quantitative hydrogenation and peracid titrations. As dehydrogenation of (II) with Pd-C (30%) furnished cadalene (III) in quantitative yields as the only product of dehyrogenation (TLC on silica gel layers impregnated with sym trinitrobenzene, mp and mixture mp of the picrate with an authentic sample 116°) it was clear that the reaction product (II) contained the intact cadinane skeleton.

Oxidation of (II) with Jones reagent afforded a crystalline keto ether (IV, $C_{16}H_{26}O_2$, mp 121°) $\gamma c = O$ 1680 cm^{-1}, λmax 230 nm (This value is lower than expected for this chromophore, but is in good agreement with that observed [λmax 233 nm $\varepsilon 13{,}800$] for the oxidation product[3] of dihydro Khusinol which has an identical chromophore) ε 15,500, whereas prolonged oxidation of (II) with active manganese dioxide afforded only the parent compound back. These observations showed that Jones oxidation of (II) is attended by a double bond shift to afford an α, β-unsaturated ketone (IV). The NMR-spectrum of the oxidation product clearly demonstrates that it should be represented by (IV).

These spectral features and chemical reactions clearly established structure (II) for the reaction product. This structure could also be confirmed by the NMR-spectrum of (II) which displayed 2 doublets (3 H each) at 0.74 and 0.9 δ (J = 6 c/s) assignable to an isopropyl group situated in an assymetric environment. The singlet at 1.29 δ and 3.25 δ could be assigned to C-C-CH_3 and OCH_3, respectively. The broadened singlet at 1.63 δ could be assigned to a C = C-CH_3 grouping. The one proton signals at 4.72 (multiplet) and 5.3 δ (narrow multiplet) are assignable to CHOH and olefinic protons, respectively.

The formation of (II) is obviously not a simple electrophilic addition as this would require the trisubstituted double bond to react first. The formation of (II) can be rationalized[3] as an initial complexing (as in V) followed by proton transfer to yield an incipient carbonium ion at C_7 which may be attacked by the nucleophile in a stepwise or concerted reaction.

Zusammenfassung. Es wird eine neuartige Reaktion an einem Terpen beschrieben.

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Effects of Cyclophosphamide on the Chemical Composition of the Glomerular Basement Membrane and Non-Collagenous Urinary Glycoprotein of the Rat

Cyclophosphamide (Cytoxan) in addition to its uses as an immunosuppressive and anticarcinogenic agent and in treatment of rheumatoid disorders has been widely employed in the therapy of the idiopathic nephrotic syndrome (INS) of childhood, or lipoid nephrosis, with encouraging results[1]. Since this disease is not immunologically mediated[2], but possible the result of metabolic derrangements, and since it has been suggested that proteinuria may result from molecular alterations in the glomerular basement membrane (GBM)[3], it is possible that cyclophosphamide may modify proteinuria by altering the chemical composition of the glomerular capillary wall. We have isolated and characterized a urinary glycoprotein (MUPpg) from normal rat urine which resembles the non-collagenous component of GBM[4,5]. We have also described alterations in the chemical composition of this glycoprotein in nephrotic rats and suggested that increased permeability of the GBM may be associated with biochemical changes in this membrane related urinary glycoprotein (MUPpg). Although cyclophosphamide does not significantly modify proteinuria induced by administration of aminonucleoside promycin (AMP) to rats, it seemed reasonable to evaluate the effects of cyclophosphamide on the chemistry of normal rat GBM and rat urinary glycoprotein.

Materials and methods. Daily 24 h urine specimens were collected for 48 h from 100 normal Sprague-Dawley rats (150 g) for isolation of normal control MUPpg. 30 normal rats from this group (normal control) were sacrificied and their kidneys used for preparation of GBM. The remainder were divided into 2 groups. Group I (35 rats) received daily i.p. injections of cyclophosphamide 10 mg/kg 5 days per week for 6 weeks. Group II (35 rats) received 1 cm^3 of saline i.p. for 5 days per week for 6 weeks.

One week after cessation of injections, daily 24 h urine specimens were collected for 48 h from Groups I and II. The urine from each group was pooled and used for preparation of MUPpg. The rats were sacrificed and the kidneys from each group pooled for isolation of glomerular basement membrane. MUPpg was isolated as previously described[4,5] by DEAE ion exchange chromatography followed by Sephadex G 200 gel filtration. Glomerular basement membrane was isolated by the method of KRAKOWER and GREENSPON[6]. Aliquots of lyophyllized MUPpg and lyophylized GBM from control and both experimental groups of animals were hydrolyzed in 6 *N* HCl for 21 h for amino acid analysis. The NCl was removed by flash evaporation and amino acid analysis was performed on a Beckman amino acid analyzer by the method of SPACKMAN, MOORE and STEIN[7]. Materials were prepared for analysis of carbohydrates by hydrolysis at 110 °C for 4 h followed by chromatography on Whatman No.1 filter paper. Hexose[8], glucose and galactose (glucostat and galactostat enzymatic methods, Worthington Biochemicals) were determined quantitatively on GBM and MUPpg from the control and both experimental groups of animals.

