bacilli (Table). Furthermore the amount of methylmercury produced by the obligate anaerobes and lactobacilli was much smaller than that by the other bacterial types tested. These observations suggest that lactobacilli and obligate anaerobes play only a minor role in the methylation of mercuric chloride in the human gut.

Using a suspension of caecal contents of the rat, we have shown that the amount of methylmercury synthesized from $HgCl_2$ by the caecal contents was higher (up to 26 ng methylmercury formed/g caecal contents) than by pure cultures of intestinal bacteria (ROWLAND, unpublished observation), so it would appear that in caecal suspensions several types of bacteria may act synergistically to synthesize methylmercury, or possibly that the methylation of $HgCl_2$ can be catalyzed by intestinal enzymes other than those of bacterial origin. These possibilities are now being investigated.

Summary. Most strains of staphylococci, streptococci, yeasts and *E. coli* isolated from human faeces, could synthesize methylmercury compounds. In contrast, few strains of obligate anaerobes could do so. Up to 6 ng methylmercury/ml were formed in 44 h from 2 µg mercuric chloride.

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Mechanism of Urea Nitrogen Binding by Proposed Oxidized Starch Gastrointestinal Absorbents

In recent years, the prospect has been considered of ameliorating the uremic state by oral administration of materials to absorb selectively the excess products of metabolism which might diffuse into the gastrointestinal tract. Reports by GIORDANO[1] and by SPARKS[2] have indicated that ingestion of polyaldehyde starch increases fecal N excretion and may lower blood urea N in animals and in man. The mechanism of this effect presumably involved complexing urea which had diffused into the gut, thus preventing reabsorption of the sizable portion of the urea pool which may be available by this route[3]. Attempts to demonstrate urea binding in vitro by various oxidized starch preparations, however, have met with variable success[4] partly because the rapid depolymerization of the polyaldehyde prevents separation of the binding product during equilibrium dialysis or gel filtration experiments. Reports of urea binding by oxidized starch have involved equilibrium dialysis experiments at high urea concentration and over long duration[1, 2, 4]. In this report, we present evidence to indicate that any apparent binding of urea by polyaldehyde starch is due to conversion to ammonium ion through cyanate ion, and that direct binding of ammonium ion is a more plausible basis for activity.

Methods. Samples of periodate oxidized starch were obtained through the courtesy of Dr. C. GIORDANO (Naples), or were prepared in our laboratories by Dr. A. BERGER (Morton Grove). Binding of small molecules was determined by equilibrium dialysis conducted in Visking dialysis bags (A. H. Thomas Co., Philadelphia, Pa., USA) of about 16 mm diameter and containing 10 ml of oxidized starch solution or suspension in ligand solution in 0.2 *M* pH 7.4 sodium phosphate buffer. Dialysis was accomplished in 50 or 65 ml of external solution identical to that inside of the bag, without the absorbent. After the appropriate time, the retentates were transferred quantitatively to tared flasks for volume determinations. The N content of retentates and diffusates were determined by the Dumas method, and after correction for volume factors, the extent of binding was calculated. For these purposes, each repeating unit of oxidized starch was taken as 160 Daltons. The binding of ^{14}C-labeled urea or cyanate (International Nuclear and Chemical Corp., Cleveland, O., USA) was determined as described or by scintillation counting.

Results. Several determinations of urea binding to oxidized starch produced the results summarized in the Table. In the equilibrium dialysis system used here, no binding was observed after a few hours of dialysis, and the results at 20 h were variable. At physiological urea concentrations, less than 0.1 mole of urea per unit of polymer was bound.

The figures for molar binding shown in the Table represented minimum values, since oxidized starch was demonstrated in our laboratory and by others[4] to depolymerize rapidly at room temperature. These depolymerized fragments were of 1200 Daltons or less, since they appeared in the included volumes of small pore polyacrylamide gel filtration columns (Size P-2, Bio Gel Corporation, Richmond, California, USA).

Previous experience in our laboratory had indicated that small molecule binding by macromolecules was a rapid process. Thus, the requirement for long time periods to demonstrate urea binding seemed anomalous, and we examined binding of related compounds, including ^{14}C-labeling to reduce the requirement for many N analyses.

Binding of urea by oxidized starch as determined by equilibrium dialysis

Urea concentration		Moles of urea bound/repeating unit[a]			
Molar	(mg/100 ml)	Naples		Morton Grove	
0.050	(300)	0.043 ± 0.010	(5)	0.032 ± 0.016	(4)
1.000	(6000)	0.432 ± 0.068	(7)	0.281 ± 0.102	(6)

[a] Mean ± SD (number of trials) for binding to 700 mg of oxidized starch in 10 ml of buffer. External solution volumes was 65 ml. There were no significant differences between sources of starch. Binding was determined by N analyses; the molecular weight of a repeating unit was taken as 160.

