because the increment in enzyme activity was mainly due to an increase in the



FIGURE 14. Induction of IDO by LPS in various organs.

amount of immunologically reactive protein.

The induction of IDO activity by LPS is most pronounced in the lung (Fig. 14). However, the enzyme activity increased at least several fold in other organs and tissues, including heart, spleen, colon and so forth.

It is also noteworthy that tryptophan dioxygenase activity in the liver decreased rather than increased with LPS treatment. In order to find out the exact location of indoleamine dioxygenase activity, we treated the lung tissue with trypsin and collagenase, fractionated and determined the cellular distribu-The indoleamine dioxygenase activity tion. was found exclusively in alveolar interstitial cells. Prostaglandins dehydrogenase, which is responsible for the inactivation of prostaglandins in the lung, is also found associated with this type of cell. Immunohistochemical studies with anti-IDO antibody also supported this conclusion.

We then looked to see whether or not the induction by LPS was specific for the indoleamine dioxygenase activity, and various other enzyme activities in the lung were determined. Enzymes supposedly involved in inflammation such as monoamine oxidase, prostaglandin cyclooxygenase, superoxide dismutase, lipoxygenase and so forth, typical lysozomal enzymes such as acid phosphatase were not induced to any significant extent (Fig. 15). The question then arises, how and why bacterial endotoxin or virus infec-

	1	Relative 10	Specific	Activity 20	30
100					ן נ
Monoamine oxidase	þ				
Prostaglandin cyclooxygenase	þ				
Acid phosphatase	þ				
Superoxide dismutase	þ				
Lipoxygenase	þ				

FIGURE 15. Induction of various enzymes in lung.



FIGURE 16. Induction of IDO by LPS and interferon in mouse lung slices.

tion induces specifically the indolearnine dioxygenase.

In an attempt to determine the mechanism of enzyme induction, we then switched to an in vitro system and exposed lung slices to LPS. As can be seen on the left of Fig. 16, when LPS was given at 0 time, indoleamine dioxygenase was gradually induced but with a brief lag period. As expected, the interferon titre increased almost instantaneously after LPS administration. When interferon itself was administered at zero time. there was no lag in the induction of indoleamine dioxygenase. Interferon appears to be an even more effective inducer of this enzyme, as shown in this figure. These time courses indicate that when LPS enters the cell, interferon is produced and then the induction of the indoleamine dioxygenase is triggered by interferon.

TABLE 4. Comparison of  $IC_{50}$ 

Agents	IDO- induction IC <sub>50</sub>	Cyclo- oxygenase IC <sub>50</sub>
Indomethacin	12 µM	$0.16-3.60 \mu{ m M}$
Phenylbutazone	$83 \ \mu M$	$7.23\mu{ m M}$
Aspirin	$120 \mu M$	36.7–150 $\mu M$
Salicylate-Na	>1  mM	$750~\mu M$
Phenacetin	$>1 \mathrm{mM}$	

TABLE 5. Comparison of  $IC_{50}$ 

Agents	IDO- induction IC50	Phosph- lipase $A_2$ $IC_{50}$
Dexamethasone	1 nM	3.6 μM
Betamethasone	0.6 nM	$3.6\mu\mathrm{M}$
Cortisone	20 nM	$120.7\mu\mathrm{M}$
Aldosterone	280 nM	
$17\beta$ -Estradiol	>1 mM	
Testosterone	>1 mM	701-004

This induction of indoleamine dioxygenase by interferon is inhibited by a number of antiinflammatory agents such as indomethacin, aspirin and so forth, compounds which inhibit the biosynthesis of prostaglandins.

Table 4 shows the  $IC_{50}$ , namely 50% inhibition concentration of various non-steroidal antiinflammatory drugs on IDO induction and the prostaglandin synthesizing enzyme, cyclooxygenase. The  $IC_{50}$  values are not exactly the same, presumably because of the difference in experimental conditions. Nevertheless, it is clear that the order of effectiveness was essentially identical in both cases indicating the possible involvement of prostaglandins in the induction of indoleamine dioxygenase.

We also looked at the effect of steroids, another group of compounds which act as antiinflammatory agents and inhibit phospholipases, thereby inhibit prostaglandin synthesis.

Again we found that the most effective inhibitors of phospholipase such as dexameth-



FIGURE 17. Virus yield in IFN-treated lung slices and IFN-mediated IDO induction.



