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Potentiation of endotoxin shock by oral L-tryptophan[1]

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BOGURHOW, IRWIN B., GEORGE D. LUDWIG, AND DANA WONTORSKY. *Potentiation of endotoxin shock by oral L-tryptophan.* Am. J. Physiol. 214(3): 525–531. 1968.—The role of protein degradation products, or metabolites formed by the action of intestinal bacteria upon them, in potentiating experimental shock was investigated. Oral or intravenous L-tryptophan was given to dogs prior to endotoxin administration. Tryptophan (an ubiquitous amino acid in proteins) and many of its metabolites have important physiologic and pathophysiologic effects. Mortality from endotoxin shock was increased following oral, but not intravenous, tryptophan. Metabolites of various tryptophan pathways were assayed in urine of dogs subjected to endotoxin shock. Oral tryptophan produced great increases in kynurenic acid, anthranilic acid, anthranilic acid-glucuronide, kynurenine, and *N*-α-acetyl kynurenine. Except for kynurenic acid, none of these metabolites increased following intravenous tryptophan. The implication of one of the above metabolites, or a combination of them, in shock potentiation was thus suggested. However, the observation that the same metabolites appeared in urine of dogs protected against shock by oral neomycin or by large doses of intravenous hydrocortisone made this possibility seem less likely. No data was obtained to incriminate other tryptophan metabolites, e.g., those produced in the tryptamine, indoleacetic acid, or serotonin pathways.

endotoxin shock, protection against; tryptophan metabolites; indoles; portal vein ammonia; portal vein serotonin

BOUNOUS ET AL. have shown that after lethal hemorrhagic shock and retransfusion there is a specific and profound depression in the oxygen uptake of the intestine (4). Associated with this is an impairment in both the synthesis of adenosine triphosphate and the rate of oxidative phosphorylation within the mucosal cells of the intestine (5, 7). The metabolically depressed mucosa then becomes abnormally permeable (6), and is subject to enzymatic autodigestion by the proteolytic enzymes present in the intestinal lumen, leading to the hemorrhagic necrosis of the intestinal mucosa which characterizes irreversible shock in the dog (3).

If the bowel is washed free of its fecal contents, the metabolic activity of the intestinal mucosa is significantly higher after lethal shock and retransfusion (5). This suggests the possibility that the metabolically damaged intestinal mucosa is subject to a deleterious influence from the bowel contents that are normally present.

Ligation of the pancreatic ducts several days before a shock experiment renders the animal resistant to what usually constitutes a lethal period of hemorrhagic shock, and also prevents enzymatic digestion of the intestinal mucosa (3). An additional mechanism by which removal of proteolytic enzymes from the gut may exert this protective action may be by preventing the formation of deleterious end products from the exogenous and endogenous proteins present in the intestinal lumen (17).

An investigation of the possible role of protein degradation products in potentiating the state of shock seemed pertinent. Endotoxin shock in the dog was selected as the experimental model. As an initial step, a study of shock potentiation was undertaken, using the amino acid, L-tryptophan, an ubiquitous product of protein digestion. Another reason for choosing this particular amino acid was that Berry and Smythe (1) had shown previously that administration of L-tryptophan potentiated endotoxin shock in mice. A sublethal dose of endotoxin was selected in order to permit observation of shock potentiation if it were to occur.

METHODS

Healthy adult mongrel dogs of either sex weighing between 13–15 kg were used. L-Tryptophan (Nutritional Biochemicals, Cleveland, Ohio) in a dose of 1 g dissolved in 150 ml of 5% dextrose in saline was administered either orally by gavage, or intravenously, to various groups of animals prior to the intravenous administration

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of endotoxin. The pH of the mixture was 5.3–5.5. All animals were lightly anesthetized with intravenous pentobarbital (28 mg/kg), employing endotracheal intubation to maintain an adequate airway. Polyethylene catheters were inserted into the aorta and inferior vena cava via the femoral artery and vein. Mean arterial pressure was determined with a mercury manometer.

