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The Effect of Various Microorganisms Found in Urinary Tract Infections on Creatinine

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=國文抄錄=

細菌性 尿道炎의 感染菌이 尿中 크레아티닌의 量에 미치는 영향

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영양실태조사 및 사람을 대상으로 하는 많은 영양적 연구에서 尿中 여러 成分들의 배설량을 측정하기 위해 만 하루의 소변을 완전히 채취하기는 매우 어려우므로 任意時間의 소변을 채취하여 사용한다. 이 경우엔 흔히 尿成分의 농도는 尿中 크레아티닌을 量을 기준으로 하여 표시되는데, 크레아티닌은 尿中 一日 배설량이 개인에 따라 일정하고 尿量에는 상관없이 비교적 일정한 속도로 배설된다고 간주되기 때문이다. 그러나 비교적 높은 발생율을 갖는 細菌在 尿道炎에서는 感染菌이 크레 아티닌을 파괴할 가능성이 있고 따라서 이 경우의 尿中 크레아티닌의 여러 用途는 비합리적으로 될 수 있다. 본 연구에서는 이 가설을 규명하려고 한다.

첫 실험에서는 感染菌이 尿素를 암모니아로 파괴함으로써 형성되는 尿의 알칼리성에 대한 크레아티닌의 安定性을 알아 보았다. 健康人의 尿中 크레아티닌과 완충액에 용해시킨 순수 크레아티닌을 pH 4.5~9.0으로 조정하여 37°C에서 6일간 배양시켰다. 잔존한 크레아티닌을 측량한 결과, 크레아티닌은 완충용액이나 尿에서 모두 산성 pH에서 보다 알칼리 pH에서 더욱 安定함을 보여주었다. 1일간 배양후엔 거의 變化가 없었고 6일 후에나 4.2~8.0%의 감소율을 나타냈을 뿐이다.

두번째 실험에서는 감염균이 크레아티닌을 成長을 위한 질소급원으로 사용하는지를 결정하기 위해 細菌性 尿道炎에서 자주 발견되는 13種類의 박테리아를 健康人의 尿와 크레아티닌을 질소급원으로 하는 合成培地에 37°C로 배양하였다.

대부분의 박테리아는 크레아티닌含量을 감소시키지 않았다. 그러나 *Pseudomonas aeruginosa*와 *Klebsiella pneumoniae*는 合成培地中의 크레아티닌을 상당량 파괴시켰고, 그 파괴율은 그들 성장율과 평행하였다. 배양 6일후에는 크레아티닌이 *Pseudomonas aeruginosa*에 의해 처음 量(500mg/100ml)의 12.8%가, *Kleobsiella pneumoniae*에 의해서는 11.8%가 감소되었다. 감소율은 크레아티닌의 처음 농도가 낮을수록 커져서 50mg/100ml일 때는 각각 21.1%와 28.2%이었다. 더우기 *Klebsiella pneumoniae*는 황산암모늄과 尿素같은 다른 질소급원이 크레아티닌과 共存할 때에도 크레아티닌을 어느 정도 파괴함을 보여 주었다.

결론으로, 細菌性 尿道炎환자의 尿의 알칼리성은 尿中 크레아티닌量에 중요한 영향을 주지 못한다. 그러나 本 硏究에서 사용된 *Klebsiella pneumoniae*와 가능하게는 *Pseudomonas aeruginosa*같은 몇 感染菌은 크레아티닌을 그들 成長의 질소급원으로 사용하여 尿中 크레아티닌量을 저하시킬지도 모른다. 특히 尿에 尿素, 尿酸같은 다른 질소급원이 크레아티닌에 비해 비교적 낮은 비율로 존재할 때에, 例를 들면 低단백食事인 경우, 감염균에 의한 크레아티닌의 파괴율이 더 클 것으로 기대된다.

INTRODUCTION AND REVIEW OF LITERATURE

Creatinine, isolated from urine by Liebig as early as 1847[1], is a urinary excretory product from warm-blooded animals including human beings. Creatinine is produced from creatine which acts as a spare-source of the energy for muscle contraction.

Thus, creatinine is regarded as an end-product of muscle metabolism. On creatinine and creatine free diets, muscle creatine is the only source of urinary *creatinine*[2].

Nutritional and metabolic interests in creatinine were originated by Folin[3], who pointed out in 1905 that on meat-free diets the daily creatinine excretion in urine was quite constant in a given individual and seemed to be related to body weight. In 1908, Schaffer[4] reported that the hour-by-hour excretion of creatinine was as stable as the day-by-day values. Since then, this work has been repeatedly confirmed and the effects of diet and exercise have been extensively reported to be insignificant[5~7]. Body weight, more precisely muscle mass, has been generally known to have quite high correlations with the urinary creatinine excretion[8]. The term 'creatinine coefficient' which expresses the level of creatinine excretion per kg of body weight was devised from that fact.

Those individual presumed constancies of creatinine excretion in urine have been utilized practically in various ways. First, the constancy of daily excretion has given great convenience to the many researchers of nutritional surveys and metabolic studies. Collection of timed or 24-hour urine is one of the difficulties and common error-makers on surveys. The urinary constant of creatinine has been a useful index to correct urine losses in incomplete urine samples or to check the completeness of 24-hour urine collections [9]. At the same time it has been satisfactorily used as a basis on which concentrations of vitamins or metabolites in urine are expressed and compared[10]. Particularly, urea *nitrogen/creatinine* ratio[11], hydroxyproline/creatinine ratio [12,13], and height/creatinine ratio[14] have been clinically proposed as simple indices of protein nutriture. Secondly, urinary creatinine excretion is used as a measure of the total lean body mass because of its direct relationship with muscle mass in both children and adults[15]. Graystone[8], who reviewed the early studies on the subject, concluded that 1 gm of urinary creatinine per day is equivalent to 20 kg of muscle mass. Finally, since creatinine is known to be metabolically inert and is concentrated to the greatest extent through the glomerulus but neither secreted nor reabsorbed significantly by the tubule, creatinine excretion is often used to measure the glomerular filtration rate as creatinine clearance (either endogenous or exogenous)[16,17].

Some recent studies have suggested that daily creatinine excretion varies significantly in the same individual and thus may not be a reliable index of urine collection or a reference standard for urinary components[18~23]. But Jackson[7], in his review, strongly recommended that creatinine excretion be utilized as a urinary standard. Many investigators have continued to measure and utilize creatinine excretion with the wishful assumption of the constancy of creatinine excretion. Thus, this subject remains important but a debatable area.

