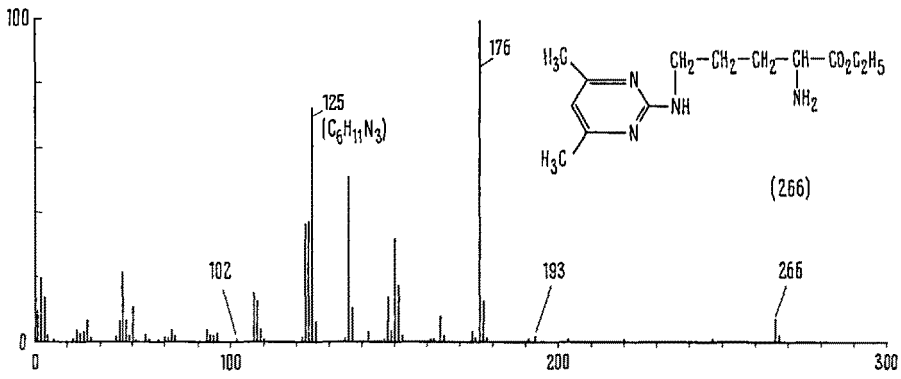

Das Massenspektrum des Äthylesters (II).

schen als ausreichend. Die Retentionszeit von (II) ist im Vergleich zu einigen anderen Aminosäureäthylestern in der Tabelle angegeben.

Retentionszeiten: (Trennsäule: Stahl, 5′ × 1/4″, 250 °C, 20% SE-30 auf Chromosorb W 60–80 mesh; Trägergas: Helium, 40 ml/min)

Methioninäthylester	1,1 min.
Hydroxyprolinäthylester	1,25 min.
Phenylalaninäthylester	1,8 min.
Tyrosinäthylester	4,15 min.
Pyrimidylornithinäthylester	7,9 min.

Die Fragmentierung wird sichtlich durch die Anwesenheit des heterocyclischen Ringes stark beeinflusst. Die meist sehr charakteristischen Fragmente von α-Aminosäureäthylestern[1], z.B. das «Ester-Fragment» $\overset{(+)}{H_2N}{=}CHCOOC_2H_5$ (m/e 102) und das entsprechende «Amin-Fragment» $RCH{=}\overset{(+)}{N}H_2$ (m/e 193), treten überhaupt nicht bzw. nur in sehr geringer Menge auf. Letzteres ist allerdings wie beim hierin vergleichbaren Lysinäthylester eine Folge der starken Eliminierung von NH_3 aus dem Amin-Fragment, so dass als dessen Folgeprodukt ein Ion der Masse 176 auftritt, welches sogar dem Basispeak des Spektrums entspricht. Die Peakgruppe m/e 123, 124 und 125 repräsentiert, wie die entsprechenden Elementarzusammensetzungen zeigen, den Aminopyrimidinrest des Moleküls, wobei zur Bildung der jeweiligen Ionen die Übertragung von 1, 2 und 3 Wasserstoffatomen auf den Heterocyclus erforderlich ist. Über die mechanistische Seite dieser ungewöhnlichen Reaktion soll in einem anderen Zusammenhang berichtet werden.

Summary. Arginine has been converted into a suitable pyrimidine derivative to permit its qualitative analysis in the presence of other amino acids by means of mass spectrometric and gas-chromatographic techniques.

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[6] Derzeitige Anschrift: F. Hoffmann-La Roche & Co., Aktiengesellschaft, Basel (Schweiz).

Evidence that Methylguanidine is Retained in Chronic Renal Failure

Monosubstituted guanidines are known to be retained in chronic renal failure (Yatzidis et al.[1]) and their concentration has been found to be proportional to that of creatinine (Giovannetti et al.[2]). It is also known that guanidine and methylguanidine exert a broad inhibitory effect on several enzymes of biological importance for man (Hollunger[3], Rajagopolan et al.[4]). Furthermore guanidine has been found to inhibit the in vitro oxygen consumption of brain slices (Lascelles et al.[5]).

Studies from this laboratory provided evidence that guanidine, monosubstituted guanidines, creatine and creatinine increase the amount of the spontaneous autohaemolysis occurring in vitro during incubation of normal blood samples, guanidine and methylguanidine being the most effective (Giovannetti et al.[2]).

In the present study attempts were made to identify guanidines which accumulate in chronic renal failure.

Material and methods. Blood samples from 10 normal persons and from 12 chronic uraemics were examined. In the uraemic plasma samples the creatinine concentrations ranged from 11–21 mg% (auto-analyzer) and those of the monosubstituted guanidines from 0.6–3.2 mg% (Yatzidis method[1]). Figures in 10 normal persons were previously found to be 0.22 ± 0.19 (Giovannetti et al.[2]).

