The Induction of Host Tryptophan Oxygenase by Staphylococcal Infection[1] (36635)

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Berry and his colleagues first drew attention to the important changes in the metabolism of tryptophan during infection (1). They analyzed the activity of tryptophan oxygenase in the livers of *Salmonella typhimurium* infected mice and found an increase in enzyme activity soon after the mice were infected, followed by a decrease in the enzyme activity prior to death. The authors believe that activation of the host defense mechanisms initially induced enzyme and that it was inhibited later because of overwhelming infection and suppression of protein synthesis. Rappoport, Lust and Beisel (2) have made similar observations in *Diplococcus pneumoniae* infected mice.

Infection with *S. aureus* has been shown to uncouple oxidative phosphorylation in mouse liver (3). One of the important coenzymes involved in the normal oxidative process is derived from nicotinamide, a tryptophan metabolite. The possible interrelationship between staphylococcal infection and tryptophan metabolism were therefore studied and the results of these investigations are reported here.

Materials and Methods. The Smith strain of hemolytic coagulase positive *S. aureus* was used to infect 30 g Swiss Webster albino male mice (Arthur Suter, Springfield, MO). The challenge dose given intraperitoneally to each mouse was 0.1 ml of a fresh overnight culture containing 10^{10} bacteria/ml (3, 4). Ten control and 10 test mice were used for each experiment in a standardized model described previously (5). Adrenalectomized mice were maintained on 1% sodium chloride solution for 1–2 weeks before being subjected to different experiments. Completeness of adrenal insufficiency was demonstrated by the water excretion test of Beatty, Bocek and Peterson (6). The various metabolites from tryptophan to niacin were tested for their relative abilities to either protect and/or to treat the infected mice. This was done by injecting the test material (0.5 ml) subcutaneously 1, 2 or 3 hr before challenge with staphylococci or 0, 1, 2, or 3 hr postinfection. The dose of the chemical chosen was as close to its LD_{50} so that a maximum effect from the treatment could be obtained, but such that no ill effects or death occurred in control mice. Nicotinamide (25 mg), kynurenine (2.5 mg), and *N*-methylnicotinamide (5 mg) were dissolved in pyrogen-free saline solution. Tryptophan (20 mg), 3-hydroxyanthranilinic acid (5 mg), quinolinic acid (25 mg), and nicotinic acid (15 mg) were dissolved in diluted NaOH solution and neutralized with HCl. All test solutions were prepared immediately prior to their use.

Tryptophan oxygenase was assayed as originally described by Knox and Auerbach (7) and modified by Berry (1). Enzyme activity was expressed as micromoles of kynurenine formed per hour per gram of liver dry weight measured at 360 nm with a Gilford model 300 spectrophotometer. Graded amounts of kynurenine sulfate were added to normal mouse liver homogenates in order to establish a standard curve.

Results. Protection and treatment of infected mice with tryptophan metabolites. Mice inoculated intraperitoneally with 10^9 cells of *S. aureus* die 270 ± 20 min later. The

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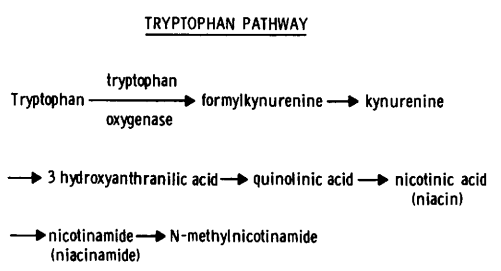

FIG. 1. Metabolic pathway of tryptophan to show intermediate products utilized in this study.

various metabolites that are formed along the pathway of conversion of tryptophan to nicotinamide (Fig. 1) were compared to see if they could protect or treat the infected mice. Since treated animals received 0.5 ml of fluid in the test solution, control animals were also injected with an equal volume of 0.1 *M* sucrose solution. Table I summarizes the differences in minutes between the lethal times of control mice (expressed as LT_{50}) and mice injected subcutaneously with the various tryptophan metabolites prior to or after challenge with *S. aureus*. Nicotinamide was the only metabolite which was successful in prolonging the life of the infected mice, averaging 42, 29 and 49 when given at —3, —2, and —1 hr, respectively. Similarly, nicotinamide was an effective treatment if given at 0 or 1 hr postinfection, but was ineffective if delayed for longer periods of time.