Creatinine excretion has been reported to be decreased in people with various pathologic conditions, muscular dystrophy, mental retardation, and renal failure as well as in over-and under-nutrition[4,5,24,25]. In fever, the creatinine elimination is increased corresponding to the rise in temperature[26]. The variation in some diseases can be explained with regard to muscle mass and efficiency and impaired glomerular function, but in others the cause is uncertain. In a group of Egyptian children with urinary tract infections, greatly lowered (about half of the normal value) urinary excretion value for creatinine have been noticed by Van Reen[27]. This is possibly due to the direct or indirect actions of the infecting bacteria, even though damaged renal filtration may be one of the reasons. In 1972, Ravnskov[28] reported the almost complete disappearance of urinary creatinine together with an unchanged serum creatinine level in a patient subjected to kidney homotransplantation and suggested the presence of a microorganism capable of decomposing creatinine. A strain of ***Pseudomonas*** capable of splitting creatinine into urea, ammonia, and carbon dioxide was isolated from human urine by Kopper[29] and Kopper and Beard[30] in 1947. The strain closely resembled ***Pseudomonas aeruginosa***, one of the prevalent bacteria in urinary tract infection, in cultural and biochemical characteristics, though differed from it in its ability to break down creatinine. Several bacteria which can degrade creatinine have

also been isolated from soil[31~34]. In addition, enzyme activity decomposing creatinine has been observed from rat feces[35] and from the colon flora of rats fed creatinine[36]. Therefore, microorganisms of urinary tract infections could degrade urinary creatinine, cause invalid estimations of urinary creatinine, and compromise its various usages, that is, as an index of urine collection, creatinine ratios, a measure of muscle mass, and creatinine clearances. These measurements have been used undoubtedly in people with urinary tract infections. Urinary tract infection, defined as bacteriuria with or without signs or symptoms of inflammation, affect all age groups and both sexes, particularly females. About 1 percent of school-girls and 5 to 12 percent of women have bacteriuria[37]. As a matter of fact, to my knowledge, there has been no report on this point except the one by Cattell *et al.*[38]. They incubated ninety different urine samples infected with bacteria commonly encountered in patients with urinary tract infections without finding any effect on the reduction of urinary creatinine content.

Therefore, research should be carried out on this subject to clarify the effect of urinary infection on the level of urinary creatinine. In the present study the followings were investigated: first, whether alkalinity of medium can affect the amount of creatinine since urine from patients with urinary tract infections can be alkaline mainly due to ammonia produced by bacterial decomposition of urea; secondly, whether bacteria, commonly found in urinary tract infections, utilize or decompose creatinine.

MATERIALS AND METHODS

I. Creatinine Analysis

Creatinine was analysed using a Technicon Autoanalyser II (Technicon Instruments Co., Tarrytown, N.Y.). The method is based on the Jaffe reaction [39,40] and the absorbance was measured at 505 nm in a 15 mm flowcell.

Organisms in the sample, if present, were removed by centrifugation at 2000 rpm for 20 minutes. The clear solutions were then diluted with distilled water to have a creatinine concentration of 5 to 10mg/100 ml before being applied to the Autoanalyser.

II. Collection of Urine Samples

Three 24-hour urine samples were obtained from normal persons. They did not take any medicine including antibiotics during collection. The urine samples were collected under the toluene, and the subjects were requested to keep the bottles refrigerated after each voiding.

III. Effect of Alkalinity on the Stability of Creatinine

Creatinine in buffer solutions

Samples of 50, 100 and 200 mg of creatinine (anhydrous, Sigma Chemical Co., St. Louis, Mo.) were dissolved in separate 50 mls of distilled water. The pH of each solution was adjusted to 4.5 with HCl and NaOH. To these solutions was added a buffer of pH 4.5 to bring the volumes up to 100 ml, making the final concentrations 4.4, 8.8, and 17.6 nM (equivalent to 50, 100, and 200mg/100ml). For buffered creatinine solutions with the pHs of 6, 7, 8, and 9, the same procedures were followed. For pH 4.5, 6, 7, and 8, potassium phosphate buffers (0.2M) were prepared and for pH 9 a borate buffer (0.2M) was prepared according to the AOAC methods[41].

Twenty-five ml aliquots of each buffered creatinine solution were distributed into 50-ml culture tubes with screw caps (25×150 mm, Corning Glass Works, Corning, N.Y.) and sterilized by autoclaving at 121°C for 15 minutes. After cooling at room temperature, all solutions were incubated at 37°C. After 0, 1, 2, 4, and 6-days of incubations, 2.5 ml of each solution was pipetted out aseptically and frozen until creatinine could be analyzed.

Urinary creatinine

Five 45-ml aliquots of each urine sample were adjusted, with NaOH and HCl, to pH 4.5, 6, 7, 8, and 9, respectively, and the volumes were brought up to 50 ml with distilled water. They were sterilized by filtration through a Millipore membrane, 0.45 mμ in pore size (Millipore Filter Co., Bedford, Mass.). Their 25-ml aliquots were aseptically distributed into 50-ml culture tubes which were sterilized by autoclaving at 121°C for 15 minutes. After 0, 1, 2, 4, and 6-days of incubations at 37°C, 2.5ml of each

solution was pipetted out aseptically and frozen until analyzed for creatinine.

Ⅳ. Bacterial Utilization of Creatinine

Cultures

Organisms used are commonly encountered in urinary tract infections [38,42,43]. Pure cultures of *Escherichia coli*, *Proteus mirabilis*, *Proteus morganii*, *Proteus rettgeri*, *Proteus vulgaris*, *Proteus inconstans*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* Groups D, *Streptococcus faecalis* and *Candida albicans* were obtained through the courtesy of Dr. S. Gaines, the Department of Tropical Medicine and Medical Microbiology, University of Hawaii. The cultures were maintained by transferring every three weeks as stock cultures in Cystine Trypticase Agar (BBL, Becton, Dickinson and Co., Cockeysville, Md.). After incubation for 24 hours at 37°C, they were kept at room temperature.

Inocula

The suspensions of 24-hour old bacteria, freshly prepared, were used as inocula. one loopful of the stock cultures was transferred on the slant of Tripticase Soy Agar (BBL, Becton, Becton, and Co., Cockeysville, Md.) in 16×125 mm tubes and grown at 37 °C for 24 hours. Then, 4ml of sterile physiological saline (0.85% NaCl) was pipetted onto the slant, and the growth in it was suspended.

Media

The bacteria were grown aerobically in urine and liquid synthetic media containing creatinine as a sole nitrogen source.

Urine samples were sterilized by Millipore filtration and refrigerated before use. During filtration, urine was kept cold by covering the container with shredded ice. Twenty five ml aliquots of sterilized urine were distributed into 50-ml culture tubes.

The synthetic media were freshly prepared on the bases of Dubos and Miller[31] and Miller *et al.*[44]. The media used throughout contained: creatinine 5 g; glucose 5 g; NaCl 5 g; potassium phosphate buffer (0.2M, pH 7.0) 25ml; tap water to 1,000 ml. Twenty five ml aliquots of the medium were distributed into 50-ml culture tubes, autoclaved at 121°C for 15 minutes, and cooled at room temperature. Glucose was autoclaved separately and added aseptically prior to inoculation. The media were aseptically supplemented with a vitamin mixture (1ml/100ml of the medium) or both vitamin mixture and amino acid mixture (2 ml/100ml of the medium) for some organisms in order to meet their growth requirements[45,46]. The vitamin mixture was prepared in the laboratory[47] and the amino acid mixture (BME AA solution, 50×) was obtained from the Flow Lab. (Rockville, Md.).

