CHAPTER I

INTRODUCTION

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It is well-known that protein is a vital constituent of living cells and that protein or its precursors are necessary not only for the growth of the living organism but also for the constant renewal of tissues.

The demonstration of the importance of protein for the animal body by Magendie (1783-1855) naturally led to an interest in the fate of the protein ingested. It was conclusively demonstrated by Atwater (1899) that nitrogen is not metabolized via the respiratory route and the catabolism of protein is almost entirely accounted for by the nitrogenous products in feces and urine with small amounts being lost through other secretions such as sweat, tears and saliva and desquamation of cells. The major route for the excretion of catabolic products of protein was recognized as the kidney.

The nitrogenous nature of urine is rather obvious from the nature of its degradatory products. Urea came to be recognized as a major excretory product, the other constituents identified being ammonia, uric acid and creatinine.

Since the nitrogenous products in urine represent the end products of protein catabolism, it was natural to speculate whether the rate of protein catabolism is related to the overall rate of metabolism as reflected by energy

expenditure. On this basis, exercise which may increase energy expenditure enormously should result in an increased catabolism of proteins. However, this was not found to be the case (Kocher, 1914; Chambers and Milhorat, 1928; Mitchell and Kruger, 1928; Ramiah, Sundaram and Narayanayya, 1939; Kehar, Mukherjee, Murty and Sen, 1943) although some recent studies appear to have reopened the question (Gontzea, Sutzescu and Dumitrache, 1974; Gontzea, Sutzescu and Dumitrache, 1975; Consolazio, Johnson, Nelson, Dramise, and Skala, 1975).

On the other hand, the amount of nitrogen excreted in the urine was found to vary with the amount in the diet (Folin, 1905). It was also observed that even on diets providing no protein, a certain minimum quantity of nitrogen was eliminated from the body (Smuts, 1935; Smith, 1926; Bricker and Smith, 1951). This was taken to represent the obligatory loss of nitrogen from the body during the course of tissue breakdown and renewal.

Folin (1905) was the first to enunciate in clear terms that not only the amount of nitrogen in the urine but also its composition varies with the amount of protein in the diet. He found that whereas the excretion of urea varies markedly with the same, that of creatinine shows much less variation. He came to the concept of dichotomy in protein metabolism,

one component representing the catabolism of 'exogenous' nitrogen, and the other component 'endogenous metabolism' the former being exemplified by urea and the latter, by creatinine. Although it is now known that aminoacids, whether derived from the diet or tissue catabolism, enter a common free amino acid pool, the concept that nitrogen metabolism consists of two components, namely, an obligatory component representing the inevitable cost of tissue repair, and the other the availability of 'luxus aminoacids' for metabolic purposes remains essentially valid as pointed by Mitchell (1962, 1964) and has formed the basis for computing protein requirements for maintenance (FAO, 1965; FAO, 1973).

The obligatory requirements for protein are sought to be determined in terms of nitrogen lost by the animal on a protein free diet (Smuts, 1935) or on a diet containing a minimum quantity of completely utilizable protein such as egg albumin (Mitchell and Kruger, 1928; Young, Taylor, Rand and Scrimshaw, 1973). However, this ignores the fact that turn over rates for protein like those for any other nutrients are likely to be influenced by previous dietary history.

The concept of minimum obligatory requirements for protein led to the continuing attempts to link it with the minimal or basal level of overall metabolism (Table 1). This relation was originally demonstrated by Terroine and

Table 1 : Endogenous nitrogen excretion in adults.

Investigators	no. and sex	age (years)	weight (kg)	height (cm)	surface area (sq.m.)	BMR (Calories)	days of low or no protein diet	urinary nitrogen (mg) per			
								day	kg	sq.m.	basal calorie
Murlin et al (1946)	5 M	adults	63	171	1.7	1400	4	1976	31	1140	1.4
Mueller and Cox (1947)	4 M	adults	80	180	2.0	2441	4-6	3662	46	1850	1.5
Gopalan et al (1966)	4 M	28	46	163	1.5	1223	5-7	1710	37	1170	1.4
Young and Scrimshaw (1968)	8 M	19	73	178	1.9	1636	7-10	2680	37	1410	1.6
Calloway and Margen (1971)	13 M	27	71	179	1.9	1875	13-15	2410	38	1280	1.4
Scrimshaw et al (1972)	83 M	21	73	179	1.9	1536	10-14	2690	37	1410	1.8
Huang et al (1972)	50 M	24	55	-	-	1416	-	1815	33	-	1.3
Inoue et al (1974)	9 M	adults	63	-	-	-	11-15	2108	33	-	-
Nicol and Phillip (1976)	9 M	26	54	-	-	1427	-	1836	34	-	1.3
Rawley et al (1948)	10 F	adults	-	-	-	-	4-6	-	29	-	1.2
Bricker and Smith (1951)	25 F	23	58	165	1.6	1277	10-14	1454	25	900	1.14
Scrimshaw et al (1976)	7 F	76	60	157	1.6	991	7-10	1440	24	910	1.4
Jourdan et al (1974)	6 F	35	123	164	2.22	1947	12	2460	19	1060	1.2

M, Male; F, Female

Sorgmatter (1927) and further developed by Sorgmatter (1928), Smuts (1935), Brody (1945) and others. An overall correlation has also been found in different species (Table 2).

The major excretory product of protein catabolism varies in different classes of animals being ammonia, in aquatic animals; urea, in mammals, amphibians, and also a species of reptiles (Turtles); and uric acid, in avians and other classes of reptiles (Snakes and lizards) (Table 3).

Following the suggestion that the excretion of creatinine is essentially independent of protein intake and that it is a product of endogenous nitrogen metabolism, muscle creatine and phosphocreatine were identified as the precursors of urinary creatinine (Folin, 1905; Shaffer, 1908; Myers and Fine, 1913) the latter being the major one (Borsook and Dubnoff, 1947).

It is known that creatine is synthesized in the liver, transported by the blood and taken up by the muscle. The synthesis of creatine is believed to proceed via the pathway shown in Fig. 1. The bulk of creatine appears to be in the muscle. More than 90% of body creatine is believed to be accounted for by that in skeletal muscle (Meador, Kreisberg, Friday, Bowdoin, Coan, Armstrong and Hazelrig, 1968; Waterlow, Neale, Rowe and Palin, 1972). A higher value of 98% is reported by Best and Taylor (1967), the remaining 2% being

Table 2 : Endogenous nitrogen excretion in different species*.

	mouse	rat	guinea pig	rabbit	pig	man
number	9	23	9	10	5	8
weight (kg)	0.024	0.223	0.414	2.072	67.6	73
surface area (sq.m.)	0.0064	0.032	0.0403	0.152	1.348	1.9
basal metabolism (Cal.)						
per day	7.6	24.0	38.6	118	1293	1636
per day/sq.m.	1192	748	958	777	959	861
period required for the attainment of endogenous level** (days)	5	6	8	15	20	7-10
nitrogen excreted (mg) :						
per day	14.7	47.5	73.7	250.7	2704	2680
per kg	614	213	178	121	40	37
per sq.m.	2308	1481	1827	1652	2006	1410
per basal cal.	1.93	1.98	1.91	2.12	2.09	1.60

* data based on the study of Young and Scrimshaw (1968) for man; compiled by Mitchell (1962) from the study of Smuts (1935) for other species.

** the different species studied were subsisting on diets containing the same level of protein (approximately 10%) prior to the switch over to a nitrogen free diet.