A single lot of the endotoxin of *Escherichia coli* (Difco Laboratories, Detroit, Mich.) was used in all experiments. To induce endotoxemia, 0.5 mg/kg of endotoxin was injected intravenously, 2 hr after administration of L-tryptophan by gavage, into one group of animals. This interval time was chosen arbitrarily in order to allow sufficient time for gastric emptying, enzymatic action on the amino acid in the gut, and absorption of the metabolic products. In another group of animals, endotoxin, 0.5 mg/kg, was injected intravenously 20–30 min following an intravenous infusion of L-tryptophan; the shorter time interval was chosen because it was assumed that tryptophan degradation would proceed more quickly when the need for intestinal digestion and absorption was eliminated.

Certain metabolites of the alternative metabolic pathways of L-tryptophan metabolism (Fig. 1) were assayed in blood and urine in an attempt to determine the mechanism whereby oral tryptophan potentiated shock. Specimens of urine were obtained from the bladder by catheterization and frozen until analyzed. Chromatograms of urine for indoles and other tryptophan metabolites were obtained from at least two dogs in each group. Blood and urine specimens were collected at zero time as control specimens, immediately prior to endotoxin administration (2 hr following oral tryptophan or 20–30 min following intravenous tryptophan), and 1 hr following endotoxin administration.

Blood pressure was recorded for 3 hr following injection of endotoxin, after which the cannulas were removed and the animals returned to their cages, where food and water were available. Since the majority of animals that survived shock for 72 hr recovered completely, all animals living 3 days after endotoxemia were considered permanent survivors. Autopsies were performed at the time of death or sacrifice.

Eight groups of dogs were studied in the manner outlined according to the following specific protocols:

Group I—controls (endotoxin alone). Twenty-five anesthetized dogs were used to establish an LD_{50} dose of endotoxin.

Group II—saline gavage. Ten dogs were gavaged with 150 ml of 5% dextrose in saline 1.5 hr prior to anesthesia and 2 hr prior to endotoxin, and then observed.

Group III—pretreatment with intravenous tryptophan. Nine anesthetized dogs received a 15-min infusion of L-tryptophan as outlined above. Endotoxin was given 20–30 min after completion of the infusion, and the animals observed.

Group IV—tryptophan gavage. Five dogs were gavaged with tryptophan. Anesthesia was given 1.5 hr later, following which they were returned to their cages.

Group V—tryptophan gavage plus endotoxin. Sixteen dogs were anesthetized 1.5 hr after tryptophan gavage. Endotoxin was administered 2 hr after gavage, and the animals observed.

Group VI—neomycin. In order to evaluate the role of intestinal bacteria in tryptophan degradation, and the effectiveness of poorly absorbed antibiotics in protecting from endotoxin shock, nine dogs received 4 g of neomycin and 10 ml of magnesium sulfate daily for 5 days. The drugs were manually inserted into the esophagus of the dog. On the 4th and 5th days an additional 20 ml of magnesium sulfate was given by gavage. On the 6th day tryptophan was administered by gavage. The animals were anesthetized 1.5 hr later, preliminary measurements were taken, and a stool culture obtained. Endotoxin was given 2 hr after gavage.

Group VII—hydrocortisone (50 mg/kg). To ascertain whether pretreatment with pharmacologic doses of hydrocortisone (Solucortef, Upjohn) would alter the metabolism of tryptophan in the gut, and to determine whether this might be related to the protective action of hydrocortisone in endotoxin shock, six dogs were given 50 mg/kg hydrocortisone intravenously immediately after tryptophan gavage. They were anesthetized 1.5 hr later and given endotoxin 2 hr after gavage.

