kinase step can also be demonstrated. Indeed, PONCE et al. pointed out that 'inhibition' of these enzymes by 2,3-DPG was 'competitive' with Mg^{++}.

Although the effect effect of 2,3-DPG on these enzymes is merely due to Mg^{++} binding, this does not mean that it could not be physiologically significant. The total intracellular magnesium concentration of erythrocytes is only approximately 4.5 mM [14,15] and this small amount of magnesium is largely bound to ATP, a powerful magnesium chelating compound (dissociation constant $= 1.81 \times 10^{-5}$ M [12]) and 2,3-DPG. It is possible that, in spite of the high intracellular potassium concentration of red cells, 2,3-DPG may, under physiologic circumstances, produce some degree of modulation of magnesium requiring reactions [16].

Zusammenfassung. Der Einfluss von 2,3-DPG auf die Erythrozytenenzyme PFK, 6-PGD, GPI und PK ist nochmals untersucht worden. Unterschiede zu bereits publizierten Ergebnissen werden auf Grund unterschiedlicher experimenteller Bedingungen und verschiedener kinetischer Eigenschaften der Enzyme erklärt.

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Precipitating Antibody Against Core Glycolipid of *Enterobacteriaceae*

The cell walls of most *Enterobacteriaceae* possess a common 'core' glycolipid moiety which consists of 2-keto-3-deoxyoctonate (KDO) linked to lipid A [1,2]. Endotoxic properties of bacterial lipopolysaccharides have been related to the biologic activity of lipid A [3,4] and there is evidence that anti-KDO-lipid A antibodies are protective in man [5].

This report details methods for producing and demonstrating precipitating antibody against the core glycolipid moiety, as expressed in *S. minnesota* 595 chemotype 'Re'. The organism, whose cell wall consists principally of KDO-lipid A linkages [3,6], was obtained from Dr. OTTO WESTPHAL, Max Planck Institute for Immunobiologie, Freiburg (Germany). Albino New Zealand rabbits (2.5 to 4 kg), were immunized with suspensions containing 10^9 organisms/ml which were boiled for 1 h at 100 °C. Suspensions were injected i.v. according to the following schedule: 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml, 1.6 ml (followed by repetition of the last dose) at intervals of 5 to 7 days. Optimal antibody production was found in animals given more than 10 injections. Albino rabbits were also immunized with a 1 ml suspension of heat-killed organisms mixed with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan).

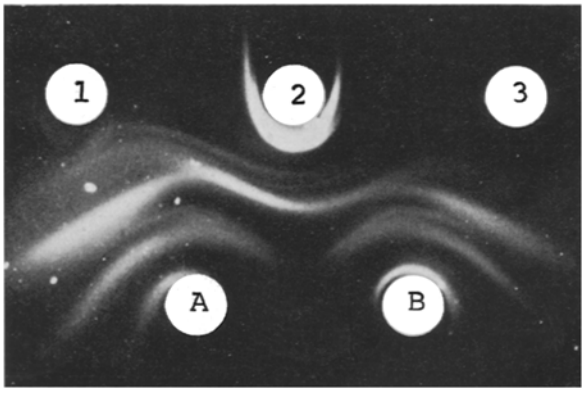

Fig. 1. Diffusion of *S. minnesota* 595 chemotype 'Re' antiserum against crude and purified antigens. A) Serum from rabbit immunized by i.v. route. B) Serum from rabbit immunized with heat-killed bacteria and Freund's complete adjuvant. Wells 1 and 3: Crude (freeze-thawed) antigen. Well 2: Purified antigen prepared by phenol-chloroform-petroleum ether extraction.

Lapine antibodies were raised against the following organisms by an initial i.m. injection of approximately 10^8 boiled bacteria followed by 4 to 6 i.v. injections of the same numbers of organisms every 5 to 7 days: *Escherichia coli* 014 (ATCC-19110), *Klebsiella pneumonia* type I, *Proteus rettgeri* type 80, and *Serratia marcescens* 01.

Crude antigenic preparations from all bacteria were prepared by methods identical to making the antigens used in immunizations except that they were additionally frozen at −80 °C and thawed 10 times. Antigen suspensions were centrifuged at 500 × g for 15 min and the supernatant used in immunodiffusion studies. Extraction of the glycolipid of *S. minnesota* 595 chemotype 'Re' was accomplished using the phenol-chloroform-petroleum ether method of GALANOS et al. [7] which yielded a water soluble antigen suspension containing 75 μg/ml of glycolipid.

The immunodiffusion method was that previously described using 1% agarose (Mann) thinly coated on glass slides [8]. Figure 1 demonstrates that serum from rabbits immunized by either method formed precipitin bands against the purified glycolipid preparation and crude *S. minnesota* antigen. In general sera from animals immunized by the i.v. route with 10 injections produced more intense bands of precipitation. A strong line of identity is observed between wells containing glycolipid extract and the crude supernatant antigen. A strong precipitin band close to the well containing the phenol chloroform-petroleum ether extract may have been due to antigen aggregation secondary to the extraction process. Figure 2 demonstrates precipitating antibody resulting

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from diffusion of immune *S. minnesota* serum against *S. minnesota* (crude), *Klebsiella pneumonia* type 1, and *Serratia marcescens* 01. Two lines of identity are observed as were observed when the antiserum was tested against the *Proteus rettgeri* type 80 antigen, *E. coli* 014, and the lipopolysaccharide of *E. coli* 0111 derived from Boivin-type extraction (1000 µg/ml, Difco Laboratories, Detroit, Michigan). Conversely, antiserum produced against *Klebsiella pneumoniae* type 1, *Proteus rettgeri* type 80, and *Serratia marcescens* 01 all formed at least 2 precipitin bands against the crude or purified *S. minnesota* antigen. Precipitating antibody could be removed by absorption with whole *S. minnesota* organisms or latex particles coated with purified glycolipid. No precipitin bands were formed between *S. minnesota* antiserum and the purified lipopolysaccharides of *Pseudomonas aeruginosa* types 1 through VII[9,10].

