The Role of 5-Hydroxyindole Metabolism in the Taxonomy and Ecology of *Alcaligenes faecalis*

by

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INTRODUCTION

A. The Broad Realm of Serotonin Research

The story of serotonin is a fascinating chapter in the progress of medical science, and few of the final conclusions have yet been written. From a physiologically active but unknown substance studied by two investigators fifteen years ago to a known crystalline compound- a possible regulator of brain function, peristalsis, and blood pressure- studied by hundreds of investigators today, serotonin has illustrated a pattern of intensive research, that of trying to make the unknown known, and then of ascribing to the known a rational practical place in the realm of reality. Serotonin continues to be widely investigated pharmacologically, biochemically, anatomically, physiologically, and medically, but as yet its true function in normal body processes is unknown.

The history of serotonin actually spans almost one-half century. A powerful vasoconstrictor substance obtained from extracts of platelets has been known for at least 40 years (34). However, the chemical nature of this substance was not known until 1948, when Rapport, in Page's laboratory, succeeded in purifying the vasoconstrictor in beef serum, serotonin, and identifying it as 5-hydroxytryptamine (52, 53). In 1952, Erspamer (21), who had been working for 15 years with a biologically active extract from the gut which he called enteramine, also identified his substance as 5-hydroxytryptamine. Thus, extracts were purified from two different sources in the body and found to be the same compound.

Serotonin (designated hereafter as 5HTM) is now known to be widely distributed in nature. It has been found in bananas in a concentration of 3.7 mg. per banana (75). It has been reported in venoms of the toad, scorpion, and wasp (69, 1, 35). It occurs in nettle stings, for which

an old remedy, dock leaves, seems to have been at least partially rationally used; dock leaves contain a 5HTM antagonist (15, 10). Octopods have 5HTM in their posterior salivary gland in a concentration of 1 mg. per gram tissue (21). It is found in similar high concentrations in amphibian skin (19).

In mammals, including man, it is found in diverse places, the highest concentration being in the enterochromaffin cells of the intestinal tract. Here the 5HTM exists in a concentration of 1 - 10 micrograms per gram tissue (17). It is also found in relatively large amounts in blood platelets (19, 49), lung (78), spleen (19), mast cells (3), and gray matter of nervous tissue, particularly hypothalamus (7, 68).

The assay of 5HTM has been accomplished by biological, chemical, and physical methods. Biological methods were first used. These included: 1) contraction of a uterus from an ovariectomized rat brought into estrus by injection of ovarian hormone (19), 2) contraction of a strip of rat colon (18), 3) contraction of isolated mollusc heart (68), and 4) contraction of a strip of fundus from the rat stomach (74). Chemical methods, developed mostly by Udenfriend and his group (73), depend on primary extraction with organic solvents and then determination of the 5-hydroxyindole structure by reaction with 1-nitroso-2-naphthol to form a purple colored complex which is measured colorimetrically. Physical methods include both absorption of ultraviolet light, and fluorescence in ultraviolet light (73). A spectrophotofluorimeter has been devised, using the latter principle, and has found wide use (8).

The metabolic pathways of 5HTM synthesis and degradation have largely been characterized by a group of investigators at the National

Institutes of Health, (Shore, Udenfriend, Weissbach, and co-workers) (71). The generally accepted pathway is as follows (figure 1). Tryptophan, the dietary precursor, is absorbed in the intestinal tract. In the enterochromaffin cells, it is thought that hydroxylation of tryptophan occurs in the 5 position to give 5-hydroxytryptophan (17, 71, 44). However, the evidence for the hydroxylation is the most unsatisfactory of all the steps in the metabolic scheme. Most of the evidence has been obtained from radioactive C^{14} studies, in vivo (71). No in vitro evidence for enzymatic hydroxylation has yet been found. The next step in the pathway, for which there is substantial evidence, is decarboxylation of the side chain of 5-hydroxytryptophan by a decarboxylase enzyme resulting in 5HTM (13, 25). This enzyme has been purified and studied extensively, and is thought to be the main agent for the formation of 5HTM. The circulating platelets are thought to absorb the 5HTM from the enterochromaffin cells and distribute it throughout the body (25). The degradation of 5HTM is accomplished by a nonspecific amine oxidase, for which tyramine and tryptamine are also substrates (4, 5, 30, 61). The enzyme is found in many tissues, particularly liver and lung. It converts 5HTM to 5-hydroxyindoleacetaldehyde which is rapidly broken down to 5-hydroxyindoleacetic acid by a nonspecific aldehyde dehydrogenase (66). 5-hydroxyindoleacetic acid (5HIAA) is then excreted in the urine. In spite of the fact that only 25-75% of injected 5HTM, as studied in many animals and man, is recoverable in the urine as 5HIAA (20, 66, 71), it is generally accepted that this metabolic pathway accounts for the majority of 5HTM breakdown. However, three other possibilities have been suggested: 1) 5HTM is broken down by other yet unidentified pathways (17, 49, 60, 20); 2) 5HTM is conjugated with glucuronic acid (77);

3) 5HIAA is broken down by the body (17). However, it is generally considered at the present time that 5HIAA is not degraded by mammalian tissue (17, 49, 71), although little work has actually been done on this problem.

Much biochemical and pharmacological study of 5HTM action has been done indirectly by the use of chemical antagonists and metabolic inhibitors. Woolley (59, 80) has done considerable work in this field, especially with synthetically-produced compounds which closely resemble 5HTM in structure, especially the methoxyserotonins, such as 1-benzyl-2-methyl-5-methoxytryptamine (BAS). Other compounds that have been widely used are iproniazid (11, 49), an amine oxidase inhibitor, and chlorpromazine (2) and lysergic acid diethylamide (LSD) (17, 49), inhibitors at unknown sites of the effects produced by administered 5HTM.

The pharmacology of 5HTM has been extensively studied in dogs, cats, rats, and man (24, 54, 31, 49). From these studies, various investigators have formulated theories concerning its role in the body. Page (40) regards 5HTM as functioning mainly to control arteriolar tone. However, 5HTM has no constant effect on arterial blood pressure, often giving biphasic results. Recent work, however, has indicated that 5HTM may act as a homeostatic controller of blood vessel tone, causing either an increase or decrease in blood pressure, depending upon the pre-existing tone and caliber of the vessels (49). Erspamer (21) believes 5HTM helps to control kidney function by an antidiuretic action. He has shown 5HTM to exert this effect in physiological concentrations in rats, but this has not been confirmed by other investigators (17). Dalgliesh (17) believes the primary effect of 5HTM is on smooth muscle,

and very likely in the maintenance of normal peristalsis in the gut. Recent work on the effects of administered 5HTM on intestinal motility seems to substantiate this hypothesis (12, 28, 31).

Woolley and Shaw believe 5HTM is most likely a mediator of normal brain function (80). Bases for this suggestion are many. Antagonists of 5HTM, such as yohimbine, medmain, and LSD are powerful hallucinogenic agents (63). The injection of 5-hydroxytryptophan (5HTR), the precursor of 5HTM, results in a marked increase in free brain 5HTM and resultant sensorial effects similar to LSD (72). Reserpine, a potent tranquilizer and antihypertensive agent, brings about the release of 5HTM from all body depots, including brain, platelets, and intestinal tract (51). This results in a decrease in "bound" 5HTM in the brain, and an increase in the "free" 5HTM. Moreover, the tranquilizing effect of reserpine is related not to its own concentration, but to the concentration of "free" 5HTM in the brain (11). Iproniazid further accentuates the effect, because it blocks the conversion of 5HTM to 5HIAA and thus further prolongs the high concentrations of "free" 5HTM in the brain (11). Chlorpromazine, also a potent tranquilizer, is also a 5HTM antagonist, but as yet the exact mechanism of antagonism is poorly understood (2). Thus far, however, no consistant quantitative differences from normal in 5HTM metabolism, as measured by 5HIAA excretion in the urine, have yet been determined in patients with mental illness, such as schizophrenia (22, 36), except by one investigator (38). However, as pointed out by many of the workers in this field, 5HIAA determination may not be an adequate index of nervous tissue metabolism of 5HTM (22). Other as yet unknown metabolic pathways of 5HTM breakdown may be important, and their measurement may give a more adequate index of nervous tissue function.

5HTM has also been suggested as having a role in the hypersensitivity phenomenon. It has been postulated that the anaphylactic response is mediated through 5HTM in rats (56) and mice (78) in contrast to histamine in guinea pigs (56, 78). Mice previously immunized with pertussis vaccine have been shown to be markedly sensitive to the toxic effects of injected 5HTM (37, 45). It has been noticed that humans with atopic dermatitis (14) or carcinoid tumors (55) react differently from normal humans in response to intradermal 5HTM. Patients with allergic asthma have been found to respond to 5HTM aerosols by developing attacks of dyspnea while controls were unaffected (32). These findings point to some role for 5HTM in the realm of allergy.

The practical medical importance of 5HTM is limited to patients with a metastasizing carcinoid tumor. This unique opportunity for the study of 5HTM physiology in vivo is afforded when the argentaffin cells of the intestinal tract undergo neoplasia with the production of a carcinoid tumor. These tumors produce large quantities of 5HTM and, when metastasized, produce the resultant "malignant carcinoid syndrome." This symptom-complex, due to high levels of circulating 5HTM, is characterized by transient flushing of the face and extremities, chronic diarrhea, respiratory distress, and valvular disease of the right side of the heart. These patients excrete large, pathognomonic amounts of 5HIAA in the urine. The values range from 100-800 mg per 24 hours, as compared to normal values of 0.5-13.0 mg per 24 hours (62). Only a few other medical conditions exist in which the daily 5HIAA excretion is altered. Patients with "collagen" diseases were found to have somewhat low values (29). Patients with active nontropical sprue were found

to have increased excretion levels (28). The role of 5HTM in the production of symptomatology in these other conditions is not known.

B. Bacterial Metabolism of 5-hydroxyindoles

Reports of experiments on the bacterial metabolism of the 5-hydroxyindoles have been exceedingly sparse. The formation of small amounts of 5HTR from tryptophan has been shown by Mitoma et al (44) to occur in the bacterium Chromobacterium violaceum. The evidence for the occurrence of the hydroxylation reaction in the bacterium is somewhat stronger than any so far reported for mammalian tissues. However, in this bacterium 5HTM is not formed from 5HTR, which Mitoma suggested might rather be a precursor of the purple pigment characteristic of this species.

Weissbach et al (76) reported that a suspension of human fecal bacteria were capable of the decarboxylation of tryptophan to yield tryptamine in small amounts. The bacteria were also able to produce small amounts of indole-3-acetic acid, resulting from transamination of tryptophan with alpha-ketoglutarate.

Other observations on bacterial metabolism of these compounds have been made in this laboratory (48). Several organisms were isolated from various soils on the basis of their ability to derive all of their carbon and nitrogen for growth from their degradation of 5HTM. Further studies showed that most of these organisms metabolized 5HTM to 5HIAA, with profound degradation of the latter product. Three of the isolates were identified as strains of Alcaligenes faecalis, a bacterium which occurs not only in soil, but also as a nonpathogenic intestinal organism and occasionally as a partner in mixed infections. The recognition of 5HTM metabolism by strains of A. faecalis suggested the following

possibilities: first, that this metabolic property might be a species characteristic of this organism; second, that other intestinal organisms might also be capable of this metabolism; and third, that A. faecalis or other intestinal bacteria might contribute to the pattern of 5HTM production or degradation in the body. The first two of these possibilities were explored in this thesis.

C. Studies on Alcaligenes faecalis

Previous experimental work on the bacterial species A. faecalis has also been sparse. Since its discovery in 1896 by Petrushky (50), there have been many publications on its isolation from pathologic processes, such as enteric-like fevers, abscesses, meningitis, cystitis, and arthritis. However, the species itself has never been well studied taxonomically or metabolically. Sarkar et al (57) recently attempted a more complete study of the species, and found much taxonomic variation within the large number of cultures studied. They found, among other things, that A. faecalis was pathogenic for mice when injected with gastric mucin, but not for guinea pigs or rabbits. This suggested to us the possibility that the ability to metabolize 5HTM by A. faecalis strains might contribute to their ability to infect and kill mice. The exploration of this possibility is described in this thesis.

A. faecalis is reported to occur in the normal intestinal flora of from 0 to 19% of humans (58). The incidence of A. faecalis in the rat intestine has not been previously reported. It was decided to undertake first a study of the incidence of A. faecalis in a hospital and clinic population, and second a study of the incidence of A. faecalis

in the rat intestine, to find out whether an increase in the amount of 5HTM in the intestine itself might bring about a selective increase in the number of 5HTM metabolizing bacteria, and more specifically of A. faecalis.

The underlying question in the series of experiments reported in this thesis was essentially this: what is the significance of 5HTM metabolism by A. faecalis, considered from metabolic, taxonomic, and ecological points of view? These three aspects are closely related, since ecological and taxonomic patterns are based on metabolic differences among organisms. The investigations here reported were devised to study whether the metabolism of a small group of compounds, the 5-hydroxyindoles, might serve as a critical factor in the definition and the environmental behavior of the bacterial species, A. faecalis.

MATERIALS AND METHODS

A. Metabolism of 5-Hydroxyindoles by "Intestinal" Bacteria

1. Bacterial Cultures

A total of 42 cultures of "intestinal" bacteria were examined for their ability to metabolize 5HTM. Nineteen of these cultures were obtained as members of the genus Alcaligenes from soil enrichment isolations, stool cultures, various departmental stock culture collections[1], and the American Type Culture Collection. All cultures received as

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1. These cultures were obtained from Dr. Charles A. Evans of the University of Washington, Dr. Owen B. Weeks of the University of Idaho, and Dr. Herman C. Lichstein of the University of Minnesota, as well as from the culture collections of Dr. Evelyn L. Oginsky and of the Department of Bacteriology of the University of Oregon Medical School.

Alcaligenes were subjected to routine taxonomic procedures for identification, according to Bergey's Manual (9) and to Conn (16). The 23 other cultures were obtained from the departmental culture collection at the University of Oregon Medical School. They included 2 strains of Escherichia coli, 5 Aerobacter aerogenes, 2 Proteus vulgaris, 1 Proteus morganii, 1 Pseudomonas aeruginosa, 2 paracolon (strains Jackets and Gasser), 1 each of Salmonella typhimurium, Salmonella paratyphi, Salmonella schottmuelleri, Salmonella enteriditis, Salmonella london, Salmonella newport, Shigella flexneri (Oxford), Shigella flexneri (Weber), and Shigella sonnei. The stock cultures were maintained on nutrient agar.