Group VIII. In order to determine whether the enteric degradation of orally administered tryptophan would release serotonin and ammonia into the splanchnic circulation, four dogs were subjected to sterile implantation of siliconized polyethylene portal vein catheters 4 days prior to the shock experiment. Portal venous blood was obtained for serotonin and ammonia determination at zero time as controls, 2 hr after tryptophan gavage and 1 hr after endotoxin. Serotonin concentration was determined in whole blood by the method of Waalkes (22). Blood ammonia was determined by the method of Seligson and Hirahara (19) as modified by Reinhold and Chung (18).

Paper Chromatography of Urinary Indoles and Tryptophan Metabolites

Two dimensional paper chromatograms were run according to the method of Jepson (13). Isopropanol:ammonia:water (200:10:20) were used as the first solvent, and butanol:acetic acid:water (120:30:50) as the second solvent. The chromatograms were dried in air and examined for fluorescence in ultraviolet light. One set of papers was sprayed with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) to demonstrate urinary indoles. Another set of papers was dipped in Ekman's reagent to detect diazotizable primary aromatic amines. Sulfanilic acid was used for the detection of xanthurenic acid, and ninhydrin-pyridine solution, followed by viewing under ultraviolet light, for the detection of kynurenic acid. Standard amounts of indoles and various intermediate metabolites in the tryptophan-nicotinic acid pathway, as well as metabolites in other pathways of tryptophan metabolism, were run concurrently (Fig. 1). Ten microliters of untreated urine from representative