Bacterial reduction of urinary creatinine and creatinine in synthetic media

In order to study bacterial reduction of creatinine, 0.5 ml of the inoculum was aseptically pipetted into each tube of either urine or the synthetic medium, which then was incubated at 37°C for 6 days. Every 24-hours, 2.5 ml of the medium was aseptically pipetted out from each tube after careful shaking. After autoclaving at 121°C for 15 minutes, turbidity of the synthetic media was measured by using a Coleman Junior Spectrophotometer (Coleman Instruments, Inc., Maywood, Ill., cell size: 7×75mm) at 625 nm for the bacterial growth determination. The turbidity of urine media was measured prior to being autoclaved because autoclaving caused precipitates of phosphates in urine, which interfered with the turbidity measurements. Control medium, uninoculated with any bacterium, was used as a blank. The same sample used in the turbidity measurements was kept frozen for the creatinine analysis. The amount of creatinine remained after bacterial growth was compared to that of control.

Ⅴ. Effect of Other Nitrogen Sources on the Bacterial Utilization of Creatinine

Since *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* reduced creatinine in greater amount than other organisms, these two organisms were tested.

As nitrogen sources other than creatinine, ammonium sulfate and urea were simultaneously used in such amount that the ratio between ammonium sulfate nitrogen and urea nitrogen was 1 to 7. This ratio has been found between the total nitrogen except urea and creatinine nitrogen and urea nitrogen in the urine from people on normal diets[48]. The creatinine

Table 1. The Ratio Between Creatinine Nitrogen and Other Nitrogen Added to the Synthetic Media

Creatinine N /Other N	g/1,000 ml of the medium		
	Creatinine	$(NH_4)_2SO_4$	Urea
Creatinine only	2.5	0	0
1/1	2.5	0.43	1.74
1/2	2.5	0.86	3.48
1/4	2.5	1.72	6.96
1/6	2.5	2.58	10.44
1/10	2.5	4.30	17.40

concentration of the synthetic media used above was changed to 2.5g per 1,000 ml, then both ammonium sulfate and urea were added at various ratios between creatinine nitrogen and other nitrogen (Table 1). Urea was sterilized through Millipore filtration and added aseptically before inoculation. After inoculation and 0, 1, 3, and 6-days of incubation, 2.5ml of the medium was pipetted out, autoclaved, and subjected to growth measurement and creatinine analysis

Ⅵ. Statistics

Three replicates of all experiments with duplicate determinations were obtained and subjected to statistical analyses using the Wang Computer, 600 series (Wang Laboratories, Inc., Tewksbury, Mass.). Student 's t test was used to determine the significance of differences between means.

RESULTS

Ⅰ. Stability of Creatinine in Alkaline Medium

The effect of the pH of medium on the recovery of creatinine

It was necessary to confirm that the pH of the icreatinine medium does not affect creatinine determination using the Technicon Autoanalyzer II Technaque. In order to do that, the effect of the pH of the medium on the recovery of creatinine was examined by dding 50mg, 100mg, and 200mg of creatinine to 100ml of buffer solutions with pH ranges of 4.5 to 9.0. The results are shown in

Table 2. Effect of the pH of Medium on the Recovery of Creatinine

pH of Medium	Percent recovery of creatinine added to 100ml of buffer solutions		
	50mg	100mg	200mg
4.5	98.6±1.29*	99.5±0.69	102.2±0.76
6.0	98.4±1.33	101.7±0.55	103.1±0.51
7.0	100.8±1.43	101.9±0.54	101.6±0.27
8.0	102.8±1.31	100.7±0.39	102.4±0.77
9.0	101.4±0.70	100.9±0.78	101.5±1.16

*Mean±SEM.

Table 2. Recoveries of creatinine averaged 101.2±0.12 (mean±SEM) percent, and there were not any significant differences in the recoveries regardless of the pH of the media. Recovery of creatinine, when 50mg of creatinine was added to buffer solution, showed slightly lower values in acidic media than in alkaline media. The differences were not significant. Therefore, the pH of media did not influence the creatinine determination.

The rate of creatinine loss

The rates of creatinine loss when solutions were incubated at 37°C at various pHs ranging from 4.5 to 9.0 are shown in Table 3 (creatinine concentration =50mg/100ml), Table 4 (creatinine concentration= 100mg/100ml), Table 5 (creatinine concentration=200 mg/100ml), and Table 6 (urinary creatinine). These results show that the decomposition rate of creatinine is very slow at body temperature. Most of the creatinine, added to buffer solutions or present in urine, was recovered without significant changes due to pH after one-day of incubation. The amount of creatinine remaining ranged from 94.4 to 100 percent of the initial creatinine content. At up to 4-days of incubation, creatinine remaining in buffer solutions was not significantly affected by pH. After 6-days of incubation, greater losses of creatinine were observed and the loss of creatinine was larger at acidic pH than at alkaline pH. An average decrease of 15.2 percent of the initial creatinine content was observed at pH 4.5 and a 6.3 percent decrease at pH 9.0 after 6 days. This tendency was consistent at all concentrations of creatinine in buffer solutions but

Table 3. Effect of pH on the Rate of Creatinine Loss in Buffer Solution (Creatinine Concentration=50mg/100ml)

pH of buffer solution	Creatinine remaining after incubation at 37°C (mg/100ml)				
	Days of Incubation				
	0*	1	2	4	6
4.5	46.3±0.68** (100)***	45.0±1.19 (96.2)	42.4±1.60 (91.6)[a]	40.2±0.45 (87.0)	39.3±0.96 (84.8)
6.0	47.2±1.27 (100)	45.9±1.56 (97.2)	45.0±1.51 (95.4)	41.7±0.59 88.8)	40.8±0.94 (86.4)
7.0	46.7±0.56 (100)	44.6±1.07 (95.6)	44.6±0.60 (95.6)	42.4±1.62 (90.8)	40.8±0.94 (87.4)
8.0	47.2±0.93 (100)	44.6±2.07 (94.4)	41.6±1.59 (88.2)	42.2±1.37 (90.4)	41.9±0.49 (88.8)
9.0	43.2±0.59 (100)	42.4±1.27 (99.2)	41.5±1.57 (96.0)	40.6±0.98 (94.0)	40.3±0.52 (93.2)[b]

*The differences in the initial creatinine contents at the various pHs are due to the effect of autoclaving.

**Mean±SEM.

***The creatinine remaining after incubation as percentage of the initial creatinine content.

[a]After 2 days : The value at pH 4.5 was significantly different from that at pH 9 ($p<0.05$).

[b]After 6 days: The value at pH 9 was significantly different from those at pH 4.5 ($p<0.05$), pH 6 ($p<0.05$), pH 7($p<0.05$), and pH 8 ($p<0.05$).

