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IMMUNOCHEMISTRY OF THE COMMON ANTIGEN OF ENTEROBACTERIACEAE (KUNIN)

Relation to Lipopolysaccharide Core Structure

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An antigen common to most members of the family Enterobacteriaceae was first described by Kunin and coworkers (1, 2) and confirmed by Whang and Neter (3) and by Vosti et al. (4). Common antigen (CA)[1] was detected by passive hemagglutination with rabbit antiserum to *Escherichia coli* 014. This antiserum agglutinated red cells coated with crude lipopolysaccharides from most *E. coli* O serotypes (1) and from some species of *Salmonella, Shigella, Proteus,* and *Aerobacter* (2). Aoki et al. (5) demonstrated the presence of CA in the cell wall of species of *E. coli, Klebsiella-Aerobacter,* and *Proteus* by the indirect immunofluorescence technique using anti-*E. coli* 014 antiserum. Only immunization with *E. coli* 014 and to a lesser extent with *E. coli* 056, 0124, and 0144 gave rise to antibodies against CA (1). Whang et al. (6) also demonstrated that the immune response to CA was markedly suppressed when ethanol-soluble CA from *S. typhimurium* (7) was injected together with lipid A from *E. coli* or *Salmonella.* Hemagglutination-inhibition experiments indicated that the CA activities of lipopolysaccharide preparations from different *E. coli* O serotypes varied (8, 9). *E. coli* 014 contained large amounts of common antigen. Kunin (8) also demonstrated high amounts of CA in rough mutants of *E. coli.* He found CA to be heat stable at neutral pH, resistant to trypsin, and also resistant to periodate oxidation.

Recent studies from this laboratory have demonstrated that the common enterobacterial antigen is immunologically related to the colon antigen of ulcerative colitis. Thus, hemagglutination between serum from ulcerative colitis patients and red cells coated with colon antigen extracted from germfree rats was inhibited by *E. coli* 014 lipopolysaccharide preparations (9). Furthermore, hemagglutination between *E. coli* 08-sensitized erythrocytes and rabbit anti-*E. coli* 014 serum was inhibited by germfree rat colon antigen (9). Since type 08 and 014 specificities are unrelated, the latter experiments indicate that CA in *E. coli* 014 is the antigen which cross-reacts with colon antigen. In order to

[1] *Abbreviations used in this paper:* CA, common antigen; HU, hemagglutinating units; KDO, ketodeoxyoctonate; LPS, lipopolysaccharide.

establish the structural basis of this cross-reaction we initiated a study of the chemical nature of CA as it appears in *E. coli* 014.

Materials and Methods

Bacteria.—*E. coli* 014:K7:H-(SU 4411/41) was kindly provided by Dr. F. Ørskov, International *Escherichia* Center in Copenhagen. *E. coli* 08 and *E. coli* 075 were obtained from Dr. K. Lincoln, University of Gothenburg, Gothenburg, Sweden. The latter two strains were isolated from patients with urinary tract infections. The bacteria were grown for 18 hr at 37°C on a synthetic substrate containing sodium lactate as the carbon source (10). Extraction of lipopolysaccharide (LPS) from bacteria washed twice with phosphate-buffered saline, pH 7.3, was performed by the hot phenol-water procedure of Westphal et al. (11). All LPS were treated with ribonuclease from bovine pancreas (type XII-A, Sigma Chemical Co., St. Louis, Mo.). Fractionation of the acetic acid hydrolysate of *E. coli* 014 LPS was performed on a Sephadex G-50 column (88 × 1.5 cm) with pyridine acetate (pH 5.4) as eluant (12). Downwards flow was maintained at a constant rate with a peristaltic pump. The same material was also gel filtered in phosphate-buffered saline, pH 7.3, on a calibrated Bio-Gel P-10 column (Bio-Rad Laboratories, Richmond, Calif.) (85 × 1.5 cm). Dextran fractions of defined molecular weights were used for calibration.