Triplicate studies demonstrated the reproducibility of the carbohydrate analytic methods with the following

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standard deviation of the method: Hexose 1.05%, glucose 0.035%, galactose 0.051%. Since urine and GBM specimens were pooled, composition of the groups were compared but intra-group variability could not be evaluated.

Results. The amino acid composition of MUPpg and GBM from both experimental groups and the controls showed no significant differences. We have previously reported the amino acid composition of GBM and MUPpg in detail[5]. The Table summarizes quantitative differences in the carbohydrate composition of GBM and MUPpg from experimental and control groups. The total hexose, glucose and galactose in MUPpg were increased about 20% in the cyclophosphamide rats as compared to untreated (control) and saline treated animals. The quantity of MUPpg excreted was similar in the 3 groups (Table).

Similar increases (15% in total hexose, glucose and galactose) were observed in the GBM isolated from cyclophosphamide treated rats compared to controls and saline treated rats without significant changes in the glucose: galactose ratio (Table).

Discussion. The pathogenesis of lipoid nephrosis remains an enigma but remissions commonly accompany corticosteroid or cyclophosphamide therapy. There is evidence that proteinuria regardless of pathogenic mechanism is probably a function of increased glomerular permeability[9-13] which may depend on the molecular arrangement of the basement membrane, related basement membrane glycoproteins[3] or epithelial or endothelial lining[14-16]. The alterations in carbohydrates in GBM and MUPpg associated with treatment with cyclophosphamide suggest that cyclophosphamide may alter the chemical composition of this membrane related glycoprotein both in situ on GBM and in the urine. MISRA[3] has indicated that prednisone may decrease proteinuria in the nephrotic syndrome in man by alteration of the carbohydrate composition of GBM.

Quantitative differences in carbohydrates of GBM and MUPpg in control and experimental groups

	Control group	Group I[a] Cyclophosphamide treated	Group II[b] Saline treated
Total hexose MUPpg (%)	15	18	14.7
Glucose MUPpg (%)	4.4	5.2	4.38
Galactose MUPpg (%)	1.05	1.53	1.06
Average MUPpg (mg animal/day)	0.6	0.7	0.75
Total hexose GBM (%)	5.6	6.8	5.65
Galactose GBM (%)	2.3	3.1	2.31
Glucose GBM (%)	2.0	2.7	1.92

[a] Received cyclophosphamide 10 mg/kg i.p. 5 days a week for 6 weeks.
[b] Received saline 1 cm³ i.p. 5 days a week for 6 weeks.

These studies suggest that one of the mechanisms by which cyclophosphamide may bring about improvement in the effectiveness of the protein filtering capability of the glomerular capillary wall is through modification of the chemistry of this class of constituents of the wall. The precise role of these components in the maintenance of GBM integrity to protein and the mode of synthesis and degradation remain unknown. Further evaluation of these important questions seem indicated on the basis of the observations reported in these studies. Also, the beneficial effect of cyclophosphamide on other diseases affecting membranes may be due to a similar mechanism.

Résumé. La cyclophosphamide est un agent efficace dans le traitement de la néphrose lipoidique et de plusieurs maladies rhumatoides qui attaquent la membrane basale glomérulaire (MBG). Des analyses chimiques ont été effectuées sur la MBG et sur la glycoprotéine urinaire chez des rats traités et non traités. Entre les deux groupes on n'a pas constaté de différence dans la composition des amino-acids, mais de grands changements dans le glucose et le galactose de la MBG et la glycoprotéine urinaire ont été observés chez les rats traités à la cyclophosphamide; cette drogue semble donc capable d'altérer l'intégrité de la MBG.

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Triglycerides and Other Lipid Classes in Human Atherosclerosis

Data so far available do not make clear whether triglycerides, or cholesterol, or both these lipid classes, are significantly involved in changes in lipid metabolism brought about by atherosclerosis[1-7]. The purpose of this investigation was to define the role played by individual plasma lipid classes in atherosclerosis.

Materials and methods. The investigation was carried out on plasma from 25 atherosclerotic patients in compar-