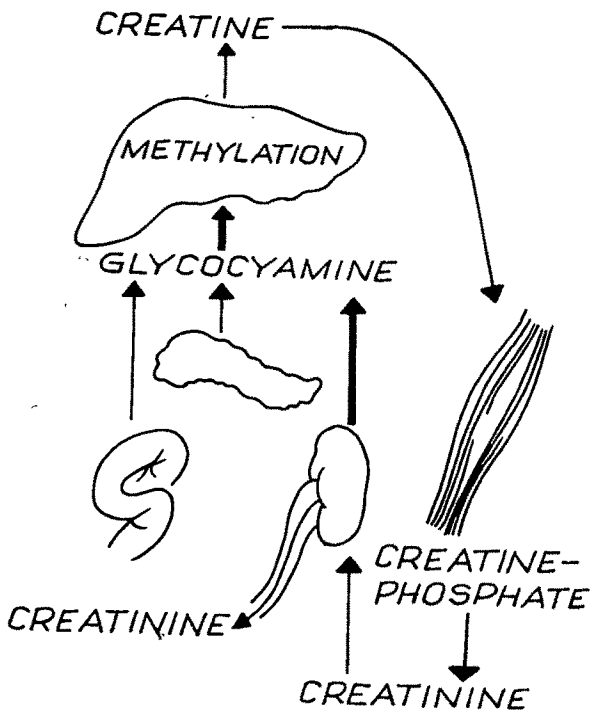
Table 3 : End products of protein and purine metabolism in different organisms correlated with the occurrence of enzymes (West, Todd, Mason and Van Bruggen, 1966).

	metabolic end products of		liver Arginase	Ornithine cycle	Xanthine oxidase	uricase	Allantoinase Allantoicase
	protein	purines					
mammals :							
man and apes	urea	uric acid	+	+	+	-	-
others	urea	allantoin	+	+	+	+	-
birds :	uric acid	uric acid	-	-	+	-	-
reptiles :							
snakes and lizards	uric acid	uric acid	-	-	+	-	-
turtles	urea ± uric acid	allantoin ?	+*	+*	+	?	?
amphibians	urea	urea	+	+	+	+	+
fish :							
elasmobranchii	urea	urea	+	+	+	+	+
teleostei	ammonia	urea	+	-	+	+	+

+, present; -, absent

* in some species

FIG.1: FORMATION, METABOLISM AND EXCRETION OF CREATINE AND CREATININE. (TYLER, 1972)



distributed in brain (1.5%), skin and other tissues (0.5%). Skeletal muscle contains 5-10 mg % creatinine and 300-500 mg % of creatine (Best and Taylor, 1967; Albanese and Orto, 1967). Creatine in muscle is reversibly phosphorylated to creatine phosphate which serves as a reserve of high energy phosphate bond transferable to adenosine and its mono- and di-phosphates. A small part of the creatine (about 1-2 %) seems to be irreversibly converted to creatinine by a non-enzymatic reaction (Bloch and Schoenheimer, 1939) but as mentioned earlier, creatine-phosphate seems to be the major precursor (Borsook and Dubnoff, 1947). Although muscle appears to reconvert creatinine to creatine in vitro, this reaction does not appeaf to operate in vivo on the basis of studies carried out using labeled creatinine (Bloch and Schoenheimer, 1939).

The daily output of creatinine in urine has been found to be constant in a number of studies (Shaffer, 1908; Albanese and Wangerin, 1944; Tanner, Healy, Whitehouse and Edgson, 1959; Kennedy, 1961; Doolan, Alpen and Theil, 1962; Lee and Lucia, 1963; Tolbert and Watts, 1963; Kiriyama and Ashida, 1964; Nakagawa, Takahashi, Suzuki and Kobayashi, 1964).

Attempts have been made to correlate the excretion of creatinine to basal metabolism and it has been suggested that about 1 mg of the same is excreted per basal calorie (Palmer, Means and Gamble, 1914) (Table 4). On the other hand,

Table 4 : Creatinine excretion of adults on protein free or low protein diet*.

Investigators	no. and sex	creatinine excretion (mg) per				
		day	kg	cm	sq.m.	basal calories
Young and Scrimshaw (1968)	8 M	1740	24	9.8	916	1.1
Calloway and Margen (1971)	13 M	1590	23	8.9	846	0.9
Scrimshaw et al (1972)	83 M	1720	24	9.6	900	1.1
Huang et al (1972)	50	1265	23	-	-	0.9
Nicol and Phillip (1976)	9 M	1296	24	-	-	0.9
Bricker and Smith (1951)	25 F	978	17	5.9	604	0.8
Scrimshaw et al (1976) (elderly)	7 F	676	11	4.3	428	0.7
Jourdan et al (1974) (obese)	6 F	1340	11	8.2	604	0.7

M, Male; F, Female

* except where otherwise indicated, the studies were on young adults.

details given in Table 1.

creatinine excretion is believed to reflect the turn over of creatine in the body and to depend, therefore, on muscle mass. Myers and Fine (1913) suggested that within species "the constancy of muscle creatine offers satisfactory explanation for the constancy in daily elimination of creatinine". In this connection, the contribution of muscle total body weight does not vary remarkably between different species (Table 5) and the composition of muscle also seems similar (Beach, Munks and Robinson, 1943). Comparative data on creatinine excretion in different species are limited but the same suggest some correlation with both muscle mass and basal metabolism (Table 6) but much greater variability is observed in terms of either standard. The constancy of creatinine excretion must, therefore, deemed to hold only 'within' species, although in some large animals such as pigs and cattle creatinine excretion per kg body weight appears to be comparable to that in man (Mitchell, 1962 pp 141). It has also been suggested that creatinine excretion varies with body weight $^{0.9}$ whereas endogenous nitrogen excretion varies with a much smaller exponential power of body weight (Munro, 1969) (Table 7).

In human subjects on a diet free of creatine and creatinine, the amount of creatinine excreted daily in the urine has been found to vary with amount of creatine in the

Table 5 : Proportion of skeletal muscle in the bodies of various animals (Munro, 1969).

species	sex	body weight (kg)	muscle weight as % body weight
Bat	M + F	0.006	42
Shrew	M + F	0.007	40
Mouse	M + F	0.014	43
Hedgehog	M + F	0.07	37
Rat	M + F	0.24 - 0.49	42 - 45
Guinea pig	M	0.400	46
Rabbit	M + F	1.1 - 2.7	34 - 54
Cat	M + F	2.4 - 2.8	45 - 50
Monkey	M	3.9	54
Dog	M + F	20.4	43
Pig	M + F	35 - 271	31 - 41
Man	M + F	60 - 67	38 - 44

M, Male; F, Female.

Table 6 : Creatinine excretion in different species* on protein free diet**.

	mouse	rat	guinea pig	rabbit	pig	man
number	9	23	9	10	5	8
weight (kg)	0.024	0.223	0.414	2.072	67.6	73
surface area (sq.m.)	0.0064	0.032	0.0403	0.152	1.348	1.9
basal metabolism per day (Cal.)	7.6	24.0	38.6	118.0	1293.0	1636
muscle mass (kg)***	0.011	0.1	0.186	0.9324	30	33
Creatinine excretion (mg) per :						
day	1.94	9.14	14.08	95.31	1318.0	1740
kg	81	41	34	46	19.5	24
sq.m.	304	286	349	627	978	916
basal calorie	0.255	0.381	0.365	0.808	1.019	1.10
Creatinine-N as % total nitrogen	4.9	7.2	7.1	14.1	18	24.1

contd...

Table 6 : contd.

	mouse	rat	guinea pig	rabbit	pig	man
	values as percentage of values for man					
basal metabolism	0.466	1.47	2.36	7.2	79.0	
muscle mass	0.033	0.30	0.56	2.83	92.0	
Creatinine excretion :						
per kg	338	171	142	192	81	
per basal calorie	23	35	33	74	93	

* data based on the study of Young and Scrimshaw (1968) for man; compiled by Mitchell (1962) from the study of Smuts (1935) for other species.

** periods are more comparable from the fact that species were subsisting on diets containing the same level of protein (approximately 10%) prior to the removal to a nitrogen free diet.

*** muscle mass is calculated as 45% of the body weight.

Table 7 : The influence of body size on the metabolism of energy and protein (Munro, 1969).

measurement	exponent $Wt.^{x}$	units per kg		
		rat (200 g*)	dog (10 kg*)	man (70 kg*)
basal metabolism (kcal/day)	0.73	108	38	23
endogenous urinary nitrogen (mg/day)	0.72	230	77	45
total body protein synthesis (mg N/day)	0.76	1010	620	218
muscle weight (gm)	0.99	454	428	417
creatinine in urine (mg N/day)	0.90	14	-	8

* body weight

body (Hoberman, Sims and Peters, 1948; Hoberman, Sims and Engstrom, 1948), the turnover rate of endogenous creatinine being constant over a period of 39 days, amounting to 1.64 % per day. A range of 1.5 % to 3.5% is suggested by isotopic studies on species as varied as the mouse (Fitch, Oates and Dinning, 1961), rat (Bloch and Schoenheimer, 1939; Cohn, Simmonds, Chandler and Du Vigneaud, 1946; Meador et al, 1968; Waterlow et al, 1972) and man (Hoberman et al, 1948a; Hoberman et al, 1948b; Benedict, Kalinsky, Scarrone, Wetheim and Stetten, 1955; Fitch and Sinton, 1964; Fitch, Lucy, Bornhofen and Dalrymple, 1968; Kreisberg, Bowdoin, and Meador, 1970; Crim, Calloway and Margen, 1972; Crim, Calloway and Margen, 1976). The studies on man include one on children (Picou, Reeds, Jackson and Poulter, 1976).

