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.

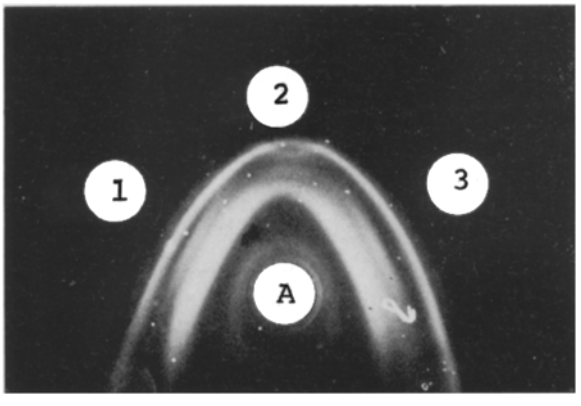

Fig. 2. Precipitation of *S. minnesota* 595 chemotype 'Re' antiserum with antigens of other enteric bacilli. A) Serum from rabbit immunized i.v. with heat-killed *S. minnesota*. Well 1: *Klebsiella pneumoniae* type 1 antigen. Well 2: *S. minnesota* antigen. Well 3: *Serratia marcescens* 01 antigen.

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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[9] M. W. FISHER, H. B. DEVLIN and F. J. GNABASIK, J. Bact. *98*, 835 (1969).
[10] S. HANESSIAN, W. REGAN, D. WATSON and T. H. HASKELL, Nature New Biol. *229*, 7, 209 (1971).
[11] R. SCHNEERSON, M. BRADSHAW, J. K. WHISNANT, R. L. MYEROWITZ, J. C. PARKE and J. B. ROBBINS, J. Immun. *108*, 1551 (1972).
[12] D. L. KASPER, J. L. WINKELHAKE, W. D. ZOLLINGER, B. BRANDT and M. S. ARTENSTEIN, J. Immun. *110*, 262 (1973).
[13] P. MINDEN, J. K. MCCLATCHY and R. S. FARR, Infect. Immun., *6*, 574 (1972).
[14] C. M. KUNIN, M. V. BEARD and N. E. HALMAGYI, Proc. Soc. exp. Biol. Med. *111*, 160 (1962).
[15] S. R. FARR, J. infect. Dis. *103*, 239 (1958).
[16] This study was supported by a general development grant from the National Institutes of Health No. GRSG RR-5354, a grant from the National Institute of Allergy and Infectious Disease No. A1-11271, and a U.S. Public Health Service Training Grant No. A1-309.

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.

Correlations between individual rat serum levels of orosomucoid, haptoglobin and seromucoid

Glycoproteins		Rat population	n	r	p	$y = ax + b$
x	y					
Orosomucoid	Hp	Normal controls	10	0.928	< 0.001	$y = 0.89x + 0.06$
		All experimental rats	47	0.844	< 0.001	$y = 0.72x + 0.42$
Orosomucoid	Seromucoid	Normal controls	10	0.887	< 0.001	$y = 1.42x + 0.41$
		All experimental rats	46	0.454	< 0.01	—

x, y, correlated values; n, number of values; r, correlation coefficient; p, level of significance; $y = ax + b$: equation of regression line.

2 µl of serum, or orosomucoid standard solution ranging from 0.4 to 5 g/l, were put in the wells. For normal sera, 6 µl of serum or orosomucoid standard solution ranging from 0.10 to 1.5 g/l were necessary (Figure 1).

Hp levels ranging from 0.05 to 0.7 g/l were measured by a modification[11] of the automated method described by one of us[12].

Seromucoid was measured by a modification of the technique of HUERGA et al.[8]: precipitation in 0.6 *M* perchloric acid of the serum diluted to 1/30 was effected at 4 °C: the perchlorosoluble protein-bound hexose was determined by the orcinol sulfuric reagent[13] with galactose standards. Total proteins were determined according to GORNALL[14].

Polyarthritis was induced in 40 SPF Sprague-Dawley rats, weighing from 165 to 190 g (Iffa-Credo, 69 St Germain sur l'Arbresle). Food and water were supplied ad libitum. Adjuvant was prepared as follows: 90 mg of dessicated *Mycobacterium butyricum* (Difco) were suspended, homogenized and sterilized in a mixture of mineral oil, 40 ml. 'tween 80', 10 ml, and NaCl 0.9%, 40 ml. 0.1 ml per 100 g weight was injected into the right posterior foot pad. The rats were then randomly divided into 8 groups of 5 animals which were sacrificed respectively on days 2, 4, 7, 9, 11, 14, 18 and 24 after the adjuvant injection, 2 control groups of 5 animals injected with saline being sacrificed on days 2 and 24. Just before sacrifice, the rats were scored visually. For each paw, 0 was given to normal aspect, 1 to erythma, 2 to oedema, 3 to pseudo-phlegmonous aspect and 4 to necrosis. For the tail, the presence of one nodule was noted 1, several nodules 2, and rigidity of the whole tail 3. For each ear, purpuric points were noted 1 and nodules 2. The maximum score was 23. Then the rats were bled from the abdominal aorta under ether anesthesia and the sera frozen at −20 °C.