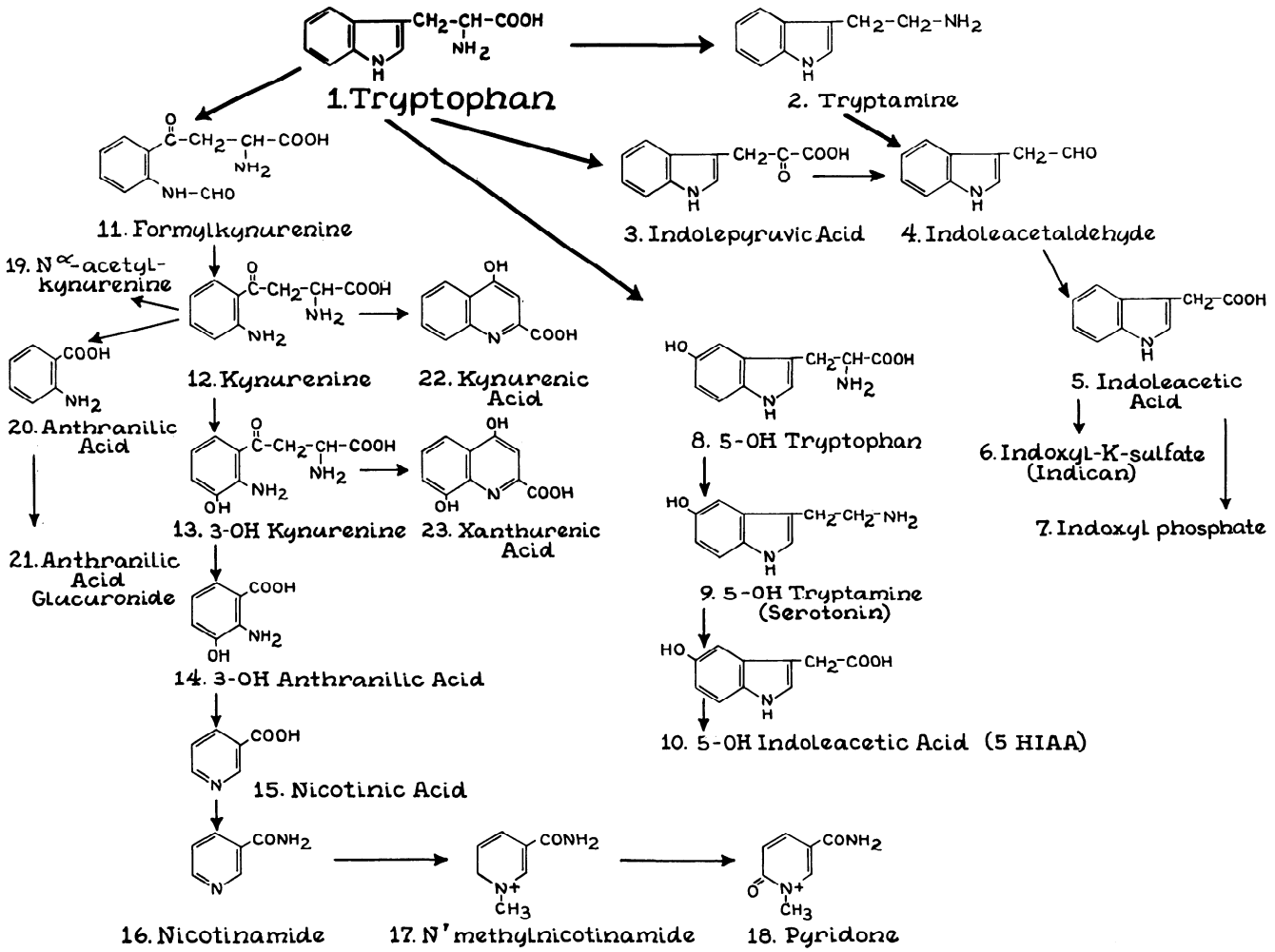

FIG. 1. Pathways of tryptophan metabolism.

animals in each experimental group were applied to one set of papers to search for gross changes. Subsequently, an amount of each urine specimen equivalent to 0.06 mg of creatinine was applied to the paper in order to eliminate differences that might arise from variable urinary volumes.

RESULTS

Survival Experiments

The survival experiments are summarized in Table 1.

Control group (endotoxin alone). Mortality in dogs given 0.5 mg/kg of endotoxin alone, and those pretreated with saline by gavage, followed by the same dose of endotoxin, approached an LD_{50} as determined at 72 hr. Following endotoxin, all animals demonstrated in varying degrees the characteristic response in arterial pressure (16). This consisted of a prompt and precipitous fall in the arterial blood pressure followed in a few minutes by a rise which never reached the preinjection level. The control mean arterial pressures ranged between 135–150 mm Hg, and fell to 26–58% of the control values. At autopsy all control animals dying of endotoxin showed hepatic congestion and pulmonary atelectasis. Hemorrhagic necrosis of the intestinal mucosa tended to be less in dogs given this dose of endotoxin, than in those dogs observed in this laboratory that were given an LD_{65} dose of endotoxin.

Intravenous tryptophan (followed in 20–30 min by endotoxin). The intravenous administration of 1 g of L-tryptophan alone caused small (± 5 mm Hg) or no change in control mean arterial pressures (135–160 mm Hg). When this was followed in 20–30 min by endotoxin, the course and autopsy findings were similar to the control group of dogs. Since the survival rate was similar to the control group given endotoxin alone, there was no evidence of shock potentiation.

Tryptophan gavage (followed by endotoxin in 2 hr). Orally administered tryptophan alone caused no apparent ill effects on the dogs' behavior. However, when given as pretreatment 2 hr before endotoxin, there were no permanent survivors. This enhancement of mortality was statistically significant compared to the results in dogs with saline gavage or tryptophan intravenously ($P = < .01$). However, two of the dogs survived for 48 hr, and hemorrhagic necrosis of the intestinal mucosa was not observed in five of the dead animals. The blood pressure

TABLE 1. *Summary of survival experiments*

Group	No. of Dogs	No. of Permanent Survivors
I Controls; endotoxin only	25	12
II Saline gavage plus endotoxin	10	5
III Tryptophan intravenously plus endotoxin	9	4
IV Tryptophan gavage; no endotoxin	5	5
V Tryptophan gavage plus endotoxin	16	0
VI Intestinal antibiotics, tryptophan gavage, and endotoxin	9	4
VII Hydrocortisone (50 mg/kg), tryptophan gavage, and endotoxin	6	6

responses were similar to that seen in the nonsurviving group of control dogs (given endotoxin alone).