Although creatinine excretion may not vary with day to day variation in protein intake, it may be modified by the rate of turnover of protein as evidenced by some of the earlier studies on starvation (Benedict, 1915), semi-starvation (Keys, Brozek, Henschel, Michelsen and Taylor, 1950) and protein deprivation (Smith, 1926). As early as in the forties, Beard (1945) questioned Folin's concept of constant creatinine excretion on the basis of evidence reviewed by him. But his views do not seem to have received the consideration they deserve.

The so-called constancy of creatinine excretion has led to the use of the same as a check for authenticity of 24 hr. collections of urine for other estimations (Wilson et al, 1964; Krehl and Hodges, 1965). However, a number of studies suggest that this assumption may not be altogether warranted (Vestergaard and Leverett, 1958).

Vestergaard, Leverett and Grangeburg (1958) found the constancy to hold only for 5 out of 18 normal individuals they studied. It was found to be indeed poor in some of the subjects studied. A similar pattern was found by Paterson (1967).

Creatinine excretion has been found to vary with age being less in children (Macy, 1942; Clark, Thompson, Beck and Jacobson, 1951; Stearns, Newman, McKinley and Jeans, 1958) and particularly low in infants (Fenner, Lange, Monkeimeier and Ohlenroth, 1974; Talafant, Hoskova and Pojerova, 1977). This has been attributed to a lower muscle mass (Stearns et al, 1958). The need for caution in using creatinine excretion or creatinine clearance in children for assessing the excretion of other metabolites (Applegarth and Ross, 1973), fluid and electrolyte homeostasis (Bernhardt, 1974) and the dosage (Drabowski, 1957) and elimination of drugs (Sertel and Scopes, 1973) has been pointed out (Talafant et al, 1977).

As pointed out by Pierro and Johnson (1970) the so-called constancy is a statistical concept in man (Paterson, 1967; Scott and Hurley, 1968) and in the rat (Kumar, Land and Boyne, 1959). The original notion of the constancy of creatinine excretion might have been the result of observations on selected individuals because some individuals do appear to show a such constancy. It would only be natural to regard the variability in others as due to unreliability of the collection. Even under careful conditions of study, however, some individuals seem to show much more variation than others (Curtis and Fogel, 1970; Vestergaard and Leverett, 1958). The daily variability either in the same individual or between individuals seems to be of the order of 5-15 % in different studies (Best, 1953; Bleiler and Schedl, 1962; Doolan et al, 1962; Oomen, 1967; Bray, Schwartz, Rozin and Lister, 1970; Pierro and Johnson, 1970). A larger variation of upto 20-30 % has been found in hospital patients (Edward, Bayliss and Miller, 1969) and a figure of 25% derived from the data of Folin (1905). A similar variation has been found in rats (Kumar et al, 1959; Das and Waterlow, 1974). A significant day to day variation is observed in buffalo calves (Chetal, Mehra, Nath and Ranjhan, 1975).

Diurnal variation in creatinine excretion has been found to be of the same order as day to day variation (Best, 1953), the excretion being low during sleep (Mills, 1964). The

variation has been found to be greater with 4 hr. collections consecutively made over 24 hr. period than with 24 hr. collections (Ram and Reddy, 1970). The data of this study also suggest a diurnal variation.

The rate of creatinine excretion has been found to vary from one voiding to the next in adults (Best, 1953; Hegsted, Gershoff, Trulson and Jolly, 1956; Vestergaard and Leverett, 1958; Plough and Consolazio, 1959; Curtis and Fogel, 1970; Pasternak and Kuhlback, 1971; Rubin, 1971; Lis, McLaughlin, McLaughlin, and Hackbeil, 1972), adolescents (Clarke, Cosgreve and Morse, 1966) and children (Ram and Reddy, 1970; Kularni and Kilgore, 1973). The excretion per minute was found to vary as much as 300-500 % of the 24 hr. value in several children (Lewis, Bunker, Getts and Essien, 1975) being less in the first voidings on rising and more in the midmorning and midafternoon voidings. The elimination of creatinine has also been found to vary to some extent with urine volume (Cramer, Cramer and Selander, 1967; Chattaway, Hullin and Odds, 1969; Szadkowski, Joergensen, Essing and Schaller, 1971) but no such relation has been found in other studies (Tanner et al, 1959; Paterson, 1967; Ritchey et al, 1973).

As already mentioned, the apparent constancy of creatinine excretion has led to attempts to correlate the same with endogenous nitrogen metabolism, basal metabolism and

muscle mass. These studies have been extended to other indicators of these factors such as lean body mass, body size as determined by surface area, height and weight.

Varying values for the correlation coefficients between creatinine and lean body mass determined by different methods have been reported, the same being 0.61 determined by skinfold (Best, Kuhl and Consolazio, 1953), 0.73 (Muldowney, Crooks and Bluhm, 1957), 0.65 determined by total body water (Edwards and Whyte, 1959), 0.83 by densitometric measurements (Miller and Blyth, 1952) and 0.87 by anthropometry (Doolan et al, 1962). The correlation was found to hold in ~~these~~ obese subjects after making correction for adiposity (Muldowney et al, 1957; Bray et al, 1970). In the study of Bray et al (1970), the apparently low creatinine coefficient (mg per kg body weight) of 10 found in obese subjects rose to 21 after correction for adiposity.

From the comparative studies on normal and patients suffering from muscular dystrophy, Ryan and his associates (Ryan, Williams, Ansell and Bernstein, 1957) concluded that creatinine excretion is related to cell mass rather than lean body mass.

The values for creatinine excretion may be confounded by energy restriction because of the resulting negative nitrogen

balance and loss of muscle involved (Scrimshaw, Taylor and Young, 1973).

As early as 1913 (Myers and Fine, 1913), it was suggested that muscle mass could be predicted from the daily output of creatinine. This postulate is based on the observation that urinary creatinine is the sole breakdown product of creatine (Bloch and Schoenheimer, 1939). However, no accurate method is available for measuring muscle mass in man. A limited number of whole body dissection studies have been performed in autopsies of well-nourished and severely malnourished children (Garrow, Fletcher and Halliday, 1965; Picou, Halliday and Garrow, 1966; Halliday, 1967).

The direct method has limitations because it is difficult to ensure complete dissection of muscle tissue. Variable amounts of water and other fluids are lost during and after dissection and studies are done at varying times after death, making it difficult to extrapolate these findings to the dynamic situation in the living individual.

Attempts have been made to derive ~~eanating~~ equations in relation muscle mass ~~to~~ and creatinine excretion (Talbot, 1938; Miller and Blyth, 1952; Best et al, 1953; Muldowney et al, 1957; Ryan et al, 1957; Chinn, 1966; Chinn, 1967; Graystone, 1968; Cheek, 1968; Kreisberg et al, 1970).

Many studies including some recent ones on malnourished children have emphasized the value of urinary creatinine output as a measure of muscle of mass (Standard, Wills and Waterlow, 1959; Picou, Alleyne, and Seakins, 1965; Alleyne, 1968; Alleyne, Viteri and Alvarado, 1970; Cheek, Hill, Cordano and Graham, 1970; Viteri and Alvarado, 1970).

Appreciable individual variations can, however, be expected with regard to muscle mass, creatinine content of muscle and percentage turnover. Even if it is assumed for practical purposes that all urinary creatinine is derived from muscle, the question still arises as to how much creatinine corresponds to how much muscle. Chinn (1967) on the basis of earlier literature (Shaffer, 1908; Burger, 1919; Talbot, 1938; concludes that 1 mg of creatinine corresponds to 15-20 g of muscle, on the basis of observation that adult man excretes 20-25 mg creatinine per kg body weight per day and that muscle forms 40% of body weight. A similar value was derived in a more recent study (Cheek et al, 1970).

The reasonable assertion that the relationship between muscle mass and creatinine excretion is relatively invariant also tends to obscure the fact that it may be affected not only by the plane of nutrition but also by age. It has been established by several investigators (e.g. Stearns et al, 1958)that children excrete much less creatinine in relation to

body weight. This could be due to a smaller proportion of muscle mass, a lower creatinine concentration of the same and/or its small turnover. A comparison of the study on adults by Kreisberg et al (1970) and on children by Picou et al (1976) made in Table 8 suggest that the former two may be the major factors.

Preliminary studies on children with N^{15}-creatine suggested that the variation in creatine turnover between individuals is fairly large with a mean of 2.15 and a standard deviation of 0.42 (Picou et al, 1976). The reported values for the turnover of creatine in man are of the same order of magnitude 1.63 ± 0.2 (Kreisberg et al, 1970). However, a much smaller variation is suggested by isotopic studies in which the turnover rate is found to be 1.69 ± 0.06 % (mean ± sd.) (Crim et al, 1976).

