A Method for Separation of Some Peptides from Human Urine* (32986)

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Urinary peptides are of interest because of what they may suggest of renal physiology and metabolism, as well as their possible biologic roles. This report describes the separation from normal human urine of a group of acidic peptides, apparently different from those hitherto reported (1–3).

Methods. Fifty ml of fresh urine, adjusted to pH 2.8, was applied to a 100 ml Dowex 50W-X8 (AG,H^+) column, followed by 100 ml of water. The effluent was swirled with 100 gm of the weakly basic resin IR-4B(OH^-) (Rohm and Haas) and the supernatant fluid rotary evaporated at 60°C to about 20 ml. The concentrate was put through a 2 × 12 cm Dowex 1-X8(OH^-) column, followed by 200 ml of water; the effluent was discarded. The column was eluted with 100 ml of 5 *M* acetic acid and the eluate rotary evaporated at 60°C. The residue was dissolved in 3 ml of water and 1 ml was applied to a 0.63 × 130 cm column of AG1-X8(-325 mesh, spherical, acetate form) (Bio-Rad). The column was eluted at 0.88 ml/min, 60°C, with water for 1 hour, 0.2 *N* acetic acid for 1.5 hours, 0.4 *N* acetic acid for 1.5 hours, and finally 1.0 *N* acetic acid for 8 hours. A stream splitter divided the column effluent, part of which was collected in 10-min fractions while the rest went through a peptide detector (4), omitting a nonhydrolysis line since the peptides are not detectable with ninhydrin before hydrolysis. The column was ready for use again after washing with water for 3 hours.

Contents of tubes from the fraction collector corresponding to some of the larger peaks were dried at 110°C, the residue was hydrolyzed in boiling water for 20 hours in 6*N* HCl, evaporated, dissolved in pH 2.8 citrate buffer, and analyzed with an amino acid analyzer (5).

Results. A typical pattern obtained with the peptide detector is illustrated in Fig. 1. Free sugars emerge mixed with peaks 1 and 2. Carbohydrate, presumably peptide-bound, could also be detected with anthrone in tubes corresponding to peaks 5, 6, 12, 13, 15 (or 16), 17, 21, 22 (or 23), and 26.

Table I gives the approximate proportions of various amino acids found in some of the components. Glucosamine would be largely destroyed by the hydrolytic conditions used, so the proportions given for it are minimal. Peak 3 is composed mostly of free taurine. The qualitative amino acid composition of these peptides is similar; no imino acids, and generally no amino acids eluting beyond valine were found, except for an unidentified peak eluting 20 min after ammonia seen in the hydrolyzate of components 2 and 5.

Discussion. A preliminary concentration of the urinary peptides was attempted by use of a procedure for isolation of sialic acid-containing compounds (6): 1200 ml of urine was stirred with 150 gm of Darco G-60 charcoal, which was then washed and eluted with 80% ethanol. The unhydrolyzed eluate gave a typical aminogram for urine (5) with the amino acid analyzer. After then putting it through the present separation procedure as described, the sixteen peptide components seen were in insufficient quantities for amino acid analysis, indicating inefficient peptide recovery from the urine.

The qualitative similarity of these peptides may be to some extent a reflection of common origin and of their resistance to enzymatic attack (7, 8). Their relative ninhydrin negativity is ascribable to their chain length. Those components eluted from the AG1 column with water are probably a mixture of the longest chain peptides and appear to contain a very considerable part of the glucosamine in urine, all of which is in the "bound" form.

Summary. A method is described for sepa-

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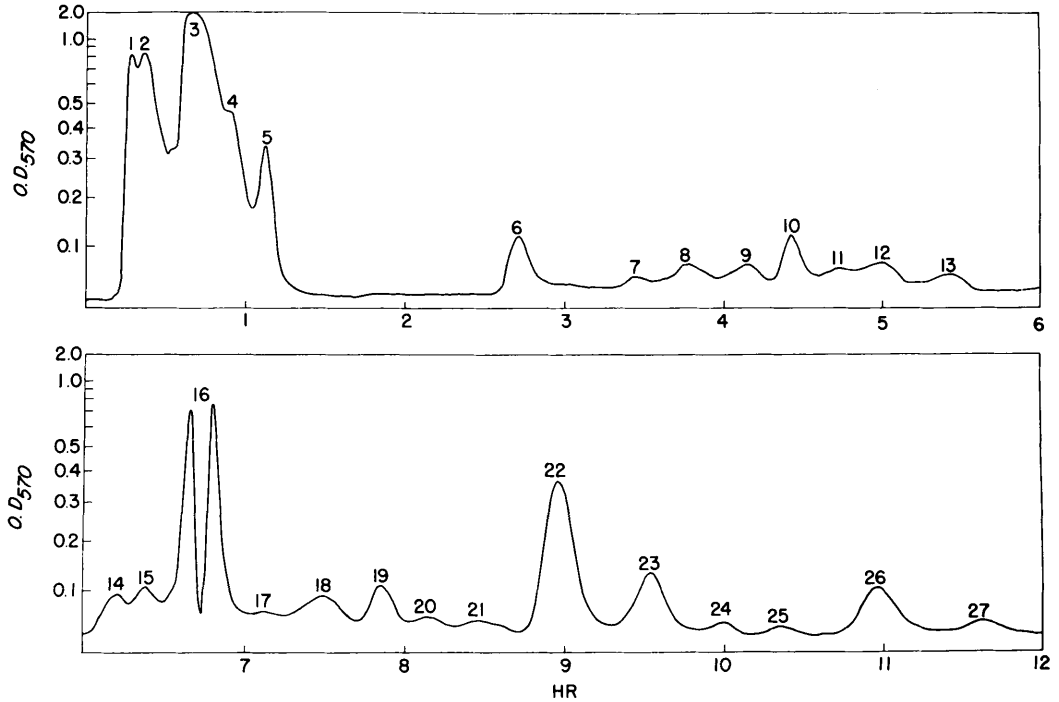