Pretreatment with neomycin and tryptophan gavage. In animals given tryptophan by gavage followed by endotoxin, survival approached LD_{50}, paralleling the control group (endotoxin alone). Thus, there was no evidence of shock potentiation in these animals, despite the finding that none of the stool cultures was completely sterile. Arterial blood pressure responses and autopsy findings were similiar to the control group of dogs.

Hydrocortisone. All dogs pretreated with 50 mg/kg of hydrocortisone and tryptophan gavage survived. The immediate fall in blood pressure following endotoxin administration (10–17% below control values) was not as marked as in dogs given endotoxin alone, and there was a return to normal levels within 30 min. The dogs rapidly recovered from anesthesia. No gross abnormalities were observed when the animals were sacrificed.

Portal Vein Serotonin and NH_3

Table 2 shows the results of serotonin and ammonia determinations on portal venous blood from dogs with chronically implanted portal vein catheters. There was no significant change in portal serotonin concentration 2 hr after tryptophan gavage. Serotonin levels fell significantly 1 hr after endotoxin administration ($P = <.02$). There was a tendency for the portal venous ammonia concentration to fall 2 hr after tryptophan gavage ($P = <.10$), and for the ammonia concentration to rise 1 hr after endotoxin ($P = <.10$). However, there was one exception in five in both categories, and with this small number of animals, these trends are not statistically significant.

Paper Chromatography of Urinary Indoles and Other Tryptophan Metabolites

Control urine specimens showed the following compounds in two dimensional paper chromatograms stained with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) (EBR); indoxyl potassium sulfate (indican), indoleacetic acid (IAA), urea, and smaller amounts of tryptophan, 5-hydroxyindoleacetic acid, an unidentified primary diazotizable amine (yellow with EBR, magenta with Ekman's reagent), and a compound that displayed staining properties and R_F values closely approximating citrulline (Table 3 and Fig. 2*A*).

Two hours following oral tryptophan (1 g in 150 ml dextrose and saline), the urinary chromatograms (Fig. 2*B*) showed all of the above-mentioned compounds without significant quantitative increase, but in addition, large amounts of kynurenic acid, anthranilic acid and its glucuronide, kynurenine, and *N*-α-acetyl kynurenine. The papers were checked (by appropriate stains and running of authentic pure compounds) for 3-hydroxyanthranilic acid, formylkynurenine, xanthurenic acid, nicotinic acid, and nicotinamide. None of these were present in sufficient concentration to be detected in urine specimens representing 2 hr of excretion when

TABLE 2. *Changes in portal vein serotonin and ammonia*

Dog No.	Portal Vein Serotonin, μg/ml			Portal Vein Ammonia, μg/ml		
	Control	2 Hr after tryptophan gavage	1 Hr after endotoxin	Control	2 Hr after tryptophan gavage	1 Hr after endotoxin
207	0.18	0.21	0.02	4.86	3.22	7.76
360	0.26	0.22	0.09	3.52	2.10	3.18
388				3.60	3.80	7.30
602	0.27	0.30	0.04	2.18	1.13	4.03
Mean	0.236	0.243	0.05	3.54	2.563	5.567
SD	0.0489	0.0489	0.0173	1.0948	1.1874	2.3001

TABLE 3. *Staining properties and R_F values of urinary tryptophan and its metabolites*

	R_F Values	EBR	Ekman
Urea	42, 50	Yellow	
Tryptophan (1)	25, 50	Purple	
Indoleacetic acid (5)	34, 86	Purple	
Indican (6)	56, 46	Orange	
Indoxylphosphate (7)	4, 35	Gray-blue	
5-OH indoleacetic acid (10)	17, 77	Blue	
Citrulline (?)	5, 20	Yellow	
Kynurenine (12)	17, 53		Purple
N-α-Acetylkynurenine (19)	8.5, 48	Orange-yellow	Magenta
Anthranilic acid (20)	34, 95	Orange	Purple
Anthranilic-glucuronide (21)	30, 82	Orange-yellow	Magenta
Kynurenic acid (22)*	37, 60		