Several studies suggest a correlation between creatinine excretion and basal metabolism (Palmer et al, 1914; Smuts, 1935; Talbot, 1936; Talbot, Worcester and Stewart, 1939; Correa, 1944; Bricker and Smith, 1951; Miller and Blyth, 1952; Young and Scrimshaw, 1968; Calloway and Margen, 1971; Scrimshaw et al, 1972; Huang et al, 1972; Jourdan et al, 1974; Nicol and Phillip, 1976; Scrimshaw et al, 1976) and it is well-known that the latter is related to surface area (Mitchell, 1962).

Table 8 : Creatine kinetics in children and adults*.

	children	adults
no. of subjects	8	4
Sex		M + F
Age (years)	1.4	48
Isotopic creatine used	N^{15}	C^{14}
Muscle	mean ± S.D.	
Creatine concentration (mcg/mg)	3.12 ± 0.43 (2.25 - 3.59)	4.3 ± 0.17 (4.2 - 4.5)
Mass (kg)	1.9 ± 1.2 (1.0 - 4.9)	28.0 ± 8.5 (20.8 - 40.3)
mass as % body weight	21.8 ± 7.8 (15.0 - 37.0)	40.0 ± 9.5 (29.0 - 50.0)
mass equivalent to 1g creatinine	18.6 ± 6.6 (13.8 - 31.9)	16.2 ± 1.89 (14.5 - 18.6)
creatine pool (g)	5.6 ± 2.4 (3.0 - 11.0)	120.0 ± 36.7 (89.0 - 173.0)
creatine turnover (%)	2.15 ± 0.42 (1.53 - 2.63)	1.63 ± 0.2 (1.38 - 1.88)

* compiled from the data of Picou et al (1976) for children and Kreisberg et al (1970) for adults.

range given in parentheses.

Not surprisingly, attempts also have been made to correlate creatinine excretion directly with basal metabolism.

Macy (1942) found a close relationship between surface area and creatinine excretion in children. Creatinine per sq. m. of surface area was found to be more in men (929 mg) than in women (722 mg) (McMillan and Reid, 1965). Doolan et al (1962) found a highly significant correlation (r = 0.89) between creatinine excretion and surface area in adult men and women. A lower correlation coefficient of 0.591 was found in military personnel by Best et al (1953) with 4 hr. collections of urine. The correlation has been found to be lower still with wide variations in the degree of muscular development (Ryan et al, 1957) and to be even negative in obesity (Bray et al, 1970).

Surface area is determined by body size which is largely determined in man by height and weight and attempts have also been made to relate the same to both. The creatinine height index (CHI) has been defined as milligrams of creatinine excretion per centimeter of height per unit time. This index is believed to be a better measure than that per unit weight since it is not affected by variations in adipose tissue (Arroyave and Wilson, 1961; Arroyave, 1962; Arroyave, 1966; Viteri and Alverado, 1970; Viteri, Alvarado and Alleyne, 1971; Viteri, 1972). Moreover, this method is claimed to have the

advantage that information on the exact age of the child, which is difficult to obtain in poor rural areas, is not essential (Arroyave, 1966; Viteri, Alvarado and Alleyne, 1971; Viteri, 1972). The creatinine-height index has been used as an indirect measure of the relative mass and the degree of protein depletion and repletion in malnourished children (Alleyne, Viteri and Alvarado, 1970; Viteri and Alvarado, 1970; Viteri, Alvarado and Alleyne, 1971).

Although 24 hr. collections are preferred, shorter collection periods have been used at the loss of some precision (Arroyave, 1962; Widdowson and McCance, 1970; Viteri, Alvarado and Alleyne, 1971; Viteri, 1972). Attempts also have been made to compare the creatinine excretion of malnourished children with the expected values for normal children of the same height (Assessment of Protein Nutritional Status, 1970; Viteri and Alvarado, 1970; Viteri, 1972).

Limitations in the use of the urinary creatinine-height index have also been pointed out (Assessment of Protein Nutritional Status, 1970; Mendez and Buskirk, 1971; Viteri, Alvarado and Alleyne, 1971; Viteri, 1972; Annon, 1971). In the study of Lewis et al, (1975) the index was found to vary from 50 to 150 % of the norms proposed in malnourished children suggesting a 300 % variation as against 30% variation

found in well nourished children (Viteri, Mata and Behar, 1973). Creatinine-height indices of normal children in this study (Lewis et al, 1975) were lower than the norms derived by Viteri and Alvarado (1970) from creatinine data of Stearns et al (1958) and the height data of Stuart and Stevenson (1954).

Questions arise regarding the feasibility and validity of using timed or untimed collections of urine for the assessment of nutritional status. Would one 24 hr. sample be adequate? Would randomly voided urine samples be acceptable? Although the former would seem preferable, Watson and Langford (1970) pointed out two rather obvious difficulties in 24 hr. collections. First, it is both physically difficult and some times embarassing for people to make such collections when they are away from home. Secondly, it is difficult to ascertain the completeness of the 24 hr. collection. Obvious problems with random specimens include physical activity and liquid consumption prior to the collection and the time of day when the collection is made, as both these factors may markedly affect the concentration of the metabolite. These limitations are sought to be reduced to some extent by the collection of a fasting sample.

The justification for using creatinine as a yard stick in random samples is that the concentration of a metabolite

in urine per unit volume may not give us a correct picture as urine volume is highly variable. On the other hand, the excretion of creatinine is believed to proceed at a fairly uniform rate regardless of fluctuations in urine volume (Wilson et al, 1964; Krehl and Hodges, 1965).

It is generally assumed that the amount of water soluble vitamins excreted in urine represents the spill-over after meeting the requirements of the body and can,therefore, be taken as an index of vitamin status. However, 24 hr. collections, or at any rate collections over an extended period of time have to be made to get reasonable estimates of vitamin excretion. On the other hand, the amount of vitamin excreted in relation to that of creatinine excreted permits extrapolation from 24 hr. value on the basis of norms available for creatinine excretion and thereby set criteria for the assessment of vitamin status. Such an approach has been made in the case of thiamin, riboflavin, N'methyl nicotinamide, pyridoxine and vitamin C.

Various biochemical procedures have been proposed for detecting thiamin deficiency or assessing thiamin status (Wuest, Furness, White and Beckel, 1962; Pearson, 1962a; Pearson, 1962b; Manual of Nutrition Surveys, ICNND, 1963; Goldsmith, 1964; Pearson, 1966; Pearson, 1967; Sauberlich, 1967; Landen, 1972). The most commonly used procedure has been

the measurement of urinary levels of thiamin (Darby et al, 1953; Manual of Nutrition Surveys, ICNND, 1963; Goldsmith, 1964; Brin, Dibble, Peel, McMullen, Bourquin and Chen, 1965; Krehl and Hodges, 1965; Shimazano and Katasura, 1965; Thanangkul and Whitakar, 1966; Pearson, 1967; Sauberlich, 1967; Henshaw, Noakes, Morris, Bennoin and Gubler, 1970; Nutrition Survey Reports, 1957-1972; The Ten State Nutrition Surveys, 1972). Findings from various investigations point to the existence of a reasonably close correlation between thiamin status and excretion of thiamin in urine. Typical values at varying levels of dietary intake are 100, 40-90, 5-25 mcg for intakes of 0.5, 0.3-0.4 or 0.2 mg per 1000 calories (Pollack, Ellenberg and Dolger, 1941; Elson, O'Shea, Nicholson and Choronock, 1942; Mason and Williams, 1942; Melnick, 1942; Oldham, Davis and Roberts, 1946; Mickelson, Caster and Keys, 1947; Horwitt, Liebert, Kreisler and Wittmann, 1948; Spector, Peterson and Friedemann, 1954; Williams, 1961; Pearson, 1962a; Pearson, 1962b; Manual of Nutrition Surveys, ICNND, 1963; Shimazano and Katasura, 1965; Pearson, 1966; Pearson, 1967; FAO/WHO, 1967; NRC, 1968; Sauberlich, Stevens and Herman, 1970). In beri-beri the excretion is less than 15 mcg (Spector et al, 1954; Williams, 1961). A correlation between the urinary excretion of thiamin per gram of creatinine and thiamin intake has been observed

(Krehl and Hodges, 1965; FAO, 1967; Sauberlich et al, 1970). However, it is observed that children have a markedly higher levels of thiamin excretion than adults, when expressed on creatinine basis (Pearson, 1962; Pearson, 1966) presumably because of the lower creatinine excretion (Stearns et al, 1958). This was taken into account by Pearson (1962, 1966) who formulated different standards for different ages.

