fibers are not independent of time. Similar kinetics have been seen in frog[6] and crab[7] muscle fibers, and are most simply interpreted on the theroy that the bulk of the injected ^{45}Ca is fairly quickly bound or sequestered. That the large fall-off in the rate constant for Ca^{2+} efflux during the initial phase of the experiment is not the consequence of a deteriorating Ca^{2+} pump is indicated by the fact that reintroducing the microinjector into the fiber during the early part of the slow phase of Ca efflux fails to modify the behavior of ^{45}Ca emergence. On the other hand, it could be argued that the initial rate constants for ^{45}Ca efflux are high because of the contraction of these fibers following loading with radiocalcium. This would be explained as being the result of the squeezing out of ^{45}Ca mainly from the T-system.

The experiments with caffeine show that barnacle fibers are not as sensitive to the alkaloid as crab muscle fibers. It will be remembered that CALDWELL and WALSTER[3] found crab fibers to always shorten when treated internally with caffeine, even in concentrations as low as 1 m*M*. This is not true of barnacle fibers, since concentrations as high as 100 m*M* usually caused only local, weak contractions. This point is worth emphasizing in view of the conclusion by AXELSSON and THESLEFF[1] that internal application of caffeine to frog muscle does not cause a contraction. It is thus quite possible that differences in experimental results may not just be related to species differences but also to the presence of a non-uniform population of fibers within the same muscle bundle.

One explanation for the failure of caffeine in both instances to exert an effect on the radiocalcium efflux as great as that observed in crab muscle fibers[11] is as follows. Caffeine, be it applied externally or internally, mobilizes a large fraction of the 'bound' calcium, resulting in considerable dilution of the injected ^{45}Ca by the inactive calcium. Thus failure to see a fall in the rate constant for ^{45}Ca efflux could mean that the Ca^{2+} pump is not easily saturated or that the action of caffeine is actually rather marked. On this view the effect of injected caffeine is only small in comparison to that of externally applied caffeine because of a physically weaker ejection of the accumulated ^{45}Ca from the region of the T-system[13].

Zusammenfassung. Die Befunde ergeben, dass einzelne Muskelfasern der Entenmuschel durch Mikroinjektion mit ^{45}Ca aufgeladen werden können. Eine Umspülung der Muskelfasern mit einer coffeinhaltigen Lösung bewirkt ein stärkeres Ausströmen des Calciums als eine Mikroinjektion des Coffeins in die Fasern direkt.

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[13] Acknowledgment. This work was supported in part by grants from the Medical School Research Committee, the Graduate School Research Committee, the Wisconsin Heart Association and the Office of Naval Research. We wish to acknowledge our indebtedness to Mr. GEOFFREY CHAMBERS for technical help.

Modification of Hexose of a Glycoprotein Obtained from the Urine of Aminonucleoside of Puromycin Nephrotic Rats

Recently we described the isolation and purification of a glycoprotein from rat urine (MUPpg) with chemical and immunologic characterestics similar to the non-collagen-like glycoprotein of glomerular basement membrane (GBM)[1,2]. Several investigators have demonstrated alterations in the chemical composition of GBM in nephrotic animals[3–11]. Aminonucleoside puromycin (AMP) consistently produces a non-immunologic nephrotic syndrome in rats when administered either by a single large injection or by daily intraperitoneal injections[12,13]. This disease is associated with altered glomerular capillary permeability[14–16], which may occur as a result of alteration in basement membrane chemical composition[6]. The present study was designed to determine whether quantitative or qualitative alterations in MUPpg occurred in association with the development of AMP nephrosis.

Materials and methods. Two consecutive 24 h urines were collected from 50 Sprague-Dawley rats (150 g) at the beginning of the study. Urine volumes were recorded and urinary protein excretion measured. The urine samples were pooled and MUPpg, a glycoprotein similar in chemical composition but of a larger molecular weight then the non-collagenous GBM glycoprotein described by KEFALIDES[17] was isolated by ion exchange chromatography on Diethylaminoethylcellulose chromatography (DEAE) followed by Sephadex G 200 gel chromatography as previously described[1,2]. Following baseline studies, 2 groups of animals were used.

Group I. A) 15 rats in this subgroup received a single large dose (10 mg/100 g body wt.) of aminonucleoside of puromycin (AMP Sigma Biochemicals, St. Louis, Missouri). B) 15 rats received daily i.p. injections of AMP (2mg/100g body wt.) for 10 days.

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Group II. A) 10 animals in this subgroup received a single dose (1 cm³) of pyrogen free physiologic saline. B) 10 animals received daily i.p. injections (1 cm³) of pyrogen free saline for 10 days.

