digests of the potentiator A and the synthetic peptide showed similar patterns on the paper chromatography.

Of the 5 peptides isolated from the venom of *Agkistrodon halys, blomhoffii,* this peptide had the weakest bradykinin-potentiating activity on guinea-pig ileum[2]. This weak activity may be because, unlike potentiators B, C and E, it lacks prolylprolyl sequence at the C-terminus. This was supported by the fact that the synthetic peptide, Pyr-Gly-Arg-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Pro, has strong bradykinin-potentiating activity on guinea-pig ileum. The amount of the synthetic peptide to potentiate 2-fold action of bradykinin on guinea-pig ileum was 0.16 nmole/ml, while that of synthetic potentiator A was than 33 nmole/ml.

Zusammenfassung. Strukturaufklärung eines Bradykinin-potenzierenden Peptids, Peptids A, aus dem Gift von *Agkistrodon halys blomhoffii.*

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Increased Urinary Excretion of a Basement Membrane Like Glycoprotein in Acute Uranium Nephropathy*

Urinary glycoproteins which cross react immunologically with antigens in glomerular basement membranes (GBM) have been isolated in several species[1-4]. Quantitative and qualitative alterations in these proteins have been described in immunologic and chemical injury and appear to reflect GBM damage[5,6]. In previous communications we have purified and immunologically characterized a major protein of rat urine (MUP), a GBM like glycoprotein, and demonstrated alterations in this protein by drugs and chemicals[7,8].

Uranium poisoning is a model of non-immunologic mediated renal injury. As well as acute tubular injury, uranium produces glomerular lesions with deposits of randomly oriented fibers on the endothelial side of the lamina densa[9,10]. This study was done to examine rat MUP during acute uranium nephropathy.

Materials and methods. Fifteen Sprague Dawley rats, 300 g, were used in this study. On day 0 baseline 24 h urine samples were collected from each animal. 10 animals received 14 mg/kg of uranyl nitrate hexahydrate as a single s.c. dose 24-h urine specimens were collected daily over the next 3 days. Urine protein excretion was determined using sulfosalicylic acid. Daily excretion of MUP was quantitated by radial immunodiffusion using concentrated urine samples. On day 5 all animals were sacrificed and serum creatinine determinations performed. Sections of kidney were fixed in formalin for light microscopy. Rat MUP was isolated and purified as previously described[7], and used to prepare standards for radial immunodiffusion.

Antisera to rat MUP was prepared in rabbits by weekly immunization. The antiserum was absorbed with lyophylized rat serum and its monospecificity confirmed by immunoelectrophoresis against concentrated rat urine. A single precipitin band was obtained against purified rat MUP and against concentrated rat urine. No precipitin band occurred with rat serum. The antisera was incorporated into agar and radial immunodiffusion plates prepared.

One ml concentrated normal rat urine and concentrated uranium rat urine containing a total protein level of 20 mg/ml was used for chromatography. Sephadex G 200 gel chromatography was performed on a Pharmacia column, 0.9×85 cm, and eluted at a flow rate of 5 ml/h. 2 ml fractions were collected and read at 280 nm in a Leitz spectrophotometer. Agarose gel and immunoelectrophoresis were performed on urine from normal and uranium treated animals.

Results. The uranium treated animals showed marked renal morphologic alterations varying from acute tubular necrosis with moderate to severe diffuse mesangial proliferation to cortical necrosis. Mean creatinine levels in control animals was 0.8 mg/100 ml S.D. $\pm$ 0.1 as compared to 12.2 mg/100 ml S.D. $\pm$ 3 in diseased animals.

Daily urine volume, urine protein and MUP excretion are shown in the Table. Excretion of MUP increased almost 50% during the first 24 h after uranium injection. This increase was significantly different from the controls at the 95% confidence limit. Over the next 2 days MUP excretion returned to normal. As the animals became moribund and oliguric on day 5, MUP excretion dropped. The control animals showed no significant alterations in protein, volume or MUP during the course of the study.

No unique MUP fragments were recognized in the urine of uranium rats as compared to normals by immunoelectrophoresis using antisera to rat MUP. Migration of

Average total daily urine protein, MUP and volume in baseline and uranium treated rats.

