CREATINE AND CREATININE*

VICTOR C. MYERS

The first review of the literature on creatine and creatinine after the advent of the Folin colorimetric method was presented by Professor Mendel[22] before a joint meeting of the Section on Physiology and Experimental Medicine of the American Association for the Advancement of Science and the biochemical, physiological and bacteriological societies in Baltimore in 1908. Although only four years had elapsed since Folin introduced his new method, it had already stimulated a sufficiently large number of investigations to warrant a symposium and general review of the subject. In his review Mendel presented a critique of investigations already reported, and made pertinent suggestions as to fruitful lines of future research. It will be of interest to consider some of these remarks in the light of the twenty-three years of subsequent investigation.

In discussing the enzymatic transformations of creatine and creatinine proposed by Gottlieb and his co-workers, Mendel comments, "truly a bewildering array of enzymatic processes". Referring to the creatine-feeding experiments of Folin and of Klercker he questioned their conclusion that there is no conversion of administered creatine to creatinine.

Turning from critique to suggestions regarding new investigations the following pertinent comments may be quoted: "How constant is the creatine content of adult muscle; and is it altered during activity? In the light of the meager and conflicting data available today, a satisfactory answer cannot be given to these questions. Yet they are of fundamental importance for any adequate discussion of the rôle of creatine. Mellanby's convincing experiments on isolated muscle showed that muscular work leaves creatine unaffected, as does the survival of muscle." . . . "In this connection it is of interest that according to Urano, the creatine of the muscle appears to be held in some non-diffusible form in the contractile tissue and is only released when the integrity of the muscle bundles is impaired." And again, "At the outset we need to know more definitely about the possible distribution of creatine in the tissues and

* From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

blood; and above all, whether the creatine content of the muscle is normally a constant, as some maintain, or subject to variations incident to activity, growth or atrophic changes."

It will be noted in the pages to follow that these remarks of Mendel were quite prophetic of the turn investigations on creatine and creatinine were to take. He suggested the analysis of both muscle and blood, and, from his appreciation of the pioneer work of Urano in Hofmeister's laboratory on the diffusibility of creatine and phosphoric acid from muscle, a paper overlooked by many subsequent investigators, he foreshadowed the work of Fiske and Subbarow on phosphocreatine twenty years later.

In a monograph on creatine and creatinine published in 1928, Andrew Hunter[19] presents a very complete and masterly review of the literature on this subject. Titles to nearly 850 papers are given in the bibliography, covering chiefly articles which have appeared in the quarter-century following the introduction of Folin's colorimetric method.

In the limited space available for the present review an attempt will be made merely to indicate the important steps of progress in the solution of this baffling problem. Literature reference will be given only to some of the more important papers which have appeared since the publication of Hunter's monograph.

It has been customary to attribute all recent progress to the introduction of the simple and accurate method for creatinine by Folin in 1904. While it is undoubtedly true that this simple colorimetric method made possible many new investigations, it necessitated other fundamental work on Folin's part, requiring quite as much chemical ingenuity as the large scale use of Jaffé's color reaction for creatinine. Although reference is seldom made to this work in this connection, it would appear quite as fundamental and deserving of credit as the method itself. Previous to Folin's time pure preparations of creatine and creatinine were not available, this statement being particularly true for creatinine. Folin developed methods of preparing both of these substances in pure form. Although these methods were subsequently improved by S. R. Benedict, they served Folin's purpose at the time, and permitted his making fundamental and necessary observations on the methods for creatinine and creatine. From feeding experiments in which some of this material was subsequently employed, he must have soon realized that many of the statements of the older workers regarding the metabolism of creatine

and creatinine were due to the fact that they had worked with mixtures of these two substances.

A summary of the important new facts on creatine and creatinine antedating 1928 will be given on the basis of observations conducted on the urine, muscle and blood, following in a large measure their historical presentation.

Urine

Constancy of the Daily Creatinine Output. With the aid of his new colorimetric method, Folin (1905) was the first to point out that the amount of creatinine excreted in the urine on a meat-free diet is quite independent of either the amount of protein in the food or of total nitrogen in the urine, the amount excreted from day to day being practically constant for each individual, thus pointing conclusively to its endogenous origin. The constancy of this creatinine excretion for man and several species of animals has been fully confirmed by many subsequent investigators. In the writer's experience the absolute constancy of the creatinine excretion appears to be more marked in some individuals than in others.