Table 4. Effect of pH on the Rate of Creatinine Loss in Buffer Solution (Creatinine Concentration=100mg/100ml)

pH of buffer solution	Creatinine remaining after incubation at 37°C (mg/100ml)				
	Days of Incubation				
	0	1	2	4	6
4.5	95.7±1.32* (100)**	94.9±0.84 (99.2)	86.9±2.58 (90.8)	81.2±4.88 (84.8)	79.3±3.96 (82.9)
6.0	95.3±1.15 (100)	93.5±0.45 (98.1)	89.1±1.63 (93.5)	81.6±3.01 (85.6)	79.3±2.11 (83.2)[c]
7.0	93.1±1.74 (100)	89.6±2.66 (96.2)	85.6±2.62 (92.2)	81.6±2.42 (87.6)	79.3±2.11 (85.2)[d]
8.0	93.5±2.61 (100)	89.6±3.49 (95.8)	89.6±2.27 (95.8)	89.6±1.21 (95.8)[b]	87.1±0.76 (93.1)
9.0	85.6±3.36 (100)	84.7±3.14 (98.9)	84.7±1.46 (98.9)[a]	81.2±2.26 (94.9)	79.8±0.71 (93.2)

*Mean±SEM.

**The creatinine remaining after incubation as percentage of the initial creatinine content.

[a]After 2 days : The value at pH 4.5 was significantly different from that at pH 9 ($p<0.05$).

[b]After 4 days : The value at pH 8 was significantly different from those at pH 6 ($p<0.05$) and pH 7 ($p<0.05$).

[c]After 6 days : The value at pH 6 was significantly different from those at pH 8 ($p<0.01$) and pH 9 ($p<0.01$).

[d]After 6 days : The value at pH 7 was significantly different from those at pH 8 ($p<0.05$) and pH 9 ($p<0.05$).

Table 5. Effect of pH on the Rate of Creatinine Loss in Buffer Solution (Creatinine Concentration=200mg/100ml)

pH of buffer solution	Creatinine remaining after incubation at 37°C (mg/100ml)				
	Days of Incubation				
	0	1	2	4	6
4.5	192.9±4.34* (100)**	186.6±1.65 (96.8)	185.1±0.97 (96.0)	175.3±3.60 (90.9)	167.2±3.41 (86.7)[a]
6.0	184.4±4.07 (100)	181.3±3.81 (98.3)	175.8±6.87 (95.4)	172.2±7.70 (93.4)	167.2±3.41 (89.2)
7.0	184.2±4.34 (100)	179.9±5.02 (97.7)	178.0±4.92 (97.7)	171.8±5.55 (93.3)	166.1±4.47 (90.2)
8.0	190.0±0.47 (100)	180.0±7.06 (94.0)	182.8±2.39 (96.2)	181.8±1.29 (95.7)	176.7±1.80 (93.0)
6.0	174.0±4.64 (100)	173.0±3.82 (99.5)	172.6±5.06 (99.1)	168.6±3.63 (96.9)	165.2±3.70 (94.6)

*Mean±SEM.

**The creatinine remaining after incubation as percentage of the initial creatinine content.

[a]After 6 days: The value at pH 4.5 was significantly different from those at pH 8 ($p<0.05$) and pH 9 ($p<0.05$).

Table 6. Effect of pH on the Rate of Creatinine Loss in Human Urine*

pH of urine	Creatinine remaining after incubation at 37°C (% of the initial content)				
	Days of Incubation				
	0	1	2	4	6
4.5	100.0	95.6±1.36**	93.9±1.64[c]	87.0±1.46[e]	84.9±1.51[g]
6.0	100.0	97.3±0.32	95.9±1.25[d]	91.7±0.56[f]	87.5±0.87[h]
7.0	100.0	97.6±1.12	99.4±0.66	95.1±0.74	92.0±0.83[i]
8.0	100.0	99.3±0.31[a]	100.4±0.56	96.0±0.64	94.9±1.11
9.0	100.0	100.0±0.49[b]	98.8±1.13	97.7±1.33	95.8±1.29

*The initial creatinine contents in three urine samples were 142.3, 113.5, and 68.7mg/100ml.

**Mean±SEM.

[a]After 1 day : The value at pH 8 was significantly different from those at pH 4.5 ($p<0.05$) and pH 6 ($p<0.01$).

[b]After 1 day : The value at pH 9 was significantly different from those at pH 4.5 ($p<0.05$), pH 6 ($p<0.001$), and pH 7 ($p<0.05$).

[c]After 2 days : The value at pH 4.5 was significantly different from those at pH 7 ($p<0.05$), pH 8 ($p<0.01$), and pH 9 ($p<0.05$).

[d]After 2 days: The value at pH 6 was significantly different from those at pH 7 ($p<0.05$) and pH 8 ($p<0.01$).

[e]After 4 days: The value at pH 4.5 was significantly different from those at pH 6 ($p<0.05$), pH 7 ($p<0.001$), pH 8 ($p<0.001$), and pH 9 ($p<0.001$).

[f]After 4 days : The value at pH 6 was significantly different from those at pH 7 ($p<0.01$), pH 8 ($p<0.001$), and pH 9 ($p<0.001$).

[g]After 6 days : The value at pH 4.5 was significantly different from those at pH 7 ($p<0.01$), pH 8 ($p<0.001$), and pH 9 ($p<0.001$).

[h]After 6 days: The value at pH 6 was significantly different from those at pH 7 ($p<0.01$), pH 8 ($p<0.001$), and pH 9 ($p<0.001$).

[i]After 6 days: The value at pH 7 was significantly different from that at pH 9 ($p<0.05$).

was more obvious in creatinine present in urine medium. Even after one-day of incubation, creatinine decreased more in acidic urine than in alkaline urine. Significant positive correlations existed between pH and the stability of creatinine in urine. The correlation coefficients between pH and the creatinine remaining in urine after 1, 2, 4, and 6 days of incubation were 0.630, 0.596, 0.837, and 0.836, respectively. The correlation coefficients were all significant at a p value less than 0.001. This indicates that the stability of creatinine is pH-dependent with a greater stabiliity at alkaline pH.

Ⅱ. Bacterial Utilization of Creatinine

Creatinine in urine medium

The creatinine concentration remaining in urine after incubation with individual bacterial strains at 37°C is expressed as a percentage of the creatinine content originally present in urine samples (Table 7). Table 8 shows the bacterial growth in urine medium expressed as absorbance at 625 nm. All bacteria showed good growth. The turbidities ranged from 0.005 to 0.308 after one-day of incubation. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* continues to grow until the last day of incubation, while others had already reached stationary phase after one-day of incubation. Their growths were estimated as 10 to 1,000-fold using a standard calibration curve of the turibidities and the viable bacterial counts of mixed cultures. For *Klebsiella pneumoniae*, a standard calibration curve was prepared separately because of its slimy appearance. The turibidity determinations of *Proteus* species seemed to overestimate growth due to the precipitates formed. *Proteus* produces ammonia and thus an alkaline condition in urine.

In spite of the good growth of organisms, creatinine content generally did not decrease significantly compared to that of uninoculated urine after 3 to 6 days of incubation with bacteria. With several organisms the creatinine contents were lower than that of controls, but the differences were not statistically significant. An exception is the creatinine content of urine samples incubated with *Pseudomonas aeruginosa* which showed slight but significantly lower values than control on the second ($p<0.01$, decreased amount=1.8%) and the fourth ($p<0.05$, decreased amount=4.6%) days of incubation. The creatinine levels on the third and sixth days after incubation were not lower than that of control, thus the decrease could be due to analytical errors.