R_F values found using isopropanol-NH_3-H_2O (200:10:20) as the first solvent, and butanol-acetic acid-H_2O (120:30:50) as the second solvent, respectively. The number in parentheses after each compound refers to the number in the schema of tryptophan metabolites shown in Fig. 1. The compounds were visible when sprayed with either Ehrlich's benzaldehyde reagent (EBR, *p*-dimethylaminobenzaldehyde, 1% in 1N HCl) or Ekman's reagent (2N HCl:acetone:Na nitrite, 5% in H_2O—5:45:1, and then ethyl α-naphthylamine, 5% in ETOH). * Kynurenic acid was visible only when sprayed with ninhydrin-pyridine solution (0.2% ninhydrin in acetone with few drops pyridine) and viewed with an ultra-violet lamp (light blue fluorescence).

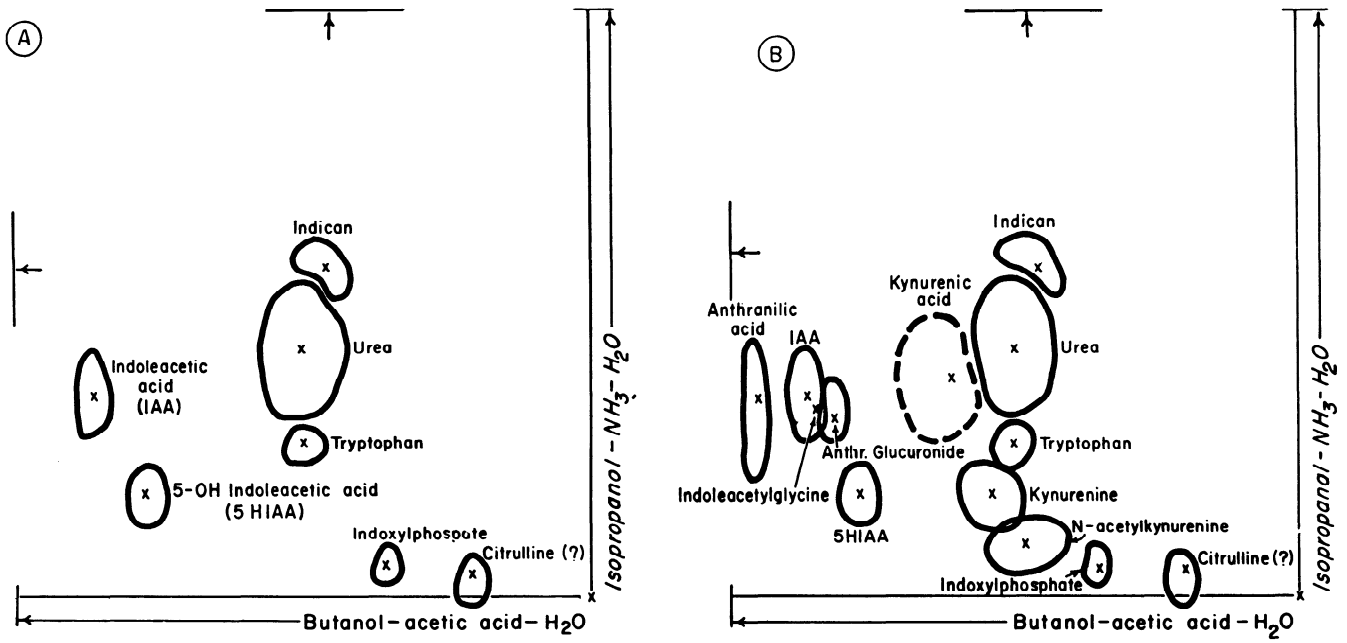

FIG. 2. *A*: Paper chromatogram showing indolic compounds and other tryptophan metabolites excreted in the urine of control dogs given no tryptophan load. Control animals excreted urea; tryptophan (1); indoleacetic acid (5); indican (indoxyl potassium sulfate) (6); indoxyl-phosphate (7); and an unknown compound producing a yellow spot moving very close to authentic citrulline. Numbers in parentheses, in *A* and *B*, refer to the number assigned to the compound in the schema of tryptophan metabolites shown in Fig. 1. *B*: Paper chromatogram showing indolic compounds and other tryptophan metabolites excreted in the urine of dogs given a 1-g oral tryptophan load. The same compounds shown in the chromatogram of control urine specimens appear here, but are not increased. In addition, there is a large amount of kynurenic acid (22), and significant (but less than kynurenic acid) amounts of the following: kynurenine (12); anthranilic acid (20); anthranilic acid-glucuronide (21); and *N*-α-acetyl kynurenine (19). Urine from animals administered tryptophan intravenously did not differ from that from control animals (no tryptophan load), except for slight increases in the tryptophan and indoxyl-phosphate excretion and a large amount of kynurenic acid.

applied to the paper in amounts equivalent to 0.06 mg of creatinine.

When endotoxin was given alone without tryptophan, urinary chromatograms of 2-hr samples showed no differences from control urines. When endotoxin was given following oral tryptophan, the chromatograms appeared to be similar to those of the urine from animals given oral tryptophan alone.

