Urine Peptidase Activity Following Single and Multiple Oral Doses of Streptokinase. (29309)

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Enhanced urinary peptidase activity was observed in rats given oral doses of streptokinase and proteases(1). The underlying mechanism was obscure because, in all probability, neither kinases nor proteases penetrate the gastrointestinal tract(2,3). Later, rat kidney homogenates showed augmented plasminogen activator and peptidase activity following orally given streptokinase(4). The probable mechanism appeared to be conversion of gastric juice plasminogen pro-activator into plasminogen activator, systemic absorption of plasminogen activator, activation of renal plasminogen into plasmin, and plasmin activation of renal pro-peptidases into peptidases(4).

The present study was undertaken to ascertain the effect of orally given streptokinase upon urinary peptidase activity in man. Following single and multiple doses of streptokinase in man, urinary peptidase activity appears to increase significantly.

Materials and method. This study comprised 20 healthy male students and technicians whose ages varied from 21 to 38 years. Leucine aminopeptidase activity was measured according to Goldbarg and Rutenberg(5). Carboxypeptidase B was measured according to Folk *et al*(6). Determinations are made after 10 minutes of incubating urine samples with hippuryl-l-arginine.

Procedure. A. Single dose study. Breakfast is omitted. After voiding and discarding the urine sample at 8 a.m., the subject drinks two 8 oz glasses of water. Control urine samples are obtained and saved at 9 a.m. and 10 a.m. Following the 10 a.m. voiding, the subject drinks another two 8 oz glasses of water and is given two 10,000 unit streptokinase tables or 2 identical appearing placebo* tablets. Urine samples are then obtained at 11 a.m. and 12 p.m.

* Placebo tablets contain all ingredients except the 10,000 units of streptokinase in "Varidase" tablets.

To determine the extent of spontaneous fluctuation in urine volume and peptidase activity, each subject follows the above procedure, on placebo tablets, for 3 successive mornings. On the fourth morning the test procedure is followed, except that a single dose of streptokinase is given with 2 glasses of water after the 10 a.m. voiding. On the fifth morning the procedure is reversed to determine the significance of time of enzyme administration. Following the 8 a.m. voiding, streptokinase tablets are taken with the first 2 glasses of water. The rest of the procedure remains unchanged. Urine volume and peptidase assays are done on each urine sample obtained.

B. Multiple dose study. Ten healthy male students, ages 23-27 comprised this study.

Breakfast is omitted. The 7 a.m. urine sample is discarded. At 8 a.m. a urine sample is obtained, after which the first 10,000 unit streptokinase tablet is taken. Streptokinase tablets are again taken at hourly intervals for 5 hours. Urine samples are obtained at hourly intervals from 8 a.m. to 8 p.m. Each subject drinks one 8 oz glass of water at 7 a.m. and at the time of taking each tablet.

Results and discussion. Urinary leucine aminopeptidase activity appears considerably increased following a single dose of oral streptokinase. Baseline urinary control studies over a 3 day period show marked fluctuations in urinary volume but only slight aminopeptidase activity changes. The mean range of urinary volume in the 3 morning specimens during this pre-treatment control period was 72-164 ml; for aminopeptidase activity the range was 2.5-2.7 LAP units. On the fourth day, pre-treatment control urine volumes were 197 and 74 ml at 9 and 10 a.m. respectively and 122 and 112 ml after streptokinase. Leucine aminopeptidase levels rose from 2.9 and 2.6 units per ml to 9.7 and 4.8. Similar changes developed in the cross-over experi-

TABLE I. Urinary Volume and Leucine Aminopeptidase Activity After a Single Dose of Oral SK (Mean Values).

Day of test	Treatment	9 a.m. Vol (ml)	9 a.m. Amino-peptidase (unit/hr)	10 a.m. Vol (ml)	10 a.m. Amino-peptidase (unit/hr)	11 a.m. Vol (ml)	11 a.m. Amino-peptidase (unit/hr)	12 p.m. Vol (ml)	12 p.m. Amino-peptidase (unit/hr)
1–3 (pre-treatment control period)	2 glasses water at 8 a.m. and 10 a.m.	149 (76–351)	2.5 (2.1–3.4)	164 (46–294)	2.7 (2.2–3.7)	72 (59–203)	2.7 (2.4–4.1)	128 (65–295)	2.6 (2.2–3.4)
	2 placebo tablets at 10 a.m.	S.D. 50.2	S.D. .5	S.D. 42.8	S.D. .6	S.D. 41.6	S.D. .8	S.D. 43.6	S.D. .6
4	2 glasses water at 8 a.m. and 10 a.m.	197 (64–320)	2.9 (2.6–3.4)	74 (52–148)	2.6 (2.4–3.3)	122 (63–210)	9.7 (8.3–11.2)	112 (70–185)	4.8 (3.9–5.4)
	2 Varidase tablets at 10 a.m.	S.D. 54.1	S.D. .6	S.D. 19.6	S.D. .6	S.D. 34.2	S.D. .3 P = <.005	S.D. 25.6	S.D. .5 P = <.01
5	2 glasses water at 8 a.m. and 10 a.m.	114 (55–330)	9.6 (8.2–12.3)	98 (172–238)	5.8 (4.6–6.7)	124 (84–226)	2.7 (2.2–3.2)	111 (88–178)	3.0 (2.4–3.4)
	2 Varidase tablets at 8 a.m.	S.D. 28.8	S.D. .45 P = <.001	S.D. 17.3	S.D. .7 P = <.05	S.D. 44.6	S.D. .6	S.D. 22.4	S.D. .7

Range in parentheses.

ment performed on the fifth morning (Table I).