Creatinine in synthetic medium

Table 10 shows the bacterial growth in synthetic medium and Table 9 shows the creatinine content, expressed as percentage of the initial creatinine content, after 1 to 6 days of incubation with individual bacterial strains at 37°C. All organisms grew well giving turbidities of 0.002 to 0.070 after 2-days of incubation. Their growths were estimated as 10 to 50-fold. Most of the bacteria tested reached their stationary phase on the first or the second day, but *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* kept on growing until the last day of incubation. Their growths increased up to 500-fold and 200-fold, respectively.

Up to 6-days of incubation, none of the bacteria tested except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* reduced the creatinine content in synthetic media significantly compared to control, uninoculated synthetic medium. The creatinine content continuously dropped in the presence of the above two organisims until the last day of incubation. After 6-days of incubation, 12.8 percent (60.7mg/100ml) of the initial creatinine content was lost with *Pseudomonas aeruginosa* and 11.8 percent (56.0 mg/100 ml) was lost with *Klebsiella pneumoniae*. The rate of creatinine destruction was parallel to the growth of both bacteria.

The amount of creatinine lost and bacterial growth were both less as the initial content of creatinine was lowered from 500mg/100ml to 50mg/100ml with both bacteria (Table 11). However, the percentage of the initial creatinine content destroyed was increased as the initial content was lowered.

Ⅲ. Effect of Other Nitrogen Sources on Creatinine Destruction by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Table 12 and 13 show the rates of creatinine destruction by *Ps. aeruginosa* and *Kleb. pneumoniae*

Table 7. Bacterial Utilization of Creatinine in Human Urine*

Organism	Creatinine remaining after incubation at 37°C (% of the initial content)				
	Days of Incubation				
	1	2	3	4	6
Proteus rettgeri	100.1±0.78**	96.9±1.16	95.1±0.94		
Proteus morganii	100.0±0.85	97.8±1.21	95.8±1.12		
Proteus mirabilis	104.0±2.42	98.7±2.85	101.9±3.24		
Proteus vulgaris	100.1±2.41	99.2±1.97	99.1±2.24		
Proteus inconstans	96.6±1.43	96.7±1.67	97.8±1.71		
Pseudomonas aeruginosa	101.3±2.47	95.0±0.37***	98.5±3.93	93.9±0.67****	93.2±1.72
Pseudomonas stutzeri	98.1±1.96	95.8±1.71	94.9±1.06	94.6±0.95	91.1±1.71
Escherichia coli	98.5±1.62	95.2±0.99	96.5±1.33		
Staphylococcus aureus	99.9±0.94	95.0±0.85	96.9±0.97	94.5±0.59	92.6±1.19
Klebsiella pneumoniae	98.2±0.57	95.0±0.85	95.8±1.23	95.3±1.11	91.8±1.61
Streptococcus Groups D	94.4±1.34	100.2±3.25	97.2±0.99		92.7±1.35
Streptococcus faecalis	98.1±1.01	94.5±1.07	95.9±0.76		
Candida albicans	98.1±2.01	95.4±0.87	94.9±1.33		
Uninoculated urine	99.5±1.42	96.9±0.38	97.7±2.17	97.5±1.92	94.2±1.12

*The initial creatinine content in three urine samples were 142.3, 113.5, and 68.7mg/100ml.
**Mean±SEM.
***The value was significantly different from that of the uninoculated urine ($p<0.01$).
****The value was significantly different from that of the uninoculated urine ($p<0.05$).

Table 8. Bacterial Growth in Urine Medium: Turbidity at 625 nm

	Turbidity at 625 nm after incubation at 37°C				
	Days of Incubation				
	1	2	3	4	6
Proteus rettgeri	0.274	0.325	0.219		
Proteus morganii	0.219	0.184	0.169		
Proteus mirabilis	0.308	0.261	0.255		
Proteus vulgaris	0.299	0.275	0.259		
Proteus inconstans	0.020	0.020	0.017		
Pseudomonas aeruginosa	0.030	0.043	0.090	0.110	0.097
Pseudomonas stutzeri	0.005	0.016	0.016	0.024	0.035
Escherichia coli	0.037	0.033	0.046	0.053	0.086
Staphylococcus aureus	0.140	0.142	0.093	0.112	0.149
Klebsiella pneumoniae	0.063	0.107	0.144	0.087	0.100
Streptococcus Groups D	0.013	0.015	0.010		
Streptococcus faecalis	0.016	0.015	0.012		
Candida albicans	0.012	0.010	0.009		

Table 9. Bacterial Utilization of Creatinine in Synthetic Medium*

Organism	Creatinine remaining after incubation at 37°C (% of the initial content)					
	Days of Incubation					
	1	2	3	4	5	6
Proteus rettgeri	100.1±1.63	99.6±1.29	97.9±0.86	98.6±0.62	97.0±0.31	97.3±0.57
Proteus morganii	99.8±0.11	100.5±0.68	96.9±1.30	97.5±0.18	95.8±2.45	99.0±0.85
Proteus mirabilis	100.0±1.88	100.3±2.10	100.6±2.15	98.6±1.46	97.9±1.15	99.5±2.02
Proteus vulgaris	98.8±1.66	98.6±0.83	98.8±0.85	97.5±0.98	96.3±0.79	98.8±1.42
Proteus inconstans	97.6±0.57	97.2±0.90	94.5±0.52	99.7±1.22		97.8±0.82
Pseudomonas aeruginosa	98.7±0.58	97.5±0.08	95.6±0.24***	93.0±1.09***	85.6±0.61***	83.7±1.05***
Pseudomonas stutzeri	100.7±1.15	97.7±1.22	100.2±2.28	100.6±2.13		98.1±0.76
Escherichia coli	102.0±2.40	100.0±1.74	100.6±1.81	100.1±1.17	99.4±1.30	96.5±2.24
Staphylococcus aureus	98.3±0.27	96.8±0.47	96.3±0.49	97.0±1.65	95.2±0.70	94.5±0.25
Klebsiella pneumoniae	99.7±0.36	97.4±0.24	95.3±0.38****	92.1±0.76***	86.8±0.64***	84.7±0.34***
Streptococcus Groups D	99.1±0.87	97.7±0.71	98.6±1.89	99.6±0.90	99.0±0.69	95.9±1.15
Streptococcus faecalis	100.9±1.08	98.8±1.00	100.2±0.71	103.6±0.11		101.2±0.73
Candida albicans	98.9±1.34	99.3±1.12	98.2±0.97	97.2±1.16	96.7±0.46	96.8±1.09
Uninoculated urine	99.1±0.11	97.7±0.68	97.1±1.50	98.2±0.18	96.4±0.25	96.5±0.85

*The initial creatinine content was 474.5±3.02 (mean±SEM) mg/100ml after autoclaving.
**Mean±SEM.
***Values were significantly different from that of the uninoculated synthetic medium ($p<0.001$).
****The value was significantly different from that of the uninoculated synthetic medium ($p<0.01$).