Tryptamine was not detected in any of the chromatograms. Tryptophan loading produced no significant increase in indoleacetic acid or indican in 3-hr urine specimens, and 6-hydroxyskatole was not found in any of the chromatograms. It should be emphasized that the urine specimens represented 3-hr excretions, at most, with the animal subjected to endotoxemia for 1 of the 3 hr. Many compounds mentioned above as not being found might have been present in too low a concentration to be detected, considering the amount of urine applied to the paper.

When tryptophan was given as a 15-min intravenous infusion alone, or followed in 20–30 min by endotoxin, urine collected during the succeeding hour resembled control urines, except that a spot clearly identified as kynurenic acid was now readily apparent.

Urinary chromatograms from dogs pretreated with neomycin for 5 days, and from those pretreated with intravenous hydrocortisone (50 mg/kg), resembled the chromatograms obtained from dogs subjected to tryptophan gavage. No significant decrease in indican or IAA was discerned.

DISCUSSION

The present experiments have demonstrated that the oral administration of the amino acid, L-tryptophan, potentiated the effects of a sublethal dose of endotoxin. The intravenous administration of tryptophan did not potentiate or protect from endotoxin shock. Although enzyme systems that metabolize tryptophan are present in tissues, there was no increase in excretion of tryptophan metabolites following intravenous administration of tryptophan, with the exception of kynurenic acid. It is important to emphasize that urine specimens were collected for only 1.5–2 hr following the completion of an intravenous infusion of tryptophan.

The observation that potentiation of endotoxin shock occurred only when tryptophan was given orally suggested that tryptophan metabolites produced by intestinal bacteria might be involved. Gram-negative organisms, such as *E. coli*, attack almost all of the naturally occurring amino acids by the two basic processes of decarboxylation and deamination (9).

Bacterial degradation of tryptophan produces a number of potentially harmful substances that are normally found in the intestine. A variety of these indolic compounds have been shown to depress cellular respiration

in vivo (23). Indole has been found to inhibit cellular uptake of labeled inorganic phosphate (14). Tryptamine, a potent biologically active amine, is produced by bacterial decarboxylation of the amino acid (24).