Hourly Output of Creatinine. Shaffer (1908) early came to the conclusion that the hourly output of creatinine was just as constant as the daily output. Although this appears to be true in a large measure, a number of subsequent workers believe that there is a slightly lowered rate of excretion during the night hours. In this connection Simpson (1924) has made the plausible suggestion that these minor changes in creatinine excretion may be due to variations in the urine volume when these are relatively small.

Creatinine Coefficient. While the creatinine excretion is practically constant for each healthy individual, different persons excrete different amounts, and Folin (1905) early pointed out that the chief factor determining this appears to be the weight of the person. He further noted that the fatter the subject, the less creatinine is excreted per kilo of body weight and concluded from this that the amount of creatinine depends primarily upon the mass of active protoplasmic tissue or, as Shaffer (1908) has expressed it, "Kreatinin is derived from . . . some special process of normal metabolism taking place largely, if not wholly, in the muscles. And upon the intensity of this process appears to depend the muscular efficiency of the individual." It has been found convenient to express the daily creatinine elimination in milligrams of creatinine per kilogram of body weight,

this creatinine coefficient varying between 18 and 30 in a strictly normal individual.

Muscular Work and Creatinine Output. Most of the older workers claimed that the daily output of creatinine was invariably raised by muscular work. Soon after the advent of the Folin colorimetric method, van Hoogenhuyze and Verploegh (1905), Shaffer (1908) and others conducted experiments which showed that neither increased or decreased muscular activity, uncomplicated by other factors, had any effect upon the creatinine elimination.

Decreased Creatinine Output in Wasting Conditions. It was early observed by van Hoogenhuyze and Verploegh (1905), F. G. Benedict (1907), and by others that there was a progressive and more or less regular, though slight, fall in the daily output of creatinine accompanying the fall in body weight resulting from inanition. As one might therefore expect, the creatinine output may be comparatively low in many chronic disorders, while very low figures have been found in progressive muscular dystrophy and certain other disorders involving the muscles. In general, the fall in these diseased conditions is accompanied by somewhat corresponding excretion of creatine.

Increased Creatinine Output in Fever. The excretion of creatinine has been found to be increased in fever. Here the rise in temperature is followed by a corresponding rise in creatinine output. The excretion of creatinine appears to follow closely the rise in temperature during fever, whether the hyperthermia is of infective origin or artificially induced. From this it would appear that the rise in the creatinine elimination was due entirely to the hyperthermia.

Feeding Experiments with Creatinine and Creatine. The older investigators stated that both administered creatinine and creatine reappeared in the urine as creatinine. When Folin (1906) first reinvestigated this question with accurate methods and pure creatinine and creatine, he found that 80 per cent of the administered creatinine did reappear as creatinine, but that when creatine was given in moderate amounts (1 gram to man) it not only failed to reappear as creatinine, but completely disappeared. This led Folin to the often-quoted conclusion that creatinine and creatine are independent in metabolism. van Hoogenhuyze and Verploegh (1908) were the first to assert that there was a slight conversion of administered creatine to creatinine, while somewhat later Myers and Fine (1913, 1915), and Rose and Dimmitt (1916) presented a more conclusive

demonstration of this fact. The more recent experiments of S. R. Benedict and Osterberg (1923) on the dog, and of Chanutin (1926) on man in which large quantities of creatine were consumed over a considerable period furnish unmistakable proof of the augmenting action of creatine on the creatinine output.

With the ingestion of small amounts of creatine (1 to 2 grams) by the adult human little or no creatine reappears in the urine, but with the ingestion of larger amounts, increasing amounts do reappear in the urine. However, there is always a considerable quantity which remains unaccounted for. It is particularly significant in this connection that in infants, where we normally have creatinuria and a low creatine content of the muscle, there should be an almost complete elimination of ingested creatine when the infants are on a high protein diet (Gamble and Goldschmidt, 1919).