Table 10. Bacterial Growth in the Synthetic Medium: Turbidity at 625nm

Organism	Turbidity at 625 nm after incubation at 37°C					
	Days of Incubation					
	1	2	3	4	5	6
Proteus rettgeri	0.006	0.010	0.009	0.011	0.011	0.011
Proteus morganii	0.001	0.002	0.001	0.001	0.001	0.001
Proteus mirabilis	0.009	0.014	0.011	0.014	0.013	0.013
Proteus vulgaris	0.015	0.018	0.018	0.018	0.019	0.018
Proteus inconstans	0.004	0.008	0.008	0.008		0.007
Pseudomonas aeruginosa	0.007	0.019	0.043	0.072	0.113	0.173
Pseudomonas stutzeri	0.001	0.005	0.015	0.010		0.025
Escherichia coli	0.010	0.014	0.015	0.014	0.013	0.014
Staphylococcus aureus	0.007	0.007	0.014	0.014	0.014	0.016
Klebsiella pneumoniae	0.065	0.070	0.080	0.098	0.112	0.166
Streptococcus Groups D	0.001	0.010	0.012	0.010	0.014	0.013
Streptococcus faecalis	0.020	0.016	0.013	0.006		0.011
Candida albicans	0.001	0.014	0.014	0.014	0.014	0.014

Table 11. Effect of the Initial Content of Creatinine on the Rate of Creatinine Destruction by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Organism	Cratinine destroyed after 6-days of incubation at 37°C (mg/100ml)			
	Initial content of creatinine (mg/100ml)*			
	44.4	91.6	221.4	468.3
Pseudomonas aeruginosa	8.6±1.34**(21.1)***	20.5±1.35(21.3)	25.7±0.87(11.6)	51.4±2.42(10.9)
Klebsiella pneumoniae	12.5±1.45(28.2)	15.7±0.79(17.1)	31.9±1.60(14.4)	64.2±5.02(13.7)

*Initial contents of creatinine were determined after autoclaving.
**Mean±SEM.
***The destroyed amount of creatinine as percentage of the initial creatinine content.

when ammonium sulfate and urea were simultaneously added as nit rogen sources other than creatinine.

Growth rates of the two organisms were stimulated by the addition of other nitrogen sources up to a creatinine N/urea and ammonium sulfate N ratio of 1/6, but inhibited when other nitrogen sources were added at the ratio of 1/10.

In the medium containing only creatinine as a nitrogen source, *Ps. aeruginosa* reduced creatinine by 30.3mg/100ml (12.5% loss of the initial content) after 6-days of incubation. However, when other nitrogen sources were added at any ratio from 1/1 to 1/10, *Ps. aeruginosa* did not destroy creatinine at all. In the medium containing other nitrogen sources at the ratio of 1/6, some lower values of creatinine contents than those of paralled controls appeared after 1 and 3 days of incubation, but the differences were not statistically significant ($p>0.1$).

In contrast, as shown in Table 13, *Kleb. pneumoniae* destroyed creatinine in significant amounts when other nitrogen sources were added up to the ratio of 1/4 as well as when only creatinine was added as the nitrogen source. The loss of creatinine in the medium containing only creatinine as its nitrogen source was 56.4mg/100ml (23.3% loss of the initial content) after 6-days of incubation. When other nitrogen sources were added at various ratios of 1/1, 1/2, and 1/4, the amount of creatinine destroyed after 6-days of incubation were 15.7, 22.2, and 8.2mg/100ml, respectively. The destruction rate of creatinine tended to decrease as added amount of other nitrogen sources were increased. After one-day of incubation with a ratio of 1/6, some creatinine appeared to be lost. This must be an analytical error since the difference from the control was not significant ($p>0.1$), and losses did not appear in the same medium after 3 and 6 days of incubation.

DISCUSSION

I. Stability of Creatinine in Alkaline Medium

Urine from the patients with urinary tract infections has been reported to be alkaline, while normal urine is acidic with a pH of around 6.0. For example, In *Porteus* infections, urine is consistently at about pH 8 or higher. Urine in lower urinary tract infections is at a pH ranging from 6 to 8 with predominance at about pH 7 [37,49]. This alkaline urine may be due to the bacterial decomposition of urea to ammonia. If creatinine were unstable in alkaline media, urinary tract infections could lower the urinary creatinine level through the indirect action of the infecting bacteria. Thus, the stability of creatinine was checked after incubation at acidic and alkaline pHs and at body temperature. As the result showed, creatinine is quite stable at both acidic and alkaline pHs without any great difference with one-day of incubation. On prolonged incubation, slightly more amounts of creatinine were lost at acidic pHs than at alkaline pHs, indicating that creatinine is more stable in alkaline medium than in acidic medium. Therefore, it can be concluded that the

Table 12. Effect of Other Nitrogen Sources on the Destruction of Creatinine by *Pseudomonas aeruginosa*

The ratio of creatinine N /other N	Creatinine remaining after incubation at 37°C (mg/100ml)*								
	Days of Incubation								
	1			3			6		
	C**	Ps**	C-Ps**	C	Ps	C-Ps	C	Ps	C-Ps
Creatinine only	229.9	227.5	2.4	237.0	216.4***	20.6	210.7	180.4****	30.3
1/1	226.0	226.9	0	217.1	218.7	0	202.0	203.5	0
1/2	237.1	238.5	0	239.6	238.8	0.8	215.5	214.8	0.7
1/4	231.4	231.4	0	212.1	219.1	0	209.5	212.5	0
1/6	251.7	240.7	11.0	227.4	218.9	8.6	208.6	212.6	0
1/10	225.7	225.7	0	211.9	217.7	0	207.6	218.3	0

* The initial creatinine content was 241.7±8.68 (mean±SEM) mg/100ml after autoclaving.
** C indicates the mean of creatinine contents remaining in the uninoculated medium.
Ps indicates the mean of creatinine contents remaining in the medium inoculated with *Ps. aeruginosa*.
C-Ps indicates the difference between C and Ps: the amount of creatinine destroyed.
*** The value was significantly different from that of the uninoculated medium ($p<0.01$).
**** The value was significantly different from that of the uninoculated medium ($p<0.001$).

Table 13. Effect of Other Nitrogen Sources on the Destruction of Creatinine by *Klebsiella Pneumoniae*

The ratio of creatinine N /other N	Creatinine remaining after incubation at 37°C (mg/100ml)*								
	Days of Incubation								
	1			3			6		
	C**	K**	C-K**	C	K	C-K	C	K	C-K
Creatinine only	229.9	212.0	17.9	237.0	190.5***	47.5	210.7	154.3****	56.4
1/1	226.0	210.5	15.5	217.1	213.0	4.1	202.0	186.3***	15.7
1/2	237.1	244.1	0	229.6	191.1***	38.5	215.5	193.3***	22.2
1/4	231.4	220.7	10.7	212.1	203.3*****	8.8	209.5	201.3***	8.2
1/6	251.7	220.3	31.4	227.4	227.1	0.3	208.6	214.7	0
1/10	229.2	234.6	0	215.2	229.8	0	207.6	221.2	0

*The initial creatinine content was 241.7±8.68 (mean±SEM) mg/100ml after autoclaving.
**C indicates the mean of creatinine contents remaining in the uninoculated medium.
K indicates the mean of creatinine contents remaining in the medium inoculated with *Kleb. pneumoniae*.
C-K indicates the difference between C and K: the amount of creatinine destroyed.
***Values were significantly different from those of the uninoculated media ($p<0.01$).
****The value was significantly different from that of the uninoculated medium ($p<0.001$).
*****The value was significantly different from that of the uninoculated medium ($p<0.05$).

alkalinity of urine produced in urinary tract infection does not lower its creatinine level. This agrees with the results of Drabbe and Reinhold[50] and Ellinger and Matsuoka[51]. In the former study, with two weeks incubation at 37°C, only 10 percent of the creatinine in a solution containing 0.75 percent sodium carbonate was destroyed. In the latter study, no changes in creatinine concentration were shown in both neutral and alkaline urine (up to pH 10) which were stored at 5 to 8°C. All creatinine was recovered

even after four weeks.