Urines were collected daily and volumes and quantitative urine protein excretion measured. All animals in Group IA developed proteinuria (100 mg/24 h) by day 9–12. Urine from the days of peak proteinuria was pooled and used for isolation and chemical characterization of MUPpg. On days 9 and 10 urines from 10 survivors from Group IB with edema and proteinuria (100 mg/24 h) were also pooled for analysis.

Urines from Group IIA animals were pooled on day 9 and 10 and Group IIB on day 20 and 21. None of these animals were edematous or had protein excretions greater than 8 mg/24 h. MUPpg was isolated from pooled urine of animals in Group II by methods previously described[1,2]. A modification of this technique was used on the animals in Group I. After the urine was concentrated, it was dialyzed against 0.01 *M* phosphate buffer pH 8 (rather than with 0.05 *M* phosphate buffer pH 8) and a gradient elution from DEAE ion exchange column using 0.01 *M* to 0.05 *M* phosphate buffer was employed. The fraction showing no serum proteins but cross reaction with basement membrane component by immunodiffusion using antisera to rat serum and rat GBM was placed on Sephadex G 200 and MUPpg isolated as previously described[1,2]. The MUPpg fraction from all groups was dialyzed extensively against distilled water, lyophyllized and weighed. Immunodiffusion in agar gel against antisera to rat GBM, MUPpg and normal rat serum was also performed.

Amino acid analysis[17], total hexose[18], glucose and galactose by enzymatic methods (glucostat and galactostat, Worthington Biochemicals) and sugar chromatography were performed on MUPpg from pooled urines in each group. These methods have been described by us in detail in a previous communication[2]. The standard deviation of the methods on triplicate samples were for hexose 1.2%, glucose 0.05% and galactose 0.05%. Since urines from each group were pooled individual standard deviations were not obtained. The standard deviation reported is analytical error of triplicate analyses.

Results. Rat serum proteins were not detected in the MUPpg fraction isolated from any group by immunodiffusion using antisera to normal rat serum, rat albumin and rat IgG as controls. However a single precipitin band was obtained using antisera to rat GBM and MUPpg.

There were no significant differences in the amino acid composition of MUPpg of any of the groups. The amino acid composition is the same as reported previously[2]. Glucose, galactose, mannose and fucose were detected by paper chromatography of MUPpg hydrolysates of all groups. A 4–6-fold quantitative increase in excretion of MUPpg in both nephrotic groups was observed. Total hexose was decreased and the ratio of glucose to galactose markedly altered in the nephrotic animals. These changes are summarized in the Table.

Discussion. MUPpg is chemically different from whole GBM and from the collagenous component of GBM in the lack of hydroxyproline and hydroxylysine and the quantity of glycine as well as the carbohydrate composition[2]. This glycoprotein is also chemically quite different from the purified trypsin digest of GBM, Tamm-Horsfall protein and glomerular polyanion; but most closely resembles the non-collagenous GBM glycoprotein[2]. The carbohydrate composition and molecular weight as well as the immunologic properties suggest that it represents a combination of both the collagenous and non-collagenous GBM glycoproteins but consists primarily of the latter.

Glomerular injury accompanied by proteinuria, produced either by immunologic or other mechanisms is associated with alterations in GBM chemical composition[3–11]. It has been suggested that chemical alterations of GBM may modify glomerular permeability and thus cause increase in protein excretion[6].

Electron microscopic studies have shown that Ferritin molecules (M.W. 462,000) which do not permeate the normal glomerular basement membrane penetrate the GBM of aminonucleoside nephrotic rats[14]. Other studies using beef liver catalase (M.W. 240,000) the passage of which into the urinary space of normal rats is restricted by the basement membrane and the epithelial slit pore show the catalase appearing in the urinary space in aminonucleoside nephrosis.

A decrease in glomerular sialoprotein has been observed in aminonucleoside nephrosis[8]. KEFALIDES[17] has observed a decrease in hydroxylysine and hydroxyproline and alteration in the glucose:galactose ratio in the basement membrane of aminonucleoside nephrotic rats. We have confirmed these studies[10]. BLAU observed a decrease in hydroxyproline in AMP nephrotic rats[11].