2. Media

The synthetic medium employed (0.15% K_2HPO_4, 0.05%KH_2PO_4, 0.02% $MgSO_4$, 0.05% $(NH_4)_2SO_4$) was supplemented with 0.1% yeast extract throughout all of the experiments. When solid medium was required, 1.5% agar was added. The final pH of the medium was 7.0. The substrates[2] (5HTR, 5HTM and 5HIAA) were Seitz filtered and added to the cooled sterilized medium to give a final concentration of 0.025%.

Conn's (16) two media for differentiation of "soil" from "intestinal" strains of A. faecalis had the following composition: Medium I - 0.1% $(NH_4)_2HPO_4$, 0.02% $MgSO_4.7H_2O$, 0.02% yeast extract, and 1.5% agar. Medium II - same as above with the addition of 0.15% glucose. Each of the A. faecalis cultures was streaked onto the solid media and growth was observed for 48 hours at 37C.

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2. 5HTM creatinine sulfate was obtained from the California Corporation for Biochemical Research; DL-5HTR and 5HIAA were obtained from both the Nutritional Biochemicals Corporation and the California Corporation for Biochemical Research.

3. Screening Test for 5-hydroxyindole Degradation

A screening procedure was designed to detect bacterial degradation of 5-hydroxyindoles. This consisted of incubation of the organisms with the substrate (5HTR, 5HTM, 5HIAA) and the determination of the total concentration of 5-hydroxyindoles by a colorimetric method. Eighteen hour cultures grown on nutrient agar or synthetic 5HTM agar were suspended in sterile water to a concentration of 0.5 mg bacterial nitrogen per ml, using the optical density of the bacterial suspension at 520 mμ as a measure of nitrogen content. Multiplication of this optical density value by a factor of 1.5 gave a result equivalent to the nitrogen content as measured by micro-Kjeldahl analysis. Into a 50 ml Erlenmeyer flask were placed 0.6 ml of 0.01 M 5HTM creatinine sulfate, 0.01 M 5HIAA, or 0.02 M DL-5HTR, 1.0 ml of cell suspension, and 2.4 ml of the supplemented synthetic medium, giving a total volume of 4.0 ml. The flasks were incubated in air at 37C in a Dubnoff shaker for 48 hours. At 5, 24 and 48 hours, 0.4 ml samples were removed for colorimetry and paper chromatography.

4. 5-hydroxyindole Determination by Colorimetry

The colorimetric procedure was adapted from the method of Udenfriend (73) to determine the total concentration of 5-hydroxyindoles without distinguishing among them. Into a stoppered centrifuge tube were placed 0.2 ml of the reaction sample from the flask and 1.8 ml distilled water. To this were added 1.0 ml of 0.1% nitrosonaphthol solution in 95% ethanol and 1.0 ml of 0.1% nitrous acid reagent; the tubes were stoppered, shaken, and placed in a water bath at 55 C for 5 minutes. Five ml of ethylene dichloride were then added, and the

tubes reshaken vigorously. After centrifugation for 5 minutes at 1000 rpm, the optical density at 540 mμ of the supernatant layer was determined. A standard curve, using a control flask incubated without cells, was run with each determination, because the 5-hydroxyindoles undergo slight destruction on aerobic incubation. Another control flask containing only cells and medium was used to check the possibility of endogenous formation of 5-hydroxyindoles or other reactive compounds. Descending paper chromatography was used to separate the 5-hydroxyindoles, using n-butanol: acetic acid: water (4:1:1) as solvent and short wave length ultraviolet light, diazotized sulfanilic acid (DSA) (42), and Ehrlich's reagent (18) as the visualizing agents.

5. Manometric Studies

The results of the screening procedure were confirmed with conventional manometric oxygen uptake studies in the Warburg apparatus. All cultures effecting a decrease in concentration of 5-hydroxyindoles and all cultures showing compounds other than the original substrate on the paper chromatograms were examined manometrically. Several other cultures showing no activity on the 5-hydroxyindoles were also examined to confirm the validity of the screening procedure. For these manometric experiments, cells were grown for 18 hours on the synthetic agar medium containing 5HTM, washed with distilled water, and resuspended in water to 0.5 mg bacterial nitrogen per ml. One ml of cell suspension was used per flask, with 30 μM PO_4 buffer pH 7.0, and 3 μM of 5HTM, 5HIAA, or tyramine, or 6 μM DL-5HTR; the total volume was 3.0 ml. Oxygen uptake was measured at 37C. During some of these experiments, duplicate flasks were removed at different oxygen uptake levels and their contents examined chromatographically. During some

of the experiments, metabolic inhibitors (8-hydroxyquinoline, arsenite, and iproniazid) were added to the reaction mixtures.

B. A. faecalis Infections in Mice

1. Mice

A Webster strain of Swiss white female mice, 7 - 12 weeks old, weighing 18 to 24 grams, were used throughout the experiments. The stock mice were kept in metal cages with sawdust bedding; the diet of all the mice consisted of kibbled Super Meat Dog Food ad lib. Prior to each experiment, the mice to be used were weighed, and placed in 10 inch-deep Duraglas jars with ventilated screw-top lids, each of which contained a 2-3/4" hole for ventilation and a 1/2" hole for a water bottle. Glass jar cages were used for all the acute experiments because they allowed for excellent observation of the mice during the infection and could be autoclaved in toto.

2. Experimental Infections

The experimental infections were effected in the following manner. Bacterial cultures were grown for 18 hours on either nutrient agar, or synthetic agar containing 5HTM. The cells were suspended in normal saline, centrifuged, and resuspended in saline to a concentration of 0.4 mg. bacterial nitrogen per ml. Plating experiments, using cell suspensions of known density, were done in order to determine the number of organisms at known nitrogen concentrations. Dilutions of the original suspension were made to obtain the desired number of organisms per ml.

Throughout the experiments, a 5% gastric mucin[3] suspension in

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3. The gastric mucin was obtained from the Nutritional Biochemicals Corporation.

saline was used to enhance the virulence of the bacteria (43). The gastric mucin was suspended in normal saline and autoclaved at 15 lbs. pressure for 20 minutes. Equal volumes of the mucin suspension and the final bacterial suspension were added to sterile rubber-topped vaccine bottles and shaken thoroughly. A total of 0.5 ml of this mixture was injected into each mouse.

All mice were injected intraperitoneally in the lower left quadrant of the abdomen with a 26 gauge needle. The mice were subsequently observed for morbidity and mortality over a period of 72 hours, during which time, the mice either died or recovered from their infections. Samples of heart and tail blood were obtained from freshly deceased mice and cultured for A. faecalis.

Preliminary studies using all of the A. faecalis strains were carried out to assess grossly the virulence of each individual strain. Three of the most virulent strains, ATCC 212, O-C10, and O-83, were more thoroughly investigated. The LD_{50} doses were approximated by trial and error injections of bacterial suspensions containing different numbers of organisms.

3. 5HTM Administration in Experimentally Infected Mice

The effect of 5HTM administration on experimentally infected mice was determined in the following manner. A solution of 5HTM in distilled water was made to give a total concentration of 20 mg per kg (as free base) when injected into the mouse in a total volume of 0.1 ml. The 5HTM dose was calculated from the average weight of the mice used in each particular experiment. The solution was adjusted to pH 7.0, Seitz filtered, and injected into the lateral tail vein of each mouse. The injection was made routinely one hour prior to the intraperitoneal

bacterial injection.

4. Immunological Studies

Mice surviving the acute infections were maintained in the laboratory for a period of up to 15 weeks. At different times during this period, the animals were challenged with the homologous organism in a dose twice as large as the initial infecting dose. One week after this second injection, 24 of the mice were bled from the orbital sinus by a method described by Stone (65), and the serum collected and pooled. Each "pool" consisted of sera from 8 mice which had recovered from infection by one of the three bacterial strains. Agglutinating antibodies against flagellar and somatic antigens of the homologous and heterologous strains of organisms were determined. The "immunized" mice were also subsequently challenged with the homologous and heterologous organisms in a dose three times the original infecting dose.

C. A. faecalis in Human Feces

1. Survey of Stool Specimens for A. faecalis

A survey of 87 stool specimens received at the clinical laboratories of the University of Oregon Medical School Hospitals and Clinics was done to determine the incidence of A. faecalis in the clinic population. In the first 80 determinations, a pea-sized sample of stool was suspended in 2.0 ml of sterile saline. A loopful of this suspension was streaked onto a MacConkey agar plate and incubated overnight at 37C. Any colorless colonies were transferred to Triple-Sugar-Iron (TSI) slants and incubated overnight. Only those cultures that resembled A. faecalis in their lack of sugar fermentation on the TSI agar were kept for further study. One stool sample from a patient with known malignant carcinoid (later proved by autopsy) was treated in a similar manner.

In an attempt to obtain a more selective medium for the screening of stool samples for A. faecalis, glucose was added to the MacConkey agar in a concentration of 1.0%. On this medium, all of the common intestinal gram negative rods produced red colonies, except Pseudomonas and Alcaligenes species which produced colorless colonies. One gram of each of 7 stool samples was suspended in 9.0 ml of sterile normal saline to obtain approximately a 10% suspension. One loopful of this was streaked out on the modified MacConkey agar. Any colorless colonies were further identified by the usual taxonomic procedures.

2. Fecal Enrichment Studies

Fecal enrichment studies were also done in an effort to find a procedure with a high degree of sensitivity for the detection of A. faecalis in stool. Synthetic liquid medium, without yeast extract, containing 0.025% 5HTM (as free base) was used as the enrichment medium. Seven stool samples were suspended to 1.0% and 0.1% in this media and incubated at 37C for 5 days. At 3, 7, and 24 hours, a loopful from each of the suspensions was plated on the modified MacConkey agar. At the end of 5 days, 0.1 ml of the suspension was transferred to a tube containing 9.9 ml of the 5HTM synthetic media; the new suspension was then incubated for another 14 days. At various intervals, loopfuls were streaked out on the MacConkey-glucose agar, for the detection of colorless colonies.

3. Survival of A. faecalis in Stool Specimens

The survival of A. faecalis in stool was examined by inoculating a known number of organisms into each of 2 stool specimens known to contain no A. faecalis as detectable by the above procedures. The survival of 3 strains of A. faecalis, ATCC 8750, 0-31, and ATCC 212,

was determined. Into 1.0 ml of a 1.0% fecal suspension was placed 0.02 ml of a suspension of one of the A. faecalis strains containing 0.25 mg bacterial nitrogen per ml. Then 0.1 ml of this mixture was placed into 9.9 ml of the 5HTM synthetic medium. The same procedure was then followed as above. At 8 days, a transfer was made to fresh media; the new suspensions were observed for 21 days, during which time samples were streaked out for A. faecalis.

D. A. faecalis in Rat Feces

The incidence of A. faecalis in normal rats and in rats given 5HTM orally was studied. Sixteen Sprague-Dawley female rats, weighing 200-250 grams were used for the experiment. They were kept individually in metal metabolic cages on a diet of Purina Laboratory Chow ad lib. The isolation of A. faecalis from stool was done in the following manner. Twenty-four hour fecal specimens were collected by placing a 2" x 6" wire screen within the metal collecting funnel of the cage; this allowed for the urine and food bits to pass either through or around the wire screen. A small bit (0.1 - 0.25 gram) of the 24 hour specimen was weighed and diluted with sterile saline to give a 1.0% fecal suspension. A 0.1 ml aliquot of this suspension was placed on a MacConkey-glucose agar plate and spread with a glass spreader. The plates were incubated for 24 hours at 37C. At the end of this time, the colonies were counted; all colorless colonies were replated on the same medium and then, if still colorless, further identified by the usual taxonomic procedures.

To determine the incidence of A. faecalis in normal rat feces a total of 5 fecal specimens were obtained from each of the 16 rats over

a period of 10 days. Then the animals were divided into 2 groups of 8 animals per group, and fed twice each day with solutions of either 5HTM creatinine sulfate or creatinine over a 10 day period. A 16 gauge stomach tube was used to administer 1.0 ml of solution. Group I received creatinine, 20 mg per kg for the first 7 days and then 40 mg per kg for the last 3 days. Group II received 5HTM creatinine sulfate, 20 mg per kg for the first 7 days and then 40 mg per kg for the last 3 days. On days 1, 3, 6, 8, and 10, twenty four hour stool specimens were collected and plated on MacConkey-glucose agar as previously. Again any colorless colonies were further identified.

RESULTS

A. Metabolism of 5-Hydroxyindoles by "Intestinal" Bacteria

1. Classification of Organisms

The definition of A. faecalis as given by Conn and Breed in the 7th Edition of Bergey's Manual (9), includes the following characteristics: gram-negative rods, 0.5 x 1.0-2.0μ, motile by peritrichous flagella, aerobic, non-chromogenic, litmus milk alkaline without peptonization, no detectable acid or gas from carbohydrates, no gelatin liquefaction, no indole production, no urea hydrolysis, nitrates may or may not be produced from nitrites, acetyl-methyl-carbinol not produced. The results of the taxonomic studies done on all cultures received as Alcaligenes are shown in Table I. All cultures received as A. faecalis had the following characteristics: alkaline in brom-cresol-purple milk, no fermentation of carbohydrates, and no gelatin liquefaction. However, the species designation could be truly confirmed, according to Bergey's Manual (9), for only 11 out of 16 organisms reported to be A. faecalis, and 0 out of 1 reported to be A. bookeri. The species designation for 1 A. viscolactis, and 1 A. ammoniogenes, now called Brevibacterium ammoniogenes was confirmed. Four of the reputed A. faecalis strains were eliminated by the marked discrepancy of their cell sizes from that given in Bergey's Manual: strain O-S1, cell size of 1.5 x 7.0 μ; O-C10, I-1, and I-2, cell sizes of 1.0-1.5 x 4.0-5.0 μ. One strain, O-C2, was eliminated because of polar flagellation, which is characteristic of the genus Pseudomonas. All five strains were, however, carried through the screening procedure along with the confirmed A. faecalis.