An essentially similar approach has been made towards the assessment of riboflavin status. Adult men maintained on a daily intake of 0.55 mg of riboflavin developed clinical signs of riboflavin deficiency (Horwitt, Harvey, Hills and Liebert, 1950; Horwitt, 1972). Their urinary excretion of riboflavin on this diet was approximately 50 mcg/day or 20-30 mcg/g creatinine and increased progressively with increasing intakes showing a sharp increase above 1.3-1.6 mg suggesting that at this level tissue saturation is maintained. A similar observation has been made on young women (Brewer, Porter, Ingalls and Ohlson, 1946; Davis, Oldham and Roberts, 1946). From these studies a 24 hr. urinary riboflavin excretion of less than 100 mcg was considered as an indication of dietary inadequacy (Horwitt et al, 1950). Guidelines for interpreting urinary riboflavin excretion data have been derived from these studies (Pearson, 1962a, 1962b; 1966; 1967; Manual of Nutrition Surveys, ICNND, 1963; O'Neal, Johnson and Schafer, 1970; The Ten State Nutrition Surveys, 1972).

The values for riboflavin excretion per g creatinine were found to be comparable with 24 hr. samples, in 2 hr ~~samples~~ (Du Plessis, 1967) 6 hr. (Lowry, 1952; Plough and Consolazio, 1959) and even casual samples (Lowry, 1952; Hegsted et al, 1956; Plough and Consolazio, 1959; Nutrition Surveys Reports, ICNND, 1963; Du Plessis, 1967; The Ten State Nutrition Survey, 1972).

But as in the case of thiamin, children excrete more riboflavin per gram of creatinine than adults (Pearson, 1962b; Nutrition Survey Reports of ICNND, 1963; Du Plessis and De Lange, 1966; Du Plessis, 1967; Reports of the Ten State Nutrition Survey, 1972) leading Pearson to develop a sliding scale which has been applied in numerous nutrition surveys (Nutrition Survey Reports of ICNND, 1963; Manual of Nutrition Surveys, ICNND, 1963; O'Neal et al, 1970; Reports of the Ten State Nutrition Survey, 1972).

Variability in metabolite creatinine ratios was observed by Hegsted et al (1956), Clarke et al (1966) and Kulkarni and Kilgore (1973) who found the ratios of riboflavin and thiamin to creatinine were some times in the 'acceptable' or 'high' ranges in the midmorning and midafternoon voidings whereas they had been in the 'low' or 'deficient' ranges in the first voidings in the morning. Further, in the study of Lewis et al (1975), the absolute values for riboflavin excretion were low,

but the ratio of the same to creatinine was high, presumably due to low creatinine excretions.

Similar efforts have been made to assess niacin status in terms of the excretion of its metabolites, mainly,N'methyl nicotinamide, in relation to creatinine excretion. Subjects on a daily intake of about 5 mg of nicotinic acid and 200 mg of tryptophan show a clinical signs of pellagra and excrete N'methyl nicotinamide at the rate of 0.2 mg in 6 hr. or 5 mg/g of creatinine (Goldsmith, Sarett, Register and Gibbens, 1952; Unglaub and Goldsmith, 1954; Frazier, Prather, and Hoene, 1955; Goldsmith, Rosenthall, Gibbens and Unglaub, 1955; Horwitt, Harvey, Rothwell, Cutler and Haffron, 1956). When intakes of nicotinic acid increased to 8 to 10 mg or more per day the excretion of nicotinic acid metabolites increased rapidly (Frazier et al, 1955; Horwitt et al, 1956). Data are limited on the excretion of nicotinic acid metabolites in small children. Nevertheless, information available on 7 to 15 year old children (Du Plessis, 1967) shows that with increasing age a pronounced fall in N'methyl nicotinamide level expressed in terms of creatinine is observed. In the ICNND nutrition surveys, interpretation guides for niacin status are limited to N'methyl nicotinamide excretion of adults. Here again, the interpretation may be rendered difficult because of variations in creatinine excretion. In a study on pellagrous children

(Prinsloo, Du Plessis, Kruger, De Lange and de Villiers, 1968) the ratio of N'methyl nicotinamide may cease to be a reliable index of niacin status in certain conditions. For instance, it is elevated in pregnancy (Lojkin, Wertz and Dietz, 1952; Darby et al, 1953) although the increased excretion of xanthurenic acid in response to tryptophan suggests an impaired conversion of tryptophan to niacin. A similar increase in pellagrins has been reported in studies in Hyderabad (Annual Report of National Institute of Nutrition, 1973) and has been attributed to the failure of quinolinic acid to get converted to NAD.

In the case of pyridoxine a satisfactory intake of the vitamin, 1.5 mg per day, is associated with a urinary excretion of 35 to 55 mcg per day or 20 mcg or more per gram of creatinine (Yess, Price, Brown, Swan and Linkswiler, 1964; Miller and Linkswiler, 1967; Linkswiler, 1967; Recommended Dietary Allowances, NRC, 1968; Canham, Baker, Harding, Sauberlich and Plough, 1969; Sauberlich, Canham, Baker, Raica, and Herman, 1970; Sauberlich, 1970; Donald, McBean, Simpson, Sun and Aly, 1971; Sauberlich and Canham, 1973). Here also a graded scale that takes into account the age differences has been evolved.

No such norms have been developed for pantothenate. In normal subjects amounts excreted have been found to be 7.4 mg/day

and 2.4 mg/day in studies on men and women respectively. The values were 5.3 mg and 2.5 mg when expressed per gram of creatinine (Cohenour and Calloway, 1972).

Similarly, the excretion of hydroxyproline which is believed to be determined by rate of growth as well as body weight has also been sought to be expressed in relation to creatinine. Hydroxyproline is present only in collagen and is formed by hydroxylation of proline after it has been incorporated into peptides. During the course of collagen synthesis some hydroxyproline containing peptides are released and excreted in the urine. The amount of hydroxyproline excreted is related to growth and is found to be reduced in malnourished children (Picou, Alleyne, Waterlow and Seakins, 1965; Picou, Alleyne and Seakins, 1965; Whitehead, 1965; Anasuya and Rao, 1966a; Anasuya and Rao, 1966b; Howell, Wharton and McCance, 1967; Le Roy, 1967; Whitehead, 1967; Rutishauser and Whitehead, 1969; Waterlow, 1969; Assessment of Protein Nutritional Status, 1970; McLaren, Loshkajian and Kanawati, 1970; Prasad and Rahman, 1970; Katz, 1970; Cabacungan, Miles, Abernathy and Ritchey, 1973; Nagamine, Yamakawa, Izobe, Chinose, Nakagagami, 1973). The highest absolute hydroxyproline excretion of groups of normal subjects is seen in rapidly growing adolescents (11 to 16 years) (Crowne, Wharton and McCance, 1969; Waterlow, 1969) with a gradual reduction thereafter. The hydroxyproline index given

below has been proposed as an index of nutritional status. It can be seen that the index allows for increase in excretion per unit weight with growth and therefore obliterates sex differences (Whitehead, 1965; Whitehead, 1966; Ruishauser, and Whitehead, 1969; Assessment of Protein Nutritional Status,1970).

Urinary hydroxyproline index :

$$\frac{\mu\text{moles of hydroxyproline/ml}}{\mu\text{moles creatinine/ml/kg body weight}}$$

When the values are expressed in terms of creatinine, sex differences in adults are eliminated (Le Roy, 1967) but age differences remain. (Jones, Bergman, Kittner and Pigman, 1964; Smiley and Ziff, 1964; Whitehead, 1966; Howells et al, 1967; Crowne et al, 1969; McLaren et al, 1970).

The index proposed by Whitehead is virtually constant over the age range of 6 months to 10 years (Whitehead, 1965; Whitehead, 1966; Waterlow, 1969; Crowne et al, 1969).

To avoid spuriously high values due to the dietary ingestion of foods rich in the aminoacid, fasting samples have been recommended (Le Roy, 1967). Although the large individual variation in hydroxyproline excretion limits the use of indices based on the same, they seem to be useful for assessing the degree of malnutrition in young children or other disease conditions (Le Roy, 1967; Waterlow, 1969; Whitehead,1969).

The problem is rendered even more complicated by the observation that children suffering from kwashiorkor and infested with malaria, roundworm or hookworm may have high rather than low excretion of hydroxyproline (Whitehead, 1967; Whitehead, 1969).