The changes in urinary glycoprotein composition observed in this study may reflect alterations of the non-collagen component of GBM and of adjacent tissues. Prior studies on the non-collagenous GBM glycoprotein have not been reported. The alterations observed may either be a result of GBM damage caused by passage of large quantities of protein through glomerular diseased basement membranes or may be a result from alteration of the

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Summary of the average urinary volume, recoverable MUPpg, and the percentage total hexose, glucose and galactose from MUPpg of each group

	Baseline[b]	Group[c] IA	Group[d] IIA	Group[e] IB	Group IIB
[a] Average urine volume/animal/day (cm³)	5.2	4	5.8	9	5
[a] Average recoverable MUPpg/animal/day (mg)	0.525	4	0.7	2.5	0.8
Tota hexose (%)	14.99	12	14.7	10.34	15.1
Glucose (%)	3.57	1.2	3.02	0.87	4.37
Galactose (%)	0.85	1.3	0.89	1.07	1.06

[a] Calculated by dividing total amount by animals used. [b] Baseline-values for urine collected prior to treatment (see text). [c] Values for urine collected from rats receiving a single dose of AMP. [d] Values for urine collected from rats receiving multiple doses of AMP. [e] Values for urine collected from rats receiving multiple doses of saline.

molecular integrity of this filtration membrane and be responsible for the increased permeability of the glomerular capillary to protein. The quantitative differences in MUPpg excretion in different groups is difficult to explain but may reflect increased loss of glycoprotein components in the urine in nephrotic rats. It is also possible that the increased quantities of MUPpg result from errors in the quantitation of MUPpg excretion by the techniques used since MUPpg may be lost in the isolation procedure described.

Since the nephrosis in rats produced aminonucleoside of puromycin bears a striking clinical, morphologic and immunohistological resemblance to lipoid nephrosis in the human the mechanism of production of disease by this drug may have striking pathogenic and therapeutic application[19].

Résumé. La néphrose produite expérimentalement chez le rat par l'amino-nucléoside, qui est analogue à la néphrose lipoïdique chez l'homme, est associée à des changements de la composition des glucides d'une glucoprotéïne non collagène extraite des urines de rats ayant de la protéinurie. La composition des acides aminés n'a pas changé. Il se peut que cette glucoprotéine soit le résultat d'altérations dans la membrane basale glomérulaire, altérations qui amènent la protéinurie et peuvent être importantes dans la pathogénèse de la néphrose lipoïdique chez l'homme.

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[19] Supported by Government Research Grant USPHS 1-501-FR 05354, USPHS Training Grant HD 0060 and the Westwood Hills Junior Foundation.

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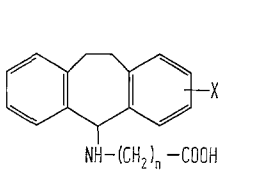
7-Aminoheptanoic Acid Derivatives as Potential Neuropharmacological Agents. I

Short chain amino-acids, such as glycine or 4-aminobutyric acid[1,2], are generally appreciated as mediators in nervous central inhibitory systems. Long chain amino-acids such as 6-aminohexanoic[3,4] and 8-amino-octanoic acids[5–10] are evaluated as possible stimulant substances acting upon the central nervous system; consequently it seemed important to study systematically a large number of aliphatic ω-amino-acids derivatives. As part of this program, we have synthetized a large number of linear ω-aminoalcanoic acids N-substituted with a tricyclic nucleus, previously known for its affinity towards central nervous system structures. This choice led us to synthetize four important series of compounds having the following 4 general formulas:



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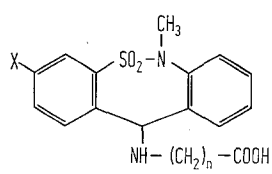

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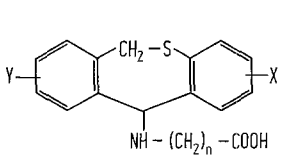
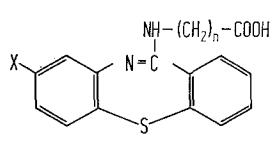


III



IV

Pharmacological screening methods. a) mice motor activity during 2 h using an actophotometer APELAB[11]; b) group toxicity in CD mice[12]; c) hyperthermia in LE rats and rabbits; d) anorectic potency in the SD rat[13]; e) antagonistic effect against reserpine or barbiturate depression or f) against reserpine-ethanol induced sleep in mice[14]; g) tail-clip test for analgesia; h) hot plate technique in NMRI mice[15]; i) antitussive activity using a citric acid spray in the guinea-pig[16]; j) cardio-vascular effects were determined in the nembutal anesthetized dog: blood pressure, heart rate, cardiac output, respiratory frequency and interactions with vaso-active mediators after i.v. injections of compounds; k) EEG studies were realized in chronically implanted rabbits and rats (CD); l) in vitro tests including ileum, vas deferens, seminal vesicle and uterus.

Results. In the series I and with n increasing, there appeared suddenly with $n \geqslant 6$ a motor stimulant effect after i.p. or oral administrations to mice. This activity slowly decreased for $n \geqslant 10$.

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