FIGURE 18. Effects of dexamethasone and IFN on IDO activity and virus growth.

asone and  $\beta$ -methasone are, potent inhibitors of IDO induction, while aldosterone and sex hormones all of which are inactive in phospholipase inhibition did not inhibit IDO induction to any significant extent. These results are consistent with the notion that prostaglandin biosynthesis is involved in the induction of indoleamine dioxygenase. We thus had a powerful tool to assess the relation between the antiviral activity of interferon and the induction of this dioxygenase (Table 5).

In an attempt to elucidate this question, we did the following experiments. We exposed mouse lung slices to various amounts of highly purified  $\alpha$  and  $\beta$  mouse L-cell derived



FIGURE 19. Biological significance of IDO induction.

interferon. After incubation with the interferon preparation at  $37^{\circ}$  for 12 hours, the slices were exposed to a suspension of influenza virus, and incubated for 1 hour, washed and the incubation continued for 24 hours. The samples were then assayed for the IDO activity and virus titre.

The horizontal axis in Fig. 17 indicates the amount of interferon added to the medium. The number of virus in the tissue and medium decreases with increase in the amount of interferon. At the same time, the indoleamine dioxygenase activity increases in proportion to the amount of interferon.

The crucial question is whether or not the indoleamine dioxygenase activity is related to the antiviral state caused by interferon. In order to answer this question, we added varying amounts of dexamethasone to the preincubation mixture to inhibit the induction of indoleamine dioxygenase. If the antiviral state due to interferon is directly related to IDO, one should observe a decrease in the antiviral activity. The results are shown in Fig. 18.

In the control experiment, IDO activity was low and the virus did replicate actively. Dexamethasone alone hardly affected both. When interferon was added, virus replication was inhibited and the IDO induction was observed. When IDO induction was inhibited by the addition of increasing amounts of dexamethasone, the interferon mediated antiviral activity was also dose-dependently

TABLE 6. Two systems in which tryptophan degradation is induced by interferon

Interferon	Agent	Host	Reference
$\alpha/\beta$	Virus	Interstitial cells of mouse lung	Our work
ĩ	Toxoplasma	Human fibroblast	E. R. Pfefferkorn (1984)

inhibited. These results strongly support the interpretation that the induction of IDO is involved in the antiviral activity of interferon, at least in this system. Under the same conditions, there was no induction of  $2' \cdot 5'$  oligo A synthetase, an enzyme reportedly involved in the antiviral activity of interferon in some other systems.

In summary, Fig. 19 shows our current working hypothesis concerning the biological significance of IDO induction. When tissues are invaded by virus, bacteria, or bacterial endotoxin, leukocytes and lymphocytes will accumulate and interferon is produced. Interferon thus produced, migrates and interacts with the cell surface of the interstitial cells and stimulates the intracellular synthesis of prostaglandins which triggers indoleamine dioxygenase induction. As a consequence of inflammation, superoxide anion is liberated and serves as a substrate for indoleamine dioxygenase. Tryptophan, an essential amino acid, is depleted and the growth of virus, bacteria and maybe certain types of tumor cells is inhibited.

Recently, Pfefferkorn of Dartmouth Medical College published a paper in PNAS, entitled "Interferon  $\gamma$  blocks the growth of *Toxo*- plasma gondii in human fibroblasts by inducing the host cells to degrade tryptophan ", in which he described the following: When human fibroblasts were treated with  $\gamma$ -interferon, the growth of Toxoplasma gondii, an obligate intracellular protozoan parasite, was blocked, and at the same time tryptophan in the host cells was degraded to generate a large amount of formylkynurenine and kynurenine.

It was proposed that  $\gamma$ -interferon induces an enzyme that degrades tryptophan and restricts the growth of this parasite. He concluded that the enzyme induced by physiological doses of  $\gamma$ -interferon described in his paper is probably "indoleamine dioxygenase." Therefore, indoleamine dioxygenase is no doubt involved in defense mechanisms of biological systems, in particular the action of interferons (Table 6).

## ACKNOWLEDGEMENT

I express my gratitude for the collaboration of Drs. Yoshida and Yasui who are responsible for the more recent biological aspects of this work and Drs. Hirata, Sono and Taniguchi for the early part of this project.