[1] H. Yatzidis, D. Oreopoulos, N. Tsaparas, S. Voudiceari, A. Stavroulakis and S. Zestanakis, Nature *212*, 1498 (1966).
[2] S. Giovannetti, P. L. Balestri, L. Cioni and M. Biagini, Clin. Sci., in press.
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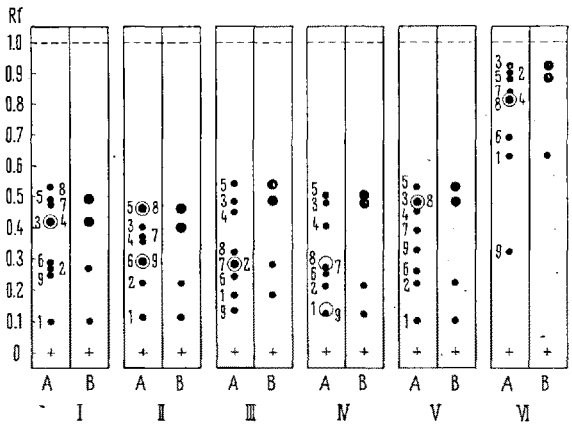
[4] K. Rajagopolan, K. V. Fridowich and P. Handler, Fedn Proc. Fedn Am. Socs exp. Biol. *19*, 49 (1960).
[5] P. T. Lascelles and W. H. Taylor, Clin. Sci. *31*, 403 (1966).

	Arginine	Creatine	Creatinine	Guanidine	Methyl-guanidine	Guanidino-acetic acid	Guanidino-propionic acid	Guanidino-butyric acid	Guanidino-succinic acid
Sakaguchi	+++	–	–	–	+++	+++	+++	+++	+++
Alcaline picrate	–	–	+++	–	–	–	–	–	–
Ninhydrin	+++	+	+	–	–	+	+++	+++	–
Alkaline ferricyanide-nitroprosside	–	++	+++	+++	+++	+++	+++	+++	+++
α-Naphthol-diacetyl	++	+++	–	+++	++	++	++	+++	++
Pentacyano-amino-ferrate	++	+++	–	+++	++	++	++	+++	++

The colour reactions of guanidines and related compounds.



A schematic representation of the chromatograms obtained with the test substances (A) and with the extracts of the uraemic plasma (B). Solvent systems: (I) butanol-acetic acid-water (80:20:20); (II) butanol-formic acid-water (63:20:17); (III) propanol-20% NH_3-water (73:20:7); (IV) pyridine-isoamyl alcohol-NH_3-water (8:4:1:4); (V) pyridine-isoamil alcohol-acetic acid-water (8:4:1:4); (VI) phenol saturated with water containing 6.3% sodium citrate and 3.7% sodium dihydrogen phosphate. Test substances: (1) arginine (base); (2) creatine; (3) creatinine; (4) guanidine (base); (5) methylguanidine (base); (6) guanidinoacetic acid; (7) guanidinopropionic acid; (8) guanidinobutyric acid; (9) guanidinosuccinic acid.

Descending chromatography on Whatman No. 1 paper was employed by using 6 of the solvent systems indicated by BLOCK et al.[6]. Guanidines, arginine, creatine and creatinine were extracted from plasma (20 ml) with the modified YATZIDIS method previously described (GIOVANNETTI et al.[2]). Attention was paid so that for chromatography the same amount of extract, corresponding to 4–5 ml of plasma, were used for normal and for uraemic plasma samples.

Chromatograms were developed with the colour reagents indicated by BLOCK et al.[6], as listed in the Table.

Guanidine base was obtained from the B.D.H. (Poole, England), creatine and creatinine from Erba S.p.A. (Milano), monosubstituted guanidines from K. & K. Lab. Inc. (New York) with the exception of guanidinosuccinic acid, which was supplied by Mann Research Lab. Inc. (New York).

Results. In all the chromatograms obtained both with normal and uraemic plasma, a spot was found having the same Rf values (Figure) and the same colour reactions (Table) as methylguanidine base added in vitro to normal blood or injected to normal dogs (as HCl), and then extracted from plasma and submitted to the chromatographic migration.

In the chromatograms obtained with uraemic plasma, this spot was much larger than in those obtained with normal plasma.

When this spot was developed with the Sakaguchi reagents and then eluted from the chromatograms (ethanol-water, 50:50 v.v.) obtained with uraemic plasma, the colorimetric evaluation showed that it represented the 88.3 ± 9.7% of the Sakaguchi positive material.

A second Sakaguchi positive spot was always found in the chromatograms and this had the same Rf values (Figure) and the same colour reactions (Table) of arginine base. Two spots were also constantly present having the same properties as creatinine and creatine (Figure and Table), and both were larger in the chromatograms obtained with uraemic plasma than in those obtained with the normal one.

In no chromatogram obtained with uraemic or normal plasma a spot was found having the same properties of guanidine base.

Conclusions. The results obtained provide evidence that methylguanidine is present both in normal and uraemic plasma and that, in the latter, it is greatly increased, accounting for most of the Sakaguchi positive material. Methylguanidine is a constituent of creatine (methylguanidinoacetic acid) and of its derivative creatinine, and this suggests that all such substances may have some common metabolic pathway.

Considering the high toxicity of methylguanidine, the suspicion is justified that it plays a role in the genesis of the uraemic syndrome as it was suggested by MASON et al.[7] many years ago[8].

Riassunto. La cromatografia su carta ha permesso di accertare che la metilguanidina è presente nel sangue umano normale ed è trattenuta negli uremici in cui rappresenta la quasi totalità del materiale Sakaguchi positivo.

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General Medical Department of the University of Pisa (Italy), 12 October 1967.

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