neurohypophyseal hormones and analogs. It appears that substances which affect the uterine motility not only change the intensity of the rhythmic contraction, but can also modify the time course of the individual contraction.

Three alternative explanations of these findings come to mind: 1. the differences in the time course of contractions are determined by the complicated nature of tonic/phasic relationships and by different receptor reserve for different substances; 2. separate receptors for different substances occur in myometrium; the very short duration of the LVP-induced contraction is particularly striking in this context; 3. the myometrium contains two populations of receptors, one for contraction and one for relaxation; different substances may have different affinities for each of the receptor populations.

The finding that the area under the contraction curve is a sensitive measure of the response of the uterus to high concentrations of hormone allowed us to use this parameter for rapidly measuring residual hormonal activities in a large number of samples after incubations of neurohypophyseal hormones with degradative enzymes, without prior dilution of samples[4]. The method was found to be valid for deamino-oxytocin and bradykinin as well as for oxytocin.

In summary, the time-response patterns of the isolated rat uterus to neurohypophyseal peptides was studied. At high doses of peptide a concentration-related effect upon the duration of the first contraction was found and the area under this contraction is useful for the bioassay of oxytocin and certain other peptides.

Zusammenfassung. Halbwertzeit und Zeit-Effekt-Integral für die erste Oxytocin-Kontraktion der isolierten Gebärmutter der Ratte wurden untersucht. Eine Verlängerung der Kontraktion wurde bei erhöhter Dosis beobachtet. Entsprechende unterschiedliche Abhängigkeiten für verschiedene uterotonisch wirkende Peptide sind deutlich. Zur Auswertung des Bioassays von Oxytoxin und gewisser Analogen wird die Verwendung des Integrals vorgeschlagen.

M. WAHRENBURG, M. KOIDA, RODERICH WALTER and V. PLIŠKA

Department of Physiology, University of Illinois Medical Center, Chicago (Illinois 60612, USA), and Institute of Molecular Biology and Biophysics, Swiss Federal Institute of Technology, 8049 Zürich-Hönggerberg (Switzerland), 8 April 1975.

The Methylation of Mercuric Chloride by Human Intestinal Bacteria

The ability of microorganisms in sediments of lakes and rivers to transform inorganic mercury to the far more toxic methylmercury and dimethylmercury is a well documented phenomenon[1,2]. There is now evidence that ingested mercuric chloride ($HgCl_2$) may be methylated, in vivo, in the rat intestine[3] and, in vitro, by human faeces[4]. However, one cannot infer from these experiments that the microbial flora of the intestine is responsible for the methylation reaction, since the gut contents contain several sources of metabolic activity other than bacteria[5]. We would like to present data on the ability of pure cultures of bacteria and yeasts, isolated from human faeces, to convert $HgCl_2$ to methylmercury.

Strains of *Escherichia coli*, streptococci, staphylococci, bacteroides and bifidobacteria were inoculated into a medium containing 0.1 *M* potassium phosphate buffer, pH 7.0, Bacto-peptone, Bacto-tryptone, yeast extract and D-glucose, each at 0.5% (w/v). Strains of lactobacilli were cultured in Oxoid MRS broth and yeasts in Oxoid Sabouraud medium. The cultures were incubated for 44 h at 37 °C under aerobic conditions, except for lactobacilli (under carbon dioxide) and bacteroides and bifidobacteria (under oxygen-free nitrogen) and in the presence of $^{203}HgCl_2$ (Radiochemical Centre, Amersham) at a final concentration of 5 µg/ml. Before use the solution of $^{203}HgCl_2$ was shaken with redistilled benzene to remove any organic impurities.

At the end of the incubation, methylmercury was extracted from the incubation mixture by the method described by WESTÖÖ[6]: the mixtures (2 ml) were acidified with 0.4 ml concentrated HCl and extracted with 2.4 ml redistilled benzene. A portion of the benzene extract was then chromatographed on silica gel G using chloroform-*n*-hexane (90:10, v/v) as developer[7]. The Rf values of methylmercuric chloride and $HgCl_2$ under these conditions were 0.85 and 0.2 respectively. The position of methylmercuric chloride was visualized by spraying with 0.04% (w/v) dithizone in chloroform and the zone scraped into a scintillation vial and counted for radioactivity. The identity of methylmercuric chloride was further confirmed by chromatography on silica gel G with *n*-hexane-acetone (70:30, v/v) as solvent[8].

Methylmercury was produced by a major proportion of strains of staphylococci, streptococci, yeasts and *E. coli*, but by only a small percentage of obligately anaerobic bacteria (bacteroides and bifidobacteria) and lacto-

Methylmercury synthesis by intestinal bacteria

Microorganism	No. tested	No. positive	Range (ng CH_3HgCl formed/ml/44 h)
Streptococci	6	4	2.1–5.8
Staphylococci	10	6	0.5–5.0
E. coli	5	3	0.9–3.0
Yeasts	9	4	0.7–1.7
Lactobacilli	9	1	0.5
Bacteroides / Bifidobacteria	10	3	0.4–0.6

The strains were incubated with $^{203}HgCl_2$ (5 µg/ml) for 44 h at 37 °C and any methylmercury formed was extracted into benzene and subjected to thin-layer chromatography as described in the text.

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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.

I. R. ROWLAND, P. GRASSO and M. J. DAVIES

British Industrial Biological Research Association, Woodmansterne Road, Carshalton (Surrey, SM5 4DS, England), 29 April 1975.

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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