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<td><strong>Author</strong></td>
<td>Galloway, Robert Menzies</td>
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CREATINE AND CREATININE

With Special Reference to the Creatinine Coefficient in Pulmonary Tuberculosis.

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Being a thesis for the degree of Doctor of Medicine in the University of Edinburgh.

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SECTION 1.

INTRODUCTION.

A sound progressive knowledge of physiology and Pathology associated with the capacity for accurate observation is the basis for all advance in clinical medicine.

"The Study of Physiology and Pathology within the past half century" wrote the late Sir William Osler (1) "has done more to emancipate medicine from routine and the thraldom of authority than all the work of all the physicians from the days of Hippocrates to Jenner; and we are as yet but on the threshold".

It was Sir William Bayliss (2) who pointed out that in regard to medical education there is some need to guard against too narrow an interpretation of what is supposed to be of direct application to clinical practice. Just as what is regarded at the present time as pure abstract or academic science may turn out to be of vital clinical importance so also clinical findings may throw light on physiological principles. It is by a correlation of clinical findings with experimental data that progress is made both in our knowledge of the normal physiological functions and in our fight against disease.

Consider how our present knowledge of the functions of the endocrine glands was obtained. It was the recognition of acromegaly by Pierre Marie, the study of its symptoms and morbid Anatomy, the surgical results of Cushing and others, together with a correlation of all the facts by the brilliant experimental work of Schafer, which have clarified our conceptions of the physiology of the pituitary. Again, as Sir Archibald Garrod (3) reminds us the gradual accumulation of facts concerning exophthalamic goitre, Gull's discovery of myxodema, Kocher's observations upon cachexia strumipriva and the effects of thyroid treatment, provide a connected story of clinical and experimental work which resulted in great physiological advancement and considerable therapeutic gain. The recent isolation of the pancreatic hormone "Insulin" by Banting and McLeod (4) is another instance of the inestimable value of the combination of experimental work with clinical findings. The story was started by Cornelius Celsus, the Roman patrician who, in the first century A.D., wrote of the disease we now call Diabetes Mellitus. It was continued by Dobson who in 1776 described diabetic urine "as if there had been sugar and honey in it". The discovery by Claude Bernard in 1857 of the glycogenic function of the liver and later, Opie's demonstration of the internal secretion of the islands of Langerhans are all chapters of the history which has culminated in the Isolation of Insulin.

Creatine and Creatinine have long been substances of interest to the physiologist and biochemist. In 1835 Chevreul (5) described and named Creatine. For many years after that date however, there was no accurate method for the estimation of either creatine or creatinine and consequently most of the earlier work done was unsatisfactory and unreliable. In 1904 Otto Folin (6) described an accurate and simple method for the estimation of Creatinine and since that time much valuable research has been done in this country, in Germany, in the United States of America and elsewhere.
The remarkable constancy of the Creatinine excretion for each individual in health was soon established. Therefore conditions in which this excretion varied became of interest and were investigated. From these enquiries into the creatinine excretion in morbid states much information was obtained. Amongst many conditions examined it became apparent that fevers and muscular dystrophies were two types of disease in which the metabolism of creatine and creatinine could be profitably investigated.

From the time of the Greek physicians we have had descriptions of the clinical features of pulmonary tuberculosis. Hippocrates described the cough, emaciation and the consumptive type. Galen (A.D. 131-201), the Roman physician who had studied in Alexandria, gave an accurate picture of the disease and recognised its contagious nature. In 1689, Richard Morton, the friend and contemporary of Sydenham wrote the first modern treatise on the subject (Phthisisologia) and described the famous triad - cough, fever and emaciation - as cardinal symptoms of pulmonary tuberculosis. Two of these symptoms, fever and emaciation are conditions in which the metabolism of creatine and creatinine is stated to be abnormal. Therefore, while the opportunity offered it was thought that it might be profitable to study the creatinine excretion in pulmonary tuberculosis. It was hoped that simple but carefully taken clinical data might help to throw light on the problem of creatine and creatinine metabolism. At the same time the possibility of the creatinine excretion having some prognostic value was considered. By the correlation of these findings with the experimental data of others it was deemed probable that some small contribution might be made in this thesis, to our knowledge both of the physiological principles governing creatine and creatinine and of the factors regulating their metabolism in pulmonary tuberculosis.
SECTION 11.

CREATINE AND CREATININE.

1. HISTORICAL

2. PROTEIN METABOLISM

α. METABOLISM OF CREATINE AND CREATININE.

A. Chemistry.

B. Distribution.

C. Possible Precursors.

D. Metabolism in Disease.

E. Theories of Normal Metabolism.
1. **HISTORICAL.**

Creatine \( \text{Kρζξσ} = \text{flesh} \) was first described in the year 1835. As its name implies it was derived from a meat extractive and Chevreul's original work on the subject was embodied in a report to the French Academy of Sciences on Commercial Meat extracts (7). He noted a resemblance between creatine and "aspargine". In 1844 Schloësberger (8) obtained the substance from the muscles of the alligator; its importance as a general constituent of muscle began to be recognised. Liebig(9) in 1847 commenced the serious chemical investigation of muscle. He found creatine in the muscles of several animals including the alligator and man. For the first time creatinine was detected in crystalline deposit from urine. He further published work describing the effects of fatigue on muscles, shewing that those performing the most work contained the most creatine. About this time Liebig carried out his classical on the hunted fox in whose muscles he found ten times the quantity of creatine present in the muscle of a resting animal - a finding which has not however been confirmed by others using more accurate chemical methods.

Heintz and Pettenkofer (10) were later workers on the subject of creatinine in the urine. They concluded that it was identical with the substance isolated from muscle by Chevreul; but in 1858 Liebig showed that, on the contrary, creatinine was an anhydride of creatine and could be prepared from the latter substance by boiling with acids. Stillings-fleet Johnston (11) also investigated these substances and concluded that creatinine obtained from creatine by boiling with acid differed from that in urine. This conclusion was proved erroneous by Toppelins and Pomerehene (12) who showed that the creatinine derived from the two sources was identical. This latter finding was confirmed by Worner (13). Poulson (14) later proved that Stillingsfleet Johnston's iso-creatinine obtained from urine was ordinary creatine contaminated with urinary pigments.

The close chemical relationship between the creatine of muscle and the creatinine of urine naturally led to the supposition that the latter was derived from the former both by endogenous metabolism in the body and by exogenous metabolism of the creatine taken as food inflesh meat. (Liebig and Ranke, quoted by Bryan McSwiney). Voit (15) dissented with reference to the endogenous conversion of creatine to creatinine. He found that tetanus produced a decrease of creatine in frogs muscle while the total quantity of creatine and creatinine was not increased. The conversion of creatine during muscular contraction into another substance not identical with creatinine suggested itself to him as a possibility.

Up to the year 1904 the method used for the quantitative estimation of creatinine was that devised by Neubauer (16). This consisted in adding Calcium hydroxide to urine, evaporating the filtrate and making it up to an eighty per cent alcoholic solution. To this was added alcoholic zinc chloride and the precipitated creatinine-zinc chloride compound weighed. In 1904 Otto Folin (17) devised
an accurate method for creatinine estimation and from that date the collection of trustworthy data commenced. In connection with the particular subject of this thesis the importance of Folin's work cannot be over-estimated; all our modern knowledge of creatine-creatinine metabolism owes its inception to his discovery.
The subject of protein metabolism can only be mentioned in such a way as to indicate the practical problems confronting the investigation of creatine and creatinine. As mentioned in the sequence, injected protein is so broken down by the gastro intestinal enzymes that it is probably absorbed as amino-acid. The fate of the absorbed amino-acid is a matter of debate. Dakin and Dudley (1913) have shown that many amino acids (glycine, alanine, valine, leucine, phenyl alanine and aspartic acid) undergo spontaneous dissociation at low temperatures into the corresponding X Ketonic aldehyde and ammonia. The reaction is no doubt reversible and in metabolism may be catalysed by an enzyme; consequently the formation of an amino acid from the hydroxy-acid seems to pass through the intermediate stage of the corresponding ketonic aldehyde.

\[ R \cdot CHNH_2 \cdot COOH \rightleftharpoons R \cdot CO \cdot CHO + NH_3 \]

The X Ketonic aldehyde may be oxidised, hydrolyzed or reduced. Thus:

\[ CH_3 \]

\[ CHNH_2 \rightarrow NH_3 + CH_3 \]

\[ COOH \]

\[ (\text{Alanine}) \]

In the organism these last reactions again may be accelerated by enzymes. After this process of deamination takes place in the body, it is presumed that the ammonia is converted into urea and excreted as such and that the fatty acid part is used for the supply of energy. It is further supposed that a certain portion of the amino acids are not thus broken down but are used for tissue repair or for growth.

The conversion of the ammonia into urea is supposed to occur in the liver (Cathcart 1912). This function of the liver is demonstrated by means of an Eck’s fistula (a communication between the portal vein and the inferior vena cava.) which results in a great increase of blood ammonia. Folin and Denis (1912) hold that the tissues themselves have the power of converting ammonia into urea - " the food protein reaches the tissues in the form of amino-acids and those amino acids which are not needed for the rebuilding of broken down body material are not rebuilt either into protein or protoplasm but are broken down and their nitrogen converted into urea". Van Slyke and Meyer (1913) contrary to Folin and Denis hold that the liver is the chief if not the only situation where deamination occurs. Amino-acids, they maintain, are taken up by all the tissues but those taken up by the liver disappears very quickly. This diminution of amino and content of the liver is accompanied by increase of blood urea. But as there is an equilibrium between amino acids and urea in blood, as the liver forms urea more amino acids pass from other tissues to restore equilibrium. It is therefore apparent that no deamination should be performed according to this theory, by any tissue but the liver.
In 1905 Otto Folin showed that some of the urinary excretions of the products of metabolism maintain an almost constant figure whilst others are much greater under a rich Nitrogen food. The constant products he found to be creatinine, neutral sulphur and, to a less extent, uric acid and ethereal sulphates. The variable products, Folin pointed out are urea and inorganic sulphates - not creatinine and probably not neutral sulphur. (18) "The fact that the urea and inorganic sulphates" he says "represent chiefly the variable Katabolism does of course not preclude the possibility that they also represent to some extent the constant Katabolism" (19). Again he continues "It is clear that the metabolic processes resulting in the end products which tend to be constant in quantity appear to be indispensable for the continuation of life; or to be more definite, those metabolic processes probably constitute an essential part of the activity which distinguishes living cells from dead ones. I would therefore call the protein metabolism which tends to be constant "tissue metabolism" or "endogenous metabolism" and the other, the variable protein metabolism I would call the "exogenous" or "intermediate metabolism".

In the same article Folin (20) went on to emphasise that the fact that urea, the chief representative of exogenous protein metabolism is not to be found in the muscles (except in infinitesimal traces) acquires a new significance from this point of view. The earlier belief was that the protein decomposition in muscles did not proceed as far as urea but only produced certain precursors of it which were afterwards elaborated in the liver into urea. But as Folin pointed out (loc.cit.) the only precursor in muscle which is found in quantities that could be considered at all adequate is creatine and he reminded us that the liver had been proved incapable of converting creatine into urea. Moreover he concluded that the perfect constancy in the creatinine elimination shown in his experiments under such different conditions excluded positively the possibility that the creatine produced in the protein metabolism of the muscles was afterwards converted into urea. The muscle creatine he maintained is excreted as creatinine.
3. METABOLISM OF CREATINE AND CREATININE.