Creatinuria. Folin (1904), in his original discussion of the subject, pointed out that although creatine is normally absent from urine occasionally small amounts may be detected. This phase of the problem received renewed interest when F. G. Benedict (1907) noted in *starvation* experiments on man that considerable quantities of creatine appeared in the urine, a fact since confirmed by a large number of investigators. Myers and Fine (1915) endeavored to prove the muscular origin of this creatine in starvation experiments on the rabbit, by showing that the reduction in muscle creatine accounted fairly well for the amount of the urinary excretion. It remained for J. L. Morris (1915) to furnish the first definite proof of the excretion of creatine by the isolation of the double picrate of potassium after the conversion of creatine to creatinine.

The discovery of creatine in the urine during starvation at once precipitated a study of the possible excretion of creatine in a variety of pathological conditions associated with *malnutrition.* F. G. Benedict and Myers (1907) observed the elimination of varying amounts of creatine in a group of undernourished insane patients. Subsequently, the excretion of creatine was reported in such conditions as exophthalmic goitre, anterior poliomyelitis, diabetes, typhoid fever, pneumonia, diseases of the liver, especially carcinoma of the liver, muscular dystrophy, etc., the excretion being particularly large in the last named conditions. Further reference will be made to the excretion of creatine in *diseases of the muscles.*

To W. C. Rose (1911) is due the credit of recognizing the fact that infants and older children normally excrete creatine. The

presence of creatine in the urine of *growing animals* and infants had been observed, but the normal character of this excretion had not been appreciated. The excretion of creatine during the period of growth has received a large amount of study, but just why immature muscle is unable to store up and hold the creatine as well as adult muscle has not been answered. The amount of the creatine output does bear some relation to the level of the protein intake, as pointed out by Denis and Kramer (1917). Harding and Gaebler (1922, 1923) in particular have devoted much time to this question in experiments on both infants and puppies.

Muscle

Creatine Content of Muscle. Liebig was the first to recognize that creatine is a constituent of the voluntary muscle of vertebrates, but the percentage figures then reported were of little value because of the inaccuracies of the method. van Hoogenhuyze and Verploegh (1905) were the first to employ the Folin colorimetric procedure for the estimation of creatine in muscle, although to E. Mellanby (1908) is due the credit of giving the subject any considerable attention. Myers and Fine (1913) were the first to call attention to the remarkable constancy in the creatine content of rabbit muscle (mixed muscle of hind legs), the average value being 520 mg. per 100 gm. They suggested this as a possible explanation of the constancy in the creatinine output, and also pointed to the fact that the creatinine elimination in a given species appeared to bear a definite relation to the percentage content of muscle creatine. This remarkable constancy of the creatine content of rabbit muscle was confirmed in experiments by Baumann (1914) in this country, by Riesser (1913) in Germany, and by Palladin and Wallenburger (1915) in Russia, these workers obtaining almost identical figures. However, the constancy in other species of animals does not appear to be quite so marked. Pekelharing and van Hoogenhuyze (1910) early recognized that "white" muscle was considerably richer in creatine than "red" muscle, and this fact probably has a bearing on the constant figure obtained for rabbit muscle, and the somewhat lower figures reported for most other animals. On this basis it has been shown that a difference exists in the creatine content of the muscles of the same animal.

Changes in the Creatine Content of Muscle. From the relative constancy in the creatine content of normal adult muscle, it is evident that the saturation point is quite fixed. No irrefutable evidence has been presented to show that there is either a change in the creatine content of muscle as the result of muscular work, or an increase in creatinine content. Changes in the creatine content have been reported, however, following starvation and after the administration of creatine and other substances. Starvation generally produces at first an increase in the creatine content of muscle (Mendel and Rose, 1911), which may be followed by a premortal fall (Myers and Fine, 1913). Both Folin and Denis (1912) and Myers and Fine (1913) showed that the administration of creatine might increase slightly, but apparently only temporarily, the creatine content of muscle. Figures for the creatine content of human muscle which have an important bearing on our theories of creatine—creatinine metabolism have been reported by Denis (1916). She found that the creatine content of the muscle of individuals dying of acute conditions was essentially normal, while in patients which had been in a cachectic condition for some weeks or months before death the creatine content was reduced both absolutely and relatively. In children the creatine content of the muscle was much lower than in the adult. These observations are in harmony with the finding of creatinuria and the low output of creatinine found in both cachectic conditions and children.

