and II) suggest that these sites have been preempted in older rats by anaphylactic antibody directed against indifferent, naturally occurring antigens. This is consistent with the demonstration that anaphylactic antibodies, acquired by active immunization, interfere with sensitization by antibodies of another specificity in the PCA reaction of the guinea pig(17). Alternatively, another immunoglobulin, such as rat IgG, may block access to sensitization sites by steric hindrance. In either event these studies demonstrate that there is a normal mechanism by which a host may develop some resistance to anaphylactic sensitization.

Summary. Suspensions of rat peritoneal cells were sensitized for either anaphylactic or complement-dependent cytotoxic release of histamine. In both cases the reactivity of the mast cells *in vitro* appeared to be influenced by the coating of rat immunoglobulin previously acquired *in vivo*. Sensitization for the complement-dependent release of histamine resulted from the interaction of rabbit anti-rat immunoglobulin antiserum with host IgG on the mast cell. Mast cells from young rats failed to participate in this reaction presumably because of an insufficient coating of IgG. In contrast, cells from young or germ-free rats had a much greater capacity for *in vitro* passive sensitization by anaphylactic antibody than cells of older rats. In older rats the sensitization sites may have been preempted by anaphylactic antibody directed against indifferent, naturally occurring antigens, or another immunoglobulin may block access to sensitization sites by steric hindrance.

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Xanthurenic Acid Dehydroxylation by Fecal Microflora. (30585)

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Takahashi and Price reported formation of 8-hydroxyquinaldic acid from xanthurenic acid *in vivo* in 1958(1). Subsequently, Kaihara and Price concluded that this reaction depends on the gut microflora since 8-hydroxyquinaldic acid was not excreted in the urine of rabbits pretreated with the intestinal antibiotic neomycin(2).

In vitro dehydroxylation of caffeic acid to

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TABLE I. Chromatography of Xanthurenic Acid and Its Dehydroxylated Derivatives.

Compound	R_f values*		UV light fluorescence	Color with DSA†
	$CHCl_3$-Ac-H_2O	20% KCl		
Xanthurenic acid	.03	.25	white	red
Kynurenic acid	.15	.40	blue	none
8-Hydroxyquinaldic acid	.57	.39	orange-red	orange-red
Quinaldic acid	.85	.77	weak	none

* Bottom phase of chloroform-acetic acid-water (2:1:1) in first direction; 20% potassium chloride in second direction.

† DSA = diazotized sulfanilic acid spray in 20% HCl followed by 20% sodium carbonate.

yield *m*-hydroxyphenylpropionic acid was recently reported by Booth and Williams(3). Rat and rabbit cecal contents and sheep rumen liquor were good sources of dehydroxylating activity. In a later report they showed that freshly voided feces from rats, humans, and chickens were also active dehydroxylating media due, presumably, to the presence of specific anaerobic microorganisms(4).

The present study reports evidence of *in vitro* dehydroxylation of xanthurenic acid in an artificial cecum.

Takahashi *et al* also reported the conversion of kynurenic acid to quinaldic acid by humans and rats(5). Therefore kynurenic acid was included in these dehydroxylation studies.

Materials and methods. The 8-hydroxyquinaldic acid was synthesized by the method described by Irving and Pinnington(6). Quinaldic acid was prepared according to the procedure of Campbell *et al*(7). Kynurenic (4-hydroxyquinaldic), and xanthurenic (4,8-dihydroxyquinaldic) acids were from commercial sources. All compounds were checked for phenolic impurities by the paper chromatographic procedure described below.

Fecal fluid was prepared by trituration of freshly voided feces in phosphate buffer (pH 7, 0.15 M). Four ml of buffer solution was used per gram of feces. After trituration, the fecal suspension was forced through a triple layer of cheese cloth. Rabbit fecal fluid was prepared only from the freshly voided night feces. Thirty-five-ml aliquots of the filtrate were used for each treatment. For cecal fluid and rumen fluid the trituration step was omitted and 1 M phosphate buffer was used to minimize dilution of the filtered fluid.

The artificial cecum was set up by bubbling hi-pure dry nitrogen through a 125-ml gas washing bottle containing buffered fecal or cecal fluid and added substrate. A constant-temperature water bath at 38°C simulated normal body temperature during the overnight (18-hour) incubation.