A. CHEMISTRY

Reference need only be made to the more important chemical relationships of creatine and creatinine. It will be remembered that proteins may be hydrolysed by means of water at a high temperature, more readily by dilute acids and most readily at the body temperature by the action of certain protease enzymes such as pepsin of the stomach and trypsin of the pancreatic juice. This disintegrating hydrolysis proceeds in stages, the products becoming less and less complex as the action progresses. The scheme of hydrolysis is - : proteins - metaproteins - Proteoses - Peptones - polypeptides - amino-acids. The amino acids are the chief ultimate units of the hydrolysis of the native proteins and yield true solutions which are assimilated by the animal body. They are all of the same type having the amino group \((\text{NH}_2)\) in the X position with regard to the carboxyl group. They are thus theoretically all derivatives of glycine \((\text{NH}_2\text{CH}_2\text{COOH})\) and may for the most part be represented by the general formula \(\text{R.CH(NH}_2)\text{COOH} \).

In the formation of the more complex products of protein disintegration the amino-acids combine in a characteristic way. They are condensed together with elimination of water to form in the first place polypeptides. The peptide linkage can be represented - COOH.....H: NH =

\[- \text{CO-NH} + - \text{H}_2\text{O}.\]

In this way many peptides have been synthetised, the pioneer in all the work being the great Emil Fischer. As before stated the simplest amino acid is glycine, having the formula \(\text{H.CH(NH}_2)\text{COOH}\). Now sarcosine is methyl glycine - \(\text{H.CH.NH(CH}_3)\text{COOH}\). It resembles glycine closely in its properties. By the action of cyanamide on sarcosine it is possible to obtain creatine.

\[
\text{NH}_2\text{CN} - \text{NH(CH}_3)\text{CH}_2\text{COOH} = (\text{NH}_2)(\text{NH})\text{C.N(CH}_3)\text{CH}_2\text{COOH}
\]

(Cyanamide) (Sarcosine) (Creatine)

Creatine crystallises from water in colourless prisms. When boiled with baryta water it is hydrolysed with formation of sarcosine and urea \((\text{NH}_2)(\text{NH})\text{C.N(CH}_3)\text{CH}_2\text{COOH} - \text{H}_2\text{O} - \text{NH}_2\text{CO.NH}_2 - \text{NH(CH}_3)\text{CH}_2\text{COOH}\).

It may be looked on as a derivative of guaindine in that one of the \(\text{NH}_2\) groups is replaced by methyl glycine (sarcosine).

\[
\text{Guanidine is } \text{H-N=C NH}_2 \quad \text{Sarcosine is } \text{H-N=CH}_2\text{COOH}
\]

\[
\text{Creatine is } \text{H-N=C N-CH}_2\text{COOH}
\]

The two hydrogens of one amino group of guaindine are replaced by the radicals \(\text{CH}_3\) and \(\text{CH}_2\text{COOH}\) respectively.
Creatinine is formed from creatine by loss of water.

\[ \text{HN} = \text{C} - \text{N(CH}_3\text{)} - \text{CH}_2 \quad \text{HN} = \text{C} - \text{N(CH}_3\text{)} - \text{CH}_2\text{O} + \text{H}_2\text{O} \]

(Creatine) \hspace{1cm} \text{(Creatinine)}

The action may be brought about by boiling with acids and reversal takes place on treatment with alkalies.

Creatinine crystallises in colourless glistening monoclinic prisms. It is much more soluble than creatine, dissolving in twelve parts of cold water more readily in warm water and most readily in warm alcohol. It is a well marked base and yields crystalline salts with acids. One of the most important salts is creatinine zinc chloride \((\text{C}_4\text{H}_7\text{N}_3\text{O})_2\text{-ZnCl}_2\)', which is formed from an alcoholic solution of creatinine upon treatment with zinc chloride in acid solution. Oxidation of creatinine with potassium permanganate yields amongst other products methyl guanidine - \(\text{NH}_2\text{C(NH)}\text{.NHCH}_3\).

Creatinine must be regarded as an internal anhydride of creatine with basic properties since the carboxyl group has disappeared.
B. DISTRIBUTION.

Creatine is a constituent present in the muscles of all vertebrates. W. Koch (21) demonstrated it in heart muscle. Creatine is not generally believed to be present in the muscles of invertebrates (Weber (22), McSwiney (23)). The percentage of creatine in various animal's muscles differs considerably. Thus Mellanby (29) found that the muscle of skate contained 0.24 per cent of creatine, frog 0.26 per cent, fowl 0.31 per cent, guinea pig 0.32 per cent, hedgehog 0.2 per cent (both in winter and summer), bullock 0.3 per cent, pigs 0.33 per cent, rabbits 0.44 per cent of creatine. The figures Mellanby obtained suggested to him that with the development from cold blooded to warm blooded animals the quantity of creatine in the muscles increases. The low percentage of creatine in hedgehog muscle he considered striking and even more remarkable its constancy throughout the year both in summer and during hibernation.

Mellanby also investigated the creatine content of rabbit and chicken muscle from the onto-genetic point of view. In the rabbit foetus he showed that the creatine increased with the advance of gestation. At the fifth day of gestation he found only 0.15 per cent of creatine in the muscle. At the tenth day he discovered 0.25 per cent, the twentieth day 0.35 per cent and at the thirtieth day 0.38 per cent. By the fortieth day of gestation the creatine content of the muscle had risen to 0.4 per cent, more nearly approximating the 0.44 per cent of the adult rabbit muscle. With reference to the creatine content of chicken muscle during incubation and after hatching Mellanby (25) found that before the twelfth day of incubation there is no evidence of creatine in chicken muscle. Towards the end of incubation he demonstrated a rapid increase in the quantity of creatine and correlated it with the growth in the size of the liver. After hatching Mellanby discovered that the percentage content of creatine in chicken muscle increases from 0.2 per cent at three days to 0.29 per cent at fourteen days.

McSwiney (loc cit (26) investigated the percentage of creatine present in muscle relative to the total dried solids. Working with rabbit muscle he found the creatine content 2.04 to 2.56 per cent of dried solids. The creatine percentage of fresh rabbit muscle he discovered to be 0.50 to 0.58 per cent - a higher figure than that of Mellanby. McSwiney further showed that the creatine content of various groups of muscles varied somewhat but he attributed the differences to connective tissue variations.

Myers and Fine (27) emphasise the fact that muscle creatine is remarkably constant for any particular species. As a result of their analyses they conclude that rabbit muscle contains 0.52 per cent of creatine, the muscle of the cat 0.45 per cent and human muscle 0.39 per cent of creatine.

Creatinine in contrast to creatine is stated never to be present in muscles in quantities capable of detection (Mellanby (28) Weber (29)). Human blood is stated to contain 2.2 mg. of creatinine in 100c.c. (McLean) (30). As far as can be ascertained from an examination of the literature there is no proof that the blood contains creatine.
Creatinine is a normal constituent of urine. Maclean (loc.cit) found that the urine, in a healthy young adult male, contained 89 mgr. per 100 cc. of urine. Therefore in the human subject it would appear that the kidney is capable of concentrating the blood creatinine forty times. The average daily urinary excretion of creatinine in an adult is about 1.25 grams but it varies chiefly with the weight of the individual and to a lesser extent with the sex, the male excreting rather more proportionately than the female. Creatine does not usually occur in human urine. In young children however, the urinary excretion of creatine (creatinuria) is normal and there is moreover an almost complete absence of creatinine in the urine (loc.cit). Creatinuria also occurs consistently in the puerperium (Steenbock & Gross (32) Shaffer (33)). After prolonged fasting creatine appears in the urine (Cathcart; (34), Steenbock & Gross (35)). Cathcart further showed (loc.cit) that the creatinuria of starvation is stopped by taking starch but not fat.

In the bird creatine is not changed to creatinine but is excreted unchanged in the dejecta as an end product of muscular metabolism. This fact was pointed out by Noel Paton (36) who suggested that in birds the creatine output might be taken as a measure of muscle catabolism.

W. Koch (37) has shown that the excretion of creatinine is as constant in the dog as in man. Furthermore he found that the excretion per Kg. of body weight was about the same for the dog as for man (24-26 mg for dog; 26-36 mg (or man)).

The relationship between the creatine of the muscles in different animals and their normal output of urinary creatinine has been the subject of investigation by Myers and Fine (38). They found that those species with muscles richest in creatine showed a greater excretion of creatinine in the urine. Thus in the rabbit, the creatinine coefficient (mg. of creatinine Nitrogen eliminated per Kg. of body weight - vide, seq.) is fully one third higher than that found in man and various experimental animals.

<table>
<thead>
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<th>Animal</th>
<th>Creatine Content of Muscle</th>
<th>Creatinine Coefficient</th>
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<tr>
<td>Rabbit</td>
<td>0.52%</td>
<td>14.3</td>
</tr>
<tr>
<td>Man</td>
<td>0.39%</td>
<td>9.0</td>
</tr>
<tr>
<td>Dog</td>
<td>0.37%</td>
<td>8.4</td>
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</table>

To summarise, creatine is found in the muscles of all vertebrates but as far as is known does not occur in invertebrate muscle. The muscle percentage of creatine is constant for each type of animal but it varies in the different types. Creatinine is not found in the tissues, other than blood of any animal. Creatinine is a normal constituent of urine. Creatine appears in the urine during growth, in the puerperium and after prolonged fasting. In the bird creatine is not changed to creatinine but is excreted unaltered as an end product of muscular metabolism. Finally those species of animals showing the highest percentage of muscle creatine show the highest excretion of creatinine in the urine.
Much work has been done in the hope of finding a precursor of creatine, or a source whence the amino-acids which go to build it up might be derived.

W. Koch (39) points out that creatinine, as far as we know, is the only constant constituent of urine which contains a methyl group attached to Nitrogen, while lecithin and the closely related Kephalin are, with the exception of small amounts of caffeine in tea and coffee, the only articles of diet which contain such groups attached to Nitrogen. It is stated by Koch that there is some experimental evidence to show that methyl groups are transferred as a result of reactions going on in almost every cell of the animal body. He quotes Albanese (40) who showed that caffeine is changed to dimethyl xanthine in man and to monomethyl xanthin in the dog. In order to attempt to demonstrate some relationship between lecithin and Kephalin and creatine - creatinine metabolism Koch carried out a series of feeding experiments on a dog. Periods of diet rich in lecithin and Kephalin were succeeded by periods low in these ingredients. As a result of his work he concluded that (41) under ordinary conditions of diet the methyl groups of the lecithin and Kephalin ingested can all be accounted for by the methyl groups of the creatinine excreted. With an excess of lecithin and Kephalin this was not the case, although the creatinine was undoubtedly increased. This augmentation he regarded as being due to the lecithin and Kephalin of the ingested egg and not to any other constituent of the diet. The value of Koch's observations, however, seem doubtful. The average increase on which he based his deductions was in one case only 0.001 grams and in the other 0.014 grams of urinary creatinine per twenty four hours. Moreover, his estimations were made with an arrangement of Nessler glasses, a Dubosq colorimeter being unavailable. Koch made the interesting enquiry (loc. cit) (42) whether, under a lecithin free diet long continued, the amount of creatinine could finally be reduced or whether, in its stead, glyco-cyanidine (which only differs in lacking the methyl group) would be excreted.