Under pre-treatment control conditions, urinary peptidase activity of each individual in the study remains remarkably constant from hour to hour. Following single doses of streptokinase, however, there is the following consistent response: a significant increase in aminopeptidase activity (p less than 0.001) in the first hour specimen, and a less considerable but still significant (p less than 0.01) increase in aminopeptidase activity in the second post-treatment samples (Table I).

Following multiple doses of oral streptokinase (10,000 units per dose for 5 doses) urinary carboxypeptidase B activity increases strikingly and appears to be maintained for several hours after the final dose (Table II).

TABLE II. Hourly Urinary Carboxypeptidase B Activity After Multiple Doses of Oral SK.

Time	Treatment	Carboxypeptidase B activity (units/hr)	Range	P
8 a.m.	—	102	78- 210	—
8:05 a.m.	SK			
9 a.m.	SK	153	96- 347	<.01
10 a.m.	SK	378	184- 682	<.001
11 a.m.	SK	564	217- 843	<.001
12	SK	673	281- 907	<.001
1 p.m.	—	894	307-1428	<.001
2 p.m.	—	1026	284-1888	<.001
3 p.m.	—	845	193-1459	<.001
4 p.m.	—	637	206-1133	<.001
5 p.m.	—	441	184- 746	<.001
6 p.m.	—	371	86- 552	<.001
7 p.m.	—	156	69- 337	<.001
8 p.m.	—	113	81- 246	<.01

In an earlier publication, one of us (I.I.) indicated the probability that once activated, the plasminogen to plasmin reaction proceeded auto-catalytically(7). This could account for the rather remarkable enhancement in urinary carboxypeptidase activity well beyond the last dose of streptokinase(8).

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Viral Inhibitors of Biological Origin. II. A Viral Inhibitory Factor Obtained from *E. coli* 0111 and Inhibition of Viral Replication by Nucleic Acid Derivatives.* (29310)

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Suppression of viral multiplication by crude materials derived from a variety of sonically disrupted bacteria has been described(1,2). The inhibitory material from each bacterial species tested suppressed certain viruses without affecting the multiplication of others. Thus, while arboviruses are inhibited by material derived from an unclassified Corynebacterium, ECHO 11 is suppressed by material from *Corynebacterium diphtheriae* (both non-toxigenic and intermedius strains) and vaccinia is suppressed by material derived from various *Escherichia coli* serotypes and *Salmonella typhimurium*. The present report is concerned with further characterization of the material from *E. coli* 0111, using the multiplication of vaccinia virus in primary human amnion cell cultures as an indicator system. Preliminary observations concerning viral inhibition by nucleic acid derivatives are also included in this report.

Preparation. An inoculum of *E. coli* 0111, obtained from Dr. J. G. Michael, was grown in one liter of Bacto nutrient broth for 18 hours. The entire culture was used to prime a Biogen containing 40 liters of medium consisting of 7 g K_2HPO_4, 3 g KH_2PO_4, 0.5 g sodium citrate. 2 H_2O, 1 g $(NH_4)_2SO_4$, 0.12 g $MgSO_4$, and 5 g glucose in each liter of water. After 24 hours' growth at 37°C the culture was centrifuged and dried at room temperature. Sixty g of dried *E. coli* 0111 were obtained in this manner. Acetone soluble substances were removed from the dried culture by 3 extractions with 600 ml of acetone. Following this the bacteria were redried at room temperature. The acetone extracted bacteria were taken up in 100 ml of water and the insoluble residue was discarded. The water soluble material was dialyzed against running tap water for 24 hours. The dialysand was concentrated by lyophilization and then dissolved in 100 ml of distilled water. Microsomal elements were discarded after being sedimented by centrifugation at 35,000 rpm for 1 hour in the #40 head of a Model L ultracentrifuge. The final material was separated by further centrifugation of the supernatant fluid at 35,000 rpm for 3 hours and collection of the viscous green fluid in the lower halves of the centrifuge tubes. Lyophilization of an aliquot of this collected fluid enabled the determination that 5.5 g of material were obtained from the initial 60 g of dried *E. coli*.

Physicochemical properties. The inhibitory activity against vaccinia virus was destroyed by heating at 100°C for 5 minutes but was not affected by heating at 56°C for one hour and at 37°C for 3 hours. The activity was lost following dialysis against a citric acid disodium phosphate buffer at pH 2.3. Treatment of the active fraction with either DNAase or RNAase at a final concentration of 1 μg/ml for 3 hours at 37°C did not alter the viral inhibitory effect. The activity was destroyed by exposure to twice crystallized trypsin at a final concentration of 0.12% for 3 hours at 37°C. These findings are consistent with the characterization of the original crude preparations of the soluble ma-

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