After the overnight incubation, the contents of each gas bottle were acidified to pH 2 with concentrated HCl, saturated with solid sodium chloride, and gently extracted by hand in a separatory funnel with four separate equal volumes of ether. The combined ether extracts were evaporated to dryness by vacuum distillation under nitrogen. The residue was taken up in 2 ml of acetone and a suitable aliquot (usually 0.1 ml), was then separated by ascending 2-dimensional paper chromatography as described previously(3). Since kynurenic and quinaldic acids give no color with diazotized sulfanilic acid-sodium carbonate, exposure of the chromatogram to iodine vapor was used to produce brown spots which indicated the presence of these acids.

Results. The chromatographic properties of the authentic samples of xanthurenic, 8-hydroxyquinaldic, kynurenic, and quinaldic acids are tabulated in Table I. Chromatographic examination of the ether extracts of fecal incubates to which xanthurenic acid had been added established that 8-hydroxyquinaldic acid had been formed. Nothing was detected in the area corresponding to 8-hydroxyquinaldic acid in chromatograms of control incubates of fecal fluid without added xanthurenic acid or in incubates of xanthurenic acid without fecal fluid.

For comparative purposes urine collected from rats fed xanthurenic acid (400 mg%),

was acidified, saturated with sodium chloride, ether extracted, and aliquots of the ether solubles examined chromatographically. Positive evidence of 8-hydroxyquinaldic acid was detected. Urine from rats with unsupplemented diets contained no detectable 8-hydroxyquinaldic acid.

Appreciably more xanthurenic acid was dehydroxylated in the artificial cecum containing rabbit fecal fluid than was formed *in vivo* and excreted in the urine of rats. For example, 30 mg of crystalline 8-hydroxyquinaldic acid was isolated from a 400 ml rabbit fecal fluid incubate to which 100 mg of xanthurenic acid was added. The crystalline material was obtained first by extraction of the ether solubles with chloroform followed by elution of the appropriate areas from papergrams representing the chloroform solubles of the ether extract of an acidified 40-hour incubate. The physical properties of the isolated crystalline material were identical with those of synthetic 8-hydroxyquinaldic acid.

Rat and human fecal fluid suspensions, sheep rumen fluid, and rat and rabbit cecal fluids were capable of converting xanthurenic acid to 8-hydroxyquinaldic acid in the artificial cecum. The conversion, however, was far less that that by rabbit fecal fluid.

Dehydroxylation of kynurenic (4-hydroxyquinaldic) acid to produce quinaldic acid was also observed when rabbit or rat fecal suspensions were incubated with kynurenic acid. However, the quinaldic acid formed, as judged by its reaction on papergrams to iodine vapor, was limited to traces (less than 1%) of the kynurenic acid added. When kynurenic acid was fed to rats (150 mg) no quinaldic acid could be detected in the urine.

Discussion. The artificial cecum technique provided direct *in vitro* evidence for the dehydroxylation of xanthurenic acid to yield 8-hydroxyquinaldic acid. These results agree completely with those of Kaihara and Price (2) who abolished the excretion of 8-hydroxyquinaldic acid in the urine of rabbits by pretreatment with neomycin. Our results confirm that intestinal microorganisms are responsible for the dehydroxylation of xanthurenic acid. Pertinent to these findings is the report by Bryan *et al*(8) that certain tryptophane metabolites, including xanthurenic and 8-hydroxyquinaldic acids, may be of causal significance in the etiology of human and bovine bladder cancer.

The ortho dihydroxy configuration in caffeic (3,4-dihydroxycinnamic) acid which was previously reported to undergo dehydroxylation(3), is obviously not essential for dehydroxylation, since xanthurenic acid has a 4,8-dihydroxy grouping. The relatively poor yield of quinaldic acid when kynurenic (4-hydroxyquinaldic) acid was either fed to rats or incubated in the artificial cecum with rabbit fecal fluid suggests that the monohydroxy configuration in kynurenic acid is a poor substrate for dehydroxylation. Further, no quinaldic acid was formed when 8-hydroxyquinaldic acid was the substrate. A critical evaluation of substrate specificity, however, must await the isolation and culture of the specific microorganisms responsible for dehydroxylation.

Summary. In vitro dehydroxylation of xanthurenic acid has been demonstrated in an artificial cecum charged with rabbit fecal fluid. The dehydroxylated product, 8-hydroxyquinaldic acid, was isolated and crystallized. The results clearly indicate that intestinal microorganisms are responsible for the dehydroxylation reaction. Dehydroxylation of kynurenic acid to produce quinaldic acid was only qualitatively detectable in the same system.

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