Autolysis of Muscle. The conception of Gottlieb and Stangassinger (1907) that the transformations of creatine and creatinine are brought about under the action of the enzymes, creatase and creatininase, receives very little acceptance at the present time. As pointed out by Myers and Fine (1915), Hoagland and McBryde (1916), Hammett (1921), Hahn and Meyer (1923), and others, in the autolysis of muscle creatine is gradually converted into creatinine, until an equilibrium point is reached, but, in the absence of bacterial action, there is no loss of creatinine. Hahn and Meyer feel convinced that the only catalytic agents which are concerned in the process are the hydrogen and hydroxyl ions. In connection with their experiments, Myers and Fine called attention to the fact that the experimentally observed rate of creatinine production in autolyzing muscle (about 2 per cent per day) fully accounts for the rate of creatinine excretion.

Creatinine Content of the Muscle. Even after the advent of the Folin colorimetric method many investigators still questioned the

presence of creatinine in muscle. However, Shaffer (1914), Folin (1914), and Myers and Fine (1914, 1915) were able, with a suitable application of the Jaffé color reaction, to show the presence of creatinine in muscle, when every practical precaution had been taken to prevent a secondary production of creatinine. The amount present is larger than that found in other organs of the body including the blood, and suggests the probable formation of creatinine from creatine in the muscles. Only with the suppression of renal activity, preventing the removal of creatinine from the blood, does the amount in the blood exceed that in the muscle.

Creatine Content of Cardiac Muscle, Smooth Muscle, Brain. In comparison with striated muscle, cardiac muscle is definitely lower in its creatine content, 200-300 mg. compared with 350-520 mg., and the content is still lower in smooth muscle, 30-100 mg. In the brain the amount of creatine is surprisingly large, the concentration being appreciably higher in the cerebellum than in the cerebral hemispheres. As pointed out by Harding and Eagles (1924), the concentration of creatine in the brain appears to be a constant for each species. For seven human brains they found an average of 123 mg. in the cerebral hemispheres and 176 mg. in the cerebellum.

Blood

Creatinine Content of Blood. As early as 1910 Shaffer and Reinoso suitably applied the Jaffé color test to blood to ascertain the creatinine concentration in a few specimens, but it was not until 1914 that any considerable data on the preformed creatinine of blood were presented by Folin and Denis, Shaffer, and Myers and Fine. Roughly the figures which have been reported for normal human blood fall in the range 0.5-2.5 mg. per 100 cc. There has been much criticism of the methods employed for the estimation of creatinine in blood, and it seems quite likely that the figures reported for normal blood are considerably too high. Furthermore, Behre and Benedict (1922) have even questioned the presence of creatinine in blood, owing to the chemical behavior of the "so-called" preformed creatinine in blood filtrates. However, there are observations which lead us to believe that if creatinine is not present in blood as such, it is present as a closely related precursor. While the amount of creatinine is very small in normal blood, it (this chromogenic substance)

accumulates in the blood and tissues with impairment in renal function. As first pointed out by Myers and Lough (1915), a rise in the blood creatinine above 5 mg. is of grave prognostic significance and portends an early fatal termination, unless the retention is due to some very acute renal condition. The blood creatinine never exceeds 5 mg. unless there is a marked impairment of renal function, but in the terminal stages of renal disease it may exceed 30 mg.

Creatine in Blood. The data available for blood creatine have likewise received adverse criticism. The figures which are given for normal whole blood are considerably higher than those for creatinine. It is generally held that the creatine is chiefly present in the cells, and that when there is an appreciable amount of creatine in the plasma, creatinuria occurs. With retention, creatine also accumulates in the blood, and Behre and Benedict (1922) have reported reliable figures reaching nearly 20 mg. in dogs after ureteral ligation.

A summary of some of the most important facts which have been recorded on the metabolism of creatine and creatinine up to 1928 has been given above. These facts suggest the following interpretation. The constancy in the output of creatinine would appear to depend upon a small but equal constant transformation of creatine to creatinine, the bulk of the creatinine formation taking place in the muscles since they contain the bulk of the creatine. There is no irrefutable evidence to indicate that an enzyme is concerned in this transformation, nor would an enzyme appear to be necessary. Just what purpose, if any, the conversion of creatine to creatinine serves is not clear. It may be noted that even in pure aqueous solution there is a gradual transformation of one compound to the other until an equilibrium point is reached. In this connection it is significant that the only condition which will bring about an immediate rise in the creatinine output is a rise in the body temperature. It might be that creatinine represents the wastage in this equilibrium reaction between creatine and creatinine, and that the creatinine thus formed is picked up by the blood-stream and carried to the kidney for excretion.