Incidentally, the ratio of hydroxyproline to creatinine in amniotic fluid has been suggested as an index of gestational age (Nagase, 1976). A similar use has been made of the ratio of estriol to creatinine in assessing the maturity of the fetus (Barnard and Logan, 1971, 1972). The values were found to increase progressively in normal pregnancy and to decrease in toxemia, preeclampsia and eclampsia. The ratio is believed to indicate the condition of the feto-placental unit and the kidney function of mother in high risk pregnancies (Sulovic and Poljakovic, 1976).

The excretion of other metabolites has also been sought to be correlated to creatinine excretion and they include steroids (Kenny, Richards and Taylor, 1970; Galal, Salem, Mostafa and Morcos, 1976; Galal and Salem, 1977; Murawski, Jones and Reddy, 1970), non-nitrogenous organic acids (Aksu, Morrow and Barnes, 1974), 3-methyl histidine which is an indication of protein catabolism during starvation (Young, Haverberg, Blimazes and Munro, 1973), Cyclic AMP (Arima, Okazaki and Kitahara, 1977) and uric acid (Stuber and

Paksi, 1976). The ratio of uric acid to creatinine has been found to decline with age till 11-13 years and to increase thereafter, but this pattern is not found in mentally retarded children who show a higher values with age but a small and steady decline (Stuber and Paksi, 1976).

Since the nitrogen excreted in urine represents a more variable component such as urea the amount of which is influenced by protein intake, the proportion of urea nitrogen to total nitrogen has been taken as an index of the adequacy of protein intake. By the same token this ratio should reflect, in groups of growing animals fed isonitrogenous diets, the amount of protein not utilized by the animals for protein synthesis. On similar considerations, the ratio of sulphur and creatinine in urine has been taken as an index of protein status as the former is derived from sulphur aminoacids (Assessment of Protein Nutritional Status, 1970; Simmons and Bohdal, 1970; Simmons, 1973).

When an individual is switched from a normal to a low protein or protein free diet, urinary nitrogen decreases, the component most markedly affected being urea (Patwardhan, 1963; Kopple and Coburn, 1973). The picture is complicated in starvation (Benedict, 1915; Runcie and Hilditch, 1974) and semi-starvation (Keys et al, 1950) although even here some adaptation is evident with the progress of starvation (Owen, Felig, Morgan, Wahren and Cahill, 1969).

Several studies show variations in protein intake to be associated not only with those in urea excretion, but also protein turnover as might be expected, the same being ~~begin~~ faster on a high protein diet (Yuile et al, 1959). This has been found to be the case in the rat (Steinbock and Tarver, 1954; Jeffay and Winzler, 1958), the dog (Yuile, Lucas, Olson and Shapiro, 1959) and man (Dean, 1961; Reddy, Belavady and Srikantia, 1963).

Platt (1954, 1958) found the ratio of urea nitrogen to total nitrogen to be lower in poorly nourished children and proposed the use of this ratio to assess protein nutritional status, specially of population groups. Arroyave (1961) and Beydoun et al (1972) found the index to hold in fasting and casual samples. The ratio is found to be particularly low in children suffering from kwashiorkor (Edozien and Phillips, 1961) and to increase with dietary rehabilitation (Vasantgadkar et al, 1963; Schendel and Hansen, 1965; Ramakrishnarao, Puri and Balakrishnan, 1973), the low ratios persisting, however, for a long time. In contrast, an almost immediate response is found after rehabilitation following acute protein deficiency (Wadsworth, 1959; Kiriyama, unpublished).

The proportion of urea nitrogen to total nitrogen appears to be less in kwashiorkor than in marasmus on the basis of the values of 40, 44 and 50% reported for the former (Edozien and

Phillips, 1961; Schendel and Hansen, 1965; Ramakrishnarao et al, 1973; Vasantgadkar et al, 1963) and 65% reported for the latter (Vinnodini Reddy et al 1963).

The excretion of urea has also been found to depend on the supplies of food energy (Calloway, 1975). However, Jourdan et al (1974) found no differences in obese subjects when the food energy derived from a diet adequate in or free from protein was restricted to half of the required intakes.

In the studies of Rose and his associates (Rose, Johnson and Haines, 1950; Rose, Haines, Warner and Johnson, 1951) on aminoacid requirements, an increase in urea excretion was found with the omission of essential aminoacids such as valine, threonine and methionine but not with histidine. In rats fed diets limiting in essential aminoacids, urea nitrogen was found to decrease with the addition of the first limiting aminoacids (Kiriyama et al, 1963; 1964; Fuwa, 1964). Both these observations are consistent with expectations. Incidentally, the essentiality of arginine for the growing rat is suggested by the increase in urea excretion following its omission (Milner, Wakeling and Visek, 1974).

Protein utilization has been found to be efficient during pregnancy in both rat (Boyne, Chalmers and Cuthbertson, 1953; Pike, Suder and Rose, 1954) and man (Beaton, 1960; Jayalakshmi,

Venkatachalam and Gopalan, 1959; Rao and Rao, 1974). A decrease in the percentage of urea nitrogen from 67% and 68% in the first and second trimesters to 51% in the third trimester was observed by Rao and Rao (1974). The last figure compared with 50% reported by Platt, Stewart and Every (1958). A higher figure of 62% is reported by Reddy (1964) and lower figure of about 39% by Chaudhury (1970) who found a further reduction in toxemia of pregnancy.

Since urea nitrogen depends on the quality and/or quantity of dietary protein whereas creatinine excretion is believed to be not significantly influenced by either, the ratio of urea nitrogen to creatinine has been used similarily. Under laboratory conditions a change in protein intake is associated with marked changes in this ratio (Albanese and Orto, 1967; Kiriyama, 1970; Simmons, 1972; Beydoun, Cuenca, Evans and Aubry, 1972). When the technique has been applied in the field, variable results have been reported (Dugdale and Edkins, 1964; Bjørnsjø, Belew and Zaar, 1965; Arroyave, 1966; Du Plessis, DeLange and Fellingham, 1966; Du Pleasis, 1967; Fry, Fox and Fry, 1968; Luyken, Dubois, Leegwater, Pikkar and Van Staveren, 1970; Simmons and Bohdal, 1970; Nutrition Survey Reports, ICNND, 1957-1972, Simmons, 1972).

The need for caution in the use of this ratio has been pointed out not only because the excretion of creatinine is related to age, sex, diet, muscle mass and other factors (Powell, Plough and Baker, 1961; Pollack, 1970), but also because of its being influenced by amount of protein intakes. The index although it may not necessarily reflect the actual protein status of an individual in the face of such variations (Shendel and Hansen, 1965; Simmons, 1972), seems to serve as a valid index when applied to population groups. For instance, in a private kindergarten in Nairobi, attended by African, Asian and European children, the ratio was found to be similar in urban African children and European children (14 and 17) (Bohdal and Simmons, 1967), but to be lower (6.6) in children from many poor rural locations (Simmons and Bohdal, 1970). The values for each group were consistent with the results of dietary investigations (Bohdal et al, 1968; Simmons and Bohdal, 1970).

The ratio of urea nitrogen to creatinine has also been used for nutrition surveys of pregnant women in central America (Beaton, Arroyave and Florus, 1964). Taylor and Swartwout (1967) found the ratio to be 3.0 in the low income group as compared to 6.1 in the high income group and to be influenced by age and parity. Beydoun et al (1972) made similar observations but failed to observe any effects of age and parity. Chaudhury (1970) used this ratio in the investigation of the etiology of toxemia of pregnancy.

Arroyave and his associates (Arroyave, Janson and Torrico, 1966; Arroyave, Gullerimo and De Funes, 1967) further propose that the differences in urea excretion are maximized by restricting water intake, whereas the reverse is suggested by Simmons (unpublished) and Kenney, (1954). The former finding is not surprising since under conditions of water restrictions the loss would represent an obligatory loss against a concentration gradient.

Thus, the excretion of creatinine and other nitrogenous constituents and the relation they bear to each other have been held to be of significance for the assessment of protein nutriture. This approach is based on the invariant nature of creatinine excretion and has given rise to catagorical assertions as in standard books. To cite only one among the many instances to be found : "The daily output of creatinine in the urine is constant for the individual, amounting ~~to~~ from 1.5 to 2.0 g for men and from 0.8 to 1.5 g for women. This corresponds to about 2% of creatine in the body. Unlike the excretion of urea, which is derived largely from exogenous sources, the creatinine output is practically independent of the protein level of food (Best and Taylor, 1967).