Conn (16) developed two media which demonstrated that the addition of glucose to a medium containing inorganic nitrogen markedly improved the growth of "soil", but not "intestinal" A. faecalis. Growth of the 11 strains of A. faecalis on these media showed that there were 5 "soil", 3 "intestinal", and 3 indeterminate forms. (Table 2)

2. Colorimetric Methods

Standardization of the method for 5-hydroxyindole determination demonstrated a straight-line standard curve. Neither the media nor the cells interfered significantly with the color reaction. (figure 2A) All three of the 5-hydroxyindoles studied gave similar standard curves. (figure 2B) This substantiated the method as being a non-specific one, merely estimating the total 5-hydroxyindole concentration.

The concentration of 5-hydroxyindoles in the control flask without cells decreased only slightly over the 48 hour incubation period. This was apparently due to spontaneous destruction of the 5-hydroxyindoles on aerobic incubation. Estimation of the number of bacteria during the 48 hour incubation, as determined by plating methods and optical density, showed that little, if any, multiplication occurred in the flasks.

3. Metabolism of 5-hydroxyindoles by A. faecalis

The screening procedure demonstrated that all 11 A. faecalis strains metabolized 5HTM. (Table 3) Of these, 6 also metabolized 5HIAA, and of the latter, 2 metabolized 5HTR. On the basis of 5HTM and 5HIAA metabolism, 3 large groups could be distinguished. The experimental results on which this group differentiation was based will be presented as the data obtained on representative members of each group.

Group I was able to metabolize rapidly both 5HTM and 5HIAA, as well as tyramine. The results obtained in the screening procedure with strain ATCC 8750 grown on nutrient or synthetic 5HTM agar, figure 3A, demonstrated that the concentration of 5-hydroxyindoles decreased on incubation. Adaptation of the organisms by prior growth on 5HTM was evidenced by the much faster decrease in 5-hydroxyindole concentration with such cells. The oxygen uptake during 5HTM and 5HIAA metabolism by strain O-83, another characteristic member of Group I, is shown in figure 3B. The average total oxygen uptake of Group I organisms was 12.7 µM O per µM 5HTM and 9.4 µM O per µM 5HIAA. The average $Q_{O_2}(N)$ for 5HTM was 569 and for 5HIAA was 585. The values for the individual members of Group I are given in Table 4.

The chromatographic pattern during the metabolism of 5HTM by strain ATCC 8750 is presented in figure 4A. As 5HTM decreased in concentration, the accumulation of 5HIAA became readily apparent. This, however, declined after reaching a peak. At 200 µL oxygen uptake, another DSA-reacting spot with an Rf of 0.10 appeared. It represented a bright yellow-green pigment, not further identified, that transiently appeared during the course of the oxidation of both 5HTM and 5HIAA by only 2 of the strains, ATCC 8750 and O-390. This color was not demonstrated in the endogenous control flasks. By the time oxygen uptake had ceased, no spots could be visualized by ultraviolet or DSA spray. The changing chromatographic pattern during the manometric studies of 5HTM oxidation by other members of the group are summarized in Table 5.

Group II, consisting of only one organism, strain O-390, was found by the screening procedure and manometric experiments to be similar to

Group I in that 5HTM, 5HIAA, and tyramine were rapidly metabolized. Paper chromatography, however, revealed a marked difference in Group II (figure 4B). When samples were taken at the same levels of oxygen uptake as in figure 4A, the 5HTM decreased in concentration considerably more slowly than before. More importantly, there was no demonstrable accumulation of 5HIAA at any time. Only the yellow-green pigment, with Rf 0.10, appeared transiently. Accumulation of 5HIAA could be demonstrated only when 2.0×10^{-4}M arsenite or 2.67×10^{-6}M 8-hydroxyquinoline were present in the reaction flask. It has been previously demonstrated that these concentrations inhibit 5HIAA degradation (48). The oxygen uptake, averaged over several experiments, was 13.5 µM O per µM 5HTM, 10.8 µM O per µM 5HIAA, and 12.3 µM O per µM tyramine. The $Q_{O_2}(N)$ for 5HTM was 356, for 5HIAA was 376, and for tyramine was 116.

Group III gave still a different pattern. Figure 5A represents the results of the screening procedure with strain ATCC 212, a member of Group III. The concentration of 5-hydroxyindoles did not change significantly from the control flask. However, as is evident by figure 7, 5HTM was oxidized with a $Q_{O_2}(N)$ of 80 and a total oxygen uptake of 1.6 µM O per µM substrate. There was no appreciable oxidation of 5HIAA. Interval sampling for chromatography revealed the disappearance of 5HTM and the accumulation of 5HIAA which remained as the final end product (figure 4C). The oxygen uptake values for the other members of the group are found in Table 6. The chromatographic patterns during the manometric studies for other members of the group were identical with that of ATCC 212 shown in figure 4C. Tyramine was oxidized in a manner identical to Groups I and II.

4. Effect of Metabolic Inhibitors on 5HTM Metabolism

The effects of metabolic inhibitors on the oxidation of 5HTM was studied in a comparative manner. It was thought that if organisms from Groups I and II possessed truly different enzyme systems for the breakdown of 5HTM, their response to inhibitors might be different. Studies were carried out on strains O-31 and ATCC 8750, members of Group I, and O-390, the lone member of Group II. Arsenite in a concentration of 2×10^{-5}M to 2×10^{-3}M or 8-hydroxyquinoline in a concentration of 2×10^{-5}M were added to the Warburg reaction flasks 20 minutes before the addition of 5HTM. The results of this study of strains O-31 and O-390, shown in figure 6, demonstrated that the oxidation by O-390 was considerably more sensitive to arsenite and 8-hydroxyquinoline than that of O-31. At a concentration of 2×10^{-4}M, arsenite inhibited 5HTM oxidation by O-390 at about 80 µL oxygen uptake. Paper chromatography at this time revealed a substantial pile-up of 5HIAA, which normally was not accumulated in this organism. Similarly, 8-hydroxyquinoline effected the accumulation of 5HIAA. Arsenite inhibition of 5HTM oxidation by O-31 required a higher concentration (2×10^{-3}M) and resulted also in the pile-up of 5HIAA. 8-hydroxyquinoline was only slightly inhibitory at these concentrations.

Iproniazid (Marsilid) is known to inhibit amine oxidase. Its effect on 5HTM oxidation by strains ATCC 8750 and O-390 was studied. Iproniazid in a concentration of 6×10^{-3}M was added to the flasks prior to their incubation during the routine screening procedure (figure 7). It was evident that at this concentration, iproniazid inhibited oxidation in both organisms equally well.

5. Metabolism of 5HTR

Metabolism of 5HTR was shown by only 2 of the 11 A. faecalis, strains ATCC 8750 and O-390, members of Groups I and II. After 24 hours incubation in the usual screening procedure there appeared an intense brownish coloration in the reaction flask. Colorimetric determination showed that one-half of the DL-5HTR had disappeared (figure 8). Paper chromatography at this time revealed only 5HTR (presumably residual D-isomer) and three new spots, discussed later, which have not yet been identified. The addition of iproniazid to the flasks in a concentration of 6×10^{-3} M did not affect the metabolism of 5HTR. Manometric studies showed that both organisms demonstrated only slow oxygen uptake with 5HTR: over a 4-hour period, the oxygen uptake was only 1.4 µM O per uM substrate for ATCC 8750, and 0.7 µM O per µM substrate for strain O-390. Neither 5HTM nor 5HIAA could be demonstrated to accumulate as an intermediate during these incubations.

6. New Unidentified Chromatographic "Spots"

A number of unidentified spots were seen during the metabolism of 5-hydroxyindoles by strains ATCC 8750 and O-390. As previously mentioned, a spot with an Rf of 0.10 was seen during the oxidation of 5HTM and 5HIAA by these strains. This spot gave a bluish fluorescence, reacted with DSA to form a grayish-purple color, and with Ehrlich's reagent to form an orange color. The other 3 spots seen were unreactive with DSA and Ehrlich's reagent, but all fluoresced under ultraviolet light. A brightly-blue fluorescent spot with an Rf of 0.19 was seen when ATCC 8750 was incubated with either 5HTR, 5HTM, or 5HIAA, but not in synthetic medium alone. This spot was also seen when strain O-390 was incubated with 5HTR. Two less intense spots,

produced when ATCC 8750 or O-390 were incubated with 5HTR, had Rf values of 0.29 and 0.70. Using identical chromatographic techniques and visualization by ultraviolet, DSA, and Ehrlich's reagent, some possible intermediates in 5HTR breakdown had the following Rf values: 5HTM - 0.33, 5HIAA - 0.64, 5-hydroxyanthranilic acid - 0.55, DL-5-hydroxykynurenine - 0.17, 5-hydroxyindole, 0.84. It was shown during these experiments, that the DSA spray was an equally, if not more, sensitive method of detecting 5HTM than ultraviolet fluorescence; concentrations of 5HTM that gave a just recognizable color with DSA did not recognizably fluoresce under ultraviolet. This would tend to exclude 5HTM or other 5-hydroxyindoles in small quantities as being responsible for producing any of the above spots. A number of other sprays which give colors with complex phenols, aromatic acids, indoles, and amino acids, were used to try to further characterize the 3 fluorescent spots: sucrose, ammoniacal $AgNO_3$, methyl red, ninhydrin, isatin and dichromate formaldehyde (6); dimethylaminocinnamaldehyde (26). None of the sprays produced a recognizable color with any of the 3 spots.

7. Metabolism of 5-hydroxyindoles by Other Organisms

Of the other confirmed Alcaligenes strains, A. viscolactis showed no ability to metabolize 5HTM, at either 37C or 25C. B. ammoniogenes did, however, resemble Group III in its metabolism of 5HTM very slowly to 5HIAA, with no further breakdown of the latter.

No metabolism of 5HTM or 5HIAA could be demonstrated with any of the 5 organisms received as Alcaligenes, but not so confirmed. (Table I)

Of the other gram-negative rods examined, none of the strains of E. coli, P. aeruginosa, P. vulgaris, P. morganii, or paracolon metabolized 5HTM. Four of the 5 strains of A. aerogenes accumulated small amounts of 5HIAA after 24-48 hours incubation during the screening procedure. Since the total concentration of 5-hydroxyindoles did not change, 5HIAA metabolism was not indicated. Two of the 6 Salmonella species, S. typhimurium and S. paratyphi, produced small amounts of 2 DSA-reacting compounds with Rf values of 0.14 and 0.44 on incubation with any of the three 5-hydroxyindoles. However, these were shown to result from endogenous metabolism and not from metabolism of 5-hydroxyindoles. The other 4 Salmonella species were also negative on all 3 of the 5-hydroxyindoles. No metabolism of 5HTM by the 3 species of Shigella could be demonstrated.

B. A. faecalis Infections in Mice

Because A. faecalis has been shown to be pathogenic for mice (57), it was considered that the ability of the organisms to metabolize 5HTM might be intimately associated with their ability to kill the mice. The results of this investigation are presented below.

1. Virulence of A. faecalis Strains in Mice

A very rough estimate was made of the virulence of the 11 A. faecalis strains and one strain, O-C10, which was similar to A. faecalis except for cell size and its lack of ability to metabolize 5-hydroxyindoles. Approximately the same number of organisms of each strain was suspended in saline and then mixed with the mucin suspension and injected into 2 mice per strain. The results are shown in Table 7. It is evident that the virulence of the strains differed. Cultures of heart and tail blood taken from animals newly deceased were strikingly

positive for the injected organism. The number of organisms injected was considerably higher (approximately ten times) than that used in later experiments.

Three of the strains, O-83, ATCC 212, and O-C10 (not A. faecalis) were chosen for further study because first, they had been previously demonstrated to differ in their metabolism of 5-hydroxyindoles, and second, they were among the most virulent strains as determined by this survey.

2. LD_{50} Doses

An approximate LD_{50} dose was determined for each of the 3 strains, (Tables 8, 9, 10). It is seen that strains ATCC 212 and O-C10 had LD_{50} doses that were very similar; however, that of O-83 was considerably higher. It was also evident that neither saline nor mucin alone caused any mortality or morbidity when injected into mice. Throughout most of the following experiments, the following infecting doses of organisms were used: ATCC 212: 200×10^6 organisms in 0.25 ml; O-C10: 200×10^6 organisms in 0.25 ml; O-83: 700×10^6 organisms in 0.25 ml.

3. Effect of Mucin on Virulence.

The effects of mucin on the virulence of these organisms was studied in strain ATCC 212. Groups of mice were injected with the same number of bacteria in the same total volume. However, saline was used to replace the mucin in half of the groups. The results are shown in Table 11. It is obvious that the organisms were less virulent when mucin was not added to the injection mixture.

4. Endotoxin Effect

The possibility of an "endotoxin effect" was considered as a mechanism for fatalities during the mouse infections. To investigate

this possibility, a suspension of strain ATCC 212 was boiled for 15 minutes to kill all of the organisms. This heat-treated suspension in mucin was injected into the mice. (Table 8) No fatalities resulted, and indeed no morbidity occurred.

5. 5HTM Injections in Normal Mice

The effects of intravenous 5HTM (20 mg per kg) alone were studied. An injection into normal mice resulted in a constant, reproducible syndrome. Almost immediately after the injection, the mice became extremely lethargic and quiet, lying prostrate on the floor of the cage, only moving at the insistance of the investigator. Gait was somewhat unsteady, breathing somewhat slowed. They remained in this state of quietude for about 45 minutes and then began to regain rapidly their former activity. By one hour, most of the noticeable signs of 5HTM effect were gone, and the intraperitoneal injections of bacteria were then made. Intravenous injections of either creatinine or 5HIAA in the same dosage resulted in no noticeable behavioral changes in the mice.

6. Effect of 5HTM on the Mortality Rates of Infected Mice

The effects of intravenous 5HTM on the mortality rates of the experimentally infected mice are shown in Table 12. The statistical analyses show that 5HTM had no measureable effect on the killing rates of infected mice, as measured by these methods.

In all of the preceding experiments, the intravenous 5HTM injections were given 1 hour before the intraperitoneal bacterial injections. The effects of various other time intervals between injections are shown in Table 13. Unfortunately, during this experiment, the number of mice killed by the same dose of organisms was higher than usual.