Search was made, therefore, for a number of tryptophan metabolites that conceivably might be implicated in the mechanism of shock potentiation (refer to Fig. 1). Urine from animals given oral tryptophan, which potentiated endotoxin shock, contained large amounts of kynurenic acid, anthranilic acid, anthranilic acid-glucuronide, kynurenine, and *N*-α-acetyl kynurenine. In contrast, urine from animals given tryptophan intravenously, which did not potentiate shock, contained none of these compounds except kynurenic acid. This suggested at first that one of the above compounds, or several in combination, might be implicated in the potentiation of endotoxin shock. However, the finding of these metabolites in urine from animals that were protected against shock potentiation by administration of neomycin orally for 5 days, or by intravenous administrations of large doses of hydrocortisone, provided evidence against the involvement of the above-named tryptophan metabolites in the mechanism of shock potentiation. The possibility remains that hydrocortisone may have protected by preventing some deleterious action of one of the metabolites, and oral neomycin may have effected a quantitative decrease in one or more of them, without completely eliminating them from the urine.

Since 3-hydroxyanthranilic acid, formylkynurenine, nicotinic acid, and nicotinamide were not found, it is unlikely that any of these can be implicated. Lack of a tryptamine spot and failure of indoleacetic acid or indican to increase following tryptophan plus endotoxin tend to exclude these metabolites. The increase of tryptophan metabolites (compare *A* and *B*, Fig. 2) excreted by the dogs following oral administration of tryptophan in this study agrees in all respects with results obtained by Brown and Price (8). They have shown that dogs excrete little orally administered tryptophan as *N*-methyl-2-pyridone-5-carboxamide. Therefore, it seems unlikely that this pyridone could be implicated in the potentiation of endotoxin shock by oral tryptophan.

Lack of a rise in portal blood serotonin and failure to find serotonin or an increase in 5-HIAA in the urine mitigates against the possibility of that pathway of tryptophan metabolism being involved.

Thus, in these studies it was not possible to identify any of the above-named tryptophan metabolites as being responsible for the observed potentiation of endotoxin shock by oral tryptophan. The possibility remains that an unidentified metabolite might be implicated, or that a toxic metabolite might be detected only in the intestinal contents or in the portal venous blood.

The failure of portal venous serotonin levels to rise after the oral administration of 1 gram of tryptophan is not unexpected. Much larger doses have been shown to produce a barely significant rise in urinary 5-OH-indoleacetic acid (20). The fall in portal serotonin concentration after administration of endotoxin is in agreement with the experiments of Jacobson et al. (12) and conflicts with the findings of Davis et al. (10). In one dog, arterial serotonin concentration was determined and showed the same pattern as that obtained from portal vein blood.

The tendency for the portal venous ammonia concentration to fall 2 hr following the oral administration of tryptophan, and before endotoxin was given was unexpected. A rise in ammonia concentration in the portal vein blood might have been anticipated by virtue of the tryptophanase reaction whereby bacteria degrade tryptophan to indole, pyruvic acid, and ammonia (11). The tendency for the portal venous ammonia concentration to rise 1 hr after endotoxin administration may be a reflection of an abnormal permeability of the intestinal mucosa, an early indication of barrier function failure.

Some investigators have shown that dogs can be protected from lethal doses of endotoxin by pretreatment with pharmacologic doses of cortisone (15). In the present study, dogs were protected completely from the action of orally administered tryptophan plus sublethal doses of endotoxin by pretreatment with large doses of hydrocortisone.

The protective action of cortisone has been ascribed to its ability to induce de novo synthesis of certain liver enzymes (1, 2), to stabilize lysosomal membranes (25), to decrease the total peripheral resistance (15), and to increase protease inactivation (21).

Work in rodents suggests that the cortisone protection from endotoxemia is related to the induction or stabilization of hepatic tryptophan pyrrolase (2). Preliminary work in this laboratory suggests that this is not the mechanism of protection in dogs. Small doses of cortisone (5 mg/kg) induced liver enzyme in two of four animals,[2] but failed to protect any of four additional dogs from the lethal effects of tryptophan gavage plus sublethal doses of endotoxin.

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