At any rate it appears quite clear that there is a fairly definite relation between the creatine content of muscle and the creatinine output. Since the potential muscular efficiency appears to depend upon the creatine content of the muscle, the creatinine output should therefore furnish quite as good an index of this as the muscle creatine. This might be considered an amplification of Shaffer's original statement in this regard.

Important Observations Since 1928

It would appear, however, that a number of the most vital problems still remained unanswered up to 1928. Creatine is a characteristic constituent of vertebrate muscle, and in normal muscle it is maintained at a fixed level. It must, therefore, play some vital rôle, but the data thus far presented furnished no evidence of its function. What is the rôle of creatine? Some years ago Folin and Denis (1914) stated that creatine was probably a post-mortem product and did not occur as such in living muscle. Reference has been made to the work of Urano (1908), and others have made suggestions of a similar nature. At the time of their muscle analyses Myers and Fine (1914) carried out potassium analyses on the muscle extracts and also made an effort to estimate the phosphorus content as well. The figures for phosphorus were not very satisfactory, and since the figures for potassium did not do more than show a measure of parallelism with the creatine they were not published. In 1922 the writer stated, probably without proper justification, " . . . it may be said that there are many observations which lead one to believe that glycogen, creatine, phosphoric acid and potassium are closely associated in active muscle".

From the observations which have been reviewed it would seem quite clear that creatine must be the mother substance of creatinine, but what is the mother substance of creatine? The amino acid arginine has been suggested as structurally the most probable precursor by a number of workers, and some evidence has been presented to show that it exercises an influence in this regard. Other amino acids, such as cystine and histidine, have also been shown to influence the creatinine output. It cannot be stated, however, that we have any conclusive observations on this point.

The question whether creatinine as such actually occurs in blood was raised by the observations of Behre and Benedict (1922). Since creatinine occurs in urine, it is obvious that it must either be carried to the kidneys in this form or in some form (other than creatine) which can be readily converted to creatinine by the kidneys. It seems very important that this question should receive a conclusive answer.

Observation on the Conversion of Creatine to Creatinine. During the past four years further observations have been presented to support the thesis that creatinine is formed from creatine. Rose, Ellis and Helwing[28] have reported some very interesting observa-

tions on two subjects, one male and one female. After a preliminary period of two weeks, creatine was taken in daily doses of 1 gm. for seven weeks, and the output of creatinine (and creatine) followed for five weeks more. No creatine was excreted by the male subject, but the female subject excreted 6.0 gm. of the ingested creatine. Of the retained creatine the male subject excreted 33 per cent as extra creatinine during the twelve weeks, while the female subject excreted 42 per cent.

In a study of the influence of the level of the protein intake on the conversion of creatine to creatinine, Bohlman[4] found that the feeding or intravenous administration to two dogs of creatine in amounts comparable to the daily creatinine output had no influence on the excretion of creatinine, although considerable amounts of the administered creatine reappeared in the urine. After about two weeks of high protein feeding and administration of creatine the excretion of creatinine began to increase and in some cases remained increased for a few weeks after the high protein diet was discontinued. This seems to be direct evidence of the conversion of creatine to creatinine and is also indicative of the relation of this conversion to the existing level of the metabolism of protein.

Nature of the Blood Creatinine. Gaebler has devoted considerable attention to a study of the nature of the blood creatinine. In collaboration with Keltch[17] he described a new method of isolating creatinine from blood as a complex acid formed by precipitation with picric and phosphotungstic acids. After being released from this precipitate the creatinine was ultimately isolated as creatinine potassium picrate. From their observations they concluded that creatinine was present in large amounts in blood during experimental and nephritic retention. In a more recent paper Gaebler[16] comes to conclusions somewhat more in harmony with those of Behre and Benedict. He presents data which support the view that the creatinine-yielding substance in normal blood (while not creatine) is not creatinine itself. He was able, however, to go a step beyond this and isolate creatinine. In these experiments Gaebler employed Lloyd's reagent to adsorb the creatinine, as first suggested by Behre and Benedict. After being released the creatinine was ultimately isolated as creatinine potassium picrate. With this procedure he found that the blood of normal dogs contained a substance, other than creatine, which yielded creatinine in isolation experiments, and that a similar substance was present in human blood, the amounts present in corpuscles and plasma being approximately equal. Impairment of