The killing rates in the 6 hour time interval groups were no different from the control group of mice, but the killing rate at the 1 hour interval seemed to be significantly different from all 3 of the other groups. However, as was shown previously, no significant difference on 5HTM treatment existed when larger numbers of mice were used. (Table 12).

The experimental design was modified by adapting the organisms to 5HTM before their injection into the mice, since in this state the bacteria would be able to utilize 5HTM with increased initial rapidity, and thus have greater survival value. The bacteria for this experiment were grown for 18 hours on 5HTM synthetic medium and then injected, along with mucin, into the mice. The results are shown in Table 14. In these experiments, there was a significant difference in survival with strain ATCC 212 infections between the 5HTM injected mice and the control uninjected mice, indicating that the 5HTM injected mice were "protected". However, in this experiment, as in the previous ones, the control mice did not receive any intravenous injections, but only intraperitoneal bacterial injections. It was thought that perhaps the trauma of the injection and/or the volume of injected material might influence the killing rate. Therefore control experiments using creatinine and 5HIAA as the intravenously injected substances were carried out in an attempt to substantiate this possible 5HTM effect. These results are also shown in Table 14. It is evident that there was now a wide range of control values, and much overlapping between groups, statistically. Larger numbers of mice would be necessary to separate the groups statistically, if this indeed could be possible.

7. Immunological Studies

It has been previously shown by Sarkar et al (57) that mice infected with A. faecalis developed an immunity to subsequent infections. Immunological investigations were done in this laboratory by injecting all the survivors of previous experiments with twice the dose of organisms previously used. The results, shown in Table 15, indicate that immunity did develop in these surviving animals. The group of mice that was previously injected with only heat-killed organisms was also protected, demonstrating that heat-killed bacteria were also effective immunizing agents. Mice which had been previously injected with 5HTM as well as bacteria showed a response identical to the mice which had not received 5HTM. The group of mice previously injected with only 5HTM and no bacteria were afforded no protection against this dose of bacteria, as might have been expected.

The same group of investigators (57) working with A. faecalis infections in mice had reported that no cross-immunity developed between members of this species. This problem was studied in this laboratory by injecting the now well-immunized mice used in the above experiment with the 2 heterologous organisms. A dose three times that originally used was employed in this experiment. The data, (Table 16), suggest that there was cross-immunity between O-83 and both O-C10 and ATCC 212, but none between ATCC 212 and O-C10.

These results were supplemented with agglutination experiments, using convalescent serum of the mice and H and O antigenic suspensions of the three A. faecalis strains. The results are shown in Table 17. No serological cross-immunity could be demonstrated; all of the antisera agglutinated only the homologous organisms.

C. A. faecalis in Human Feces

Using routine MacConkey agar plating methods, 82 stool samples were negative for the presence of A. faecalis. The stool sample from the patient with malignant carcinoid also did not contain A. faecalis; it did contain Proteus mirabilis and Escherichia coli. Both of these organisms were examined with the screening procedure for the breakdown of 5-hydroxyindoles and found to be negative colorimetrically and chromatographically.

Further studies of 7 additional stool samples, using the glucose-supplemented MacConkey agar were no more successful in demonstrating A. faecalis. The latter 7 stool samples which were subjected to fecal enrichment studies with 5HTM also proved to be negative for A. faecalis. The addition of A. faecalis suspensions to stool samples known not to contain these organisms resulted in the disappearance of the added A. faecalis, in spite of the fecal enrichment methods with 5HTM designed to bring about their selective increase.

D. A. faecalis in Rat Feces

The incidence of A. faecalis in feces from 16 normal rats was found to be zero (Table 18). The oral administration of creatinine to 8 of these rats made no difference in the fecal analyses for A. faecalis. During the administration of 5HTM creatinine sulfate 6 colorless colonies were obtained from 3 of the 8 rats on days 8 and 10 of the experiment (Table 18). However, further taxonomic identification showed that none of these colonies were A. faecalis. They were all eliminated by some of the following variations from the Bergey definition of A. faecalis: production of soluble green pigment; late fer-

mentation of glucose and xylose; inability to grow well on nutrient agar at either 37C or 25C; inability to turn brom-cresol-purple milk alkaline; discrepancy in cell size. However, the screening procedure for degradation of 5HTM was done on all 6 cultures. Only one culture, which possessed a soluble green pigment and was probably a Pseudomonas, produced small quantities of 5HIAA from 5HTM, with no degradation of the latter evident on the screening procedure. The other 5 cultures showed no ability to metabolize 5HTM whatsoever.

Neither the doses of 5HTM nor creatinine produced any gross behavioral changes in the rats.

DISCUSSION

A. Taxonomy of A. faecalis

The characteristics of A. faecalis used for classification are essentially all negative ones, and therefore the classification of a culture as A. faecalis is primarily a process of elimination, and is thus subject to many difficulties. Conn (16) undertook a study of the genus Alcaligenes, with special emphasis on those organisms of soil origin to which the name A. faecalis has been ascribed. Many of his cultures which fit the species designation, including those from the American Type Culture Collection, showed a metabolic pattern different from those Conn felt were "true" A. faecalis. He showed that addition of glucose to a medium containing inorganic nitrogen improved the growth of "soil", but not "intestinal" A. faecalis. However, no definite conclusion was drawn about whether only one or both types constituted the real type species. The findings of this study indicated that there is "serious question as to whether anyone knows just what Alcaligenes faecalis is." (16)

Sarkar et al (57) did an extensive taxonomical study of 200 reported A. faecalis isolated from hospital patients. They used as their criteria for classification: "gram-negative bacilli or coccobacilli growing profusely on MacConkey plates(s), fermenting no sugars in 72 hours, and turning litmus milk alkaline (or rarely making no change in it.)" They found much heterogeneity within this large collection, including much morphological and flagellar variation, some late glucose fermentation, and some gelatin liquefaction. In spite of this nonconformity to Bergey's classification, they did not exclude any organisms from their original designation, but rather suggested that all these

differences were variations within the species. The possibility that many of these cultures might have belonged in groups such as the non-pigmented pseudomonads was not even mentioned. In many clinical laboratories, the same few criteria used by Sarkar et al are applied in the definition of a culture as A. faecalis. Such limited taxonomic study no doubt results in many false classifications of isolates.

In the studies done in this laboratory, the cultures eliminated from this species differed markedly from the Bergey definition (9) in cell size or flagellar arrangement. Two non-motile strains, S-1 and ATCC 4741 were included, believing that this one criterion was probably not enough to eliminate them from the rest of the organisms which they closely resembled, and not enough to place them in A. metalcaligenes since they grew very well at 37C, which the latter organism does not. Admittedly, this selection of what constituted an A. faecalis was rather restrictive. Less rigorous morphological criteria would not have excluded the other 5 of the original strains, and as a result, the number of cultures not metabolizing 5HTM would have definitely been increased. One such organism, O-C10 which closely resembled the "true" A. faecalis except in cell size, was demonstrated to induce cross-immunity with A. faecalis O-83 by protection tests; however no serological cross-immunity existed. It might be argued that this cross-immunity would tend to place O-C10 in the species. It is possible, however, for cultures to have related antigenic groups but be totally unrelated as to species designation (79). With our rigorous critera for defining A. faecalis, it would appear that metabolism of 5-hydroxyindoles is a species characteristic, although the number of strains tested is admittedly small for a definitive conclusion. 5-hydroxyindole

metabolism may thus provide a positive confirmatory criterion for taxonomic distinction of an organism as A. faecalis, in addition to the orthodox and essentially negative characteristics. However, the ability to metabolize 5-hydroxyindoles does not per se identify an organism as A. faecalis in the absence of other criteria, since it has been found that some Pseudomonas cultures also exhibit this metabolic property (48).

B. 5-hydroxyindole Metabolism

The finding of 3 different groups of 5HTM-metabolizing A. faecalis strains is compatible with a theory of genetic mutations within the species resulting in loss of enzymes capable of breaking down the various 5-hydroxyindoles. Group I apparently is able to metabolize 5HTM by means of the enzyme amine oxidase with the production of 5HIAA, and also to degrade 5HIAA, a feat many believe the mammalian system unable to accomplish (16, 44, 64). Group II, consisting of only one culture, is able to metabolize 5HTM through a pathway not normally involving amine oxidase and 5HIAA production. Group II is also able to metabolize 5HIAA rapidly. It is thus similar to a culture of Pseudomonas, strain 121, studied previously in this laboratory by Oginsky et al (48), which also metabolizes 5HTM without normally accumulating 5HIAA. 5HTM oxidation by both 121 (48) and O-390 in these experiments is much more sensitive to inhibition by arsenite and 8-hydroxyquinoline than is 5HTM oxidation by strains in which 5HIAA accumulation apparently results from amine oxidase activity (e.g. Group I organisms). Furthermore, this marked sensitivity to arsenite and 8-hydroxyquinoline of 5HTM oxidation by these two organisms is comparable to the marked sensitivity of 5HIAA oxidation (48). These data would suggest that the predominant

pathway of 5HTM metabolism in 121 and O-390 is not the amine oxidase pathway, but rather involves a mechanism resembling that of 5HIAA degradation. It may be that these organisms normally metabolize only a small fraction of 5HTM *via* amine oxidase to 5HIAA, as the result of a suppressive mutation. In the presence of arsenite or 8-hydroxyquinoline this would become the only mechanism for 5HTM metabolism, and 5HIAA would accumulate, as indeed it does. The comparable inhibition of both Group I and Group II 5HTM oxidation by iproniazid does not necessarily imply amine oxidase function in either group, since Oginsky *et al* have found that 5HIAA oxidation is also inhibited by iproniazid (48). It is of considerable interest that alternate routes of 5HTM metabolism in mammalian tissues have been postulated by a number of investigators (17, 49, 60, 20, 77). Characterization of the mechanisms of 5HTM metabolism by strains O-390 and 121 would further assist in clarifying this problem.

Group III cultures do appear to metabolize 5HTM by the amine oxidase pathway, but do not possess the enzyme system capable of breaking down 5HIAA, perhaps because of a genetic loss mutation. Their 5-hydroxyindole metabolism resembles the accepted route of 5HTM metabolism in mammals, which also results in 5HIAA as an end-product.

Metabolism of 5HTR, demonstrated by 2 *A. faecalis* strains, appears to take place through a different pathway than has heretofore been described. None of the compounds one might propose as intermediates in a 5HTR degradative pathway were found (13, 47). Two of the fluorescent spots had Rf values very close to those of 5HTM and 5HIAA; however, neither of them could be visualized with sprays that react with these compounds at concentrations visualized by fluorescence.

There were no other intestinal organisms tested which could metabolize 5HTM with the rapidity and completeness of A. faecalis. B. ammoniogenes and A. aerogenes were the only other cultures found capable of metabolizing 5HTM; they both demonstrated only slow breakdown of 5HTM to 5HIAA. The fact that the enzymes for the metabolism of 5-hydroxyindoles are present in A. faecalis in significant concentrations further differentiates them from other common intestinal gram-negative rods.

Differential growth response on Conn's media 1 and 2 would suggest that the cultures included not only soil and intestinal organisms, but also another group which Conn found consisted of organisms growing equally well in both media, classified as "similar to A. faecalis." (16) One organism, O-62, which would be designated as intestinal in origin by this test, was isolated by soil enrichment. Most of the other strains used were from culture collections of long standing, and their origins lost in antiquity. These findings suggest that this criterion of Conn for characterization of A. faecalis cultures may not be valid.

There is generally little interest in the study of this group of organisms, probably because they seem to be very inactive on the usual media used for their classification. There is also little general acceptance of really rigorous rules for defining this organism, morphologically and metabolically. It seems that re-evaluation of the whole genus by new metabolic approaches would be highly desirable.

C. A. faecalis Infections in Mice

A study of the virulence of A. faecalis in mice has been reported only by Sarkar et al (57). They studied 12 of their 200 cultures and

found them all to be of equal virulence. The minimum lethal dose (MLD) for all of the 12 strains was 125 x 10^6 organisms when injected with mucin. There was no mention, however, of the strains, ages, or weights of mice used. The virulence of A. faecalis in this laboratory varied with the particular strain. However, for the 3 more virulent cultures studied in detail, the LD_{50} was between 200 and 700 x 10^6 organisms, indicating that either the mice were more resistant or the strains less virulent than those of Sarker.

Immunological studies by the same investigators showed that formalin-killed bacteria produced adequate protection of the mice against one MLD of the organisms. They were able to determine no cross-immunity among the various strains; each antiserum was able to agglutinate only the homologous strain. Results in this laboratory demonstrated that an effective immunity was produced either by injection of heat-killed bacteria or by recovery from an active infection. Challenging the immunized mice with the heterologous strains demonstrated a cross-immunity between strains O-83 and O-C10, and O-83 and ATCC 212, but not between strains ATCC 212 and O-C10. This would suggest that O-83 had common antigens with both O-C10 and ATCC 212, but that the latter two had dissimilar antigens. Agglutination studies, however, showed no cross serological reactions between any of the three. This would suggest that antibodies against O and H antigens are not intimately connected with the ability of the animal to withstand infection, and that these antigens are not primarily responsible for the virulence of the bacteria.

The effect of mucin on bacterial virulence was first described by Nungester et al in 1932 (46). They showed that gastric mucin enhanced the virulence of Pneumococcus, Streptococcus, and Staphylococcus in mice.

Later, other workers noticed a similar effect in mouse infections with Meningococcus and H. influenza (23, 43). This effect has never been explained, but it is thought to be the result of a protective coat which the mucin provides for the bacteria (46). The results obtained from this study are in agreement with those of the above investigators and of Sarkar et al, since a much larger number of organisms was needed to kill the mice when no mucin was used. Because all of the blood cultures were heavily positive for the injected organism, and because heat-killed bacteria produced no morbidity or mortality in mice, it can be assumed that the mortality caused by injections of A. faecalis was due to multiplication of the organisms.