Jaffé (43) obtained an increase in the muscle creatine of young rabbits after feeding with glycocyamin. Mellanby (44) also augmented the creatine content of chicken muscle by feeding with glycocyamin. He pointed out however that the muscle of the growing animal shows a normal increase of creatine content until a certain saturation point is reached. Therefore neither his nor Jaffé's results with glycocyamin are of value.

The amino-acid arginine \(((NH)(NH_2)C.NH.CH_2.CH_2.CH(NH_2).COOH)\) has been considered by a number of workers a possible precursor of creatine. It is generally accepted as axiomatic that creatine is derived from protein. Arginine has attracted so much attention because it is contained in all the known proteins and like creatine is a derivative of guanidine. To begin with, direct experimentation by the feeding of arginine was rather limited but considerable work was done with transfusion and injection. Inouye found an increase in creatine upon adding arginine to liver extract and also when arginine was perfused through the surviving liver - suggesting that the liver is capable of converting arginine into creatine. Myers and Fine (46) on feeding rats with edestin (which
contains 14% of arginine) found a slight increase in the muscle creatine, but they drew no conclusions. Thompson (47) has reported numerous experiments in the course of which arginine was injected into rabbits, dogs and ducks; he was able to observe an increase in creatine excretion and muscle creatine content in most instances. He failed to obtain any increase by oral administration.

Gross and Steenbock (48) showed that in the pig creatinuria can always be induced by feeding sufficient protein, irrespective of the sex of the animal or the acidity or alkalinity of the diet. Consequently they regarded it as a valuable animal for metabolic experiments bearing on the subject under review. They tried (49) the oral administration of arginine and found that, in sufficient amounts, it augments the creatine excretion in the pig. They further came to the conclusion that the creatinuria induced by casein feeding (in pigs) appears to have its origin largely in the formation of creatine from arginine; but that the acidity of the phosphoric acid split off no doubt contributed to the creatinuria as a result of the stimulation of endogenous metabolism. They further demonstrated that in pigs cystine feeding causes creatinuria only when the sulphuric acid formed by the oxidation of its sulphur is left unneutralised; when neutralised, the creatinuria promptly disappears. On the other hand neutralisation of acidity does not prevent the creatinuria called forth by casein or arginine feeding.

Jaffé (50) found no increase in urinary creatine following the feeding of arginine to the rabbit. This is in accord with Thompson's finding who only obtained an increase after parenteral injection of arginine. No increase of creatine formation was discovered by Baumann and Hines (51) after injecting arginine into animals. Von Hoogenhuyze and Verploegh (52), seeking for a precursor of creatine among the protein cleavage products, got negative results following the ingestion of casein and gelatin, proteins relatively rich in arginine.

It is reported by Harding and Young (53) that cystine administration increases creatinuria in young dogs.

Reisser (54) augmented the muscle creatine content of rabbit by injecting (with urea) choline and betaine. He got similar results with sarcosine and urea.

Baumann and Hines (55) failed to obtain decisive results in perfusion experiments with sarcosine, betaine, choline and methyl guanidine.

Gibson and Martin (56) investigated a subject suffering from Progressive Pseudo-hyperthrophic muscular dystrophy. Such cases show a high degree of creatinuria (vide seq.). As a result of the observations made they concluded that in their patient
1. The creatine and to a lesser extent creatinine excretion was increased as a result of a greater protein intake.
2. The substitution of the arginine, rich protein edestin for a part of the protein failed to increase creatine excretion.
3. Hordein added to the diet increased the total Nitrogen and urea but was without effect on creatine.
4. Ingested sarcosine, asparagine cystine and histidine gave negative results.
D. METABOLISM IN DISEASE.

Before going on to discuss the creatine-creatinine metabolism in disease reference must be made to the occurrence of creatine in the urine. As already stated it is not usually present except in early life, in the puerperium and during starvation. Certain other facts have been elicited -

(i) Creatinuria is more easily established in normal adults (older children, women, adult males, after creatine administration) on a high protein diet than on a low protein intake (Folin and Denis (57) Denis and Minot (58))

(ii) In established creatinuria the creatine excretion is increased in going from a low protein to a high protein diet (Levene and Kristeller (59); Denis and Kramer (60))

(iii) In established creatinuria ingested creatine is largely or completely recovered in the urine, in part as creatinine (Levene and Kristeller loc. cit).

Gibson and Martin (loc. cit) (61) in a case of Progressive Pseudo hypertrophic muscular dystrophy, where there is a much diminished creatinine coefficient and a high degree of creatinuria, showed that:

(i) Ingested creatine is promptly and completely eliminated chiefly as creatine, in part as creatinine

(ii) Creatine and, to a lesser extent, creatinine excretion increased as the result of a greater protein intake.

The Metabolism of creatine and creatinine is of some interest in disturbances of the thyroid gland. Cramer and Krause (62) noted the appearance of creatine in the urine after thyroid feeding. Later (63) the same two observers investigated the effect of thyroid administration in man, first on a protein rich diet and secondly on a protein poor diet. With the protein rich diet they found that thyroid administration was followed by a slight increase in creatinine excretion and by the appearance of creatine in the urine. These effects were completely absent on the protein poor diet. Gross and Steenbock (64) investigated the effect of thyroid feeding to pigs on a nitrogen free diet. It must be remembered (vide ante) that in the pig the establishment of creatinuria is relatively easy. They found under the circumstances of their series of six experiments on five pigs that the administration of sheep's thyroid with the food called forth a marked stimulation of creatine.

They found also that this is accentuated when probable creatine precursors (e.g. cascin in their experiments) from exogenous sources are available. Froeschbach (65) reported a low output of creatinine in exophthalmic goitre. In a similar case of Grav'e's disease in a man Denis (66) found that creatinuria could be produced by protein feeding.

Associated with thyroid disturbance is the metabolism of creatine and creatinine in changed carbohydrate metabolism. Cathcart (67) found that creatine appeared in the urine after the withdrawal of carbohydrates from the diet. Krause and Cramer (68) showed that creatine appeared in the urine in diabetes mellitus and in phlorizin diabetes. It was noted by Thompson (69) that in three cases of diabetes examined there was an excretion of
creatinine in the urine, apparently increasing with the severity of the glycosuria. A marked diminution in the output of creatinine was also seen. Thompson found that in the mildest of his diabetes cases the addition of creatine to the food in 1 grm doses had no pronounced effect on the output of sugar, whereas the administration of creatinine in doses of 0.15 to 0.18 grams, whether hypodermically or with the food, increased the output of sugar by nearly 50%.

The excretion of creatinine and creatine in the urine during the course of acute infectious fevers has been studied by many workers. Van Hoogenhuyze and Verploegh (70), Shaffer and Coleman (71), Hawk (72), McClure (73) and others are agreed in the general statement that, in the course of acute infectious fevers, there is an increased output of creatinine and uric acid and that creatinuria sometimes occurs. Shaffer and Coleman (loc. cit) investigating the effects of high caloric diets rich in carbohydrate on the protein metabolism of patients with typhoid fever, found that these diets retarded or prevented the loss of body protein. C. W. McClure (loc. cit) (74) made enquiry into certain products of protein metabolism in six cases of enteric fever, four cases of pulmonary tuberculosis, four cases of lobar pneumonia, one case of sub-acute rheumatic fever and one case of streptococcic broncho-pneumonia, all of which were on a high caloric diet rich in carbohydrates. He came to the following conclusions -: 1. that there is no constant relation between the presence of pyrexia and creatinuria.

II. that the presence of creatinuria, although usually occurring coincidently with loss in body weight, is not definitely related either to the rapidity or the amount of weight los.

III. that during periods of pyrexia creatinuria may occur unaccompanied by disturbances in the excretion of uric acid and creatinine.

IV. that in the cases observed disturbances in the excretion of creatinine are not necessarily associated with an increase of the body temperature and that such disturbances may occur independently of anomalies in the excretion of uric acid and creatine.

Graham and Poulton (75) working with normal men in whom pyrexia was induced by exposure to unusually high temperatures found that the loss of body protein could be retarded or prevented during periods of pyrexia by the use of high caloric diets rich in carbohydrates.

Pathological Conditions involving the Muscular System are of importance from the point of view of our present considera- tion. In 1907 Spriggs (76) investigated the creatinine elimination in such diseases and noted a low excretion in all forms associated with diminution of the volume of muscle. He found the creatinine coefficient 2.2 and 4 in two cases of muscular dystrophy (decrease of muscular bulk); 1.9 in a case of amyotonia congenita; 5.6 in a case of myasthenia gravis; normal (7.1) in a case of locomotor ataxia; slightly high in two cases of tetanus (7.8 and 9.3). Levene and Kristeller (77) showed that in diseases of the muscular system associated with
dissolution of muscular tissue and with diminution of muscular activity, not only the exogenous but also the endogenous creatine follows an abnormal catabolism. In such conditions (locomotor ataxia 5 cases, Atrophy 1 case, Progressive muscular atrophy 1 case, Atktier Poliomyelitis 2 cases, Muscular dystrophy 4 cases, Dystrophy 1 case) the output of creatinine is low and of creatine high. In some cases, they found, a high protein content of the food causes an increase in the output of both creatine and creatinine. In the case of Progressive Muscular Atrophy, where there is an extreme degree of dissolution of muscular tissue, along with creatinuria they noted a normal creatinine output. When the muscular disease was associated with exaggerated activity (Tremor 2 cases, Spastic paralysis 1 case) the catabolism of endogenous creatine generally preserved its normal course.

Some interesting observations were made by Gibson and Martin (78) on creatine formation in cases of Progressive Pseudo-hypertrophic muscular Dystrophy. They confirmed the fact that in this condition there is a much diminished creatinine coefficient and a high degree of creatinuria. In one particular case they tried the effect first of a low protein diet and then of a high protein diet on creatine and creatinine elimination and came to the following conclusions:

i. Ingested creatine is promptly and completely eliminated, chiefly as creatine, in part as creatinine.

ii. Creatine and to a lesser extent creatinine excretion is increased as the result of a greater protein intake. They concluded that in such cases the power to convert creatine, both preformed and produced in intermediate metabolism, is markedly impaired.

In Pulmonary Tuberculosis various workers made observations which are recorded in Section III of this thesis (p.32). McClure (79) found either a complete absence or only a trace of creatinuria in the four febrile cases of Pulmonary Tuberculosis which he investigated. In the writers work (80) the creatinine output in pulmonary tuberculosis the excretion was found to be generally subnormal (0.91 grams with a creatinine coefficient of 7.19). With absence of systemic disturbance, whilst the daily excretion of creatinine remained below normal the creatinine coefficient was slightly above normal (8.26) (81) As systemic disturbance developed the coefficient become less (6.25). With the systemic disturbance becoming grave the coefficient rose again to 9.56 only to fall again in the last month of life to 4.87. There was found to be some evidence that, with loss of weight, the creatinine excretion tended to rise, and, per contra, that with increase of body weight the excretion tended to fall.

A rise of creatinine output in alcoholic and maniacal conditions was observed by Von Hoogenhuyze and Verlroegh (82) Benedict and Myers (83) on the other hand, found in their studies on the composition of the urine of twenty five insane women that the form of insanity had no marked influence on the creatinine elimination. In the observations recorded in this thesis alcoholic excess was in one instance followed by a striking rise in the creatinine excretion (84) Cinchonin poisoning (causing convulsions) was found by Weber (85) to produce a rise in creatinine elimination.
Von Hoogenhuyze and Verploegh (loc.cit) noted in some patients with diseases of the liver a low creatinine output; but in other cases it was normal and not infrequently the urine showed an excessive creatinine output. Work on the creatine creatinine metabolism in Diseases of the Liver was also published by Mellonby (86) Cases with cirrhosis of the liver, venous engorgement of the liver due to mitral stenosis, and carcinoma of the liver were the subjects of his research. He came to the following conclusions:

(i) The excretion of creatinine in people suffering from liver affections is markedly subnormal.