Effect of nicotinamide on the growth of S. aureus *in vitro*. Since nicotinamide seemed to have a significant protective effect on the staphylococcal infected mice, its effect on the *in vitro* growth of *S. aureus* was evaluated. The first experiment was designed to determine if a bactericidal amount of nicotinamide or nicotinamide induced substance appeared in the liver following subcutaneous injection. Two hours after mice received 25 mg of nicotinamide, their livers were removed and homogenized in 5 ml of sterile water for each liver. Then 10^7 cells of *S. aureus* were added to the livers and incubated at 37° using normal mouse liver homogenates with similar numbers of added *S. aureus* as controls. The bacterial titers of each mixture were determined at various times during incubation. No differences in the bacterial titers between the two groups were noted up to 5 hr after the onset of incubation, at which time the bacterial counts had reached 5×10^8. In the second experiment 10^4 cells of *S. aureus* were added to normal mouse liver homogenates along with or without 25 mg of nicotinamide. Table II shows the actual number of *S. aureus* recovered by dilution and plating. The mixtures were incubated at 37° and their bacterial titers were determined at various times of incubation. These results are

TABLE I. Effect of Tryptophan Derivatives on the Lethality of *S. aureus* Infected Mice.[a]

	Time of treatment in relation to infection (hr)						
	—3	—2	—1	0	1	2	3
Tryptophan	—13	+3	—6	+5	+10	—15	—1
Kynurenine			—7	+5	—19	+3	—2
3-OH anthranilinic acid	+2	+3	+21	+20	+12	+27	+25
Quinolinic acid	—20	—25	+19	—12	+1	+7	+16
Nicotinic acid (niacin)	—25	—29	—6	—9	—17	—20	—8
Nicotinamide (niacinamide)	+42[b]	+29[b]	+49[b]	>79[b]	>102[b]	29	+1
N-Methylnicotinamide	—1	—4	—5	+11	+16	+4	+10
Nicotinamide adenine dinucleotide (NAD)	—8	—15	+8	+10	29	+5	

[a] The results are expressed as differences in the mean time (min) of death between infected but untreated mice and mice treated with the tryptophan metabolite at various times before or after intraperitoneal injection of 10^9 *S. aureus*. Each value represents an average of three separate experiments. Treated, uninfected mice were never observed to have any ill effects. Experimental details are listed in the Methods section.

[b] Significantly different from control $p \lesssim .05$.

TABLE II. Effect of Nicotinamide on the *in Vitro* Growth of *S. aureus*.[a]

Time (hr)	Normal liver homogenates + *S. aureus* (titer)	Normal liver homogenates + 25 mg nicotinamide + *S. aureus* (titer)
0	2.5×10^4	1.5×10^4
3	3.5×10^5	1.0×10^5
5	8.0×10^7	5.0×10^7
8	1.5×10^7	3.0×10^7

[a] Twenty-five milligrams of nicotinamide were added to normal mouse liver homogenates *in vitro* with 10^4 *S. aureus* cells and the mixture was incubated at 37° for varying times. Control mixtures were incubated without the presence of nicotinamide.

shown in Table II and again, nicotinamide did not seem to have a significant effect on the growth of *S. aureus*.

Tryptophan oxygenase activity in livers of staphylococcal infected mice. Mice were injected intraperitoneally with 10^9 cells of *S. aureus* and at various times after inoculation the livers were removed and assayed for tryptophan oxygenase activity. Enzyme activity increased over 113% in the first 40 min but by the end of 1 hr, it had decreased to below control levels (Fig. 2). A subsequent 77% increase over control values occurred 240 min after infection at a time when the animals were close to death.

Adrenalectomized mice were similarly infected and the livers were removed and assayed for tryptophan oxygenase activity using uninfected adrenalectomized mice as controls. No significant increase in the activity of tryptophan oxygenase could be demonstrated during the 4 hr of infection. It should be noted that the tryptophan oxygenase activity in the livers of adrenalectomized mice is approximately 1/2 of that present in the livers of normal mice.