II. Bacterial Utilization of Creatinine

Since creatinine is an organic and nitrogenous compound present in the largest amount, next to urea, in urine, it was assumed that the infecting bacteria of urinary tract might consume creatinine in the course of their growth and thus reduce the urinary ceratinine level. In fact, some studies have suggested that several organisms utilize creatinine as their energy source through energy-yielding catabolism of creatinine or through the assimilation of the creatinine into their cells [56,57]. To investigate this, 13 kinds of bacterial strains found in urinary tract infections were individually incubated in human urine at 37°C. Upon 3 or 6 days of incubation, none of them reduced the creatinine content significantly compared to control, uninoculated urine. Their growths were 10 to 1,000 times. This agrees with the result of Cattell *et al.* [38].

The spontaneous disappearance of creatinine in uninoculated urine could be due to the oxidation of creatinine or to its transformation into creatinine[35,50].

More careful re-examination might be worthwhile with *Pseudomonas aeruginosa* since slight, but significantly lowered values of urinary creatinine content (1.9% and 3.6% loss of the initial content) appeared after 3 and 6days of incubation, respectively. Also, in a previous study, a strain of *Ps. aeruginosa* was isolated from human urine and was found to be capable of splitting creatinine[29,30].

From the above results, it is likely that most of the urinary tract infecting bacteria do not utilize creatinine significantly in normal urine from healthy people. However, this and Cattell *et al.*'s study may have overlooked the effect of bacteria on creatinine because the organisms might attack other nutrients prior to creatinine in the course of their growth. In fact, human urine is regarded as a complete medium for bacterial growth and has various kinds of organic materials easily utilizable by organisms[52]. Creatinine comprises only about one-twentieth of the total amount of urinary organic materials. Eighty to 90 percent of the organic material in urine is urea, the principal end-product of protein metabolism. Urea is the primary nitrogen source for *Proteus* species in urine since they have strong urease activities[53]. If urea and other organic substances are insufficient for bacterial growth, bacteria may be forced to attack creatinine. Such conditions could be found because the excretion of urea and other organic materials are affected by diet, while the creatinine excretion remains relatively constant.

Based on this hypothesis, bacteria were cultivated in limited synthetic media with creatinine as a single nitrogen source. Glucose was supplied as a carbon source, otherwise bacterial growth did not take place. In these simple media, all bacteria tested could grow, while no growth occured in the same media which did not contain creatinine. This indicates that creatinine as a nitrogen source could support the growth of bacteria, but bacteria except *Ps. aeruginosa* and *Kleb. pnemoniae* seemed to be very limited in their ability to utilize creatinine. *Ps. aeruginosa* and *Kleb. pneumoniae* grew quite well and thus reduced the creatinine content in the synthetic medium. Their creatinine decomposing effects are attributed to an adaptive enzyme system, 'creatininase'[30,34,36].

These two bacteria, capable of decomposing creatinine, were further tested to determine whether their capability could be expressed in the presence of nitrogen sources other than creatinine. *Ps. aeruginosa* did not reduce the creatinine content when other nitrogen sources were added, indicating that the organism prefers other nitrogen sources to creatinine. *Kleb. pneumoniae* could decrease the creatinine content but only when a creatinine N/urea and ammonium N ratio of less than 1/4 was present. This suggests the possibility that *Kleb. pneumoniae* may decrease the creatinine content in urine from people whose protein intakes are so poor that their urines contain non-creatinine nitrogenous materials in relatively low ratio to creatinine.

In one study, very low-protein and high calorie diets produced urine whose creatinine nitrogen/other nitrogen ratio was about 1/6[48]. To clarify thipossibility, more studies should be continued, particularly with the consideration that *Kleb. pneumoniae*

is responsible for more than 10 percent of the total incidence of urinary tract infection[43]. Whenever the urinary creatinine level is measured in patients with urinary tract infections and low values are obtained, the possibility of bacterial decomposition of creatinine should be considered.

CONCLUSION

Alkalinity of urine from people with urinary tract infections does not have a significant influence on the urinary creatinine level. However, some bacterial strains responsible for the urinary infections, such as *Kleb. pneumoniae* and possibly *Ps. aeruginosa* tested in the present study, may utilize creatinine in the course of their growth and thus lower the urinary creatinine level. Greater destruction of creatinine can be expected if the urine contains non-creatinine nitrogen materials at a relatively low ratio to creatinine.

REFERENCES

1) Miller, B.F. and R. Dubos: *Studies on the presence of creatinine in human blood. J. Biol. Chem., 121:447, 1937.*

2) Bloch, K. and R. Schoenheimer: *Studies on protein metabolism: The metabolic relation of creatine and creatinine studied with isotopic nitrogen. J. Biol. Chem., 131:111, 1939.*

3) Folin, O.: *Laws governing the chemical compositions of urine. Am. J. Physiol., 13: 66, 1905.*

4) Shaffer, P.: *The excretion of Kreatin and Kreatinin in health and disease. Am. J. Physiol., 23:1, 1908.*

5) McClugage, H.B., G. Booth, and F.A. Evans: *Creatinine excretion in abnormal states in nutrition. Am. J. Med. Sci., 181:349, 1931.*

6) Beard, H.H.: *Coefficients of correlation between the nitrogenous constituents of the urine after ingestion of low, normal, and high prot-ein diets. Human Biol., 7:419, 1935.*

7) Jackson, S.: *Creatinine in urine as an index of urinary excretion rate. Health Physics., 12:843, 1966.*

8) Graystone, J.E.: *Creatinine excretion during growth. In Human Growth edited by D.B. Cheek, Lea an Febiger, Philadelphia, 1968, pp. 182-197.*

9) Abernathy, R.P., S.J. Ritchey, M.K. Korslund, J.C. Gorman and N.O. Price: *Nitrogen balance studies with children fed foods representing diets of low income southern families. Am. J. Clin. Nutr., 23:408, 1970.*

10) Pollack and Herbert: *Creatinine excretion as index for estimating urinary excretion of micronutrients or their metabolic end-products. Am. J. Clin. Nutr., 23(7):865, 1970.*

11) Simmons, W.K.: *Urinary urea nitrogen/creatinine ratio as indicator of recent protein intakes in field studies. Am. J. Clin. Nutr., 25(5):539, 1972.*