Antisera.—Rabbits were primed intraperitoneally with 5 mg of lyophilized heat-killed organisms, and boostered 5 wk later by three intraperitoneal injections of 5 mg every 5th day. Blood was collected by heart puncture 6, 8, and 10 days after the last injection.

Gel filtration of antiserum was performed on a Sephadex G-200 column (Pharmacia Fine Chemicals, Uppsala, Sweden) (4 × 115 cm) using phosphate-buffered saline, pH 7.3, for elution.

Quantitative Determinations of Sugar Components.—Hexosamine was determined according to a modified Elson-Morgan procedure (13). Nitrogen was assayed by the ninhydrin method, and hexoses + heptoses by the orcinol method (13). Ketodeoxyoctonate (KDO) was determined by the semicarbazide method and by the thiobarbituric acid assay after acid hydrolysis as described by Dröge et al. (14). As pointed out by these workers, the semicarbazide assay measures the total KDO content, while the thiobarbituric acid method only detects KDO liberated after acid hydrolysis. *o*-Acyl (*o*-acetyl) was determined by the alkaline hydroxylamine method (13).

For identification and quantitation of the sugar components in the original *E. coli* 014 LPS preparation and its isolated immunologically active fragment, these materials were also analyzed by gas-liquid chromatography (15) after hydrolysis, as their alditol acetates. The identity of the alditol acetates was confirmed by mass spectrometry (16). In order to release hexosamine residues in a quantitative yield, the LPS was hydrolyzed with 0.5 N sulfuric acid at 100°C for 4 hr, neutralized with barium carbonate, concentrated to dryness, and acetylated with acetic anhydride pyridine. This procedure was repeated two times, however the acetylation step was omitted the last time. The resulting hydrolysate was then converted into alditol acetates. Sugar components in lipid A are not detected by this procedure.

Amino acid analysis was performed on a Technicon amino acid analyzer (Technicon Co., Inc., Stockholm) on samples hydrolyzed for 24 hr in constant boiling HCl at 111°C (17). We are grateful to Dr. Ingrid Lindström for performing these analyses.

Immunological Methods.—Passive hemagglutination was performed as earlier described (9). For sensitization of 0.025 ml of packed sheep erythrocytes with LPS, heated for 1 hr at neutral pH, the following optimal concentrations were used: *E. coli* 014 0.03 mg/ml; *E. coli* 08 0.05 mg/ml; *E. coli* 075 0.25 mg/ml. For hemagglutination inhibition 4–8 hemagglutinating units (HU) of antibody were used (9). Quantitative precipitin analysis (13) was carried out by a microtechnique, using the ninhydrin procedure for nitrogen determination (18).

RESULTS

Chemical Composition

The *E. coli* 014 LPS preparation used for this study was contaminated with relatively large amounts of nucleic acids. After prolonged treatment with ribonuclease, nucleic acids still accounted for 5–10% of the dry weight. No further purification was attempted. All material was of high molecular weight and eluted in the void volume from a Sepharose 4B column (exclusion limit for polysaccharides mol wt $\sim 5 \times 10^6$). The composition of this material is given in Table I. As can be seen, total carbohydrate, excluding KDO, amounted to ap-

TABLE I

Composition of E. coli 014 and E. coli 075 Lipopolysaccharide Preparations*

Compounds	% of dry weight	
	E. coli 014	*E. coli* 075
Rhamnose	0	16.5
Ribose	1.6	0.6
Mannose	0	18.6
Galactose	6.8	21.7
Glucose	14.4	6.9
Heptose‡	3.7	Trace
Glucosamine	3.5	19.8
o-Acetyl	3.2	0.3
Nitrogen	3.8	4.3
Amino acids (total)§	1.0	ND‖

* Sugars were determined as alditol acetates by gas-liquid chromatography.

‡ For calculation of heptose a response factor 1.6 was used.

§ Amino acids found: aspartic acid 0.20%; threonine 0.09%; serine 0.11%; glutamic acid 0.24%; glycine 0.25%; alanine 0.11%.