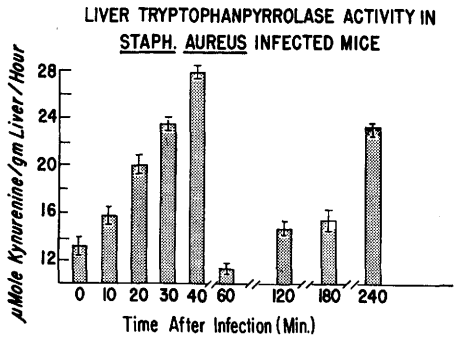

FIG. 2. Tryptophan oxygenase activity in mouse liver measured as kynurenine produced at designated times after challenge with 10^9 *S. aureus* cells in albino Swiss mice. Mean of 4 values.

Tryptophan oxygenase induction by tryptophan metabolites and other agents. Mice were given each of the tryptophan metabolites 1 hr after staphylococcal infection and the livers were assayed for tryptophan oxygenase activity 2 hr postinfection. For controls the livers of untreated mice 2 hr after infection with staphylococci were used as well as the livers of uninfected mice killed 1 hr after each animal had received a different chemical. These results (Table III) show that with the exception of quinolinic acid, all metabolites led to an increase in tryptophan oxygenase activity; the highest levels of enzyme activity being observed in mice that received tryptophan.

In similar experiments mice received sodium bicarbonate, cortisol, or glucose 1 hr after challenge with *S. aureus* and the livers were assayed for tryptophan oxygenase activity. Enzyme activity was found to be approx-

TABLE III. Effect of Tryptophan Metabolites on the Tryptophan Oxygenase Activity of Infected and Uninfected Mouse Livers.[a]

	Uninfected	Infected
Tryptophan	36.6	31.5
Kynurenine	20.7	16.0
Quinolinic acid	15.3	12.1
Nicotinic acid	21.0	22.2
Nicotinamide	20.5	20.5
N-Methylnicotinamide	23.4	22.5
Normal mouse, fasted 2 hr	13.5	
2 hr after *S. aureus* infection		14.0

[a] Mice were infected with *S. aureus* for 1 hr then treated with each of the chemicals for 1 hr before their livers were assayed for TO activity. Uninfected mice were given the chemical for 1 hr before their livers were assayed. Results are expressed as micromoles of kynurenine/hr. Mean of four experiments.

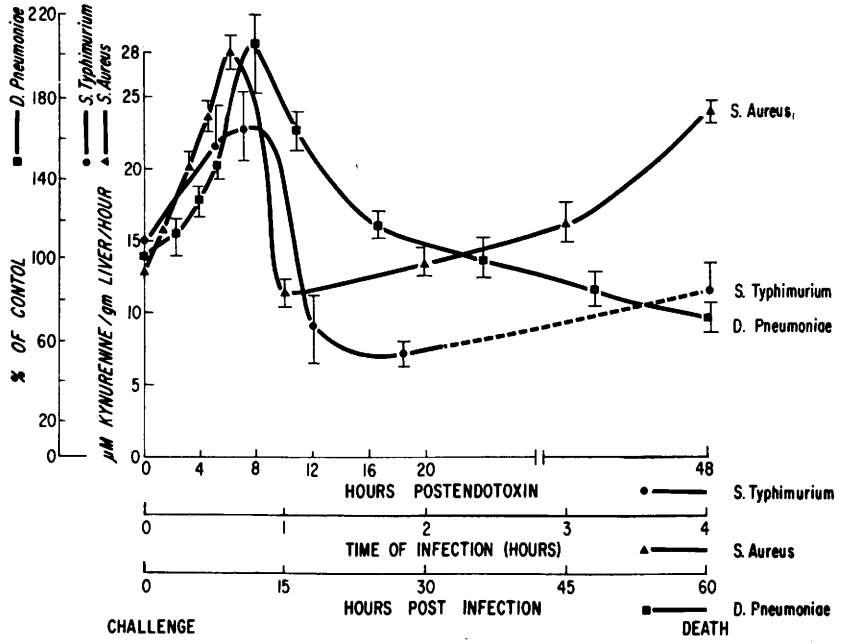

FIG. 3. Tryptophan oxygenase activity (measured as kynurenine formed) in mice challenged with three different pathogens.

imately three times greater in the livers of treated animals compared to that present in infected or uninfected mice (Table IV).