renal function was found to result in the accumulation of one or more substances other than creatine, which yielded creatinine. Isolation figures for normal blood gave about 0.5 mg. per 100 cc., while figures as high as 20 mg. were obtained in retention cases. Gaebler felt that his data were inadequate to determine whether or not the creatinine-yielding substance in normal blood was a precursor of urinary creatinine. The accumulation in early retention suggested this conclusion, but did not definitely establish it.

Storage of Creatine in Muscle of Mouse and Rat. It has been shown by Chanutin and Beard[7] that the creatine concentration of the muscle of the mouse may be raised by creatine feeding. The average creatine concentration in the muscle of 28 normal mice was 367 mg. per 100 gm., while the average obtained for 38 creatine-fed animals was 403 mg., an increase of 10 per cent. Increasing the creatine intake beyond a certain level had no further influence on the creatine content of the muscles. It is worthy of note, further, that the withdrawal of creatine from the diet and subsequent feeding of a normal diet for a short time produced no perceptible change in the excess creatine stored in the muscle. Slightly lower figures were obtained for the muscle creatine when mice were put on a diet rich in casein or edestin than when on the control diet. Since edestin is rich in arginine, it was concluded that an arginine-rich diet does not increase the concentration of muscle creatine.

In creatine-feeding experiments with growing rats (40-55 gm. in weight) Beard and Barnes[2] were able to produce a somewhat similar increase in the muscle creatine. The rats were given 0.5 or 1.0 gm. creatine mixed with the diet, and were killed 24 to 48 hours later. The average muscle creatine for 118 animals was 400 mg. per 100 gm., while for 8 animals fed creatine the average was 495 mg.

Origin of Creatine. The mechanism of creatine formation has been a much controverted question. Owing to its closely related chemical structure arginine has often been studied in this connection but with somewhat uncertain results.

From the data now available it would appear that creatine formation can be demonstrated fairly readily in conditions where a high creatine production is present and consequently a creatinuria. Gross and Steenbock (1921) in part suggested this in experiments which they conducted with thyroid feeding. Other suitable conditions which might be mentioned are phlorhizin diabetes (Benedict and Osterberg, 1914), Graves' disease, muscular dystrophy, young children and growing animals, in all of which a creatinuria is present.

As far back as 1920 Harding and Young suggested that creatine might be derived from cystine. A year later in experiments on the pig, Gross and Steenbock were able to show that both cystine and arginine served to augment the excretion of creatine. Zwarenstein[81] appears to have obtained an increase in creatinine output after the ingestion of glycine and alanine, although he does not draw this conclusion from his own data. In a recent paper Abderhalden and Buadze[1] report an increase in the creatinine output after giving both nucleic acid and histidine. More significant are the observations of Brand, Harris, Sandberg and Ringer[6]. They have shown that the administration of glycine to patients suffering from progressive pseudohypertrophic muscular dystrophy causes a 40 per cent increase in the creatine excretion. Later they reported[5] that gelatin and, to a lesser extent, edestin feeding had much the same effect. Working on growing rats, Beard and Barnes[2] have been able to show that feeding a number of different amino acids (arginine, histidine, cystine, tyrosine, phenylalanine, alanine, valine, glycine, and glutamic and aspartic acids) to growing rats will bring about an increase in the muscle creatine in all cases, the highest increases being 34 per cent with valine and 37 per cent with cystine. Increases were also obtained with choline, edestin, casein and glycocyamine, the increase in the latter case being 48.5 per cent. These observations on the increased formation of creatine are in harmony with the views expressed above. It seems hard to believe that all these amino acids are converted to creatine, and the most logical interpretation at present would appear to be that they stimulate creatine formation. Beard and Barnes also obtained increase in the creatinine excretion of the urine in adult rats after the administration of a number of these amino acids, while somewhat similar findings were obtained on the human adult after the ingestion of glycine, alanine and arginine. Small increase in creatinine excretion might be reconciled with other observations to which reference has been made and to our previous conception of the constancy in the creatinine output, but it seems hard at the present time to interpret the comparatively large increases (up to 50 per cent) reported.