‖ ND, not done.

proximately 30% of the dry weight. This was in sharp contrast to a lipopolysaccharide preparation from *E. coli* 075, investigated in parallel (Table I). The carbohydrate content of this LPS was 84%. The sugars of the *E. coli* 014 LPS were only those known to be present in the basal core structure of *E. coli* and *Salmonella*. *E. coli* 014 LPS contained about 3.2% *o*-acetyl as determined colorimetrically. This concentration was about 10 times higher than that found in *E. coli* 075 LPS. The identity of acetyl was established by gas-liquid chromatography. Nitrogen (3.8%) was higher than would have been expected for a lipopolysaccharide. Contaminating nucleic acid may account for this. As can be seen, only very small amounts of amino acids (total 1%) were present.

Immunological Properties

Rabbit antisera to *E. coli* 014 agglutinated red cells coated with LPS from *E. coli* 014 as well as from other *E. coli* 0 types, notably *E. coli* 08. This indicated that they contained antibodies to CA. Alkali treatment of *E. coli* 014 LPS (0.25 M NaOH, 3 hr 50°C) reduced its *o*-acyl content to 0.3%. As can be seen from Table II, this treatment markedly changed the immunological properties of the lipopolysaccharide. Thus, alkali-treated *E. coli* 014 LPS was a very poor inhibitor of hemagglutination between anti-*E. coli* 014 serum and red cells sensitized with untreated *E. coli* 014 LPS. In contrast, when inhibition of hemagglutination of *E. coli* 08-sensitized erythrocytes was studied, both preparations had equal and high inhibiting power. This indicated that CA specificity was not changed by alkali treatment. Both alkali-treated and untreated *E. coli* 014 LPS also inhibited hemagglutination of red cells coated with

TABLE II

Hemagglutination-Inhibition Experiment with Rabbit Antiserum to E. coli 014 and Red Cells Sensitized with LPS from E. coli 014, Alkali-Treated E. coli 014, or E. coli 08. Alkali-treated and Untreated E. coli 014, E. coli 08, and E. coli 075 LPS Were Used As Inhibitors

Red cells sensitized with	Hemagglutinating units	μg/ml needed for inhibition			
		E. coli 014	*E. coli* 014 (alkali treated)	*E. coli* 08	*E. coli* 075
E. coli 014	4	5	450	ND*	>500
E. coli 014 (alkali treated)	8	7	6	>500	>500
E. coli 08	4	2	1–2	250	>500

* ND, not done.

alkali-treated *E. coli* 014 at equal and low concentrations. Taken together, these experiments show that alkali treatment of *E. coli* 14 LPS leaves the common antigen unchanged but destroys a non-CA specificity.

Precipitin analysis confirmed these findings (Fig. 1). With untreated *E. coli* 014 LPS and anti-014 serum, approximately 5 μg of N was precipitated. However, with alkali-treated *E. coli* 014 only about 3 μg of N was precipitated from the same volume of antiserum. In the latter system, all or a considerable part of the antibody N precipitated is anti-CA antibody, since the hemagglutinating titers of the supernatants decreased gradually with increasing amounts of antigen added. At equivalence, over 95% of the antibodies reacting with *E. coli* 08-sensitized erythrocytes were removed from the supernatant. The low residual reactivity in the supernatants at equivalence may represent "natural" anti-08 antibody already present in preimmune sera (9).

Antibodies to CA could be expected to be of several immunoglobulin classes. Since differences in the number of combining sites per mole and possible dif-

ferences in affinity would complicate interpretation of the precipitin analysis, the rabbit anti-*E. coli* 014 serum was fractionated on Sephadex G-200. Almost all hemagglutinating activity to CA was found in the IgM-containing fraction. In quantitative precipitation (Fig. 2) both the IgM-containing fraction and the IgG-containing fraction precipitated alkali-treated *E. coli* 014 LPS. However, 60% of precipitating antibody N against alkali-treated *E. coli* 014 was IgM. Approximately 1/10 of the amount of antigen was needed to precipitate the