(ii) Patients suffering from cancer of the liver excrete a large amount of creatine, while in cases of cirrhosis and engorged livers the creatine excretion is not affected.

Leffmann (87) induced organic disease of the liver in a dog by poisoning it with amyl alcohol and with phosphorus. He recorded a high creatinine and a low creatine output so long as the diseased condition caused an increased nitrogen elimination. However, as soon as the nitrogen output fell the creatinine elimination was also diminished and the output of creatine rose in proportion.

Chisholm (88) states that the creatine content of muscle is reduced in malignant and in some chronic diseases of the liver, apparently owing to diminished productions.

In order to study the role of the Kidneys in creatine output Leffman (loc.cit) (89) produced lesions of those organs in dogs by poisoning them with potassium chromate. The output of creatinine in these animals was constantly lowered with the progress of the lesions; but in proportion with the fall in creatinine, the output of creatine rose so that ultimately the ratio of creatinine to creatine was 1:2. A still greater increase in the creatine output was observed after intra-vascular injection of creatine or after a beef diet. Folin and Denis (90) found that the blood creatinine is increased in uraemia. In both acute nephritis and chronic nephritis, where there is renal inefficiency McLean (91) states that the blood creatinine increases in amount. Instead of the normal 1 to 2.5 mgr. per 100 cc. of blood, it rises even up to 5 mgr.

A case of Lymphatic Leukaemia was shown by Shaffer (92) to have a creatinine coefficient of only 2.4. The subject, however, was extremely ill and died one week later.
E. THEORIES OF NORMAL METABOLISM.

Otto Folin (93), in 1905, pointed out the remarkable fact that the absolute quantity of creatinine eliminated in the urine on a meat free diet is a constant quantity, different for individuals but wholly independent of quantitative changes in the total amount of nitrogen eliminated. In six individuals of different weights, on varied diets he found that the end products of protein metabolism which tended to be constant were creatinine chiefly; then, rather less constant uric acid and the ethereal and neutral sulphur (94). He states that it is clear that the metabolic processes resulting in the end products which tend to be constant in quantity appear to be indispensable for the continuation of life (95). Creatinine in the urine he regards as one of the ultimate excretory products of metabolic processes which appear to be indispensable for the continuation of life; or, to be more definite, those metabolic processes probably constituting an essential part of the activity which distinguishes living cells from dead ones. He indicates the significance of the fact that urea is not to be found in the muscles except in infinitesimal traces. "It has been thought", he says (96) "that the protein decomposition in the muscles does not proceed as far as the urea but only produces certain precursors of it which are afterwards elaborated in the liver into urea. But the only precursor which is found in quantities that could be considered at all adequate is Kreatin, and it has been shown that the liver is not capable of converting Kreatin into urea. Moreover the perfect constancy in the Kreatinin elimination shown in these experiments under such different conditions excludes, it seems to me, positively the possibility that the Kreatin produced in the protein Katabolism of the muscles is afterwards converted into urea. The muscle Kreatin is eliminated as Kreatinin and the presence of such considerable quantities of Kreatin in the muscles, coupled with the absence of urea, as well as of any known precursors of urea, (in the light of the manifestly different laws governing their elimination) is exceedingly strong evidence against the view that the nitrogen Katabolism represented by urea takes place to any considerable extent in the muscles". Later, Folin (97) showed evidence that urinary creatinine had not a possible additional exogenous origin from ingested creatine. He found that creatine given in moderate quantities with a low nitrogen diet (starch and cream) was not eliminated at all; given in large quantities (from 5-6 grams) with a low nitrogen diet about 1 gram was eliminated unchanged, while if it be given along with food rich in proteid matter 50% was excreted unchanged in 24 hrs. The creatinine elimination was unaffected. Shaffer and Wolf (98) came to the same conclusion as Folin that the excretion of creatinine is wholly unaffected by the ingestion of creatine.

An attempt was made by Waldemar Koch (99) to show that creatinine is an index chiefly of methyl metabolism. Under ordinary conditions of diet he found that the methyl groups of lecithin and kephalin ingested could all be accounted for by the methyl groups of the creatinine excreted. With an excess of lecithin and Kephalin he could not thus account, by the creatinine excreted, for the methyl groups ingested. Koch went so far as to suggest that the constancy of the creatinine excretion during the low nitrogen periods (12-18 day) in Folin's experiments was due to the fact that in the preceding
high nitrogen diet periods his subjects received ten to twelve eggs a day. It was given by Koch as his opinion that the very slow excretion of lecithin and Kephalin from these eggs resulted in the constant creatinine excretion of Folin's experiments. Koch's theories scarcely seem to be tenable. The fact that any excess of Kephalin or lecithin in the diet could only be accounted for by a very doubtful increase in the urinary creatine make his suggestions unsatisfactory.

Philip Shaffer (100) was led to the conclusion that creatinine is not a product of total tissue catabolism but is a product of certain normal cell processes which in many diseased conditions may be extremely sluggish even though, as in exophthalmic goitre, the total tissue catabolism may be much increased. The low creatinine coefficient in all marked cases of exophthalmic goitre-subjects of which disease are specially prone to muscular weakness-is regarded as support of the hypothesis that creatinine is an index of muscular tonus or of muscular and perhaps of general cellularefficiency. Shaffer expressed no opinion as to whether creatinine arises in the Katabolic processes of all the body tissues or in the muscular tissues alone. He urged that facts support the belief that, for the muscular tissue at any rate, the amount of creatinine excreted is an index of their efficiency - not the amount of work the muscles are doing at the time but the amount of work they are capable of doing. This last proviso explained away Shaffer's previous finding (101) that the amount of muscular activity is in itself wholly without effect on the amount of creatinine excreted. Shaffer had further to make an explanation for the known increased creatinine excretion in febrile states. Obviously it cannot be supposed that fever temporarily increases the efficiency of muscle. The proffered explanation was that the increased excretion in fever might be ascribed to a pathological augmentation of the creatinine forming process, perhaps solely due to the higher temperature or to the action of bacterial toxins and coincident with the increased destruction of body protein.

Edmund I. Spriggs (102) investigated the creatinine output in certain diseases of muscle (vide. p. 15 of this thesis). As a result of his observations he concluded that creatinine is connected with the nutritional metabolism of the muscle fibre and is not a substance formed in the act of contraction; that if we liken the muscles to a machine, creatinine as a waste product would stand in relation to the structure of the machine and not to the fuel which the machine uses.

The following are the conclusions of Edward Mellanby. (103).
1. that in the formation of creatinine muscle plays a small part.
2. that the liver is intimately connected with the production of creatine and the excretion of creatinine. His reasons for this assertion were first, that in the developing chick and rabbit the accumulation of creatine in muscle and the growth of the liver occurred concurrently; second, that in his feeding experiments on chicks he found some slight evidence that food creatine can be changed to creatine and stored; third, that in no physiological experiment in his research did he ever change creatine to creatinine, confirming former experiments of Folin, Shaffer and Wolf (vide p.18 of this thesis).
fourth that creatinine is not excreted by chickens until about a week after hatching i.e., until all the muscles are saturated with creatine; similarly there is an almost complete absence of creatinine from the urine of children and puppies, with associated creatinuria. These facts Mellanby takes to show the intimate relation of creatine and creatinine for it is, he says, unlikely that an organ would commence making an entirely new substance about a week after birth. It is more probable, he thinks, that the power of making creatinine innocuous and storing it in the muscles as creatine has been reached at this age.

With reference to the liver's action Mellanby suggests that it is continuously forming creatinine from substances carried to it by the blood stream from other organs, and that in the developing muscle this creatinine is changed to creatine and stored; after the muscle has reached a saturation point creatinine is continuously excreted.

Mellanby states that the change from creatinine to creatine is in every way more likely than the change from creatine to creatinine. He regards it as more probable chemically that the ring formation of creatinine comes direct from a tissue break-down and that this ring is then hydrated to a creatine chain by muscle, than that the creatine chain is dehydrated to form the creatinine ring. From a physiological point of view he cannot think that tissues would make an innocuous neutral substance like creatine into a strongly basic substance like creatinine.

The statement of "Hoogenhuyze and Verploegh (104) that all organs have the inherent power to oxidise creatine is criticised by Mellanby. Their conclusions were based principally on observations of autolysing organs. Mellanby proved that with careful aseptic removal of rabbit muscle, after being kept in one case three days and in another case five days the creatine remained the normal 0.4%, and no creatinine could be detected. In Mellanby's words (105) "In the autolytic experiments where every opportunity for complete breakdown has been offered no change in the creatine content of the muscle can be detected. When the experiments failed by reason of the muscle becoming septic all the creatine in the muscle disappeared entirely."

Levine and Kristeller (106) reject Mellanby's views. They regard muscle creatine as the source of urinary creatinine but are confronted with the fact that urinary creatinine does not increase during exercise (vide p. 20). To explain the difficulty they quote work of Weber (107) who made experiments on the excised heart and found that when the organ contracted actively the perfusion fluid contained a higher proportion of creatinine; and that during rest the output of creatinine by the heart muscle was insignificant. These facts Levine and Kristeller take as evidence that creatinine is formed from creatine of muscle. As there is no increase of urinary creatinine during or after exercise they conclude that the creatinine formed is further oxidised by the organism. They further quote Froschbach (108) to show that in a diseased organism the rate of creatinine oxidation may exceed the rate in health. Thus, after the administration of thirty grams of beef extract a healthy man removed through the urine 1.082 gm. creatinine in excess of his normal output. A patient with muscular atrophy removed 0.352 grams, with leukaemia 0.577 grams, with exophthalmic goitre 0.368 grams and 0.600 grams after partial thyroidectomy, in excess over their normal urinary creatinine output.
Levine and Kristeller (109) find Shaffer's theories (p. 20 of this thesis) inadequate. His suggestion that creatinine output is determined by muscular efficiency does not harmonise with their findings in one case of progressive muscular atrophy where there was no marked alteration in creatinine output. Gibson and Martin (vide p. 16) have found low creatinine coefficients in cases of muscular atrophy, so this particular criticism of Shaffer's theory is unsound. Objections are raised by Levine and Kristeller to Mellanby's theory that creatinine is formed in the liver and converted into creatine in the liver. If it were true, the creatinine formation should continue to be normal so long as the liver function remained adequate, provided the muscle was healthy. But in a deficiency in the function of the muscle, a diminution in the rate of conversion of creatinine into creatine should occur with a resulting increase in the urinary creatinine. Spriggs (p. 15) Gibson and Martin (p. 16) and Levene and Kristeller themselves all show that this is not so but that on the contrary in diseases with muscular inefficiency the creatine excretion is low. One's own observations in pulmonary tuberculosis with marked muscular inefficiency and wasting confirm this finding (p. 39).