Throughout these studies, it was shown that 5HTM administration had no measureable affect on A. faecalis infections in mice. This was true whether or not the organisms were previously adapted to 5HTM, whether or not they could metabolize 5HTM and/or 5HIAA, and whether or not the 5HTM was injected before or after the bacteria. The dose of 5HTM was not varied throughout the experiments. It was felt that 20 mgm per kg was an adequate dose because it was well below the LD_{50} for mice (24, 45), and yet was well within the physiological range, producing marked effects in the mice. Whether or not 5HTM in other dosages, routes of administration, or time intervals might affect this type of infection is not known, but this seems unlikely in view of these totally negative results. The data on mouse infections make it highly unlikely that the ability to metabolize the important body constituent 5HTM, and thus to compete with mammalian tissues for an essential physiological component, is a significant factor in pathogenesis by this group of organisms. Increase of the level of available 5HTM by injection

provided no advantage to either the mouse or the bacterium.

One interesting result was obtained when 5HTM-adapted ATCC 212 organisms were injected into mice. There was a significant protection afforded from the 5HTM injection; however, this protective effect was also seen when creatinine alone or 5HIAA was injected. This would suggest the possibility that the trauma of the injection or the small added fluid volume resulted in some protective mechanism being activated, possibly by adrenal stimulation.

D. A. faecalis in Human Feces

The incidence of A. faecalis in the population studied was found to be zero. This result agrees with the results of Hirst (33), but is different from those of Trawinski and György (67) who found an incidence of 19% and Sarkar et al in India (58) who found an incidence of 3%. However, because Sarkar's designation of a culture as A. faecalis was rather non-restrictive, and included late glucose fermenters, this percentage may be somewhat high. The fecal enrichment studies in this laboratory were likewise unproductive of any strains of A. faecalis. The failure of known cultures of A. faecalis to grow during the fecal enrichment procedure would suggest that there is some factor in normal stool which inhibits the growth of A. faecalis. The nature of this inhibition has not been further studied.

Because of the difficulty in finding A. faecalis in human feces, by either routine or enrichment procedures, and because of the relative ease of finding it in soil (48), the question is raised as to whether the species designation of "faecalis" is a taxonomic misnomer. Another organism with the same species name, Streptococcus faecalis, is very easily obtainable from fecal specimens (79). Perhaps this organism

would be better understood if its name were related to its more usual environmental habitat.

E. A. faecalis in Rat Feces

Sarkar and Tribedi (58) found that the percentage of stool cultures positive for A. faecalis increased from 3.3% in normal humans to an average of 25% in convalescent enteric-like fever, cholera, and dysentery patients. We considered that the striking intestinal hypermotility of the latter two diseases might be intimately connected with an increase in 5HTM concentration in the locality of the intestine resulting in hyperperistalsis. If this were true, those organisms which could metabolize 5HTM, such as A. faecalis, would be given a biological advantage which may account for their high incidence in the feces of the previously-mentioned patients. The experiments done in this laboratory were based on the hypothesis that A. faecalis may be present normally in small numbers in the intestine, not detectable by usual methods, but the relative proportion of these organisms might be increased on 5HTM administration. The results from our experiments with rats indicate that these organisms do not appear in detectable numbers even when given this advantage. However, two factors may account for these negative results. First, there may have been no A. faecalis in the rat intestinal flora originally, and therefore it could not have appeared during the experiment. Second, the 5HTM may have been completely absorbed from the intestine before it reached the locality of the organisms. It has been shown by Lewis (39) that 5HTM given orally to humans is completely absorbed from the gastrointestinal tract within 8 hours.

SUMMARY AND CONCLUSIONS

The metabolism of 5-hydroxyindoles, particularly 5HTM, has been investigated in 42 intestinal and soil gram-negative rods, including 16 reported strains of Alcaligenes faecalis. All 11 of the 16 strains for which the species designation could be confirmed demonstrated the ability to metabolize 5HTM, 10 of them very rapidly. Six of these organisms could degrade 5HIAA equally well. All strains but one metabolized 5HTM through the 5HIAA pathway; the one exception appears to proceed through a different route, as yet unidentified. Two A. faecalis strains could also metabolize 5HTR, proceeding through pathways as yet unknown.

Some new intermediates of 5-hydroxyindole metabolism have been indicated by paper chromatography, but their identity has not yet been ascertained.

Four out of 5 Aerobacter aerogenes cultures showed a slow metabolism of 5HTM to 5HIAA. None of the other organisms, including normal intestinal gram-negative rods, Salmonella, and Shigella, had any activity on 5HTM.

The difficulty in classification of organisms in the species A. faecalis is discussed.

The possible role of 5HTM in infections of mice with A. faecalis has been studied. No definite relationship could be established. No difference in the mortality rates of infected mice was observed whether or not the organisms could metabolize 5HTM and/or 5HIAA, whether or not 5HTM was injected prior to the infection, and whether or not the organisms were "adapted" to 5HTM.

The incidence of A. faecalis in the fecal flora was studied in a hospital-clinic population and in a population of laboratory rats. No A. faecalis cultures could be found either in 87 human stool samples or in 160 fecal samples from 16 rats. The oral administration of 5HTM to the rats did not result in the appearance of A. faecalis in the fecal flora.

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TABLE 1

Classification of Cultures Received as *Alcaligenes*

Reputed species	Strain no.	Morphology[1]	Flagella	Reduction of Nitrates	Utilization of Citrate[3]	Production of H_2S	Confirmed Species
A. faecalis	O -390	Short rods	Peritrichous	-	+	+	*A. faecalis*
" "	O -31	" "	"	-	-	-	" "
" "	O -62	" "	"	-	-	-	" "
" "	O -83	" "	"	-	-	-	" "
" "	ATCC 8750	" "	"	-	+	+	" "
" "	ATCC 9220	" "	"	+	+	+	" "
" "	ATCC 212	" "	"	+	+	+	" "
" "	ATCC 4741	" "	Non motile	-	-	+	" "
" "	S -1	" "	Non motile	+	+	+	" "
" "	M -1	" "	Peritrichous	+	+	+	" "
" "	I -3	" "	"	+	+	+	" "
" "	O -C10	Long rods	"	+	-	+	- -
" "	O -S 1	Very long rods	Motile[2]	-	-	-	- -
" "	O -C2	Long rods	Polar	+	-	+	- -
" "	I -I	Large plump rods	Non motile	-	-	+	- -
" "	I -2	Large plump rods	Non motile	-	-	+	- -
A. ammoniogenes	M -2	Coccus-like rods (Gram-positive)	Peritrichous	+	-	+	*B. ammoniogenes*
A. bookeri	ATCC 9128	Short rods	Polar	+	+	+	- -
A. viscolactis	I -4	Long rods in chains	Non motile	-	-	-	*A. viscolactis*

All cultures were alkaline in brom-cresol-purple milk, showed no liquefaction of gelatin, no carbohydrate fermentation; indole-neg, methyl red-neg, Voges-Proskauer - neg.

1. All cultures were gram-negative.
2. Flagellar stains were repeatedly unsuccessful.
3. As sole source of carbon.

TABLE 2

Classification of *A. faecalis* by the Criteria of Conn*

A. faecalis Strain No.	GROWTH IN CONN'S MEDIA I	II	CONN'S CLASSIFICATION
0-390	+++	+++	-
0-31	+++	+++	-
0-62	+	+	I
0-83	+	++++	S
ATCC 8750	+	+	I
ATCC 9220	++	++++	S
ATCC 212	++	++++	S
ATCC 4721	+	+	I
S -1	++	++++	S
M -1	+	++++	S
I -3	+++	++++	-

* (16)

S = Soil

I = Intestinal

\- = Indeterminate

TABLE 3

Metabolism of 5-hydroxyindoles by *A. faecalis*

Strain	Group	5HTM	5HIAA accumulation			5HIAA	5HTR
			None	Transient	As end product		
O -31		+		+		+	-
O -62	I	+		+		+	-
O -83		+		+		+	-
ATCC-8750		+		+		+	+
S -1		+		+		+	-
O -390	II	+	+			+	+
ATCC-9220		+			+	-	-
ATCC -212		+			+	-	-
ATCC-4741	III	+ (weakly)			+	-	-
M -1		+			+	-	-
I -3		+			+	-	-

TABLE 4

Oxidation of 5HTM, 5HIAA, and Tyramine
by *A. faecalis*, Group I

ORGANISM	TOTAL OXYGEN UPTAKE (µMO/µM substrate)			OXYGEN UPTAKE RATE Q_{O_2} (N)		
	5HTM	5HIAA	TYR	5HTM	5HIAA	TYR
0 -31	12.6	8.3	11.0	690	512	700
0 -62	14.3	8.9	10.4	559	441	350
0 -83	12.9	12.1	10.8	782	770	627
ATCC-8750	12.8	9.4	11.6	535	510	570
S -1	13.1	10.3	9.5	286	352	384

TABLE 5

Chromatographic Pattern During the Oxidation of 5HTM by *A. faecalis*, Group I

	OXYGEN UPTAKE LEVEL														
	0			50			100			200			COMPLETE		
ORGANISM	5HTM	5HIAA	Other	5HTM	5HIAA	Other	5HTM	5HIAA	Other	5HTM	5HIAA	Other	5HTM	5HIAA	Other
0 -31	++++	-	-	+++	+	-	++	+	-	-	++	-	-	-	-
0 -62	++++	-	-	+++	+	-	++	+	-	-	++	-	-	-	-
0 -83	++++	-	-	++	+	-	+	++	-	-	++	-	-	-	-
ATCC 8750	++++	-	-	++	+	-	+	++	tr[2]	-	+	+	-	-	-
S -1	++++	-	-	++	tr[1]	-	+	tr	-	-	tr	-	-	-	-

++++ = Chromatographic spot at control value size and intensity
\- = No spots visualized

1= Trace
2= Chromatographic spot with R.f. of 0.10 (see text).

TABLE 6

Oxidation of 5HTM, 5HIAA, and Tyramine by
A. faecalis, Group III

Organism	TOTAL OXYGEN UPTAKE (μMO/μM substrate)			RATES Q_{O_2} (N)		
	5HTM	5HIAA	TYR	5HTM	5HIAA	TYR
ATCC 9220	1.3	0.1	8.9	103	4	355
ATCC 212	1.4	0.2	9.7	71	3	284
ATCC 4741	0[1]	0	0	0	0	0
M-1	1.7	0.3	-[2]	124	5	-
I-3	1.7	0.2	-	69	6	-

[1]The reactions progressed too slowly to be studied adequately by manometric methods.

[2]Not done.

TABLE 7

Virulence of A. faecalis Strains in Mice

A. faecalis strain	No. of organisms Injected X 10^7	Dead/Total
O-390	320	1/2
O-31	310	1/2
O-62	- *	2/2
O-83	390	2/2
ATCC 8750	400	1/2
ATCC 9220	260	1/2
ATCC 212	250	2/2
ATCC 4741	210	0/2
S-1	- *	1/2
M-1	310	2/2
I-3	140	2/2
O-C10 (not A. faecalis)	200	2/2

* Cell counts not done; optical density same as other strains.

TABLE 8

Virulence of *A. faecalis*, ATCC 212, in Mice

No. of organisms X 10^6	No. of mice used	D/T	% killed
0 (Mucin and saline only)	8	0/8	0%
2500	2	2/2	100
1250	4	3/4	75
800	2	2/2	100
500	10	10/10	100
250	12	6/12	50
200	8	2/8	25
25	2	0/2	0
200 (heat killed)	8	0/8	0

TABLE 9

Virulence of *A. faecalis*, O-C10, in Mice

No. of organisms X 10^6	No. of mice used	D/T	% killed
1000	2	2/2	100
500	10	7/10	70
250	8	5/8	63
200	8	4/8	50
25	2	0/2	0

TABLE 10

Virulence of *A. faecalis*, O-83, in Mice

No. of Organisms X 10^5	No. of mice used	D/T	% killed
1000	2	1/2	50
750	8	5/8	63
700	8	3/8	38
500	8	1/8	13
250	10	1/10	10
25	2	0/2	0

TABLE 11

Effect of Gastric Mucin on the Virulence of *A. faecalis*, ATCC 212, in Mice

No. of organisms X 10^6	D/T		% killed	
	Saline	Mucin	Saline	Mucin
2500	2/2	2/2	100	100
1250	0/2	3/4	0	75
800	0/2	2/2	0	100
500	0/2	2/2	0	100
250	0/2	1/2	0	50

T A B L E 12

Effect of Intravenous 5HTM on the Mortality Rates of Mice Infected with *A. faecalis*

A. faecalis strain	No. of organisms X 10^6	No. of mice used	5HTM injected (20 mg/kg IV)	D/T	% killed	P values
ATCC 212	200	24	-	14/24	58	
" "	"	28	+	20/28	71	>0.100
O-C10	200	8	-	4/8	50	
"	"	8	+	3/8	38	0.5000
O-83	700	16	-	5/16	31	
"	"	16	+	9/16	56	0.1426

TABLE 13

Effect of Different Time Intervals Between Injections of 5HTM and *A. faecalis*, ATCC 212

No. of mice used	No. of organisms X 10^6	5HTM (20 mg/kg) IV	IV Injection Time interval	D/T	% killed	P values
8	200	-	-	7/8	88	
8	"	+	1 hr. previous	3/8	38	0.0594
8	"	+	6 hr. previous	8/8	100	0.5000
8	"	+	6 hr. following	7/8	88	0.7667

TABLE 14

Effect of Prior Adaptation of *A. faecalis* to 5HTM on the Mortality Rates of Infected Mice

A. faecalis strain (5HTM adapted)	No. of organisms X 10^6	No. of mice used	IV Injection (20 mg/kg)	D/T	% killed	P values*
ATCC 212	200	16	-	10/16	63	
" "	"	24	5HTM	5/24	21	< 0.05000
" "	"	8	5HIAA	2/8	25	0.0965
" "	"	8	Creatinine	2/8	25	0.0965
O-C10	200	8	-	3/8	38	
"	"	8	5HTM	1/8	13	0.2846
"	"	8	5HIAA	2/8	25	0.5000
O-83	700	8	-	0/8	0	
"	"	8	5HTM	2/8	25	0.2333
"	"	8	5HIAA	2/8	25	0.2333

* As compared to control mice not receiving IV injections.