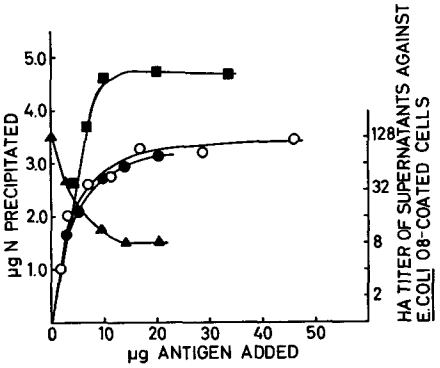

FIG. 1. Precipitation of *E. coli* 014 LPS and alkali-treated *E. coli* 014 LPS by rabbit antiserum to *E. coli* 014. 20 µl of antiserum were added to each tube; total volume/tube 200 µl. (■—■) *E. coli* 014 LPS; (○—○) and (●—●) *E. coli* 014 LPS treated with 0.25 M NaOH at 50°C for 3 hr, preparations 1 and 2, respectively; (▲—▲) hemagglutination (HA) titer of supernatants from mixtures of *E. coli* 014 serum with alkali-treated *E. coli* 014, preparation 2; sheep erythrocytes sensitized with *E. coli* 08 LPS.

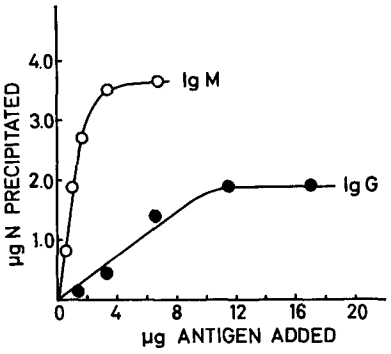

FIG. 2. Precipitation of alkali-treated *E. coli* 014 LPS by IgM- or IgG-containing fractions from rabbit anti-*E. coli* 014 serum. 30 µl of the IgM and IgG fractions were added to each tube, respectively, total volume/tube 200 µl. In relation to the original serum volume, the IgM-containing fraction was concentrated 1.9 times, and the IgG-containing fraction 1.5 times, respectively.

same amount of N from the IgM fraction than from the IgG fraction. Therefore, for all further precipitin studies the IgM-containing fraction was used as the source of anti-CA antibodies.

Hydrolysis of E. coli 014 LPS with Acetic Acid

31 mg of *E. coli* 014 LPS was treated with 1% acetic acid (pH 3.2) for 1.5 hr at 100°C. The lipid precipitate was removed by centrifugation and washed. The acetic acid soluble fraction was then lyophilized (yield 19.6 mg). This material was applied to a Sephadex G-50 column and eluted with pyridine acetate, pH 5.4 (Fig. 3). Two main carbohydrate-containing peaks were ob-

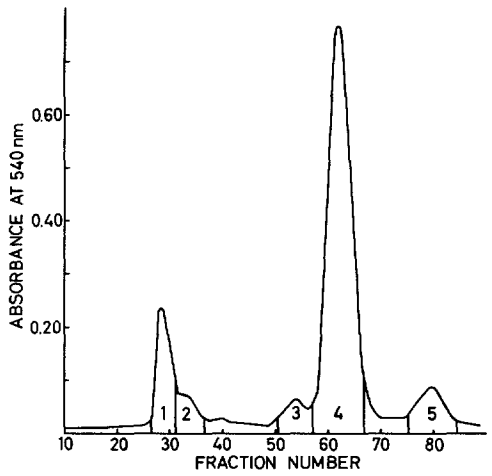

FIG. 3. Fractionation on Sephadex G-50 of water-soluble phase of *E. coli* 014 LPS, hydrolyzed with acetic acid (pH 3.2) for 1.5 hr at 100°C. Sugars determined by the orcinol reaction (OD 540 nm). The eluate was pooled in fractions as indicated in the Fig.

tained. Peak 1 was excluded from the column and thus represented material of molecular weight of $\leq$ 10,000. The major peak (fraction 4) was eluted in the molecular weight region of 2–3 $\times$ 10^3. A small peak in the monosaccharide region (fraction 5) and two small peaks (fractions 2 and 3, Fig. 3) were also recovered. In an independent experiment, the acetic acid hydrolysate of *E. coli* 014 LPS was fractionated with phosphate-buffered saline (pH 7.3) on a calibrated Bio-Gel P-10 column. Exactly the same elution pattern was obtained.