Levene and Kristeller's (110) final conclusions are that at least two factors regulate the creatinine output - first the formation of the substance very probably from creatine; and second, its further oxidation. Any disturbance of either factor may lead to an abnormal creatinine excretion. Deficiency in the second function may be partial, so that only the ingested creatine fails to be further oxidised. Whether or not the two functions are performed by one organ or by several still remains to be established by the consider that there is little doubt the muscular system takes some part in the regulation of the creatinine output. As a result of their experiments they conclude that the formation of creatine and creatinine represent two phases in the catabolism of but one substance; in most observations a fall in the creatine output was associated with an increased creatine elimination and high protein diet (creatinine free) in some patients augmented the output of both substances. The constant value of the urinary creatinine in normal men is conditioned, they think, by the high velocity of creatine combustion in health. Thus the creatinine in the urine represents only a small fraction of the creatine formed in the organism. The non-increase of creatinine output in conditions of high muscular activity may be explained by the assumption of a higher intensity in the power of the organism to oxidise creatine, although the creatine production in these conditions probably exceeds the normal limits. In support of this view Levene and Kristeller quote the administration of creatine, first to a patient with continuous tremor with a reappearance in the urine of only 48% of the ingested creatine; second, to a subject with muscular atrophy, when 90% of the creatine reappeared, showing that the formation of creatine and the rate of its further oxidation are lowered.

A new explanation of creatine creatinine metabolism was given by Thompson and Wallace (111). They worked on the basis of the facts observed, first by Polin (p. 18 of this thesis) that creatine administered to a subject on a starch cream diet is not excreted but is retained in the body; second by Cathcart (p. 10 of this thesis) that creatine appears in the urine during starvation and this output is stopped by taking starch but not fat. Thompson suggests that Polin misinterpreted his finding; he attributed the non-
appearance of creatine to the low protein content of the diet. Cathcart's finding suggests that it is associated rather with the carbohydrate in the diet. Thompson and Wallace in their experiments first starved a dog for two days and then fed it for periods of two days on various diets. They found that if 1 grm of creatine was given a rise of the starch content in the diet from fifty to seventy-five grams caused the creatine and creatinine excretion to fall about 30%. They further found that, with one gram of creatine still in the diet and the starch content maintained at fifty grams, the addition of either casein or four times extracted meat did not give any rise of creatine or creatinine excretion.

Now Cramer and Krause (112) had shown that in glycosuria there is creatinuria which increases with the severity of the conditions. Thompson and Wallace confirmed this fact and also that there is a marked diminution in the output of creatinine. They also found that whereas creatine in 1 grm. doses had no definite effect on the glycosuria, creatinine in doses of 0.15 to 0.18 grm. hypodermically or with the good temporarily increased the output of sugar by nearly 50%. Thompson and Wallace came to the view that creatine is probably concerned with the liberation of energy from carbohydrate material in muscle and that creatinine is an end product of this metabolism in mammals.

As a result of experiments on rats and cats, Cramer and Krause (113) concluded that the internal secretion of the thyroid gland has an inhibitory influence on carbohydrate metabolism. After the administration of thyroid they found the glycogen content of the liver to be less than 0.2%, compared with 0.5 to 4.36% in controls getting no thyroid. As, however, there was only a comparatively slight lowering of the tolerance for glucose they regarded the thyroid hormone as acting specifically on only one aspect of carbohydrate metabolism, in so far as it inhibits the formation and storage of glycogen in the liver. Now Cathcart (114) had shown that when carbohydrate is withheld from the diet there is a marked increase in the nitrogen excretion, even when no protein is given in the food and moreover that the increase is due almost entirely to urea and ammonia. Cathcart also showed (loc. cit) that when carbohydrate is withdrawn from the food creatine appears in the urine. It has further been shown by Thompson and Wallace (115) (p. 22 of this thesis) and by Cramer and Krause that in diabetes mellitus and phlorizin glycosuria creatine appears in the urine. As the result of experiments in which thyroid was administered with creatine, creatinine, and purine free carbohydrate diet, it was discovered by Cramer and Krause (116) that there was an increased nitrogenous output due chiefly to urea and ammonia. On a very high carbohydrate diet they found, after thyroid administration, slight increase in the creatinine excretion but no creatinuria. When the carbohydrate content of the diet was reduced the excess of creatinine continued and a small excretion of creatine occurred. In another experiment to investigate the urinary nitrogen partition on a high protein diet Cramer and Krause obtained an increased nitrogen excretion due to urea and ammonia. They also found however creatinuria and a creatinine excretion above the normal, in spite of the fact that previous to the commencement of the experiment the subject had been on a creatine and creatinine free diet for twenty four hours. They therefore again demonstrated that withdrawal of carbohydrates from the diet causes creatinuria and an increase of creatinine excretion. The similarity in the effects of thyroid feeding and the withdrawal of carbohydrates suggested to Cramer and Krause that the action of the thyroid hormone on protein
metabolism is effected partly at any rate through its action on carbohydrate metabolism. As a sequel they are of opinion that protein metabolism is probably many sided. They recognise an endogenous protein metabolism, represented by the formation of creatinine, which is not very susceptible to the influence of the thyroid hormone. But they recognise yet another form which is under the influence of the thyroid gland and which would appear to have some specially close relation to the metabolism of carbohydrate.

Paton and Mackie (117) pointed out that in the bird, metabolism of proteins is simple and that the dejecta contain no creatinine but that creatine is excreted unchanged as an end product of muscular metabolism. Bearing this fact in mind they tried the effect in the duck of excluding the liver from the circulation. They found that during the twenty four hours which the duck survived the character of the urine changed markedly. The proportion of nitrogen as uric acid decreased and that as ammonia rose. They found no effect on the proportion of creatine and therefore concluded that in the bird the liver is not connected with the metabolism of creatine - further evidence against Mellanby's theory.

In acute febrile conditions C. W. McClure (118) noticed no constant relation between creatinuria and either pyrexia or loss in body weight. He found that disturbances in the excretion of creatinine are not necessarily associated with an increase of the body temperature, nor are they in all cases related to loss of body weight. McClure (119) regards endogenous creatine and creatinine as products of the metabolism of muscular tissue. An increase in the amount of creatinine excreted may be regarded as evidence of an augmented Katabolism in muscle, or possibly of an actual destruction of that tissue. For creatinuria McClure offers two possible explanations. Firstly he suggests that it may be the result of an over production of creatine. This is the same as the explanation offered by Folin and Denis (120) who say "If so much creatine is manufactured that the muscles become supersaturated creatine is excreted by way of the kidneys. Secondly McClure is of opinion that creatinuria may be regarded as an expression of an incomplete oxidation of creatine in the body. This is identical with the theory of Mendel and Rose (121).

The theory propounded by Gross and Steenbock (112) is a very satisfactory one. As before stated they found evidence that arginine may be the precursor of creatine; in the pig it definitely is an exogenous source of urinary creatine if administered orally in sufficient amounts (p. 12 of this thesis).

Normally they believe the great bulk of creatine formed is metabolised into creatinine and thus kept from accumulating in the blood stream to the level at which its excretion would become possible. The main thesis of Gross and Steenbock (123) is that creatine may or may not be formed in direct proportion to the balance that obtains between the arginase system - destructive as far as creatine formation is concerned - and the oxidative system whereby the guanidine grouping is left intact. The arginase system resulting in the destruction of arginine with its guanidine grouping (NH2 C(NH)3NH ---- ) is very prominent, for Gross and Steenbock (124) found that the administration of arginine to the pig resulted in
creatine formation to the extent of only 3 or 4% of the theoretical possibility. With reference to the oxidative system, whereby the guanidine nucleus of arginine is left intact and the arginine possibly converted into creatine, they suggest that the active principle of the thyroid gland, which functions in oxidative reactions, takes a part. In their opinion the thyroid principle may remove arginine from the sphere of the arginase system and increase the amount of creatine formed. This is in accordance with the work of Cramer and Krause (see p.14 of this thesis) who obtained creatinuria in men and dogs as the result of artificially induced hyperthyroidism; and of Denis (see p.14 of this thesis) who reported the production of creatinuria by protein feeding in a man affected with Graves disease.

The suggestion of Gross and Steenbock (125) therefore is that creatine formation primarily depends on the balance that obtains between the arginase and oxidative system whereby arginine is destroyed. Arginine from exogenous sources, they state, is not metabolised into creatine in the same proportions as arginine from endogenous sources because this balance varies in different organs. Creatinuria they regard as the result of the accumulation of creatine up to and beyond the threshold of its excretion. Usually its accumulation is prevented by the prevalent rate of conversation of creatine into creatinine which appears to be an invariable reaction.

The study of any reaction in the animal body where only the excretory end products are available to indicate the course of the whole is bound to reveal many apparent inconsistencies. The estimation of the value of the various theories is difficult. For the most part theory has evolved as facts have accumulated.

Wolin's original theory is inadequate (p.18 ). He took no cognisance of the occurrence of creatinuria and offered no explanation for the non-increase of creatinine excretion after muscular exercise. Spriggs (p.20 ) and Shaffer (p.20 ) explain away the latter fact but make no suggestion about creatinuria. The theory of Mellanby (p.20 ) that the liver forms creatinine which is converted into creatine in the muscles, is open to severe criticism. In the first place Paton and Mackie (p.20 ) showed that exclusion of the liver from the circulation did not cause a reduction of the creatine excretion in the bird. Secondly the increased creatinine excretion which one would expect in wasting diseases of muscle - if this theory were correct - does not occur (p.15 ). Levene and Kristeller formulate an hypothesis which appears sound up to a point. They postulate two factors for the regulation of creatinine output and the possible occurrence of creatinuria - first the formation of creatinine from creatine; second the further oxidation of creatinine. A defect in the first factor may result in creatinuria; defects in either function may result in disturbance of creatinine excretion. They make no suggestion however as to the source of creatine.
The work of Cramer and Krause (p. 14) and Thompson and Wallace (p. 15) on the relationship existing between protein metabolism on the one hand, and carbohydrate metabolism and the thyroid hormone on the other, leads to the view that creatine is probably concerned with the liberation of energy from carbohydrate material in muscle, and that possibly creatinine is an end product of this metabolism. The complexity of the problem of creatine and creatinine metabolism is emphasised rather than a simple solution offered.

Of all explanations of creatine-creatinine metabolism that of Gross and Steenbock appears to be the most satisfactory and most comprehensive. They offer evidence of a precursor of creatine (p. 12). They explain the occurrence of creatinuria and the constancy of the creatinine excretion in different states of muscular activity (p. 25). A possible relationship between the thyroid hormone and creatine-creatinine metabolism is suggested (p. 25). To the writer of this thesis it appears that the hypotheses of Gross and Steenbock are the most complete and the most in accord with all the facts that are known.
SECTION III.

CREATININE COEFFICIENT IN PULMONARY TUBERCULOSIS.

1. Preamble.
2. Wasting and Fever in Pulmonary Tuberculosis.
3. Creatinine Excretion in Pulmonary Tuberculosis
   a. Historical
   b. Methods of Present Investigation.
   c. Experimental Findings.
   d. Conclusions.
1. PREAMBLE.

Research work in Pulmonary Tuberculosis may be conducted, as Sheridan Delepine pointed out, (126) on clinical, experimental, epidemiological and statistical lines. He specifies as "clinical" research work conducted at dispensaries, hospitals or sanatoria by persons conversant with clinical methods, including such physical, chemical, physiological and bacteriological methods as can be used in current clinical work. It was within the terms of his definition of "clinical" and in the laboratory which was controlled by him for so many years that the experimental work described in this thesis was performed.