		Day 0	Day 1	Day 2	Day 3	Day 4
Protein	Mean (mg)	9.3	36[a]	89[a]	111[a]	10
	Std. Error	1.6	45	18	18	10
Volume	Mean (ml)	9.7	24[a]	31[a]	24[a]	10
	Std. Error	1.5	2.7	7.0	4.8	3.5
MUP	Mean (mg)	7.0	11[a]	7.8	6.8	2.8[a]
	Std. Error	1.2	1.4	0.8	1.0	1.5

[a] Denotes statistically significant at 95% compared to control period and to control animals.

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MUP from uranium treated animals in agarose and also by immunoelectrophoresis using antisera to MUP did not differ from control urine. The elution pattern of MUP from control and normal urine on G 200 did not differ as detected by immunodiffusion of the fractions eluted.

Discussion. Renal damage has been associated with changes in the chemical composition of GBM and alterations in urinary basement membrane like glycoproteins[5,6,8,11–17]. Alterations in the chemical composition of GBM have been demonstrated in nephrotoxic nephritis[5,15–17], aminonucleoside nephrosis[6,11,12] and in cyclophosphamide[8] and corticosteroid[18] treated animals. Similar alterations have been found in urinary GBM like glycoproteins in the same conditions[5,6,8,17]. These findings suggest that urinary GBM like glycoprotein can reflect basement membrane injury.

In the present study uranium poisoning was associated with a 50% increase in excretion of major urinary glycoprotein in the rat. The reason for the increased MUP is unclear. The possibility that increased MUP is a nonspecific effect of proteinuria is unlikely as the MUP peak precedes the peak of proteinuria. The changes in urine MUP may reflect the morphologic changes in the GBM seen in uranium poisoning[9,10]. Increased MUP excretion could also be the result of damage to the tubular basement membrane[19].

Résumé. Les effets de l'uranium sur l'excrétion de la glycoprotéine urinaire principale du rat ont été étudiés chez 10 animaux. La principale glycoprotéine urinaire augmente de 50% dans les 24 h qui suivent l'administration de l'uranium.

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Decrease of Litter Size and Fetal Monoamines by 6-Hydroxydopamine in Mice

There is a rich sympathetic innervation in the female genital organs including the tubae and ovaries of many mammalian species[1]. In the ovaries of cats[2] and women[3], it is higher than in other reproductive organs. In tubes the sympathetic innervation may play a role in the motility[1], in the ovaries its physiological role is still more obscure. Because 6-hydroxydopamine rather specifically destroys the peripheral sympathetic nerve endings[4], it seemed appropriate for the studies on the role of this innervation.

Methods. Young adult female Albino Swiss mice weighting 25–35 g were used. The dose of 6-hydroxydopamine (6HD) (Fluka AG, Buchs) was either 50 + 50 mg/kg i.p. in two following days and then 50 mg/kg i.p. twice a week, or 100 + 100 and 75 mg/kg, respectively. Controls were injected with saline. Vaginal smear was taken daily for determining the effect on the estrus cycle. After about 3 cycles the female mice were put together with males for mating. Each female was kept together with 2 different males. A group of mice were killed before delivery and the rest some days after it. Ovaries, tubes, kidneys, hearts and brains were promptly frozen in liquid nitrogen, for fluorescence microscopy. Of iris a stretch preparate was immediately made and dried at the room temperature. Tubes and ovaries were freeze-dried for 3 days. The preparations for fluorescence microscopy was made according to ERÄNKÖ[5] and FALCK and OWMAN[6]. Noradrenaline (NA) and 5-hydroxytryptamine (5HT) were determined spectrophotofluorometrically recording to MILLER et al.[7].

Results. Neither the higher or lower dose had significant effect on the estrus cycle. The treated mice became pregnant at best nearly as well as the controls, but the litter size was smaller (Table II). 6HD treatment through the pregnancy decreased the NA content not only in the mother but also in the fetus and newborn mice. 5HT contents did no change significantly. (Table I and II).

Discussion. The results show that 6HD decreases the litter size in mice. The mechanism of this is not known. The decrease of NA content in the tissues of fetal and newborn mice indicates that 6HD is able to tranfer into the fetus, and thus a direct embryotoxic effect is possible. Other possibilities are changes in the uterine and placental circulation due to the direct vasoconstricting sympathomimetic action of 6HD and the sympatholysis, changes in tubal motility including the utero-tubal sphincters or the expulsion of ova.

Our preliminary results in rats suggest an abortive mechanism due to the sympatholysis[8] Rabbits, however, did not seem to become pregnant at all after 6HD treatment.

Antiadrenergic drugs have been used to block ovulation in mammals as well as in birds[9,10]. Partly these effects may be of central origin[11], but this seems not to be the case in

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