Yields and carbohydrate analysis of the fractions from the experiments of Fig. 3 are given in Table III. 36% of the material was recovered in the major peak (fraction 4). This fraction contained the highest concentrations of hexose + heptose but only trace amounts of *o*-acetyl and hexosamine. Fractions 4 and

5 both contained large amounts of KDO. However, whereas in fraction 5, KDO determination by the thiobarbituric acid assay or by the semicarbazide assay gave approximately the same results, in fraction 4 only the latter gave significant values. These findings are consistent with the assumption that fraction 5 contained free KDO (or KDO released in reactive form under the weak hydrolytic conditions used for the thiobarbituric acid assay) while fraction 4 contained KDO which is linked to the other sugars by a more acid-stable bond. Fraction 1 contained large amounts of hexosamine (and/or hexosaminuronic acid) and *o*-acetyl, whereas the KDO content was low.

TABLE III

Yields, Composition, and CA Inhibiting Activity of Sephadex G-50 Fractions of Degraded E. coli 014 Polysaccharide. Fractions Pooled As Indicated in Fig. 3

Fraction No.	Yield		Hexose + Heptose	Hexosamine*	N	*o*-Acetyl	KDO		CA‡ μg/ml needed for inhibition
							Thiobarb.	Semicarb.	
	(*mg*)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
1	3.6	20	16.1	26.4	3.7	6.0	0.1	2.0	>500
2	1.9	11	16.1	18.3	3.6	6.8	0.1	ND§	>500
3	1.1	6	28.9	11.5	4.5	2.0	2.7	ND	250
4	6.5	36	43.0	1.1	1.9	0.4	4.0	18.4	64
5	4.0	22	6.0	0.6	1.5	0.4	15.0	18.2	>500
Unfractionated	—	—	26.0	6.2	4.3	2.2	6.4	ND	250

* Calculated as glucosamine. Note that hexosaminuronic acids will also react in this determination.

‡ CA inhibiting activity was determined by hemagglutination inhibition, using 2–4 HU of the IgM fraction of anti-*E. coli* 014 serum and erythrocytes sensitized with *E. coli* 08 LPS.

§ ND, not done.

The result of hemagglutination-inhibition analysis in the CA anti-CA system is also given in Table III. As can be seen, fraction 4 was a good inhibitor of hemagglutination between anti-*E. coli* 014 antiserum and red cells coated with *E. coli* 08 LPS. Some inhibition was also obtained with fraction 3 and the unfractionated material. All other fractions were negative.

Fraction 4 was not precipitated by the IgM fraction of anti-*E. coli* 014 serum. However, fraction 4 gave complete inhibition of precipitation between these antibodies and alkali-treated *E. coli* 014 LPS (Fig. 4). Of the other fractions, only fraction 3 and the unfractionated material had some inhibiting activity.

Carbohydrate analysis of fraction 4 by gas-liquid chromatography combined with mass spectrometry showed that it was composed of galactose, glucose, and a heptose in the molar ratios 2.07:1.50:1:00. Glucosamine could not be detected

and there were only trace amounts of ribose present. The identities of the sugars were confirmed by mass spectrometry. According to the semicarbazide assay, the KDO content of fraction 4 was 18.4%. 4% of the KDO reacted with the thiobarbituric acid reagent after mild hydrolysis and was therefore not glycosidically bound to other sugars. Subtracting the thiobarbituric acid value from the semicarbazide value gave 14.4% for glycosidically-bound KDO. This corresponds to a molar ratio of 1.00:1.20 for heptose:KDO.