Throughout the centuries physicians have described phthisis and have continually been making contributions to our knowledge of the clinical features morbid anatomy and epidemiological laws of the disease. Our earliest knowledge of consumption dates from the time of the great Greek physician Hippocrates (BC460-BC377). He held that phthisis was a suppurative and ulceration of the lungs. His definition however was very comprehensive, including empyema, abscess of the lung and gangrene. Galen (AD131-201) described the disease and made a definite step forward by appreciating its contagious nature; for he says it is "dangerous to live with consumptives and with those whose foul breath imparts a heavy odour to the rooms in which they lie." In the seventeenth century, Franciscus Sylvius (1614-1672) (126) a Frenchman born in Germany who settled in Holland and for 14 years was professor of Medicine in Leyden, wrote of pulmonary tuberculosis as the "Sweating Sickness" and pointed out that the fevered state and emaciation were two of its prominent features. A few years later, in our own country, Richard Morton (129), the friend and contemporary of Sydenham, produced the first modern treatise on the subject and enunciated his now famous triad of symptoms - cough, fever and emaciation. He made an important advance in maintaining that tubercles are found in all cases of consumption. In the next century profitable investigation of the pathology of the condition began and William Stark (130) (1740-1770), having made ten post-mortem examinations, wrote a wonderfully accurate description of the morbid anatomy of pulmonary tuberculosis, incidentally giving what is probably the first description of a pulmonary aneurysm in a cavity, which caused death from haemorrhage. His works were not published until eighteen years after his death. Some thirty years later R. T. Laennec (131) (1781-1826) laid the foundation not only of our present knowledge of Pulmonary Tuberculosis but also of all modern clinical medicine. He recognised the unity of the forms of tubercle - the miliary granule, the infiltration and the caseous mass. By means of his discovery, the stethoscope, it became possible to correlate clinical features with the physical course of the disease. The Zymotic nature of tuberculosis was first definitely suggested by William Budd (132) a doctor of medicine of Edinburgh University, who pointed out the close analogy existing between pulmonary tuberculosis and enteric fever. "I now saw" he said "with a clearness which had never occurred to me before, that, with the exception of the qualification necessary for their application to a chronic disease - for the most part of slow evolution and indefinite duration - the leading conclusions to which I had been led respecting the propagation of the fever
(enteric) might be applied with the same strictness to phthisis also". In 1865 (before the publication, but after the writing of Budd's work) Villemain (133) communicated a paper in which he went a step further than Budd and gave experimental proof that tuberculosis is a specific infection, that tuberculous matter inoculated into susceptible animals is followed by tuberculosis, and that the lesions are similar to those of the naturally acquired disease. Villemain's thesis was confirmed and proved scientifically accurate in the demonstration by Robert Koch (134) of the tubercle bacillus (1882). The thoroughness of Koch's work is manifested by the fact that in the years that have elapsed the innumerable workers have amplified and extended but in no way essentially modified his original position (Osler) (135).

So through the centuries have contributions been made to the common knowledge of pulmonary tuberculosis. Hippocrates, Galen Sylvius, Morton and many others helped to make the symptoms of the disease known. Later, with the advent of Stark a reasonable understanding of the morbid anatomy of tuberculosis was commenced. Laennec made it possible for physical findings to be correlated with the course of the disease. Budd suggested, Villemain demonstrated and Koch proved the zymotic nature of tuberculosis. The modern concept of the disease cannot be more adequately expressed than in the words of Sir Robert Philip (136) who in an address to the British Medical Association at the Belfast meeting (1909) said "We have come to realize that when tuberculous disease is present in an organ, be it in the lungs or elsewhere, we have to deal with a specific disease, the local manifestations of which may be comparatively unimportant compared with the systemic effects. Commonplace as the view may appear it cannot be emphasised too much. It is lost sight of sometimes when it should be accentuated most". Then again - "The physician while making use of all bacteriological advances as aids to diagnosis cannot afford to forget that there is evidence imprinted on the patients every tissue and organ which, when read by the seeing eyes and interpreted by the trained senses, gives completeness of view otherwise unattainable." The theme running through these remarks and throughout all his teaching is that Pulmonary Tuberculosis can never be regarded as a localized disease. It must be thought of as a systemic infection with a local manifestation in the lungs, in the way that Enteric Fever is a systemic infection with local manifestations in the small intestine. Being therefore a systemic infection, pulmonary tuberculosis is accompanied by metabolic disturbances. We know that there are operative in the disease two somewhat opposing factors of undoubted importance from this point of view. Continuous pyrexia and progressive wasting of muscle mass are two very constant accompaniments of phthisis. The importance of creatinine excretion as an index of endogenous protein metabolism has been demonstrated repeatedly. The salient facts known with reference to its biochemical significance have been discussed in preceding pages. In the light therefore of the definite role of creatinine excretion in endogenous protein metabolism, and of the factors - continuous pyrexia and progressive wasting of muscle mass - of undoubted metabolic importance operative in pulmonary tuberculosis, it seemed reasonable to assume that the investigation of the creatinine excretion in the disease would be profitable.
2. WASTING AND FEVER IN PULMONARY TUBERCULOSIS.

Phthisis (φθορίς) means "wasting" or "consumption". The popular conception and oftentimes the true clinical history of active pulmonary tuberculosis consists largely of a progressive loss of weight. Emaciation is no longer regarded as a symptom of the greatest diagnostic importance for when it is a marked feature the disease is usually far advanced. Nevertheless, that active disease is usually associated with loss of tissue has been recognised for thousands of years and is the fact which is of interest in this present work.

Wingfield (137) regards weight loss in early pulmonary tuberculosis as a very inconstant sign but finds it present in the majority of cases; and if rapid and marked in a young adult thinks that it must be regarded as very important evidence in diagnosis. "Loss of weight" says Osler (138) is gradual and, if the disease is extending, progressive. The scales give one of the best indications of the progress of the patient. It is most rapid early in the disease when the patient may lose at the rate of five or six pounds a week and usually is in direct relationship to the intensity and duration of the fever. With the arrest of the progress and the fall in temperature the patient usually begins to regain weight "Fisherberg (139) says "In some cases of phthisis the emaciation is rapid and extreme; within a few months the body of the victim is reduced to a skeleton. These are the cases in which the disease runs an acute and progressive course - galloping consumption". He further (140) goes on to point out that not only is the subcutaneous adipose tissue wasting but that the Nitrogen containing muscles also vanish with astonishing rapidity. "The weakness, weariness, loss of strength and vigour of the consumptive he continues are greatly due to the muscular atrophy even in the early stages of the disease and one of the best signs of improvement is the regression in the muscular atrophy. There appears to be a direct relation between the course of the disease and emaciation. With each extension of the process in the lung, with each haemorrhage, he loses in weight and with each improvement he gains in this direction; while in guisescent cases the weight remains unaltered." The emaciation of tuberculosis is regarded by Halliday Sutherland (141) as being due to a toxaemia affecting the entire metabolism as well as the digestive functions. The same writer states that emaciation is most marked in advanced cases with profound intoxication and reiterates that it is aggravated by increased metabolism associated with hectic fever and by the deterioration of digestive functions.

Pyrexia is an even more constant accompaniment of pulmonary tuberculosis than is loss of weight. Physicians are of opinion that at some stage of every case there is a certain degree of fever. Kingston Fowler (143) states that a rise of the evening (8 or 10 p.m.) temperature to 99°F and a fall in the morning hours to 97°F or thereby is sometimes the first indication of the presence of the disease. If the temperature be recorded hourly for twenty-four hours and the results expressed as a graph he asserts (144) that the "plateau" seen between the hours of 1 p.m. and 12 midnight is almost diagnostic.
Fever is regarded as perhaps the first symptoms of pulmonary tuberculosis by Fisberg (145) who is of opinion that faulty technique in taking the temperature, especially defective thermometers is the reason why there are found so many apyretic cases of phthisis. One of the earliest changes in the temperature, as Halliday Sutherland (146) reminds us, is a rise to 99° or 100°F after moderate exercise, which increase may persist after an hour of absolute rest in bed. Some twenty years ago Daremberg (147) named such a rise "fièvre provoquée" and insisted that it was conclusive evidence of pulmonary tuberculosis.

With various types and in various stages of the disease there are often distinctive forms of temperature chart. In 1893 Kingston Fowler (148) called attention to the fact that simply by observation of the temperature in a case of pulmonary tuberculosis it is possible to determine the nature of the changes in progress in the lungs. Sir Sims Woodhead and Dr. Varrier Jones (149) have recently expressed a similar view. The consultation of authoritative opinion (Philip (150), Fishberg, Fowler, Powell and Hartley (151) etc.) and also personal experience in the largest of English Sanatoria show that the following varieties of temperature are associated with stages in the course of pulmonary tuberculosis.

1. Incipient Evening rise to 99° or 100°F; subnormal morning temperature.
2. Miliary (a) continuous 101° - 103°F
   (b) Inverse temperature (morning 103°F evening 101°F)
3. Caseous (acute pneumonic phthisis).
   (a) high remittent fever (101° - 104°F)
   (b) continuous 101 or 102°F.
4. Fibrocaseous: intermittent in various degrees.
5. Fibroid: normal or subnormal.

The cause of the pyrexia in pulmonary tuberculosis has been the subject for acrimonious controversy. One extreme view is taken rather characteristically by Kingston Fowler (152) who dogmatically states that even the hectic type of fever is not the result of a mixed infection but is due to the action of the specific organism - the tubercle bacillus. Fishberg's opinion is that the fever is due to the absorption of poisons produced by the tubercle bacilli though it may be modified by mixed infections and that it is engendered mainly by the increased production of heat - the result of complex biochemical processes having their origin in the struggle of the organism with the bacilli. At the other extreme from Fowler's view is the theory of Bonney (159) who is led to conclude that the
fever attributable to the tubercle bacillus is often of minor importance in comparison with that occasioned by other organisms. Possibly Sir William Osler, holding an opinion between the two extremes, is right when he says "Fever, one of the earliest and most important symptoms, is due to the effect on the heat centres of the toxins or materials absorbed from the tuberculous focus. Later in the disease the hectic fever is caused in part by the absorption of the bacterial products of other organisms".
3. **CREATININE EXCRETION IN PULMONARY TUBERCULOSIS.**

**A. HISTORICAL**

The records of the creatinine excretion in pulmonary tuberculosis are few and indeterminate. K.B. Hofmann (156) employing the now obsolete Neubauer method of creatinine estimation reported one advanced although afebrile case in which there was noted a slight decrease of creatinine output. In 1908 Van Hoogenhuyze and Verploegh (158) investigated the creatinine output in one febrile case (aged 72). Their patient was kept on a low protein diet and Folin's technique was employed for the estimations. They found that the creatinine excretion was 1.05 grams (average) per twenty-four hours. C. W. McClure (159 (1918) reported four febrile cases. The patients, all males, were fed on the diet proposed by Shaffer and Coleman (160) except that no purin or nuclein containing foods were given. Creatinin, creatin and uric acid were determined by the method of Folin. The first case was aged 19, apparently in stage Ls of Philip's classification. At the commencement of the observations he was febrile but later he was afebrile. The creatinine excretion was examined for thirty three consecutive days and wide ranges from .605 grams to 2.652 grams per twenty four hours were observed. There was mostly, however, an irregular increase of excretion. The second case was male aged 27 whose pulmonary tuberculosis was complicated by a haemo pneumothorax following a drunken orgie. He was under observation for thirty three days. There was again some irregular increase of creatinine excretion, however, very great range (0.624 grms to 1.976 grm. per 24 hrs.) The third case (act.28) was one of tuberculous broncho-pneumonia. He had marked systemic intoxication. In spite of high fever there was no increase of creatinine excretion during the eight days of observation. The range was from .607 grams to 1.291 grams per twenty four hours. The fourth and last case was also tuberculous broncho-pneumonia with grave systemic intoxication. In the fourteen creatinine estimations made, a slight decrease in output was observed with a range from 0.504 to 1.000 grams per twenty four hours.