FIG. 1. Representative pattern obtained with peptide detector. Only peak 3 (taurine) appeared before hydrolysis. Split in peak 16 is a consistent artifact, of unknown origin.

TABLE I. Approximate Proportions of Amino Acids Found in Some Peaks of Fig. 1.[a]

Peak no.	Tau[b]	Asp	Thr	Ser	Glu	Gly	Ala	GlcN	Val	Others
1		3	2	3	4	8	3	37	5	
2	7	4	2	4	1	10	3	80	8	Pro: 1, Ile: 1
3	++++	2	1	3	5	7	6	10	3	Tyr: 2
4		7	2	2	7	5	5	6	3	Ile: 2, Tyr: 1
5	13–15	2	2	3–4	1	2–3	1		3	Leu: 1
6		4	1	1	3	3	2	2–3		
10		5	1	1	5	8	2	2	3	Phe: 1, Lys: 1
13		2	1	1	2	4	1			Phe: 28
14		+	++	++	+		+			
16	3	4	1	1	3	6	1		2	
22		15	2			7	1		1	
23		+	+	+	+	+				
26		1		6	2	5	1			

[a] Abbreviations of amino acids according to J. Biol. Chem **241**, 527 (1966).

[b] Except for peaks 2 and 3, compound emerging at taurine position is probably not taurine.

ration from human urine of a group of peptides. These were resolved on an anion exchange column into 27 components. The approximate amino acid composition of 13 of these is reported.

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Pathogenicity of Coxsackie A-21 Virus for Suckling Mice (32987)

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The prototype strain of Coxsackie A-21 virus (Kuykendall strain), first isolated in 1955, produced a marked myositis and flaccid paralysis in suckling mice (15). Its classification as a Coxsackie group A virus was in part based on these findings. Four strains of Coe virus, recovered in 1958 by Lennette and co-workers from persons with respiratory disease, and subsequently shown to be serologically indistinguishable from Coxsackie A-21 virus, failed to produce paralysis in suckling mice even after multiple blind passages (14). Similar findings were reported by Pereira and Pereira (12) using a Coe virus strain recovered in Great Britain. In 1962 Underwood and co-workers (17) demonstrated that Coe virus could be adapted to growth in suckling and older mice after serial passage. The opportunity to investigate the effect of several newly isolated naturally occurring strains of Coxsackie A-21 virus was provided when numerous strains were recovered during an outbreak of mild acute upper respiratory illness among military personnel at Camp Lejeune, North Carolina in September and October, 1960 (2 and 7). This report describes the quantitative relationships between virus dose and the production of paralysis and myopathy in suckling mice for several naturally occurring and tissue culture adapted strains of Coxsackie A-21 virus.

Materials and Methods. Animals. General purpose Swiss strain mice approximately 24 hours old (either sex) were used. Each litter was housed in a separate cage and provided with water and Purina laboratory chow *ad libitum*.

Tissue culture. Roller tube cultures of HEp-2 and primary human embryonic kidney (HEK) cells were purchased from commercial sources. The maintenance medium for HEK cell cultures was medium 199 with 2% inactivated (30 min at 56°C) chicken serum and 0.002 *M* glutamine, and for HEp-2 cell cultures it was Eagle's Basal Medium with 5% inactivated chicken serum and 0.002 *M* glutamine (11). An antibiotic mixture was added to all media containing at final concentration: aqueous penicillin, 100 units/ml; streptomycin, 100 μgm/ml; mycostatin, 50 units/ml; and tetracycline, 100 μgm/ml. Maintenance medium was changed at 3–4 day intervals, and all cultures were incubated for 18 days at 33°C on a rotating drum revolving at 12 rph.

Virus strains. The strains of Coxsackie A-21 virus used in these studies have been described in a previous communication (11). All strains were recovered from the upper respiratory tract of patients during an epidemic of Coxsackie A-21 virus-associated mild upper respiratory illness at Camp Lejeune, North Carolina (2 and 7). Each strain was

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