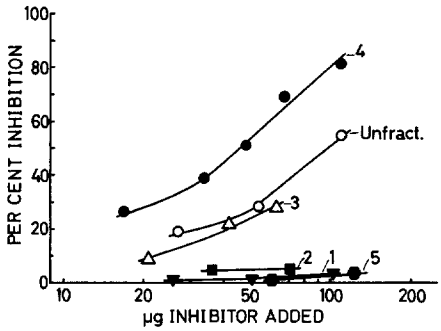

FIG. 4. Inhibition of precipitation between alkali-treated *E. coli* 014 LPS and IgM-containing fraction of anti-*E. coli* 014 serum, by Sephadex G-50 fractions 1–5 from experiment in Fig. 3. 30 μl of IgM fraction and 2.9 μg of alkali-treated *E. coli* 014 LPS were added to each tube, total volume/tube 200 μl.

DISCUSSION

By definition, CA is present in several different *E. coli* O serotypes. Furthermore, hemagglutination-inhibition experiments show that CA is present in different amounts or accessibility in different *E. coli* O serotypes: LPS from *E. coli* 014 and from certain rough mutants have high inhibiting activity while LPS from O serotypes with large and complicated O-specific side chains (e.g. *E. coli* 055 and 075) have low activity. *E. coli* 014 and *E. coli* 056, which both induce antibodies to CA when injected in rabbits, belong to chemotype I, thus their lipopolysaccharides contain only the basal core sugars (19). Gel filtration of "degraded lipopolysaccharides" from some *E. coli* strains indicated that these contain an excess of unsubstituted core (12). Hence, it is a plausible assumption that the common enterobacterial antigen is related to unsubstituted core stubs, varying in amounts or accessibility in different *E. coli* O serotypes.

In addition to anti-CA antibodies, antisera against *E. coli* 014 usually contain antibodies to the type 014-specific antigen and perhaps also against the capsular antigen K7 (see below). It is therefore not possible to use *E. coli* 014 LPS directly as test antigen for CA antibodies. However, as assessed by hemagglutination inhibition, alkali treatment of *E. coli* 014 LPS causes an almost

complete loss of non-CA activity without any detectable change in CA activity (Table II). Precipitin analysis confirms this finding. Alkali-treated *E. coli* 014 LPS precipitated only 60% of the antibody nitrogen which was precipitated by the untreated preparation (Fig. 1). The loss of non-CA specificity may be related to the liberation of *o*-acetyl, caused by alkali treatment. Probably, *o*-acetyl is part of the type 014-specific antigen. The results suggest that the antibodies which were precipitated by alkali-treated *E. coli* 014 LPS were partly if not exclusively, directed against the common antigen.

Our chemical analysis of *E. coli* 014 LPS confirmed the presence of only the basal core sugars as reported by Ørskov et al. (19). In addition our analysis showed that the preparation contained relatively large amounts of *o*-acetyl (Table I). Treatment of LPS with weak acid cleaves the acid-labile KDO–lipid A bond. This separates the polysaccharide part of the molecule from lipid A. On gel filtration of the soluble part of the hydrolysate, one would expect three fractions with different molecular weights: a fraction of rather high molecular weight, comprising the O-specific side chains attached to the core region and terminating with a KDO residue; a second fraction, constituting unsubstituted core and again terminating with a KDO residue; and finally free KDO. This pattern has been found by Schmidt et al. (12) for *E. coli* 08 LPS. Degraded *E. coli* 014 LPS gave the expected elution pattern and, in addition, two minor poorly separated components. The molecular weight of the major component was $2–3 \times 10^3$, which is in the range expected for a core fragment. The chemical composition of the first peak (probably O-specific side chains linked to the core, and possibly also K7 acid polysaccharide) was significantly different from the main peak (fraction 4). It contained high concentrations of *o*-acetyl and hexosamine (or hexosaminuronic acid), which were missing in fraction 4. The latter was rich on hexose + heptose and contained significant amounts of KDO as determined by the semicarbazide reaction. These sugars accounted for 60% of its dry weight. Closer chemical analysis of fraction 4 by gas-liquid chromatography showed that it contained galactose, glucose, and a heptose in molar ratios 2.07:1.50:1.00 but lacked glucosamine. The possibility that the material in fraction 4 was derived from the K7 antigen (20) can be ruled out since this antigen contains 2-amino-2-deoxymannuronic acid, a sugar which is detected in the Elson-Morgan hexosamine assay. Furthermore it is highly unlikely that the acid polysaccharide would have been hydrolyzed to any significant extent during the mild acid conditions used here.