Results and discussion. The data are summarized in Figure 2. The clinical manifestations of polyarthritis occured in 100% of the animals injected with adjuvant. The two classical phases of the disease were observed[15]: a primary inflammation localized to the injected paw and a secondary generalized polyarthritis beginning on day 9. The curves of orosomucoid, seromucoid and Hp in serum were strikingly parallel and showed two significant peaks corresponding to the successive phases of the experimental disease. A good correlation between the individual rat serum levels of the different glycoproteins was found, (Table). The modifications of these acute phase proteins

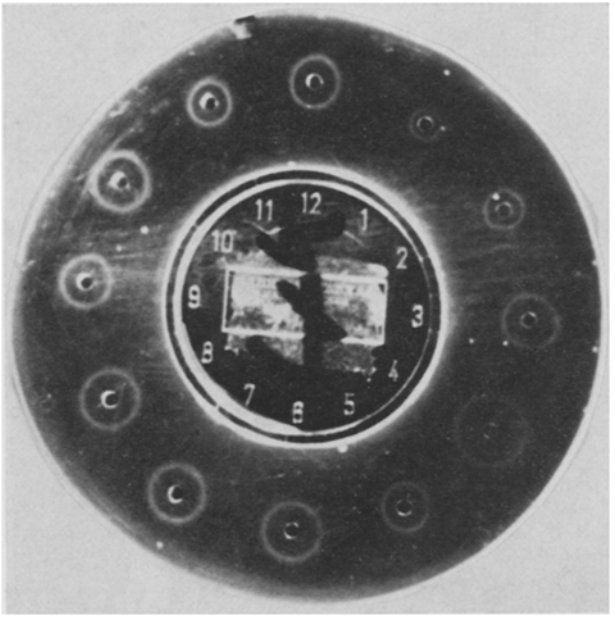

Fig. 1. Radial immunodiffusion of orosomucoïd standard solutions (1 = 0.11 g/l; 2 = 0.22 g/l; 3 = 0.57 g/l; 4 = 1.15 g/l) and rat inflammatory sera (5 to 12).

[1] B. H. WAKSMAN, C. M. PEARSON and J. T. SHARP, J. Immun. *85*, 403 (1960).
[2] E. UNANUE and F. DIXON, Adv. Immun. *6*, 1 (1967).
[3] A. H. GORDON, in *Plasma Protein Metabolism* (Eds. M. A. ROTHSCHILD and T. WALDMANN; Academic Press, New York 1970), p. 351.
[4] H. E. WEIMER, J. W. MEHL and R. J. WINZLER, J. biol. Chem. *185*, 561 (1950).
[5] K. SCHMID, J. Am. chem. Soc. *72*, 2816 (1950).
[6] M. POLONOVSKI and M. F. JAYLE, C. r. Soc. Biol. *129*, 457 (1938).
[7] C. LOMBART, M. NEBUT, M. P. OLLIER, M. F. JAYLE and L. HARTMANN, Revue fr. Etudes clin. biol. *13*, 258 (1968).
E. J. SARCIONE, Arch. Biochem. Biophys. *100*, 516 (1963).
[8] A. DUBIN, D. S. KUSHNER, H. A. DYNIEWICZ and H. POPPER, J. Lab. clin. Med. *47*, 403 (1956).
[9] R. ENGLER, F. AMPEAU, M. STERNBERG and M. F. JAYLE, Annls. Biol. clin. *29*, 257 (1971).
[10] G. MANCINI, J. P. VAERMAN, A. O. CARBONARA and J. F. HEREMANS, Prot. Biol. Fluids 11th Colloquium Bruges (Ed. H. PEETERS; Pergamon Press, Oxford and New York 1964), p. 370.
[11] C. LOMBART, R. ENGLER, M. F. JAYLE, in publication.
[12] J. MORETTI, M. WAKS and M. F. JAYLE, Annls Biol. clin. *1*, 149 (1967).
[13] H. E. WEIMER and J. MOSHIN, Am. Rev. Respiratory Dis. *68*, 594 (1953).
[14] A. G. GORNALL, C. J. BARDAWILL and N. M. DAVID, J. biol. Chem. *177*, 751 (1949).
[15] B. B. NEWBOULD, Br. J. Pharmac. *21*, 127, (1963). – F. DELBARRE, H. BROUILHET, A. KAHAN, B. AMOR, in 12 and 13e Confrontations Pharmacologiques (Doin, Paris 1968), p. 39.

may be attributed to an increased synthesis induced in the first stage by the localized inflammation and in the second stage by the generalized inflammatory polyarthritis. They were not due to any hemoconcentration, since the total proteins were not significantly modified; in fact, these glycoproteins represented at the most 5 to 10% of the total proteins. Our results are in agreement with those of GLENN et al.[16] who reported an increase of α_1 and α_2 globulins, total mucoproteins and glycoproteins. But these authors did not mention the first inflammatory peak since they began their study at day 4.