Further Observations on the Creatine Content of Muscle. Hunter[20] has presented some very interesting data on the creatine content of the muscles of fifteen different species of fish. He has verified the high values previously reported by some investigators,

and shown that the creatine content of the caudal muscle of some fish may run as high as 750 mg. per 100 gm., a much higher value than that found for mammals. The creatine content of the heart muscle is, however, lower. In two ling-cod serial analyses were made from samples of muscle from head to tail, with the result that there was revealed a progressive and quite conspicuous increase of creatine concentration from before backward, so that much the highest values were found in the powerful propelling muscles of the tail. In fish, as in mammals and birds, red muscle was found to contain less creatine than pale, and fetal muscle less than the adult.

Metabolism observations and muscle analyses in a case of myositis fibrosa in a 15-year old boy have been reported by Bodansky, Schwab and Brindley[3]. Marked creatinuria was a constant feature, and there was an almost complete inability to retain exogenous creatine for more than a very short period. However, of the fraction which found transitory storage in the tissues, a portion was apparently converted into creatinine. The muscles were found to be abnormally low in creatine and, furthermore, a relationship was found to exist between the creatine content of the various muscles and the pathological changes, in particular the degree of inflammation.

Attention has recently been given to the creatine and phosphoric acid content of various parts of the heart by Vollmer[30]. These studies portend some interesting developments. As might be expected, the creatine content of the left ventricle is considerably higher than that of the right ventricle. Seecof, Linegar and Myers[29] have collected a large number of observations on the creatine content of right and left ventricular muscle obtained at autopsy. It seems too early to draw conclusions from these observations, but the findings in certain heart disorders, for example, those associated with cardiac hypertrophy should be of decided interest. In this connection it is of interest that several years ago Palladin and Ferdmann[27] made the claim that exercise increased the creatine content of muscle.

Function of Muscle Creatine. The fact that creatine is a constant constituent of vertebrate muscle and that the concentration is maintained at a very constant level in normal adult muscle, but present in reduced amounts during growth and in disorders associated with muscular weakness has indicated that creatine must play a vital rôle in active muscle.

The discovery by Fiske and Subbarow[13], in Folin's laboratory, of phosphocreatine forms a fitting climax to the many efforts to dis-

cover the function of muscle creatine. Quite as Folin and Denis (1914) prophesied, creatine has been found to be a post-mortem product. In unfatigued living muscle it is present in combination with phosphoric acid. The developments in this field have come so rapidly since the discovery of Fiske and Subbarow, and are so close at hand, that they are hard to evaluate. This discovery has quite completely upset the current theories of the dynamics of muscular activity, and Hill[13] has just presented a most interesting review of the subject under the title, "The Revolution in Muscle Physiology". Although most of this later work has been conducted in European laboratories, the fundamental discovery was made in this country by Fiske. It seems unfortunate that Hill should give chief credit to Eggleton and Eggleton[8, 9, 10, 11]. It is quite true that these authors did recognize the presence of a labile form of organic phosphate and the fact that it played an important rôle in muscular activity entirely independent of Fiske. However, they suggested first that their so-called "phosphagen" might be a phosphoric ester of glycogen[8], and several months after the publication of Fiske's first paper they claimed[9] that the substance was of the nature of a hexosephosphate. When Fiske and Subbarow[12] attempted to apply their method for inorganic phosphorus to muscle filtrates, shortly after its publication in 1925, they noted the presence of a labile phosphorus compound. They announced the discovery of phosphocreatine in April, 1927[13], described its isolation and suggested its function in February, 1928[14], and more recently have given complete details of their work[15]. It would seem that the term phosphocreatine was more specific and correctly descriptive of the compound than phosphagen, since the compound in question is composed of phosphoric acid and creatine in molecular proportions. One term defines the compound, while the other merely suggests its action.