This populaf view ignores several observations suggesting that we may be over-emphasizing the so-called constancy of creatinine excretion. Even, Mitchell (1962) an enthusiastic

advocate of the concept, was led, on the basis of studies on muscular dystrophy, obesity and nutritional deprivation, to observe "In fact, it appears that the creatinine excretion, ~~to~~ as expressed in the creatinine coefficient is depressed by factors that seriously impairs physical fitness or the nutritional status of the individual". He, further, quotes the statement of Friedmann, Kinney, Berryman, Henderson, and Youmans (1948) that "Therefore, dietary restrictions with respect to protein and B-complex vitamins resulting in the development of deficiency symptoms may be reflected in metabolic changes associated with the formation and excretion of creatinine. The changes may persist and are not rapidly abolished during subsequent supplementation".

It is also believed that creatinine excretion is not appreciably influenced by creatine in foods as the same is largely retained in the body (Bleiler and Schedl, 1962; Schedl, 1974). The amount excreted appears to be variable. The administration of 10-20 g of creatine was followed by some increase in creatinine excretion but a large proportion was retained and was associated with an overall increase in nitrogen retention (Chanutin, 1926).

In the study of Rose, Ellis and Helming (1928) administration of 1-2 g of creatine daily to two subjects, one male and one female, was followed by a 25% increase in creatinine

excretion. An increased excretion of creatine was also found in the female but not in the male. In subsequent study on 14 subjects of varying age and sex carried out by Hyde (1942) a similar increase in creatinine excretion following the administration of creatine was found in adult men and women (n-8). No increase was found in the remaining (n-3) women, children and a male patient suffering from muscular dystrophy. In all the above studies the diets were free from meat but otherwise normal and varied.

In the studies of Crim et al (1974) young men were fed creatine in amounts approximating those present in a normal meat diet, namely, 0.23 g per day for 9 days (phase - I) followed by 10 g per day for 10 days (phase - II) and a creatine-free diet for the next 71 days (phase - III). The subjects were physically trained for vigorous exercise during phase - I in order to bring all the subjects to a similar level of fitness. Creatinine excretion increased during phase - I and was elevated further during phase - II (+ 10% - 30%) and increased levels are maintained during phase III. A control group of two subjects showed no similar variation. During the last 10 days of Phase - III isonitrogenous amounts (4 g nitrogen/day) of either equimolar mixture of arginine and glycine (precursors of creatine synthesis) or alanine were added to the diet. Creatinine excretion increased in two of

four subjects fed the precursor aminoacids but was unchanged in the other two and in the subject fed alanine.

The authors of the above study also sought to investigate creatine pool size and turnover in relation to intake. They found de novo creatine synthesis to vary from 1.1 to 1.6 % of the creatine pool in different individuals but the degradation of creatine to creatinine was fairly uniform in all the subjects being of the order of 1.7%. The pool size was also influenced to some extent by the amount of creatine in the diet and declined with a creatine-free diet. Similar observations have been made by a number of other investigators (Camara, Arn, Reimer, and Neurberg, 1951; Karambelkar, Patwardhan and Srinivasan, 1952; Best et al, 1953; Bleiler and Schedl, 1962; Ooman, 1967; Calloway and Margen, 1971).

It is commonly assumed that the amount of creatinine excreted does not vary with amount of protein in the diet, but as early as 1905, Paton reported marked changes in the creatinine excretion of dogs fed protein at varying levels. A number of studies on rats suggest a similar relation between creatinine excretion and dietary protein content (Lee and Lucia, 1963; Milenkovic, Kucukalic and Muftic, 1974; Nishiza, Shimbo, Hareyama and Funabiki, 1977) or quality (Park, 1973). No such relation was observed in other studies on rats (Das and Waterlow, 1974) and Calves (Chetal et al, 1975). Fisher (1965) reported increased creatinine excretion with low protein diet in rats.

An increase in creatinine excretion has been found following supplementation of a low protein with the precursors of creatine, namely, 5% glycine, 0.25% methionine and 0.25% arginine (Yokota, 1964) and also high protein with ~~Ag~~ Arginine + glycine (Sitren and Fisher, 1977). Similar observations were made in man (Crim et al, 1974).

Kiriyama and his associates (Kiriyama, Yagishita, Suzuki and Iwao, 1967) found creatinine excretion to vary not only with body weight and growth rate in rats but also with ~~found consistent effects of~~ aminoacid imbalances. Fisher (1965) found significant decreases on protein free diet but continuation of the same doubled the creatinine excretion in rats. A similar increase in creatinine excretion as expressed per 0.75 power of the body weight after 3 weeks on a protein free diet has been reported by Muramatsu and Ashida (1955). Kiriyama and Ashida (1964) found absolute creatinine excretion to be consistently lower in rats fed protein-free diets than in those fed casein or gluten for 4-6 days. ~~on the diet.~~

A number of studies suggest a similar relation between dietary protein and creatinine excretion in man. With a change from a normal or a low protein diet to a high protein diet creatinine excretion was found to increase to varying extents by different investigators (Barett and Addis, 1947; Vanpilsum and Siljeskog, 1958; Fisher, 1965; Mayoral, Bolanos; Lotero and

Duque , 1975; Duque, Lotero and Mayroal, 1975; Duque, Bolanos, Lotero and Mayoral, 1975). Creatinine excretion was found to decrease with decreasing levels of protein in the diet (Clark, Moon, Malzer and Fang, 1974; Vyostskii, Vlasova, Kochetkova, Usahakov, and Shishkina, 1975; Vysotskii, Agureev, Brantova, 1975) and with a restriction of both calories and protein (Manniello, Harmuth and Fryer, 1960). An increase in intake of nitrogen from 3.54 to 14.2 g/day was associated with an increase in creatinine excretion in young children (Ritchey, Derise, Abernathy and Kersuland, 1973).

In the above studies the protein content of the diet was manipulated and changes in creatinine excretion monitored. Studies have also been made of differences in creatinine excretion in subjects habitually consuming diets low or high in protein (Oomen, 1967; Tripathy, Klahr and Lotero, 1970; Mayoral et al, 1975; Duque et al, 1975a; Duque et al, 1975b). Papuan male and female subsisting on low protein diets excreted less creatinine as compared to European counterparts (Oomen, 1967). Foggy, Crooke and Fry (1968) found a greater creatinine excretion in children fed rice and wheat than in those fed rice alone. Arroyave et al (1958, 1961) found creatinine excretion to be lower in preschool children from poor families than in better nourished children with greater protein intakes. Edozein and Philips (1963) also found low levels of creatinine

excretion children taking low protein diets. Schendel and Hansen (1965) observed that in six south African infants admitted to the hospital with kwashiorkor, the mean creatinine nitrogen was 20 mg as compared to 99 mg in well-fed, healthy normal infants. Three of the protein depleted infants showed a significant increase in creatinine excretion with rehabilitation on a milk diet whereas only a slight increase was found in three infants continued on a low protein diet (presumably for a short period before refeeding was started). Increase in creatinine excretion has been reported after 2 to 8 months of treatment for kwashiorkor (Standard, Wills and Waterlow, 1959; Arroyave and Wilson, 1961). Similar increases have been reported by other investigators (Srikantia, Paragaonkar and Vinodini Reddy, 1965; Reindrop and Whitehead, 1971; Ramakrishna rao et al, 1973) and in marasmus (Vinodini Reddy et al, 1963).

Although it has been suggested that starvation does not influence the level of creatinine excretion (Van Hoogenhuyze and VerPoleger, 1905; Benedict, 1907; Cathcart, 1907; Watnabe and Sassa, 1914; Junker dorf and Lisenfeld, 1926) an analysis of the data of even the earlier studies of Benedict (1915) suggest some reduction (Tables 9 and 10). This has been confirmed by several recent studies (Wolthuis, 1961; Spencer, Lewis, Samachson and Laszlo, 1966; Krzywicki, Consolazio, Matoush and Johnson, 1968; Owen, Felig, Morgan, Wahren and

Table 9 : Changes in creatinine excretion during prolonged starvation* (Benedict, 1915).

	day of fasting			
	1st	11th	21st	31st
body weight (kg)	59.6	53.9	50.5	47.4
height (cm)		170.7		
surface area (sq.m.)	1.70	1.62	1.57	1.54
basal calories	1441	1194	1032	1072
Creatinine excretion (mg) :				
per day	1370	1000	830	810
per kg of body weight	22.9	18.7	16.4	17.1
per cm. of height	8.0	5.9	4.9	4.7
per square meter of surface area	806	617	529	526
per basal calorie	0.95	0.84	0.80	0.76

* calculated from data given by Best and Taylor (1967) and Keys et al (1950).