TABLE 15

Injection of Homologous Strains of *A. faecalis* into Mice Recovered from a Previous *A. faecalis* Infection

No. Mice	Previously received injections I.P. (Bacteria)	I.V. (5HTM)	No. of weeks since last injection	Challenging Organism Strain	No. of organisms X 10^6	D/T	% killed
12	-	-	-	ATCC 212	250	6/12	50
5	ATCC 212	+	11	"	400	0/5	0
6	" "	-	10	"	"	0/6	0
4	" "	-	4	"	250	0/4	0
6	" " (heat killed)	-	4	"	400	1/6	17
4	" "	+	2	"	"	0/4	0
2	" "	-	2	"	"	0/2	0
2	" " (5HTM adapted)	-	2	"	"	0/2	0
6	" " (5HTM adapted)	+	2	"	"	1/6	17
8	-	-	-	O-C10	250	5/8	63
4	O-C10	+	7	"	400	0/4	0
8	"	-	6	"	"	0/8	0
4	"	-	2	"	"	0/4	0
4	"	-	1	"	"	0/4	0
8	-	-	-	O-83	750	5/8	63
4	-	+	10	"	1400	4/4	100
4	O-83	-	7	"	"	0/4	0
5	"	-	6	"	"	2/5	40
4	"	+	6	"	"	0/4	0
4	"	-	4	"	"	1/4	25
3	"	+	4	"	"	0/3	0

TABLE 16

Injection of Heterologous Strains of *A. faecalis* into Mice Recovered from a Previous *A. faecalis* Infection

No. Mice	Challenging Organism Strain	Challenging Organism No. of organisms X 10^6	Previous *A. faecalis* Infection	No. of weeks Since Last Injection	D/T	% killed
10	ATCC 212	500	-	-	10/10	100
7	"	600	ATCC 212	4	1/7	14
4	"	"	O-C10	4	4/4	100
4	"	"	O-83	3	1/4	25
10	O-C10	500	-	-	7/10	70
6	"	600	ATCC 212	4	4/6	67
4	"	"	O-C10	4	0/4	0
4	"	"	O-83	3	0/4	0
8	O-83	750	-	-	5/8	63
8	"	2100	ATCC 212	4	2/8	25
4	"	"	O-C10	4	1/4	25
4	"	"	O-83	3	0/4	0

TABLE 17

Agglutination of *A. faecalis* Antigens by Serum from Mice Recovered from *A. faecalis* Infections

Serum of mice recovered from infection with	Antigen	Serum Dilutions						
		1/40	1/80	1/160	1/320	1/640	1/1280	1/2560
ATCC 212	ATCC 212-"O"	-	-	-	-	-	-	-
	O-C10-"O"	-	-	-	-	-	-	-
	O-83-"O"	-	-	-	-	-	-	-
	ATCC 212-"H"	+	+	+	+	+	+	+
	O-C10-"H"	-	-	-	-	-	-	-
	O-83-"H"	-	-	-	-	-	-	-
O-C10	ATCC 212-"O"	-	-	-	-			
	O-C10-"O"	+	+	+	+	+	+	-
	O-83-"O"	-	-	-	-	-	-	-
	ATCC 212-"H"	-	-	-	-			
	O-C10-"H"	+	+	+	+	+	+	+
	O-83-"H"	-	-	-	-	-		
O-83	ATCC 212-"O"	-	-	-	-	-	-	-
	O-C10-"O"	-	-	-	-	-		
	O-83-"O"	-	-	-	-	-	-	-
	ATCC 212-"H"	-	-	-	-			
	O-C10-"H"	-	-	-	-	-	-	-
	O-83-"H"	+	+	+	+	+	+	+
Normal mice	ATCC 212-"O"	-	-	-	-			
	O-C10-"O"	-	-	-	-			
	O-83-"O"	-	-	-	-			
	ATCC 212-"H"	-	-	-				
	O-C10-"H"	-	-	-	-			
	O-83-"H"	-	-	-				

TABLE 18

Effect of Oral Administration of 5HTM on the Incidence of *A. faecalis* in Rat Feces

Rat No.	Oral Feeding	No. of Colonies Counted on MacConkey-Glucose Agar			
		Before Feeding		After Feeding	
		Red Colonies	Colorless Colonies	Red Colonies	Colorless Colonies
1	creatinine	616	0	393	0
2	"	1905	0	1922	0
3	"	2048	0	1724	0
4	"	1653	0	1440	0
5	"	220	0	1305	0
6	"	759	0	773	0
7	"	922	0	801	0
8	"	636	0	1430	0
9	5HTM creatinine sulfate	1974	0	895	1
10	"	1329	0	1487	0
11	"	741	0	410	0
12	"	1787	0	1130	4
13	"	1021	0	640	0
14	"	733	0	448	1
15	"	403	0	776	0
16	"	644	0	644	0

Figure 1

Proposed Pattern of 5HTM Synthesis

and Degradation

SEROTONIN BIOSYNTHESIS AND DEGRADATION

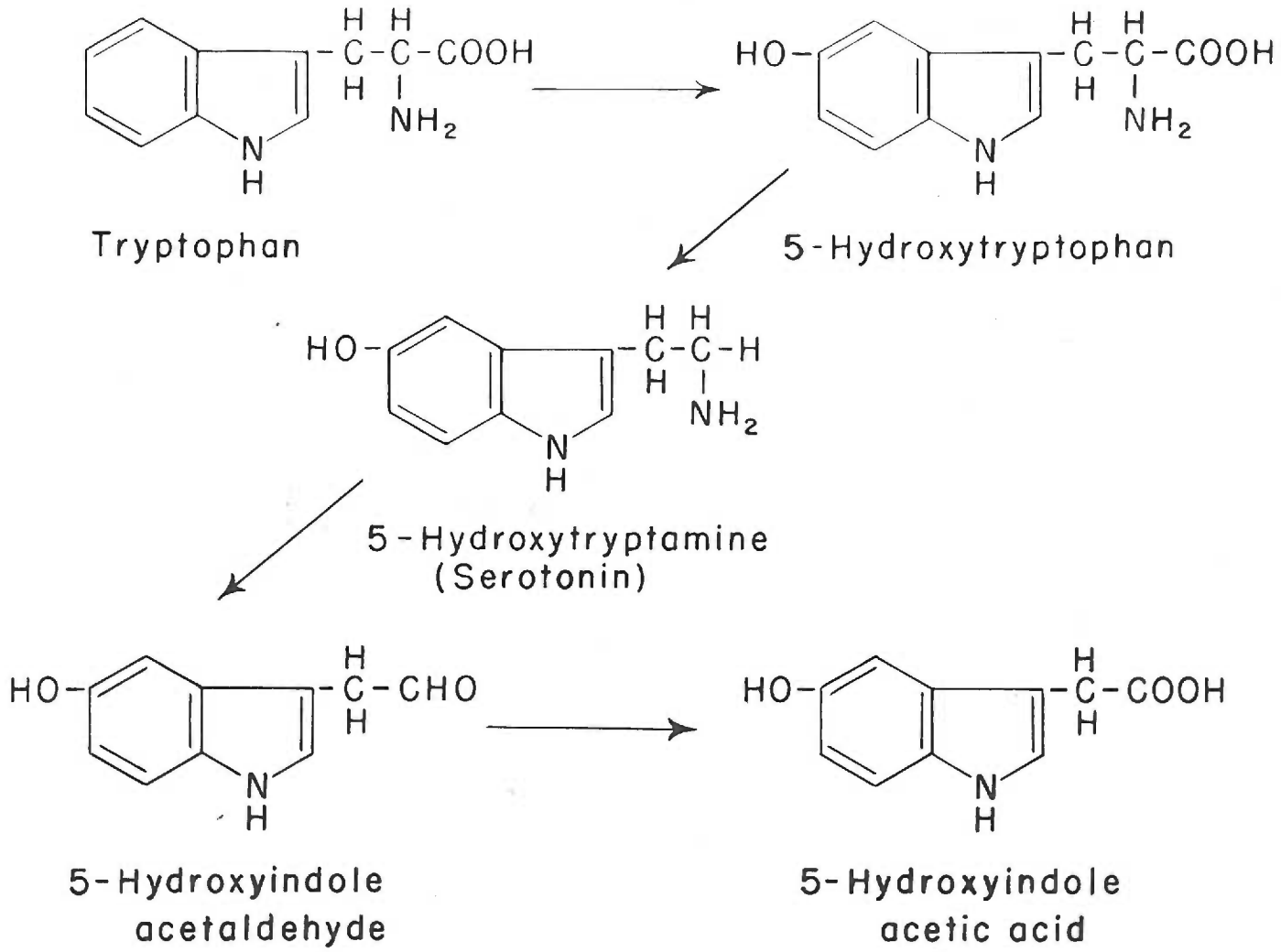


Figure 2

A. Standard Curves for the Colorimetric Determination of 5HTM.

B. Standard Curves for the Colorimetric Determination of 5HTM, 5HTR, and 5HIAA.

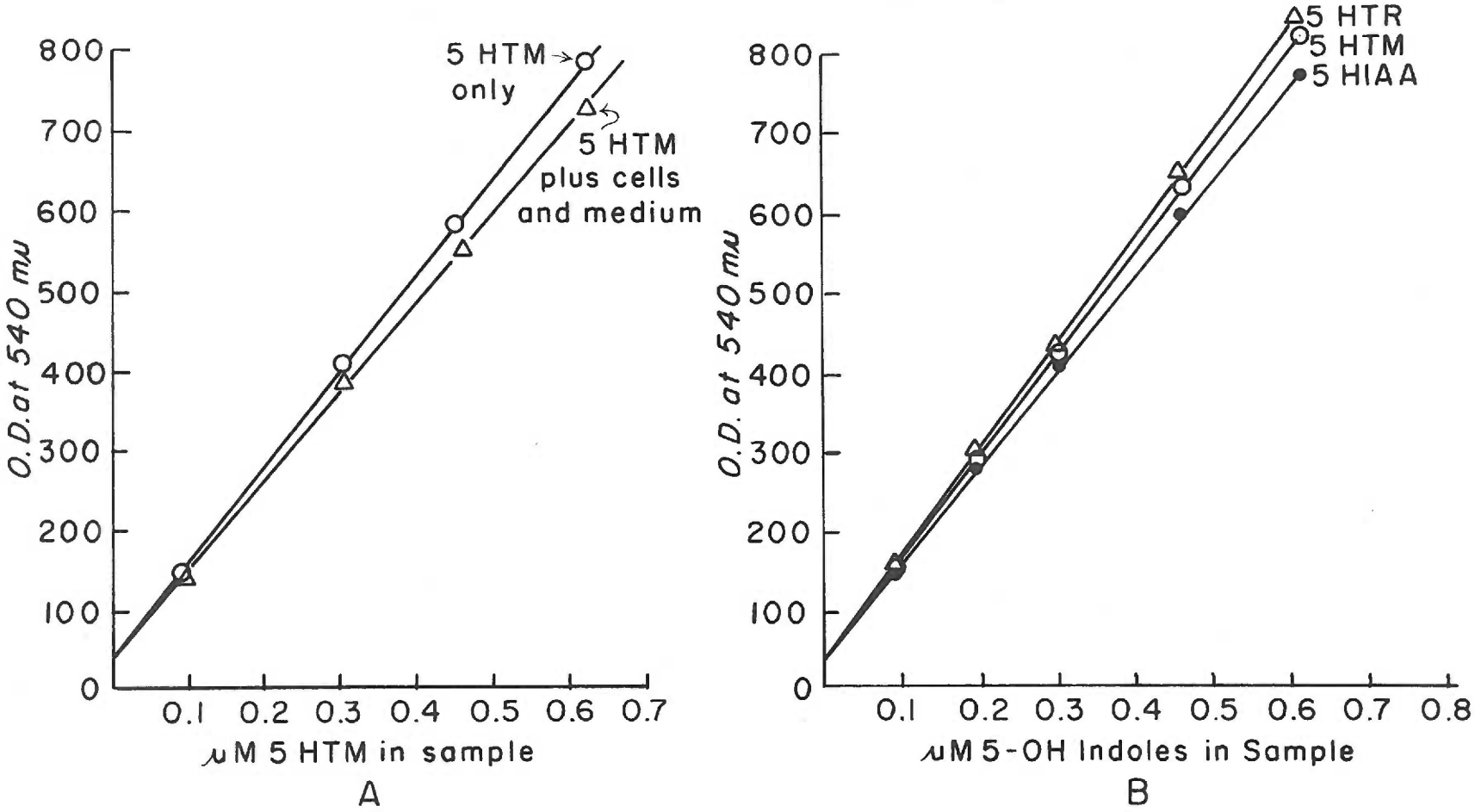
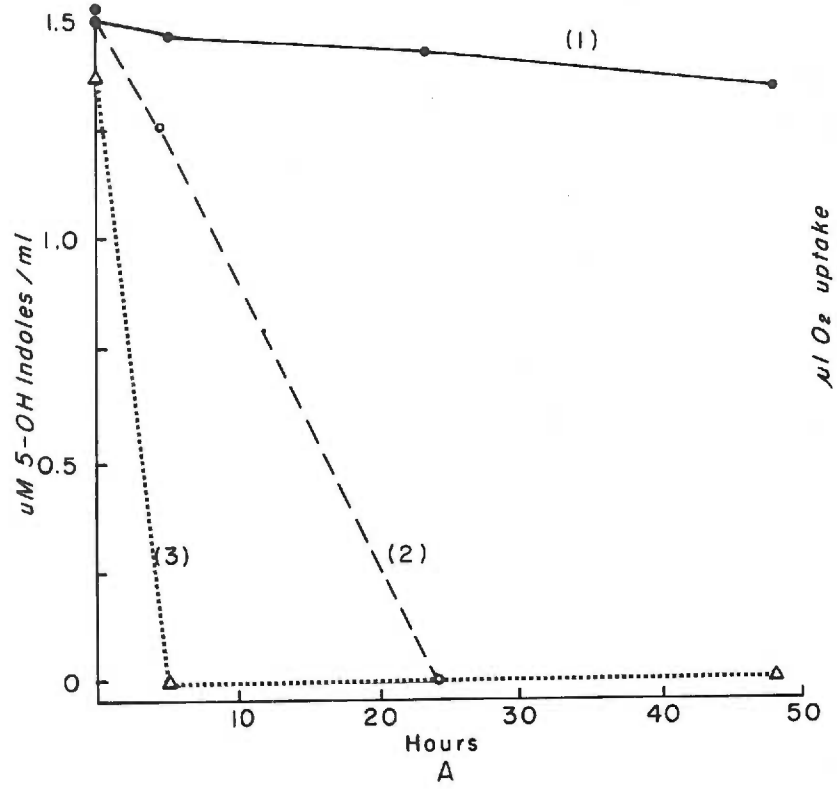

800
700
600
500
400
300
200
100
0
O.D. at 540 mμ
5 HTM
only
5 HTM
plus cells
and medium
0.1 0.2 0.3 0.4 0.5 0.6 0.7
μM 5 HTM in sample
A
5 HTR
5 HTM
5 HIAA
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8
μM 5-OH Indoles in Sample
B

Figure 3

A. Screening Procedure: Metabolism of 5HTM by A. faecalis, Groups I and II, as Demonstrated by ATCC 8750.

Key:
1. Control Flask Containing 5HTM Only.
2. 5HTM plus Cells Grown on Nutrient Agar.
3. 5HTM plus Cells Grown on Synthetic 5HTM Agar.