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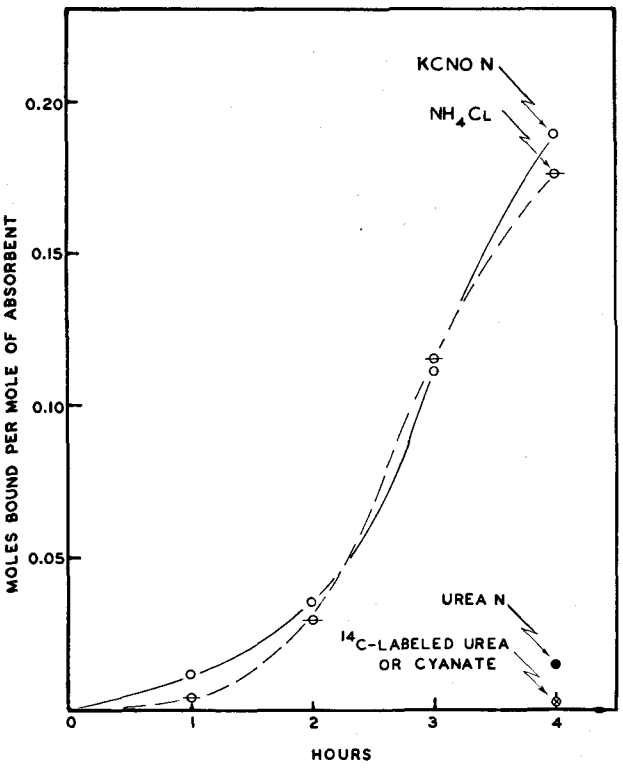
As shown in the Figure, ammonium or cyanate ion binding as determined by N analyses during equilibrium dialysis occurred more rapidly and in greater amount than did binding of urea. When determined by isotope, however, no binding of the carbon atoms of urea or KOCN was detectable. It was evident, therefore, that neither urea N nor cyanate were bound to oxidized starch as unchanged entities.

Discussion. The data presented here demonstrated that binding of urea to oxidized starch was negligible, but that binding of nitrogenous products of urea hydrolysis proceeded rapidly. Since the carbon atom of cyanate ion was not bound, and the degrees of molar binding of anionic cyanate and cationic ammonium ion were similar it was apparent that complete decomposition through cyanate was essential for N binding by oxidized starch. The kinetics of urea conversion in water to cyanate, and conversion of cyanate to CO_2 and ammonium ion have been reviewed by HAGEL et al.[5]. In the presence of polyaldehyde it seemed likely that the equilibrium was shifted towards ammonium ion formation:

$$(NH_2)\,CO \rightleftharpoons NCO^- + NH_4^+$$
$$NCO^- + H_2O \rightleftharpoons HCO^-_3 + NH_4^+$$

→ N-aldehyde oxidized starch complex

The above observations thus accounted for the long time required to demonstrate 'urea' binding by aldehyde starch preparations. Furthermore, in view of these data, a proposed mechanism for removal of fecal N by such absorbents must include conversion of gut urea into ammonium ion, a phenomenon, well characterized in man[3,6]. While the complete mechanism for increased fecal N removal by carbohydrates may not necessarily involve N-binding[7], the fact that uremic patients are unable to hydrolyze more urea than normal individuals[8] may yet leave an important therapeutic role for an innocuous gastrointestinal ammonium ion binding agent.



Time course of solute binding by oxidized starch during equilibrium dialysis. Each prospective ligand was 0.04 *M* and dialyses were performed with stirring at room temperature as described in Methods.

Summary. By use of ^{14}C label it was demonstrated that apparent binding of urea N to polyaldehyde starch was probably preceeded by hydrolysis to ammonium ion. Thus direct urea binding was not the mechanism through which ingested polyaldehyde starch might increase fecal N excretion in uremic patients.

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Further Investigations into the Effects of Baclofen (Lioresal) on the Isolated Spinal Cord

In a number of neural preparations, the effects of baclofen (Lioresal), an anti-spastic drug, are different from those of γ-aminobutyric acid (GABA), a putative inhibitory transmitter, although the two substances share a similar chemical structure[1–4]. In a previous report, it was shown that baclofen and GABA reduce the spontaneous acetylcholine (ACh) release from the isolated spinal cord but that the extent and time course of their action are different[4]. The effect of these two compounds has now been tested both on the spinal root potentials and on the electrically-evoked release of ACh, which is thought to be the neurotransmitter of motor axon collaterals[5].

Methods. Frogs (*R. temporaria*) were used. The spinal cord was removed, hemisected sagitally and placed in a 500 µl bath at 14 °C. The ventral root potential (VRP) evoked by orthodromic stimulation of the corresponding dorsal root, or the dorsal root potential (DRP) evoked by antidromic stimulation of the corresponding ventral root were recorded with Ag/AgCl electrodes and displayed on a storage oscilloscope and on a pen recorder as already described[6,7]. The ACh release was measured every 10 min as previously reported[4,6].

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