Raphael and Eldridge (1921) (161) investigated the creatinine coefficient in fifteen cases of uncomplicated pulmonary tuberculosis. Their patients were divided into three groups in accordance with Rathburn's classification (162). Organically the cases were of the moderately advanced or far advanced types but falling functionally or symptomatically into the three groups of Rathburn classification as follows:-

A. afebrile, absence of constitutional disturbance, ambulant and doing light work.

B. slightly febrile, slight constitutional disturbance; in bed one half day.

C. definitely febrile, marked constitutional disturbance; in bed entire day and general condition serious to critical.

Groups A, B and C as described in Raphael and Eldridge's paper correspond to stages L3s, L3S, L3S of Philip's classification (163).
The cases were all males and selected so as to exclude adolescence senility or obesity. The subjects were put on a strictly meat free diet and after a preliminary forty-eight hours creatinine estimations were made on successive days. Folin's technique was employed. In group A 5 subjects (av. weight 68.4 Kgs) had a creatinine coefficient of 5.5 with ranges from 3.06 to 7.56. In group B, the average weight of the five subjects was 54 Ks, and the creatinine coefficient 6.8, ranging from 5.38 to 8.34. In group C, thirteen estimations were made on five subjects whose average weight was 51.4 Kgs; the creatinine coefficient was 5.84 ranging from 3.82 to 6.91. In all three groups therefore the creatinine excretion was subnormal but it was higher in group B (i.e. LgS) than in the other two groups.
B. METHODS OF PRESENT INVESTIGATION.

Before a beginning was made with the actual examination of the creatinine excretion it seemed necessary to demonstrate that there is no great interference with renal function in the advanced stages of pulmonary tuberculosis. It is frequently stated that even in the early stages of phthisis there is an undue incidence of nephritis. Pottain (quoted by Fishberg) states that 1/5 of all consumptives have nephritis. Albuminuria with casts, phosphaturia, and polyuria are described as being unduly frequent in incipient pulmonary tuberculosis. Barbier says (139) says that albuminuria is often the only sign observed for a long time before other symptoms make their appearance. In the advanced stages of phthisis when oedema is common and amyloid change in the Kidney may occur, it is reasonable to believe that the percentage of renal inefficiency may be very great.

Creatinine is present in the blood only in small amounts. Maclean (165) in a healthy young adult male, found 2.2 mg. in 100 cc. of blood. The normal kidney cells have the power of concentrating creatinine to a very great extent, so that a given volume of urine may contain forty times the amount present in an equal volume of blood. In renal disease this power of concentration may be lost and the blood creatinine may increase to four or five times the normal (Maclean). (166). If therefore, there were a marked degree of renal inefficiency in advanced pulmonary tuberculosis creatinine retention might occur, the urinary output of the substance cease to have its ordinary significance and an additional factor would have to be considered in interpreting the findings in this thesis.

The renal function was examined in one hundred consecutive cases admitted to a hospital block. They were mostly acute advanced cases. The urea concentration test of Maclean (167) and de Wesselow was used as being the simplest efficient test. The patient is given 15 grams of urea and the bladder is immediately emptied. The specimens of urine one and two hours after the administration of the urea are examined. If the Kidneys are so able to concentrate the urea administered that the second hour specimen contains two per cent or more the renal function is held to be efficient.

The results are shown in Table 1. It seems clear that the question of renal inefficiency has no practical bearing on the point at issue. For although in the hundred tests made fifteen gave unsatisfactory results only six of these cases had absence of such signs as would exclude them from "uncomplicated" cases of pulmonary tuberculosis.

The creatinine excretion was examined in twenty four cases. They were all males between the ages of eighteen and forty-two. They were all suffering from pulmonary tuberculosis in what was, as far as could be ascertained, an uncomplicated form. In the Tjurban Gerhardt classification (168) they were all stage III; that is to say in all the cases, either more than half of a lobe of a lung was affected with severe disease, or more than a whole lobe was affected with slight disease. Symptomatically they were divisible into three groups:
(a) those with relatively slight systemic disturbance.
(b) those with mild general intoxication.
(c) those with grave systemic disturbance.

In brief the cases were in the following three stages of Philip's classification (vide ante) (a) L3s
(b) L3S
(c) L3S

The specimens of urine were collected from mid-day to mid-day, a little xylol was added as a preservative and the estimations were made within three hours of the completion of the specimen. The patients were put on a strictly meat free diet for a preliminary twenty four hours and during the collection of the urine. It has been shown by Burns and Orr (69) that on a meat free diet, the addition of half a pound of cooked meat to the diet increased the creatinine excretion in one case from 1.45 to 1.46 grams and in another from 1.6 to 1.66 grams in the twenty four hours. The further found that the increased excretion occurred within the twenty-four hours after administration of the meat. Therefore it seems reasonable conversely to assume that twenty four hours preliminary dieting is sufficient to exclude any definite effect that ingested meat may have on the urinary creatinine output.

Folin's technique was employed in the estimation of the creatinine. His own description of the method written nearly twenty years ago is as follows:

"The principle upon which this determination is based is the colour reaction given by Kreatinin and by no other normal urinary constituent with picric acid in an alkaline solution. In order to make the colour comparison accurate enough for quantitative work a high grade colorimeter is necessary. The French instrument of Duboscq, obtained through Eimes and Amend is eminently satisfactory. Half normal potassium bichromate containing 24.55 grm per litre, saturated picric acid solution containing about 12 grms per litre and 10% sodic hydrate solution are the reagents needed. Ten cc. urine is measured into a 500 cc. volumetric flask, 15 cc. picric acid and 5 cc. sodic hydrate are then added and the mixture is allowed to stand for five or six minutes. This interval is used to pour a little of the bichromate solution into each of the two cylinders of the colorimeter. The depth of the solution in one of the cylinders is then accurately adjusted to the 8 m.m. mark. With the solution in the other cylinder, a few preliminary colorimetric readings are made simply for the sake of ensuring greater accuracy in the subsequent readings of the unknown solution. The two bichromate solutions must, of course, be equal in color and in taking their readings no two should differ more than 0.1 mm or 0.2 mm from the true value (8.0mm), leaving out of consideration the very first reading made which is sometimes less accurate. Four or more readings should be made in each case and an average taken of all but the first.

At the end of five minutes the contents in the five hundred c.c. flask are diluted up to the five hundred cc. mark.
The bichromate solution is thoroughly rinsed out of one of the cylinders by means of the unknown solution and several colorimetric readings are then made at once. The calculation of the results is very simple. If, for example, it is found that it takes 9.5 mm of the unknown urine picrate solution to equal the 8 mm of the bichromate, then the 10 ccs of urine contains 10 x 8.1 - 8.4 mg. Creatinin (The calculation is based on the experimentally determined fact that 10 mg. of perfectly pure Kreatinin, give, under the conditions of the determination 500 ccs of a solution, 8.1 mm., of which has exactly the same colorimetric value as 8 mm. of half normal bichromate solution).

In his latest work Folin (172) recommends the use of a pure creatininin standard containing 1.61 grm. of creatinin zinc chloride dissolved in one litre of N Hydrochloric acid \( \frac{10}{2} \) (i.e. 1 mg. of creatininin per cc). This is treated in the same way as the unknown and used instead of N potassium dichromate as the colour standard. Hawk (173) and others recommend this method. David Burns (174) has recently devised a clinical method for the estimation of creatinin in urine, involving as apparatus only a flask, pipettes and Haldane haemoglobinometer tubes. Ingeniously simple as it is, the method is not however sufficiently sensitive for accurate work.

In this work it was decided to follow Folin's original method. Pure creatininin was not available for a standard. As Folin (175) pointed out his original method is very accurate if not more than 15 mg. nor less than 5 mg. creatininin are in the 100 cc urine taken for examination. A good Duboscq colorimeter being available, there is no reason for doubting that the results in the tables appended are trustworthy.

Whilst, for each healthy individual the creatininin excretion is practically constant from day to day, different persons excrete different amounts; and, in Folins (176) words "The chief factor determining the amount of Kreatininin eliminated appears to be the weight of the person." Folin further demonstrated that the amount of adipose tissue has to be considered, for the fatter the subject, the less the daily excretion of creatininin per unit of body weight. The amount of creatininin excreted depends primarily upon the mass of active protoplasmic tissues. Since we have no method of accurately measuring the amount of adipose tissue in various individuals the best that we can do is to express the amount of creatininin excreted per unit of body weight. Philip Shaffer (177) first described such a method of expression in 1906. Some two years later he modified his original position (178) and expressed the creatininin excreted in terms of its nitrogen, in order to be more consistent and in conformity with the expressed results of other nitrogenous substances. The ratio, milligrams of Kreatininin nitrogen per kilogram of body weight, he called the "Kreatininin Coefficient." It was decided in this thesis to express the Creatininin Excretion in terms of the Creatininin Coefficient of Shaffer.
In brief the procedure was to keep the patient for a preliminary twenty four hours on a meat free diet, to continue the same diet another twenty four hours and collect the urine. Within three hours of the completion of the specimen the creatinine was estimated. The creatine Nitrogen was calculated (i.e. .372 of the creatinine) and expressed as the Creatinine Coefficient as defined by Shaffer.
C. EXPERIMENTAL FINDINGS.

### TABLE 1.

Renal Function in Pulmonary Tuberculosis.

100 male cases examined consecutively in an acute hospital ward.

<table>
<thead>
<tr>
<th>Stage of Disease (Turban Gerhardt)</th>
<th>No. of Cases</th>
<th>No. with Efficient Renal Function</th>
<th>No. with Inefficient Renal Function</th>
<th>No. with Oedema, Albuminuria or Bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>12</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>66</td>
<td>14</td>
<td>22</td>
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<tr>
<td>TOTAL</td>
<td>100</td>
<td>85</td>
<td>15</td>
<td>24</td>
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</tbody>
</table>

### TABLE 1A

Relationship between the Albuminuria and Oedema of Advanced Pulmonary Tuberculosis and the Kidney function.

<table>
<thead>
<tr>
<th></th>
<th>No. of Cases</th>
<th>Efficient Renal Function</th>
<th>Inefficient Renal Function</th>
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</thead>
<tbody>
<tr>
<td>Albuminuria &amp; Oedema</td>
<td>11</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Oedema</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>24</td>
<td>15</td>
<td>9</td>
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<tr>
<td>Case No.</td>
<td>Date</td>
<td>Weight in Kilograms</td>
<td>Vol. of Urine in ccs.</td>
</tr>
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<td>----------</td>
<td>---------------------</td>
<td>-----------------------</td>
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**TABLE IV.**
The examination of tables II, IIII and IV, shows that the amount of creatinine excreted by persons suffering from pulmonary tuberculosis is below normal. In the twenty-four cases investigated the average daily output of creatinine was found to be 0.91 grams. Otto Folin (179) in his analyses of thirty "normal" urines found the average excretion of a healthy man to be 1.55 grams daily with range from 1.36 grams to 1.77 grams, depending on the weight of the individual.