Discussion. Stimulation of tryptophan oxygenase activity has been noted during infection of mice with *S. typhimurium* or its endotoxin (1), and pneumococci (2). Our experiments indicate that infection with *S. aureus* is also capable of inducing tryptophan oxygenase, probably by mechanisms similar to the other infections. The similarities, demonstrated by regraphing the results of these investigations, are shown in Fig. 3. (The scale in this figure has been readjusted according to the length of the lethal infection in the animals used.) As these increases in enzyme activity with infection were not observed in adrenalectomized mice, and it is known that corticosteroids can induce this enzyme, it seems reasonable to presume that the changes in tryptophan oxygenase activity accompanying staphylococcal infection in mice are mediated via the adrenal glands.

TABLE IV. Effect of $NaHCO_3$, Cortisol and Glucose on the Tryptophan Oxygenase Activity of Livers from Infected and Uninfected Mice.[a]

	Tryptophan oxygenase activity of liver (μmoles of kynurenine/hr liver)	
	Chemical in normal mice	Chemical in infected mice
Normal mouse, fasted 2 hr	14.3	
2 hr after *S. aureus* infection		15.0
$NaHCO_3$, 55 mg	38.2	42.8
Cortisol, 10 mg	44.6	41.3
Glucose, 200 mg	36.7	44.3

[a] Experimental design as in Table III. Mean of 4 values.

In addition to the induction of tryptophan oxygenase activity, cortisone has been shown to be an effective chemical treatment for *S. aureus* infection in mice (8). Glucose and sodium bicarbonate, also effective chemicals in treating this infection (5), can induce tryptophan oxygenase to equally high levels in infected and uninfected mice. In the experiments reported here, nicotinamide was found effective in prolonging the survival of infected mice and was capable of stimulating tryptophan oxygenase activity in both infect-

ed and uninfected mice. In the experiments reported here, nicotinamide was found effective in prolonging the survival of infected mice and was capable of stimulating tryptophan oxygenase activity in both infected and uninfected mice. That the mechanism of survival is not related to the increased activity of tryptophan oxygenase activity is shown by the inabilities of tryptophan and other tryptophan metabolites that are potent inducers of the enzyme to protect the mice against challenge with staphylococci.

The mechanism(s) by which nicotinamide exerts its prophylactic and therapeutic effects against staphylococcal infection remain unclear. No direct effects on the growth of staphylococci in liver homogenates are noted, nor were any indirect effects on the *in vitro* growth noted by the prior administration of nicotinamide to the animal. Nicotinamide is the direct precursor of the important coenzyme NAD and it is possible that the administration of this but not another precursor, nor NAD itself, leads to a series of events that can combat the uncoupling of oxidative phosphorylation that has been observed in the livers of staphylococcal infected mice (9). The beneficial effects of reduced ambient oxygen tension has been demonstrated in this model of infection and attributed to changes in the activity of the respiratory chain (10). It is also possible that nicotinamide exerts its protective effects against staphylococcal infection by mechanisms, as yet unknown, common to some or all of the other effective chemical agents. Further studies are necessary to clarify these possible interrelationships.

Summary. Infection of mice with *Staphylococcus aureus* leads to increased activity of liver tryptophan oxygenase, probably in an increased corticosteroid response associated with the infection. Nicotinamide was found to be effective in prolonging the survival of infected mice if given between 3 hr prior to and 1 hr after infection. None of the other tryptophan metabolites were effective against the challenge with staphylococci. Nicotinamide, as well as other chemicals which prolong the survival of infected mice can induce tryptophan oxygenase in the presence or absence of infection, but the studies reported here would seem to indicate that the mechanisms of protection do not involve changes in the activity of the enzyme.

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