These results confirm by the immunodiffusion technique that 'smooth' organisms with intact *O*-specific side chains belonging to the family *Enterobacteriaceae* still possess core antigens which will precipitate with antibody directed at the heat-stable glycolipid of *S. minnesota* 595 chemotype 'Re', a 'rough' mutant whose cell wall is principally composed of the KDO-lipid A. The significance of this detection of precipitating antibody is at least 5-fold. First, this can be a powerful tool for studying the taxonomic relationships between enteric bacteria and other microorganisms. Many antigens shared between apparently unrelated species are now being described, with the hypothesis that this may be one mechanism for the development of natural, cross-protective immunity[11–13]. Second, it becomes another method for evaluating the relationship of the 'Re' antigen to other widely shared antigens of enteric bacteria such as the 'common antigen' (CA) of KUNIN[14]. Third, it may provide the basis for developing a highly sensitive and specific assay for endotoxin by the radioimmunoassay principle, which depends on competitive binding of radiolabelled and unlabelled antigens with precipitating antibody. Fourth, it provides a basis for the quantitative measurement of antibodies against core glycolipid by the antigen binding technique[15]. Finally, the availability of precipitating antibdy against core glycolipid may be highly useful in studying the histopathology of acute and chronic gram-negative bacillary infections, particularly the localization of antigen by immunoflorescent and radiolabelled antibody techniques.

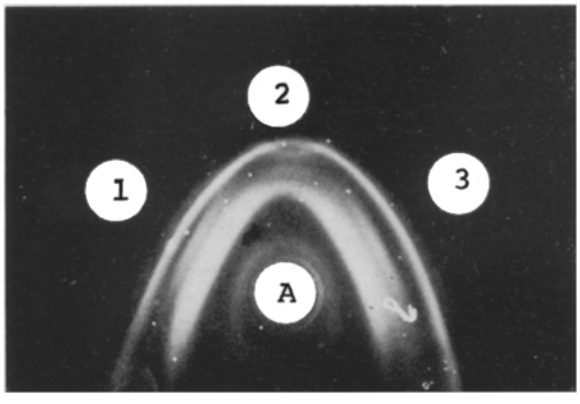

Fig. 2. Precipitation of *S. minnesota* 595 chemotype 'Re' antiserum with antigens of other enteric bacilli. A) Serum from rabbit immunized i.v. with heat-killed *S. minnesota*. Well 1: *Klebsiella pneumoniae* type 1 antigen. Well 2: *S. minnesota* antigen. Well 3: *Serratia marcescens* 01 antigen.

Zusammenfassung. Präzipitierende Antikörper gegen gereinigtes Glycolipidantigen «Re» von *Salmonella minnesota* R 595 wurden in Kaninchen erzeugt. «Re» Antiserum bildet Präzipitationslinien mit den Antigenen von *E. coli*, *Klebsiella*, und *Serratia*, nicht aber mit den Antigenen von *Pseudomonas aeruginosa*. Typenspezifische Antisera gegen die ersten drei Organismen reagierten mit den «Re»-Antigen.

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Orosomucoid, Seromucoid and Haptoglobin in Serum During Adjuvant Arthritis of the Rat

In immunopathological experimental diseases like adjuvant arthritis[1] and nephrotoxic serum nephritis[2] with inflammatory reaction, it is interesting to know the variations of the acute phase proteins[3] and to have simple specific assays for these proteins that will give quantitative inflammation criteria of the experimental disease. We were interested in two acute phase proteins: orosomucoid[4], or α_1 acid glycoprotein[5], and haptoglobin (Hp)[6], α_2 glycoprotein, both synthetized by the liver[7]. Seromucoid, or serum mucoprotein[8], a heterogenous glycoproteic fraction containing orosomucoid, was studied simultaneously. Modifications of two existing methods were made for specific assays of rat orosomucoid and haptoglobin: the first method was by radial immunodiffusion and the second by an automated procedure measuring peroxydase activity of Hp-Hb complex. Using these techniques, we studied both glycoproteins in serum during adjuvant arthritis in the rat.

Methods. Orosomucoid was kindly prepared by J. MARÇAIS from Wistar rat serum, 48 h after turpentine injection, as previously described[9]. Antiserum to rat orosomucoid was produced in rabbits (strain: 'Fauve de Bourgogne'). Orosomucoid levels were measured in rat sera by the MANCINI[10] radial immunodiffusion technique using a 1/10 dilution of anti-orosomucoid antiserum in 0.75% agarose prepared in veronal buffer pH 8.6.