Accepting the structure commonly assigned to creatine, Fiske and Subbarow[14] suggested the following as the probable formula for phosphocreatine:

$$HN=C\begin{matrix}\diagup NH.PO(OH)_2 \\ \diagdown N(CH_3).CH_2.COOH\end{matrix}$$

They[15] have been able to recover as much as 70 per cent of the labile phosphorus present in the protein-free muscle filtrate as a crystalline calcium salt. This product, which is a mixture of the secondary

and tertiary salts, can be converted to the pure secondary salt, $C_4H_8O_5N_3PCa \cdot 4H_2O$, from which, after hydrolysis, practically the theoretical amount of creatine can be obtained. From their experiments Fiske and Subbarow conclude that the labile phosphorus compound present in protein-free muscle filtrates contains nothing but creatine and phosphoric acid.

They further point out that as the second acid dissociation constant of phosphocreatine, determined by the titration of 0.005 *M* solutions of the secondary calcium salt with acid, is 2.6×10^{-5}, it follows that the hydrolysis of this substance during muscular contraction is accompanied by the liberation of a large amount of base, and consequently functions as a mechanism for neutralizing acid.

Fiske and Subbarow have recorded figures for the phosphocreatine P and inorganic P in various muscles of the cat under a variety of conditions. In the normal resting muscle the phosphocreatine P ranged from 51 to 86 mg. per 100 gm., the inorganic P from 18 to 35 mg., and the sum from 81 to 121 mg. They conclude that while there is hardly any question that the inorganic P content of the muscle in its natural surroundings is lower than indicated by the analytical results, and may therefore be well under 20 mg. in full relaxation, figures in excess of 30 mg. are sometimes found, even when no more than the usual activity during the isolation of the muscle is in evidence. Hydrolysis of phosphocreatine takes place rather rapidly when the circulation is shut off, but some may still be present after 2 hours with no blood supply. Decomposition is complete in a much shorter period when the animal is dead. The stimulation of muscle by uninterrupted tetanization of the nerve soon destroys a large proportion of the phosphocreatine even when the circulation is intact. Stimulation to complete fatigue after shutting off the blood supply destroys all the phosphocreatine, but a certain amount of resynthesis occurs in muscles which have been so treated, if they are allowed to rest for a time after removing the arterial clamp. Hydrolysis was also demonstrated after the injection of lactic acid and of potassium chloride. No increase in the phosphocreatine of muscle was demonstrable after the intravenous injection of creatine alone or mixed with sodium phosphate.

Fiske and Subbarow's work on phosphocreatine has been verified and extended in a number of directions by Meyerhof and several individuals who have done all or part of their work in his laboratory in Berlin, Lohmann, Nachmansohn, E. Lundsgaard, by the Eggle-

tons, by Hill, Palladin, Riesser and others. It is of interest in this connection that Meyerhof and Lohmann[23, 24] have shown that in crustacean muscle a combination of arginine and phosphoric acid replaces phosphocreatine. The arginine-phosphoric acid of the muscle extract undergoes enzymic hydrolysis and resynthesis like phosphocreatine, the tendency to resynthesis appearing at pH 7.0 and becoming maximum at pH 8.0.

In the performance of muscular work phosphocreatine hydrolysis does decidedly more than function as a mechanism for neutralizing acid. Meyerhof, Lundsgaard and Blaschko[25] have recently shown that the energy for mechanical work performed comes entirely from phosphocreatine cleavage. For the cleavage of phosphocreatine there has been found a heat effect of 150 calories for the enzymic process and 120 calories for the acidic process. Apparently the resynthesis of phosphocreatine under normal conditions is brought about at the expense of lactic acid formation energy. Meyerhof and Schulz[26] have recently found (in corroboration of Embden and Lehnartz) that during maximum contraction even under indirect stimulation a considerable part of the lactic acid of a brief tetanus develops during relaxation, and this is independent of temperature. The lactic acid produced during tetanus and immediately after is the same in a muscle saturated with oxygen as under anaerobic conditions.

The relations between glycogen and phosphocreatine in this connection are very interesting. Masayama and Riesser[21] have shown that the phosphocreatine: glycogen ratio is the same for the same rabbit, or the same group of muscles. Even when their absolute content varied, the ratio was still maintained the same, showing that both vary to the same degree. When isolated muscles were kept in oxygenated Ringer's solution or were fatigued, they lost simultaneously phosphocreatine and glycogen, but in the absence of oxygen the loss of phosphocreatine was greater than that of glycogen so that the ratio increased.

Further developments in the rôle which phosphocreatine plays in muscular activity will be awaited with great interest.

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