KDO was present in the ratio 1.20:1.00 as compared to heptose. The presence of both KDO and heptose strongly suggests that fraction 4 represents a core fragment. Since phosphate determinations were not performed it is not possible to draw definite conclusions as to the molar proportions of heptose to the other sugars. It is likely that some heptose is substituted by phosphate (21). This would give a low heptose content, since heptose-phosphate is not detected by

gas-liquid chromatography under the present conditions. It should also be stressed that fraction 4 may be heterogeneous and contain core structures of different chemical composition. The lack of glucosamine is of interest in relation to the studies of Schmidt et al. (12). Their results indicate that two different complete core regions could be demonstrated in LPS from different rough mutants of *E. coli* 08 strains; one class of rough mutants lacked glucosamine. As mentioned earlier, *E. coli* 08 LPS preparations contained significant amounts of common antigen (9).

As expected, fraction 4 was not precipitated by anti-*E. coli* 014 serum. However, it inhibited precipitation of alkali-treated *E. coli* 014 by the IgM fraction of anti-*E. coli* 014 serum. With the possible exception for fraction 3, the other fractions were negative in this respect. Fraction 4 was about two times more inhibitory than the unfractionated material. This shows that an enrichment of the CA determinant was achieved. The inhibiting activities of the above fractions in hemagglutination-inhibition experiments with anti-014 serum and red cells coated with *E. coli* 08 LPS were consistent with those seen in the precipitin reaction (Table III).

It may be concluded that fraction 4 contained the CA determinant. The chemical data also indicated that this antigen is part of the core structure of *E. coli* 014. Further studies are needed to establish which of the sugars of the core region participate in the reaction with antibodies to CA. The significance of the present findings in relation to the cross-reactivity between CA and the colon antigen of ulcerative colitis is obvious. The results presented here should make it possible to determine the chemical basis for this cross-reaction as defined by antibodies from patients with ulcerative colitis.

The notion that CA is related to the *E. coli* 014 core region contradicts the original conclusion reached by Kunin (8). This author isolated a CA-containing fraction from *E. coli* 014 LPS by DEAE-cellulose chromatography. CA was at least partly separated from 014 type specificity. The material contained some carbohydrate in addition to large amounts of amino acids and it was suggested that CA was of cell wall origin. However, the analytical data given for this fraction was not internally consistent. We were not able to obtain the common antigen by the fractionation procedure applied by Kunin and have at present no explanations for these discrepancies.

SUMMARY

Preparations of *E. coli* 014 lipopolysaccharide (LPS) contain a common enterobacterial antigen (CA) in large amounts or in an immunogenic form. Chemical analysis revealed, in addition to *o*-acetyl groups, only those sugars which are present in the basal core structure of the *E. coli* or *Salmonella* LPS (e.g., galactose, glucose, glucosamine, heptose, and ketodeoxyoctonate). On treatment with acetic acid (pH 3.2) at 100°C for 1.5 hr, a fragment was liberated

which after gel filtration on Sephadex G-50 appeared in the molecular weight range of 2–3 $\times$ 10^3. The fragment inhibited precipitation of alkali-treated *E. coli* 014 LPS by antibodies to CA from anti-*E. coli* 014 serum. It also inhibited hemagglutination between anti-CA antibodies and red cells coated with *E. coli* 08 LPS. Chemical analysis of the fragment indicated that it corresponded to the core region of *E. coli* 014 LPS. It contained a heptose and ketodeoxyoctonate in addition to glucose and galactose. However the fraction lacked glucosamine.

Enterobacterial CA has previously been found to cross-react with colon antigen of ulcerative colitis. These results should provide a chemical basis for further studies of this cross-reactivity.

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