B. Oxidation of 5HTM and 5HIAA by A. faecalis, Groups I and II, as Demonstrated by O-83.


1.5
1.0
0.5
0
uM 5-OH Indoles / ml
(1)
(2)
(3)
10
20
30
40
50
Hours
A

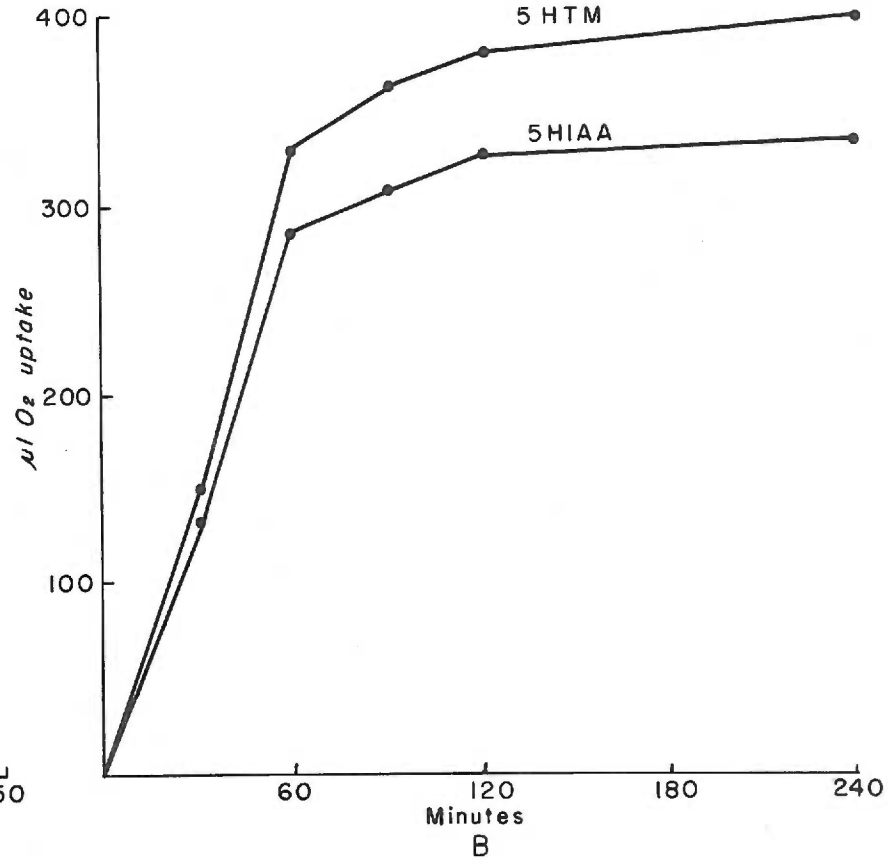

400
300
200
100
µl O2 uptake
5 HTM
5HIAA
60
120
180
240
Minutes
B

Figure 4

Paper Chromatographic Patterns During the Oxidation of 5HTM by:

A. Group I, as Demonstrated by ATCC 8750.

B. Group II, as Demonstrated by O-390.

C. Group III, as Demonstrated by ATCC 212.

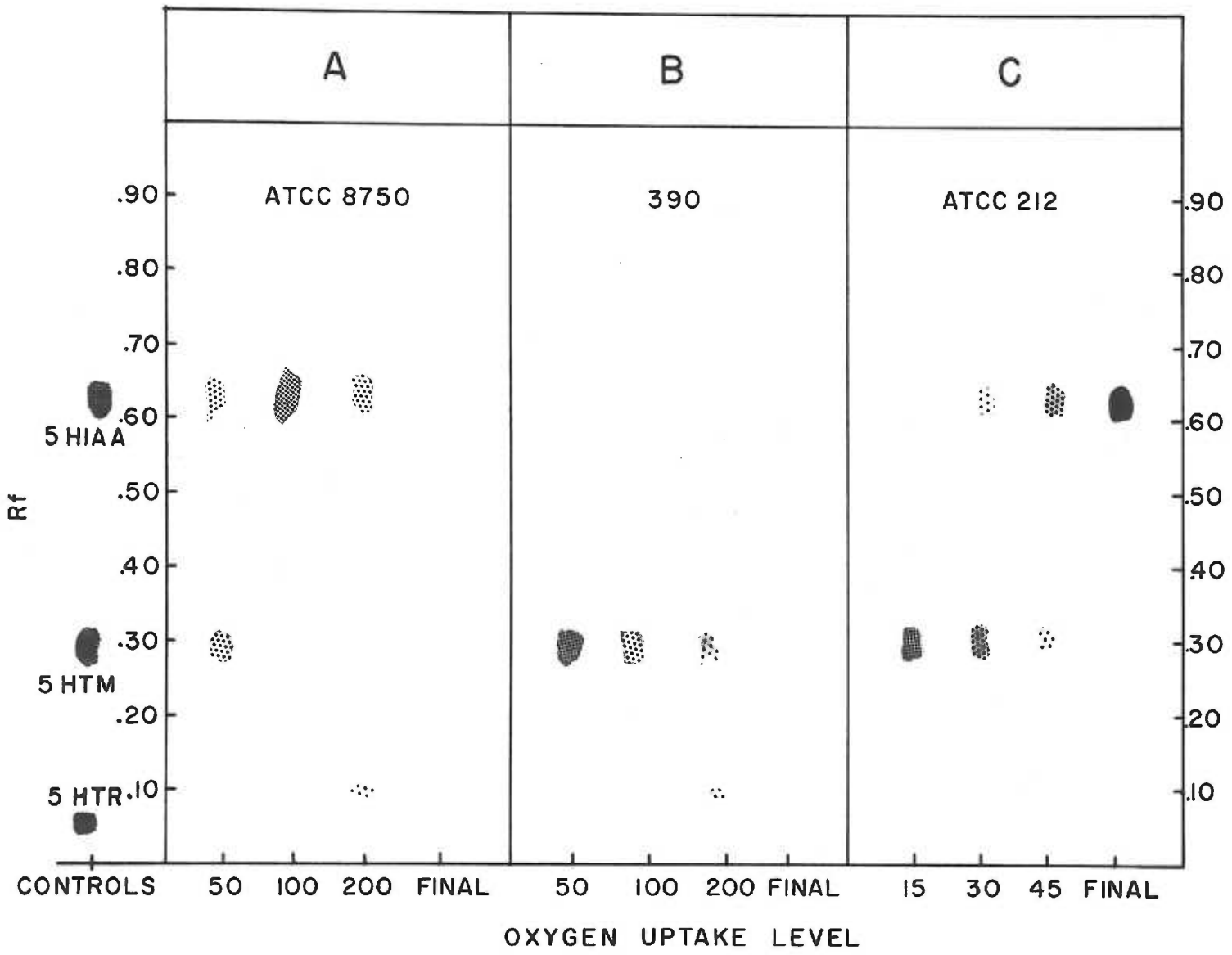

A
B
C
ATCC 8750
390
ATCC 212
.90
.80
.70
.60
.50
.40
.30
.20
.10
Rf
5 HIAA
5 HTM
5 HTR
CONTROLS
50
100
200
FINAL
50
100
200
FINAL
15
30
45
FINAL
OXYGEN UPTAKE LEVEL

Figure 5

A. Screening Procedure: Metabolism of 5HTM by A. faecalis, Group III, as Demonstrated by ATCC 212.

Key:
1. Control Flask Containing 5HTM Only.
2. 5HTM plus Cells Grown on Nutrient Agar.
3. 5HTM plus Cells Grown on Synthetic 5HTM Agar.

B. Oxidation of 5HTM and 5HIAA by A. faecalis, Group III, as Demonstrated by M-1.

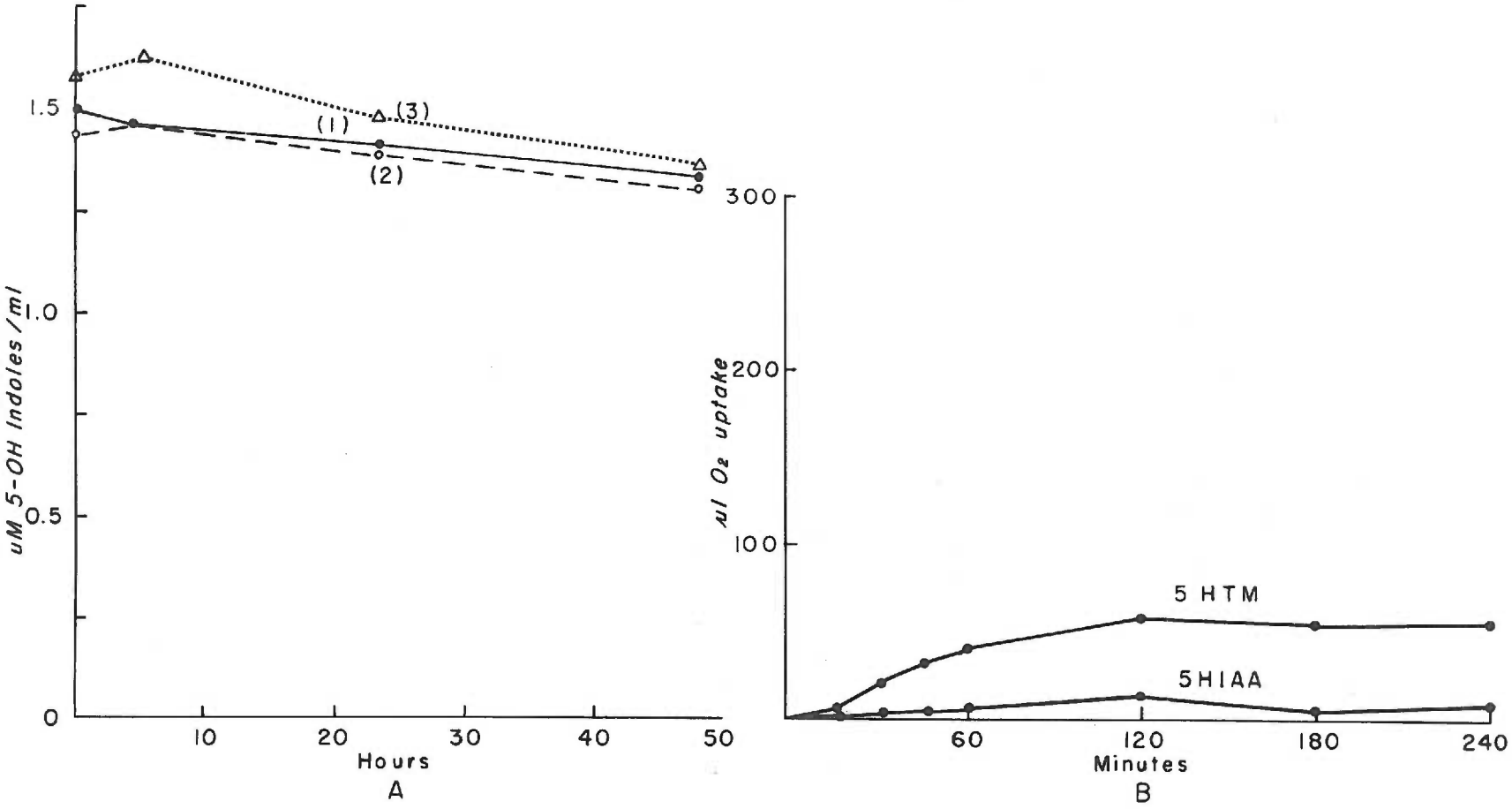

1.5
1.0
0.5
0
uM 5-OH Indoles /ml
(1)
(2)
(3)
10
20
30
40
50
Hours
A
300
200
100
µl O2 uptake
5 HTM
5HIAA
60
120
180
240
Minutes
B

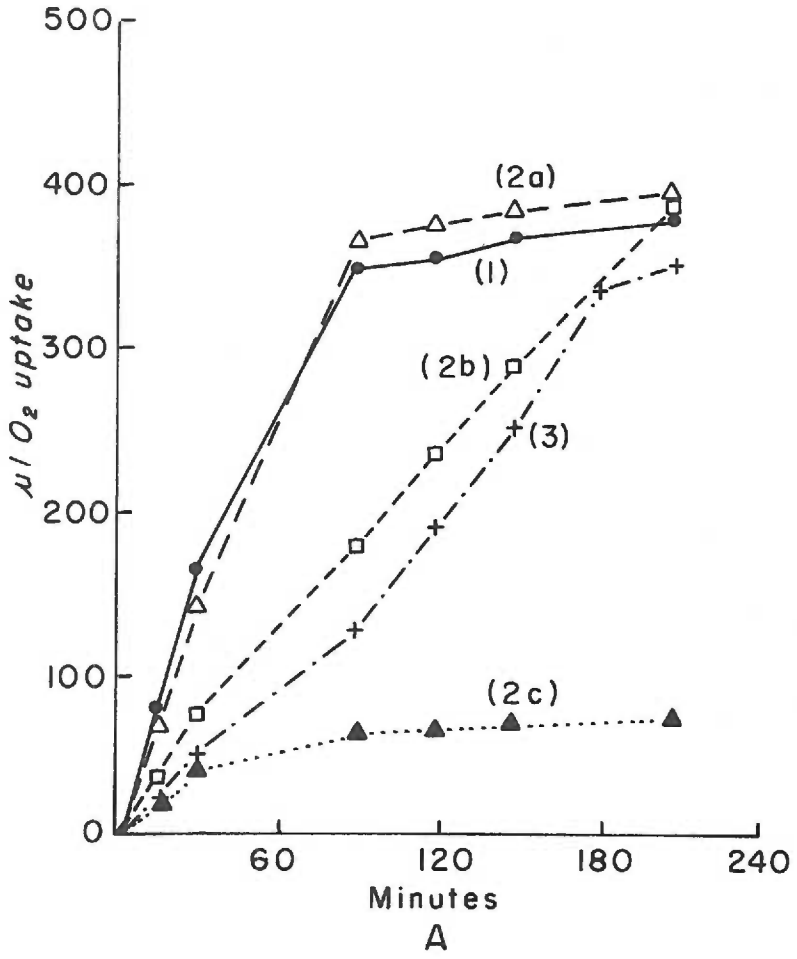
Figure 6

Effect of Arsenite and 8-hydroxyquinoline on the Oxidation of 5HTM by:

A. *A. faecalis*, 0-31

B. *A. faecalis*, 0-390

Key:
1. 5HTM
2a. 5HTM plus Arsenite 2 x 10^{-5}M
2b. 5HTM plus Arsenite 2 x 10^{-4}M
2c. 5HTM plus Arsenite 2 x 10^{-3}M
3. 5HTM plus 8-hydroxyquinoline 2 x 10^{-5}M


500
400
300
200
100
0
µl O₂ uptake
(2a)
(1)
(2b)
(3)
(2c)
60
120
180
240
Minutes
A

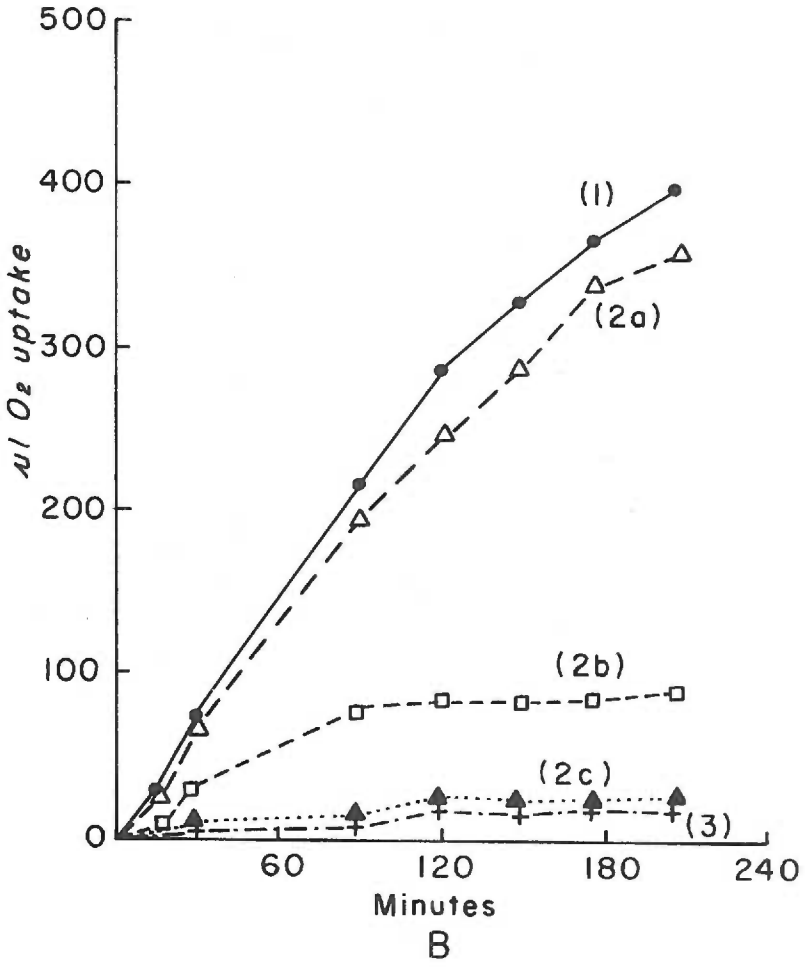

500
400
300
200
100
0
µl O₂ uptake
(1)
(2a)
(2b)
(2c)
(3)
60
120
180
240
Minutes
B

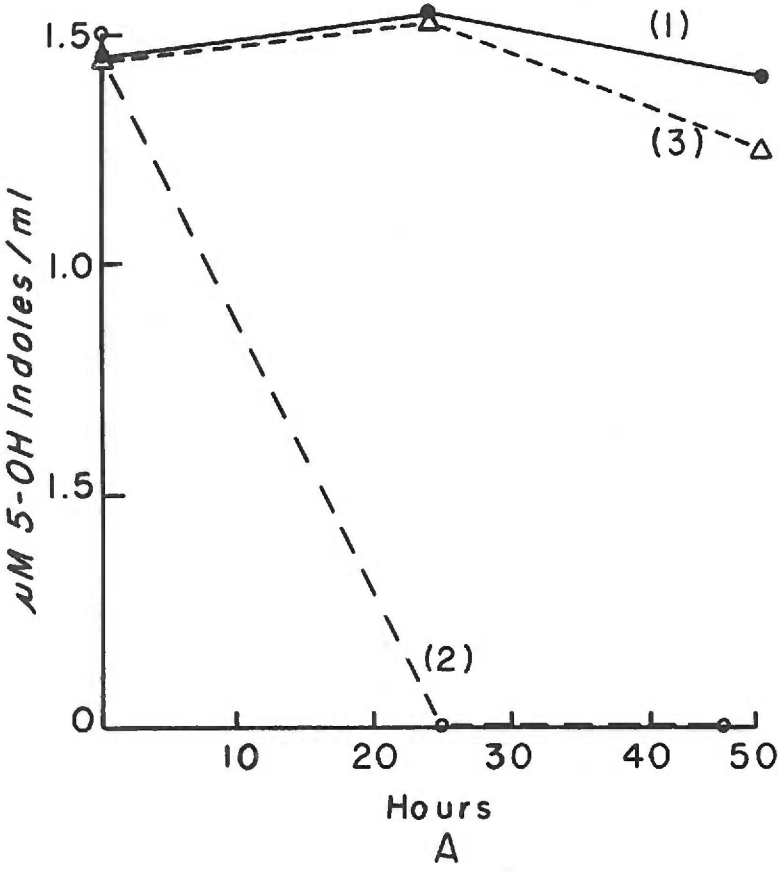
Figure 7

Screening Procedure: Effect of Iproniazid on the Metabolism of 5HTM by:

A. A. faecalis, ATCC 8750

B. A. faecalis, C-390

Key:
1. Control Flask Containing 5HTM without Cells.
2. 5HTM with Cells
3. 5HTM with Cells plus Iproniazid 6 x 10^{-3}M


1.5
1.0
1.5
0
(1)
(3)
(2)
10
20
30
40
50
Hours
µM 5-OH Indoles / ml
A

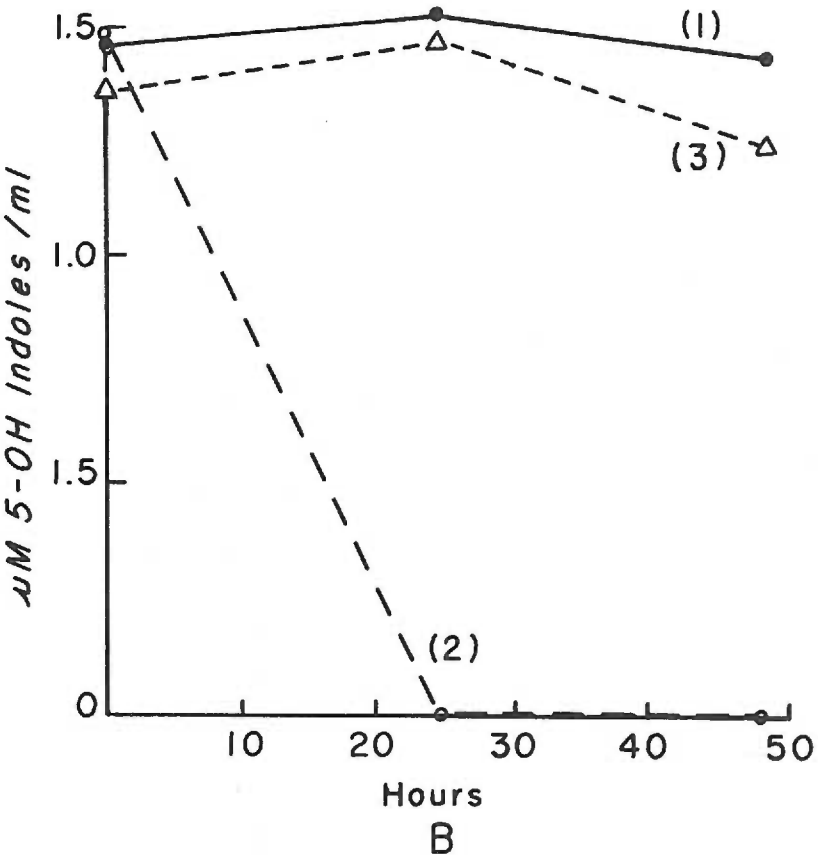

1.5
1.0
1.5
0
(1)
(3)
(2)
10
20
30
40
50
Hours
µM 5-OH Indoles / ml
B

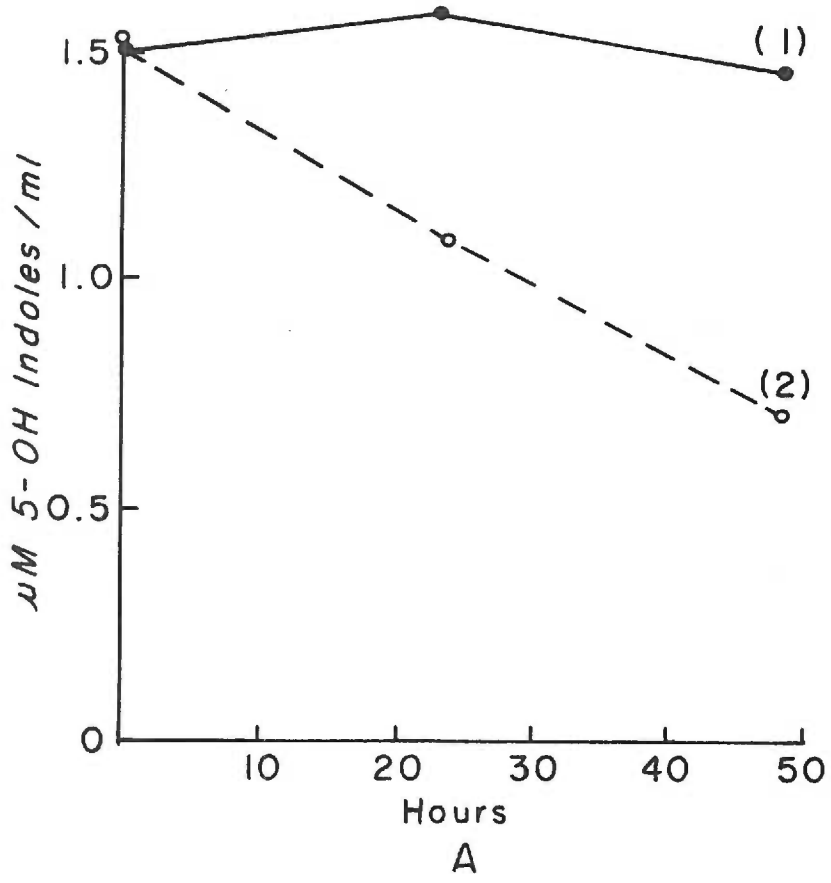
Figure 8

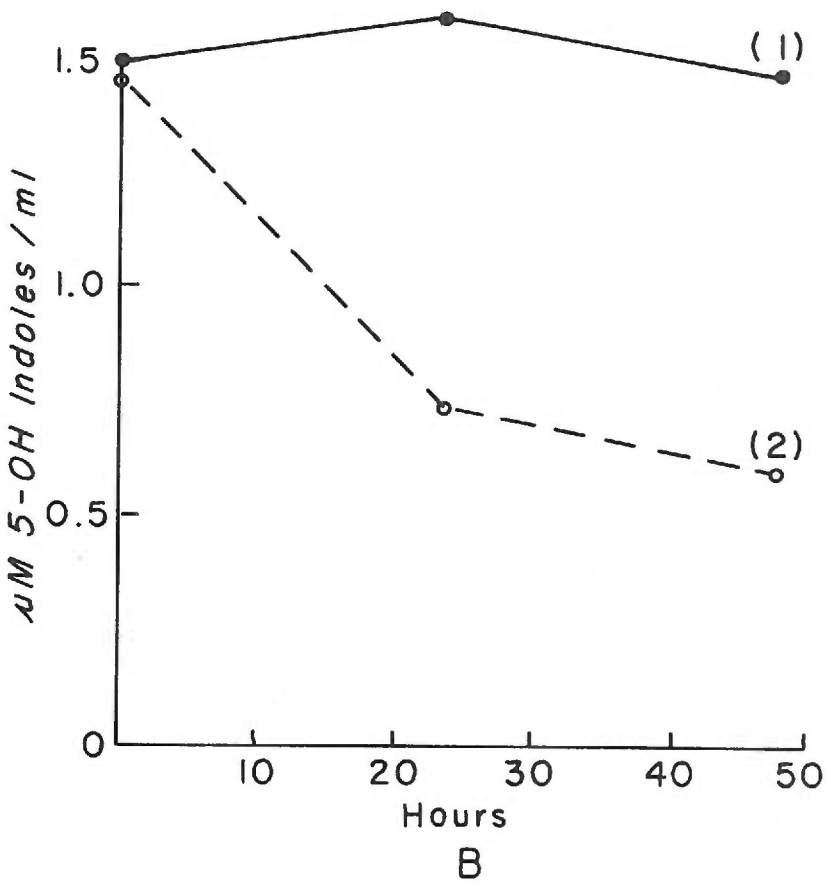
Screening Procedure: Metabolism of 5HTR by:

A. A. faecalis, O-390

B. A. faecalis, ATCC 8750

Key:
1. Control Flask Containing 5HTR without Cells
2. 5HTR with Cells


1.5
1.0
0.5
0
µM 5-OH Indoles / ml
(1)
(2)
10
20
30
40
50
Hours
A


1.5
1.0
0.5
0
µM 5-OH Indoles / ml
(1)
(2)
10
20
30
40
50
Hours
B