Table 10 : Changes in creatinine excretion in starvation and semi-starvation studies.

Investigators	No. of subjects	Sex	period of starvation (days)	weight (kg)		creatinine (mg)	
				initial	final	initial	final
				starvation			
Krzywicki et al (1968)	6	M	5	77	73	1620	1780
Adibi (1971)	6	M + F	6	-	-	1930	1350
Ohnaka (1976)	3	M	7	64	59	1960	1686
Krzywicki et al (1968)	6	M	10	77	70	1620	980
Young et al (1973)	2	M + F	20	104	93	1900*	1700
Owen et al (1969)	4	M + F	35-42	131	108	-	1747
Wolthuis (1961)	1		63	87	75	1749	1298
				semi-starvation			
Grande et al (1955)**	13	M	24	69	61.5	2140	1429

* first day of fasting.

** diet, 1000 Kcalories from only carbohydrate.

Cahill, 1969; Adibi, 1971; Peters, Grosser and Knapp, 1972; Young et al, 1973; Ohnaka, 1976). A similar decrease has been found in several studies on semi-starvation (Grande, Anderson and Keys, 1955) including the very elaborate studies of Keys et al (1950) (Table 1Ø).

It has been suggested that since creatinine excretion varies with minimum endogenous metabolism (Folin, 1905) its output may indicate the involvement of nitrogenous constituents of tissue during muscular exercise. Also, as creatine-phosphate serves as a source of high energy phosphate bond in skeletal muscle the turnover of the same and consequently the excretion of creatinine may be influenced by exercise. An increase in creatinine excretion during or after muscular exercise has been reported in a few studies (Kocher, 1914; Starling and Evans, 1956; Doolan et al, 1962; Srivastava, Mani, Soni and Bhati, 1967). However, no such increase has been found by several other investigators (Paterson, 1967; Simko, Merrifield and Stouffer, 1973; Refsum and Stromme, 1974; Crim et al, 1974, 1976). Margaria and Foa (1939) detected no increase in the elimination of nitrogen and creatinine of young men during work to exhaustion on a treadmill at grade or up grade; but the same increased with exercise at down grade. Mitchell (1962) suggested that the latter difference is not due to changes in protein metabolism, but due to kidney function

Table 11 : Changes in creatinine excretion during prolonged semi-starvation[1] (Keys et al 1950).

	week of food restriction[1]			week of rehabilitation[2]	
	control period	12th	24th	6th	12th
body weight (kg)	69.5	57.2	52.6	54.6	58.8
height (cm)			178.8		
surface area (sq.m.)	1.87	1.71	1.65	1.68	1.74
basal calories	1585	1076	967	1077	1271
Creatinine excretion (mg) :					
per day	1600	1190	990	1020	1400
per kg of body weight	23.0	20.8	18.8	18.8	23.8
per cm of height	8.9	6.6	5.5	5.7	7.8
per square meter of body surface	856	696	600	607	805
per basal calorie	1.01	1.11	1.02	0.95	1.10

1. based on data obtained for 32men whose food intake was restricted to 1583 Calories as against 3492 Calories at the start of the investigations.

2. at the end of investigation the men were rehabilitated on a diet providing 3045 Calories.

brought about by local circulatory changes resulting from increased lumbar convexity of spine (lordosis) associated with down grade walking. Nichols, Miller and Hiatt (1951) in a test on tread mill running reported that muscular exercise has no influence on the rate of creatinine excretion except for a transient depression proportional to the concomitant decrease in renal blood flow and glomerular filtration rate. A decrease has been observed during exercise by Slonim (1961) and Calloway and Margen (1971).

Similar conflicting reports have appeared in the literature on rats and farm animals. Efremov and Sakaeva (1975) found an increase in creatinine excretion with exercise in both young and old rats. On the other hand, no such increase was found in an earlier study in rats given a low protein diet providing generous amounts of food energy (Mitchell and Kruger, 1928). Vanpilsum and Seljeskog (1958) noted an increase in clearance of creatinine with exercise which average approximately 9%. In summary it would appear that inadequate intakes of energy, alteration in the kidney function and the type of exercise influence the results (Mitchell, 1962).

If creatinine excretion is influenced by exercise, it would be reasonable to expect it to be also influenced by immobilization. After 14 days of bed rest, urinary creatinine

increased in 19-26 year old human volunteers and the increase persisted even after the termination of bed rest (Pace, Grunbaum, Kodame, Rahlamann and Newsom, 1975) and may be due to the negative nitrogen balance often observed during immobilization (Glikman, Keeton, Cole, Calloway, Mitchell, Sapienza, Dyniewicz and Howes, 1948) and/or during muscular quiescence.

In conclusion, as pointed out by Waterlow (1969), "The fact that the relationship between lean body mass or muscle mass and creatinine excretion is rather constant from one species to another and in different individuals of the same species does not prove that it is also constant under different conditions of nutrition and growth. The rate of creatinine excretion must depend upon the amount or concentration of creatine in muscle, and the rate at which it is irreversibly converted to creatinine. It would certainly be unusual biological situation if both concentration and turnover rates were the same at all ages and under all nutritional conditions".

Even in the normal individual, the correlation between creatinine excretion and basal metabolism or lean body mass does not hold during the period of growth as the excretion of creatinine in relation to body weight is less in children than in adults, whereas basal energy expenditure is more. This has been attributed to the small muscle mass in children and its

lower creatine content but the possibility remains that endogenous losses of nitrogen are minimal during period of anabolism such as growth and pregnancy.

The observation made by a few investigators that the excretion of creatinine is low in poorer segments of the populations in developing countries raises questions as to whether this is because of a low plane of protein nutrition or due to a decrease in energy metabolism or a smaller proportion of muscle mass. Much more experimental work appears to be needed to clarify this problem.

In spite of several generalizations made about the constancy of creatinine output in relation to muscle mass, energy metabolism or body weight, extensive data on all these aspects do not appear to be available even for well-nourished subjects in the western world covering the period of growth, barring very exhaustive studies of Macy (1942) covering the period between 4 to 12 years of age. The paucity of information on children in countries with a low plane of nutrition is all the more striking.

Further, even when data are obtained on such subjects and they suggest low levels of creatinine excretion, the investigator is plagued by doubts about the authenticity of the 24 hr. collection. When he is reasonably convinced, others who have

not bad the opportunity to deal with similar cases remain aceptical. It becomes necessary to become doubly and trebly sure of the results obtained before attempting to perceive a pattern.

Most of the recent advances in nutrition have taken place in the affluent west even when the questions prompting the advance have arisen because of problems such as beri-beri, kwashiorkor and keratomalacia found in poor countries or regions. There is a strong tendency to assume that western patterns of growth and nutrition including physical, skeletal and biochemical aspects are valid for the whole world and that any departure represents abnormalities.

The fes studies carried out in other regions suggest differences with regard to the pattern of physical and skeletal growth (Garn, 1975) and biochemical maturation with regard to even parameters such as hemoglobin (Garn, Smith and Clark, 1975).

In this laboratory attempts are being made since past fifteen years or more to identify the degree and prevalence of malnutrition in poor rural and urban areas and to formulate and evaluate measures for the alleviation of the same. Standard indices of nutritional status were employed to assess the nutritional status before and after 'intervention' programmes

designed to prevent and treat malnutrition in young children (Rajalakshmi and Ramakrishnan, 1977). These indices included assessment of vitamin status as judged by urinary excretion of the vitamin in relation to creatinine. It turned out in these studies that although many of the children were having frank clinical symptoms suggestive of riboflavin deficiency such as pale or fissured tongue the excretion of riboflavin per gram of creatinine was quite high as compared to ICNND norms (Rajalakshmi, 1975).

In other studies concerned with ascorbic acid metabolism during pregnancy and lactation, creatinine in 24 hr. urine was determined more as a check for authenticity of the sample rather than out of any interest in creatinine per se. However, the values for creatinine were much lower than those reported even after taking reasonable steps to ensure (Rajalakshmi and Ramakrishnan, 1969) that the collections were complete (a field worker stayed with the family during the collection period).

These observations suggested that the princely values obtained for vitamin excretion in relation to creatinine are due to low levels of creatinine excretion rather than high levels of vitamin excretion. This impression was strengthened by subsequent studies in which 4 hr. collections were made at a play centre for children and the values extrapolated for 24 hrs.

These observations and the paucity of information on the excretion of creatinine and other nitrogenous constituents in relation to age, sex, plane of nutrition and energy metabolism in Indian subjects led to the present study designed to investigate the above aspects. The result of these studies are incorporated in this thesis.