12) Whitehead, G.R.: *Hydroxyproline creatinine ratio as an index of nutritional status and rate of growth. Lancet 2:567, September 18, 1965.*

13) McLaren, D.S, Loshkojian and Kanawati: *Urinary creatinine and hydroxyproline in relation to childhood malnutrition. Br. J. Nutr 24:641, 1970.*

14) *Creatinine-height index in malnourished children. Nutr. Review 29(6):134, 1971.*

15) Miller, A.T. Jr. and C.S. Blyth: *Estimation of lean body mass and body fat from basal oxygen consumption. J. Applied Physiol. 5:73, 1952.*

16) Smith, H.W.: *Clearance involving tubular excretion: creatinine. In the Kidney Structure and Functions in Health and Disease, Oxford Univ. Press, N.Y., 1951, pp. 182-194.*

17) Doolan, P.D., E.L. Alpe and G.B. Theil: *A clinical appraisal of the plasma concentration and endogenous clearance of creatinine. Am. J. Med., 32:65, 1962.*

18) Chattaway, F.W., R.P. Hullin and F.C. Odds: *The variability of creatinine excretion in normal subjects, mental patients and pregnant women. Clin. Chim. Acta., 26:567, 1969.*

19) Paterson, N.:*Relative constancy of 24-hour urine volume and 24-hour creatinine output.*

Clin. Chim. Acta., 18:57, 1967.

20) Ritchey, S.T., N.L. Derise, R.P. Abernathy and M.K. Korslund: *Variability of creatinine excretion in preadolescent consuming a wide range of dietary nitrogen. Am. J. Clin. Nutr., 26:690, 1973.*

21) Cramer, Kim, H. Cramer and S. Selander: *A comparative analysis between variation in 24-hour urinary creatinine output and 24-hour urinary volume. Clin. Chim. Acta., 15:331, 1967.*

22) Szadkowski, A.J., H.G. Essing, and K.H. Schaller: *Die kreatinin eliminationsrate als bezugrosse für analysen aus harnproben. Z. Klin. Chem. Klin. Biochem., 8:529, 1970.*

23) Curtis, G. and M. Forgel: *Creatinine excretion: Diurnal variation and variability of whole and part-day measures. Psychosom. Med., 32: 337, 1970.*

24) Goldman, R.: *Creatinine excretion in renal failure. Proc.. Soc. Exper. Biol. and Med., 85:446, 1954.*

25) Antene, I.: *Excretion of nitrogen, creatinine, and organic acids in the urine of obese and normal weight children. Helv. Pediatr. Acta., 25:482, 1970.*

26) Myers, V.C.: *Creatine and creatinine. Yale J. Biol. Med., 4:467, 1932.*

27) Van Reen, R.: *Personal communication.*

28) Ravanskov, Uffe: *Urinary tract infection with disappearance of urinary creatinine. Scan. J. Urol. Nephrol., 7:94, 1973.*

29) Kopper, P.H.: *An atypical strain of Pseudomonas aeruginosa. J. Bacteriology, 54:359, 1947.*

30) Kopper, P.H. and H.H. Beard: *Creatininase activity of a strain of Pseudomonas. Arch. Biochem., 15:195, 1947.*

31) Dubos, R. and B.F. Miller: *The production of bacterial enzymes capable of decomposing of creatinine. J. Biol. Chem., 121:429, 1937.*

32) Krebs, H.A. and L.V. Eggleston: *Bacterial urea formation. Enzymologia, 7:310, 1939.*

33) Roche, J., G. Lacombe, and H. Girard: *Sür la specficite de certaines deguanidases bacteriennes generactrices duree et sür l'argininedihydrolase. Biochim. Biophys. Acta., 6:210, 1950.*

34) Van Ejk, H.G., R.J. Vermat, H.J. Leijnse-ybema, and B. Leijnse: *The conversion of creatinine by creatininase of bacterial origin. Enzymologia, 34:198, 1968.*

35) Beard, H.H.: *The production of a creatinine oxidase and a creatine anhydras from rat feces. Arch. Biochem., 2:363, 1943.*

36) Jones, J.D. and P.C. Burnett: *Implications of creatinine and gut flora in the uremic syndrome: Induction of 'creatininase' in colon contents of the rat by dietary creatinine. Clin. Chem., 18(3) :280, 1972.*

37) Kory, M. and S.O. Waife: *Kidney and urinary tract infections. Lilly Research Lavoratories, Indianapolis, 1971, pp. 1011.*

38) Cattell, W.R., J.M. Sardeson, D. Hutchinson and F. O'Grady: *Creatinine content of urine and bacterial growth. Brit. Med. J., 2:175, 1969.*

39) Folin, O.: *Beitrag zur chemie des kreatinins und kreatins in harne. Hoppe-seyler's Z. Physiol. Chem., 41:223, 1904.*

40) Determination of creatinine (AA II-11, 2055-10-70-2): *Technicon Instruments Corporation, New York, 1970.*

41) Horwitz, W., editor: *Methods of analysis of the association of officail analytical chemists, 12th ed., A.O.A.C., Washington, D.C., 1975.*

42) Bailey, W.R. and E.G. Scott: *Microorganisms encountered in the urinary tract. In Diagnostic Microbiology, 3rd ed., The C.V. Mosby Co., Saint Louis, 1970, pp. 75-80.*

43) Braude, A.I. and J. Siemienski: *Role of bacterial urease in experimental pyelonephritis. J. Bact., 80:171, 1960.*

44) Baker, Z., B.F. Miller, M.J. Carl Allinson: *Studies on the metabolism of creatinine and creatine. J. Biol. Chem., 130:383, 1939.*

45) Buchanan, R.E. and N.E. Gibbons, editors: *Bergey's manual of determinative bacteriolo-*

gy, 8th ed. Williams and Willeins Co., Baltimore, 1974.

46) Pelczar, M.J. and J.R. Porter: *The nutrition of Proteus morganii: Amino acid and growth factor requirements. Arch. Biochem. Biophys., 2:323, 1943.*

47) Wickerham, L.J.: *A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. 52:293, 1946.*

48) Harper, H.A.: *Review of Physiological chemistry, 13th ed. Lange Medical Publications, Maruzen Co., 1971, p. 387.*

49) Brumfitt, W. and A.W. Asscher: *Urinary tract infections: proceedings of the second national symposium. London, Oxford Univ. Press, 1973, p. 203.*

50) Drabbe, C.A.J. von Frijtag and J.G. Reinhold: *Note on the stability of creatinine in neutral and alkaline urine. 39:163, 1952.*

51) Ellinger, A. and Z. Matsuoka:*Cited by Drabbe and Reinhold in Note on the stability of creatinine in neutral and alkaline urine. 39:163 1952.*

52) O'Grady, F. and W. Brumfitt: *Urinary tract infection: proceedings of the first national symposium. Oxford University Press, London and New York, 1968, pp. 3-23.*

53) Smith, D.T., N.F. Conant, and H.P. Willett, editors: *Zinsser microbiology, 14 th ed., Appleton-Century-Crofts, New York, 1968, pp. 644-645.*