No significant pathological proteinuria was observed during the course of the adjuvant arthritis, in comparison with the control groups of rats which excreted 2.3 mg proteins per day as a mean. No morphological lesions of glomeruli, tubuli, mesangium, interstitial space and vessels were noted by microscopic examination in the kidney. The contrary had been expected, since ZAHIRI et al.[17] observed histological alterations of kidney glomeruli and tubuli. However, they used a different adjuvant.

Conclusion. The kinetics of orosomucoid, Hp and seromucoid in serum during adjuvant arthritis reflect the two phases of the clinical development. Therefore, we suggest the use of these quantitative biochemical parameters to appreciate the effect of drugs or other experimental factors in this immunopathological disease. Further experiments to validate these tests are in progress.

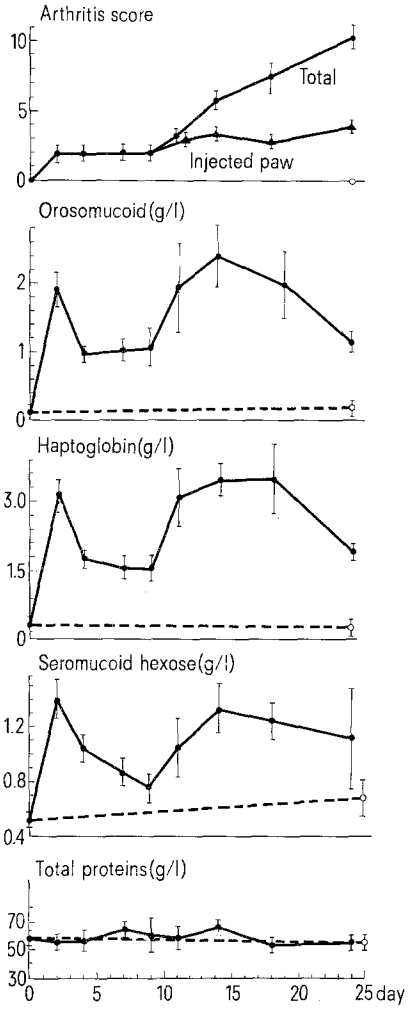

Fig. 2. Arthritis total (●—●) and partial score, limited to the injection paw (▲—▲), compared with serum orosomucoid, Hp, serum seromucoid and total proteins during adjuvant polyarthritis in the rat. ●, mean of the experimental group ± standard error of the mean; $\overset{T}{\bullet}$, mean of 24th day control group). Normal control values for 10 rats: arthritis score = 0; orosomucoid = 0.13 ± 0.03 g/l; haptoglobin = 0.27 ± 0.01 g/l; seromucoid hexose = 0.60 ± 0.06 g/l; total serum proteins = 59 ± 2.7 g/l.

Résumé. Chez le rat, au cours de la polyarthrite par adjuvant, les courbes évolutives de l'orosomucoïde et de l'haptoglobine sérique présentent 2 pics importants parallèles à ceux du séromucoïde et correspondant aux 2 phases inflammatoires de la maladie. Nous n'avons observé ni protéinurie pathologique, ni lésions histologiques rénales.

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[16] E. M. GLENN, J. GRAY and W. KOOYERS, Am. J. vet. Res. *26*, 1195 (1965).

[17] H. ZAHIRI, J. GAGNON, R. AYOTTE and C. A. LAURIN, Can. med. Ass. J. *101*, 269 (1969).

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Venular Micro-Aneurysmal Ectasia: an Accompaniment of the Cutaneous Allograft Response

Increased vascular permeability to circulating Evans' blue is a prominent and early event in the cutaneous allograft response which immediately precedes unequivocal macroscopic and microscopic evidence of the onset of rejection[1]. In the course of our studies on the vascular changes occurring at the time of this exudative response, we have noticed the development in the graft vessels of diffuse venular ectasia and in particular focal aneurysmal dilatation closely related in time to the exudative response.

Orthotopic full-thickness skin grafting was carried out using inbred strains of Wistar Albino Glaxo and Piebald Variant Glaxo rats as donors and recipients respectively. Orthotopic skin was grafted into round excised wounds (9.00 ± 0.5 mm) on the flanks of recipient animals im-