In the preceding tables the average of the Creatinine Coefficients is 7.191 as opposed to the 8.1 which Shaffer (180) gives as the normal. It is to be noted that the diminution in the absolute amount of creatinine excreted is greater than the diminution in the creatinine coefficient. The reduction of the latter figure in these cases of pulmonary tuberculosis however, is sufficient to show that there is a very definite decrease of creatinine excretion per unit of body weight in this disease.

Table II shows that the creatinine excretion in advanced pulmonary tuberculosis (stage L3S), is very much below the normal, an average of only 0.852 grams being excreted in the twenty four hours. The excretion per unit of body weight is also diminished, but not to such an extent, the creatinine coefficient being 7.363. It is seen that the creatinine coefficients vary greatly in Table II. They range from 3.0 (the specimen of urine on 28.10.24 in case 4 was probably incomplete and the creatinine coefficient of 1.1 therefore inaccurate) to 11.4. Examination of the table shows that, with one exception it is in the last month of life that the coefficient is markedly low. The exception is case 9 (C.P.) and here death was sudden, not immediately anticipated, and was due to a spontaneous pneumothorax, one of the accidents occurring in pulmonary tuberculosis. Tables are made to show more clearly this falling of the coefficient at the very end of life. Table II A consists of the results obtained for patients in stage L3S who lived at least one month after the estimations were made. Table II B gives figures showing the creatinine coefficients in the last month of life. From these two sub-divisions of Table II it can be seen that the creatinine excretion in extremely advanced phthisis is relatively high (1102 grams creatinine, 9.586 creatinine coefficient) until the last month of life when it falls very low (.676 grams creatinine and creatinine coefficient of 5.782; or if case 9 (C.P.) is excluded for reasons aforementioned the average creatinine excretion becomes .554 grams and the coefficient 4.87).

Table IIII, subjects in stage L3S of the disease again gives a low average creatinine excretion. A daily excretion of 0.853 grams and a coefficient of 6.639 are considerably less than 1.55 and 8.1, the respective figures for the normal male. The average daily excretion is reduced to 0.794 grams and the coefficient to 6.253, if the earlier figures for cases 15 (S.G.) and 17 (E.H.) whose classification was really L3S until changed by the successful induction of artificial pneumothorax, are excluded.

In Table IV referring to subjects with considerable disease in the lungs but without any marked systemic intoxication (stage L3s) the creatinine excretion approaches more nearly to the normal and the coefficient is slightly higher than normal. The creatinine
excreted averages 1.089 grams in the twenty four hours and the coefficient is 8.255.

Diagrammatically the creatinine coefficient in the stages of advanced pulmonary tuberculosis may be represented thus.

STAGES OF DISEASE.

(in last month of life)
Case No. 1

This case (A.P.) was followed at intervals for the last three months of life, the last estimation being made four days previous to death. The creatinine coefficient, somewhat above normal at the commencement of observation, fell as the end came near, although the temperature remained the high intermittent type during the whole of the patient's stay in hospital. In the sub-divisions of Table II the first two creatinine excretions come in the A section whilst the latter figures show the low creatinine excretion of the last month of life and are grouped with the B section.

Case No. 4

The excretion here was again observed for a period of three months, to within a fortnight of the patient's death. Incontinence during the last ten days of life made it impossible to collect specimens then. For the last three months of life there was a hectic temperature which only became subnormal two days before the end. The fall of creatinine excretion is seen. Again it would appear that the failure of vital powers associated with impending death is indicated by a very low creatinine excretion.

These two cases in themselves indicate very clearly that in stage 13S of pulmonary tuberculosis the creatinine coefficient is unduly high until the very near approach of the failure of the vital powers. The other cases in stage 13S all go to emphasize the same conclusion as shown by tables 11A and 11B.

Case No. 9

As has already been pointed out this patient died suddenly as the result of a spontaneous pneumothorax. This fact may explain why he was an exception to the rule that in the last month of life in pulmonary tuberculosis the creatinine coefficient is very low.

Case No. 13

This subject, an old army sergeant, had old standing fibro-caseous disease. After a long stay in the Sanatorium he was showing signs of considerable improvement. On October 6th he went out of bounds and got drunk. During the following twenty hours his creatinine excretion rose considerably. On the night of the 8th and the morning of the 9th he had nine or ten ounces of haemoptysis. Three days later (12th Oct.) there was another smaller haemorrhage. The urine specimen collected from October 14th - 15th showed a creatinine excretion more than three times greater than it had ever been before his alcoholic excess. It will further be noted that the high creatinine excretion (1.953 grams with a creatinine coefficient of 16.602) was followed by a "lag" phase in which the excretion fell to .328 grams with a coefficient of 2.689. During the period of his haemoptysis - the first he had experienced - the subject was extremely agitated. Incidentally it may be stated that from October 9th to about Oct. 20th the patient's diet was not only meat free but also very small in quantity. He refused to take anything but milk and bread with a little milk pudding.
Case No. 15. There is sufficient interest here to warrant special attention. The subject was admitted to the Sanatorium on August 7th 1924 with extensive pulmonary tuberculosis but restricted almost entirely to the left lung. He had an evening temperature of 100°F or more with a diurnal range of three degrees Fahrenheit and a pulse rate of 90 to 116 per minute. He had lost some 18 Kg weight in the twelve months previous to admission. He was classified 138. A left artificial pneumothorax was induced on August 11 1924 and in three weeks time his condition had undergone a dramatic change. He became afebrile and began to increase in weight. Unfortunately on November 1st urgent domestic affairs necessitated the patient's absence from the Sanatorium for three days during which time he probably indulged in alcohol to excess and otherwise abused himself. On returning his condition relapsed; the disease in the non-collapsed lung extended, pyrexia returned and the weight gained was lost again. The variations in the Creatinine Coefficient corresponding to the changes in the patient's clinical state can be graphically represented thus.

It will be seen that in this particular case the creatinine coefficient became lower as the pyrexia subsided and the weight increased; and that with the return of the fever and with the loss of weight the creatinine coefficient became higher again.

Case No. 17. This case resembles No. 15 except that there is no history of relapse. He was first seen on June 26th 1924. He gave a history of malaise and loss of five kilograms weight in the ten months preceding admission. Cough had troubled him for three months. He had extensive active disease, practically confined to the left side. He remained febrile and made no improvement for a month when a left-artificial pneumothorax was induced. There was rapid improvement; the fever ceased and the weight increased. The relationship between evening temperature, weight and Creatinine Coefficient can be shown graphically.
Pulmonary Tuberculosis in itself does not appear to affect the creatinine output for patients with extensive disease but no systemic disturbance show no definite change from the normal creatinine excretion. With the onset of constitutional disturbance the creatinine in the urine decreases in amount despite the concomitant pyrexia. With profound systemic intoxication the creatinine excretion rises again only to fall to a very low ebb in the last four weeks of life.

Therefore one is led to the conclusion that in pulmonary tuberculosis pyrexia is not necessarily associated with increased creatinine excretion. The fall of the output of creatinine with the onset of constitutional disturbance is associated with the tiredness and listlessness of the clinical picture. The prevalent rate of conversion of creatine to creatinine remains the same but the metabolic processes are reduced, there is less creatine formed from its probable precursor arginine and there is a consequent low creatinine excretion. The profound toxaemia of the next advancing stage causes a rapid wasting of muscle tissue and probably more of the stored creatine in muscle is converted into creatinine causing an increase of the urinary output. In the last stages of life toxaemia is still profound but the organism is so exhausted that there is metabolic response to the toxin, death is beginning to approach and the creatinine excretion falls very low.

Little of prognostic value was found. In extremely advanced pulmonary tuberculosis a Creatinine Coefficient of less than 5 means death within four weeks.

Cases 15 and 17 offer some slight evidence that loss of weight is associated with an increase of creatinine excretion.

In case 13 is one example of alcoholism being accompanied by increased creatinine excretion. The same case further shows that great psychic disturbance associated with haemoptysis is followed by a greatly augmented creatinine output.
SUMMARY.

In the introduction emphasis is laid on the importance of the correlation of clinical findings with experimental data. Our present knowledge of the thyroid gland and the recent isolation of insulin are cited as instances where such co-operative work has resulted in great therapeutic advance- ment and increased appreciation of physiological function. It was in the hope of either finding something of clinical value or of throwing some light on the problem of creatine-creatinine metabolism, that the work of this thesis was undertaken.

The second section of the thesis is a survey of the more important work already done on the problem of creatine and creatinine metabolism. A brief account of the history of the subject is first given, commencing with the original description of creatine by Chevreul in 1835 and ending with the discovery of an accurate method for the quantitative estimation of creatinine, by Folin in 1904. Protein metabolism is next briefly discussed in order to indicate the difficulties involved in the problem of creatine and creatinine metabolism. Emphasis is laid on Folin's interpretation of endogenous and exogenous protein metabolism. There follows an account of the chemical significance of creatine and creatinine. Thereafter the known facts with reference to the distribution of the two substances in the animal world are recounted. Their zoological occurrence, ontogenetic development and their distribution in the various tissues are mentioned. Next the possible precursors of creatine and creatinine are described. Lecithin and Kephalin, Glyocyamin, Arginine, Edestin (containing arginine), Cystine, Casein, Gelatin, Choline and Betaine with urea, Sarcosine and urea, Methyl guanidine, Hordeine, Asparagine, and Histidine are all recorded. Most evidence is shown in favour of arginine being a precursor of creatine and, indirectly, of creatinine. The Metabolism of creatine and creatinine in various diseases is next discussed. Reference is made to the occurrence of creatininuria in certain physiological conditions. Disturbances of creatine and creatinine metabolism are described in:

1. Affections of the thyroid gland.
2. Changed Carbohydrate metabolism.
3. Acute Infectious fevers.
4. Artificial Pyrexia.
5. Pathological conditions involving the Muscular system.
6. Pulmonary Tuberculosis.
7. Alcoholic and Maniacal Conditions.
8. Diseases of the liver.
9. Diseases of the Kidneys.
10. Lymphatic Leukaemia.

There follows a statement and critical examination of the various theories of normal creatine-creatinine metabolism. The suggestions of Folin, Koch, Shaffer, Spriggs, Mellanby, Van Hoogenhuyze and Verploegh, Levene and Kristeller, Thompson and Wallace and McClure are all described and found either unsatisfactory or incomplete. The theory propounded by Gross and Steenbock is believed by the writer of this thesis to be the most complete and the most in accord with all the facts that are known.
In section three of the thesis an account of the creatinine coefficient in pulmonary tuberculosis is given, embodying the results obtained by the writer. The evolution of the present concept of pulmonary tuberculosis as a systemic infection with a local manifestation is described in the preamble. There follows a subsection on wasting and fever in phthisis. An account of the scanty antecedent records of the creatinine coefficient in pulmonary tuberculosis is next given. A subsection on the actual experimental procedure is followed by tabulated results and descriptions of certain cases of particular interest. The general conclusions arrived at are, in the first place that the average creatinine excretion in subjects with extensive pulmonary tuberculosis is low; secondly that without constitutional disturbance the creatinine coefficient is about normal; thirdly that with onset of systemic intoxication causing pyrexia the excretion falls, only to rise again as the constitutional disturbance becomes more profound and finally to fall to a very low ebb in the last four or five weeks of life. Little of prognostic value was found. In very advanced pulmonary tuberculosis it was discovered that a creatinine coefficient of less than 5 meant death within four weeks.

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