STUDIES IN THE PHYSIOLOGY

of

CREATINE and CREATININE.

T H E S I S

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C O N T E N T S.

The studies recorded in this thesis cover a wide portion of the field of creatine-creatinine metabolism. They involve a consideration of the problem of the origin of creatine, of the endocrine factors controlling its metabolism, and of the function of creatine and creatinine in the body. In order to obtain a true perspective of the place of these investigations in relation to the physiology of creatine as a whole, it is first necessary to review briefly what is known of the metabolism and function of the two substances. And, in view of the light they throw on these questions, consideration must also be given to the chemical constitution and distribution of creatine and creatinine.

HISTORICAL:

Just over 100 years ago (1832) Chevreul reported to the French Academy of Sciences that he had discovered a new organic substance in meat. This substance he named 'CREATINE' (from the Greek κρεάς κρέατος meaning meat).

Fifteen years later Liebig (1847, 1) determined the empirical formula of creatine to be $C_4H_9O_2N_3 + H_2O$, and demonstrated that on heating with mineral acids a derivative was obtained having the formula $C_4H_7ON_3$. This substance he called 'CREATININE'. Previous to this Heintz (1844) and

/Pettenkofer

INTRODUCTION.

Pettenkofer (1844) had independently discovered a new nitrogenous constituent of human urine. Liebig (1847, 2,3) showed that this substance was identical with his 'creatinine'.

Until the close of the 19th Century a great deal of useful quantitative work was done in determining the distribution of creatine and creatinine in the animal kingdom. But it was not until 1904 that an accurate method for their quantitative estimation was introduced by Folin. The introduction of this method ushered in a new era in the study of the metabolism of creatine and creatinine and a vast literature has since sprung up round these two compounds. Nevertheless there is still much about their metabolism which remains obscure and controversial.

In this introduction it is proposed to summarise only briefly the literature up to 1926 in view of the review of the subject up to that date by Hunter (1928). Since the appearance of this admirable monograph the chief advances have been in connection with (a) the elucidation of the function of creatine and (b) the nature of the endocrine control of creatine and creatinine. These subjects will therefore be discussed in greater detail. In connection with the former, the important role of creatine in muscular activity has received much prominence during the last few years, but the various investigations into the hormonal control of creatine and

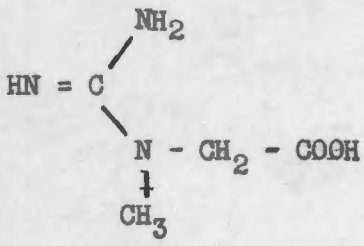
/creatinine

creatinine do not appear to have been collected and reviewed as yet.

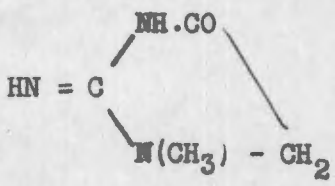
CHEMICAL RELATION BETWEEN CREATINE AND CREATININE:

There is a close structural similarity between the two substances.

CREATINE



CREATININE



Moreover, each can be converted into the other with striking ease. Thus in neutral or alkaline media the creatine-creatinine transformation is a reversible reaction, while in acid media creatine is gradually converted into creatinine.

The occurrence in the organism of two substances so closely related chemically naturally leads to the suspicion that there may be a metabolic relationship between them. The proof of this assumption has long been the subject of controversy and will be discussed in connection with the source of urinary creatinine.

DISTRIBUTION OF CREATINE AND CREATININE:

Creatine occurs predominantly in skeletal muscle and creatinine mainly in urine. Their distribution is confined to vertebrates.

Creatine - Some idea of its distribution is given by the following figures:

Striped muscle of mammals				370 - 520	MgMs.	per	cent.
Heart	"	"	"	210 - 260	"	"	"
Smooth	"	"	"	20 - 40	"	"	"
Cerebrum				100 - 130	"	"	"
Testes				80 - 100	"	"	"
Liver				16 - 37	"	"	"
Kidneys				12 - 18	"	"	"
Blood				2 - 3	"	"	"

Urine - while normal male urine on a creatine-free diet contains no creatine it occurs physiologically in the urine of children, pregnant women and in an intermittent manner in the non-pregnant adult female.

Burger (1919) has estimated that 98% of the creatine present in the human body is in muscle. Its abundance in skeletal muscle together with its invariable occurrence would appear to indicate that it has some essential function to perform. Direct proof of this, however, was not obtained until the revolutionary advances in muscle physiology during the last few years.

Creatinine - (a) In Urine - The regular occurrence of creatinine as a quantitatively important constituent of the urine indicates that it represents a waste product. In the excreta of birds and reptiles it is largely replaced by creatine.

(b) In Muscle - Whether muscle contains

/creatinine

creatinine is a matter of considerable doubt; the difficulty being to exclude a mere secondary production of creatinine from creatine. But even if creatinine is present in muscle it does not exceed 12 mgms. per 100 grams, and is thus insignificant compared to the creatine content.

(c) Other Tissues - Its presence in other tissues and body fluids such as the sweat, cerebrospinal fluid, etc. is still equivocal.

Creatine and Creatinine in the Blood: The question of their occurrence in the blood is of considerable interest in view of their probable increase in the blood stream during exercise and because of variations associated with disease.

The generally accepted figures are 1 - 2 mgms. creatinine and 3.5 - 5 mgms. creatine per 100 ccs. of normal human blood. Behre & Benedict (1922) maintain, however, that a chromogenic substance exists in blood whose reaction with picrate simulates that of creatinine and is, therefore, a source of error. More recently Gaebler (1930) has also supported the view that creatinine does not exist as such in blood.

Invertebrates - Creatine has never been shown to occur in the muscles of invertebrates, and evidence is accumulating that it is replaced in them by arginine. It is probable that as technique improves the gaps in the evidence will be filled. Thus Kutscher and Ackermann (1931) have isolated arginine by

/means

means of flavianic precipitation from two invertebrates, Lumbricus terrestis and Holothuria tubulosa, in which earlier methods had failed. This substitution of arginine for creatine in invertebrates may be not without significance as indicating a possible source of creatine in the vertebrate.

THE METABOLISM OF CREATINE AND CREATININE:

Having dealt with the chemical relationship between creatine and creatinine and with the distribution of the two, it now remains to consider,

(1) The sources from which the creatine of the body is derived,

(2) The fate of the creatine,
and
(3) Where does the creatinine of the urine come from and what is its significance.

The answer to these questions should provide a connected metabolic history of the two compounds. The truest conception of the way in which they were investigated will be obtained by considering the last question first.

The Source of Urinary Creatinine.

It has been mentioned that the constant occurrence of creatinine in the urine in notable amount indicates that it is to be regarded as a waste product; and in view of its close chemical relation to creatine the latter is its most probable source.

This was taken for granted by the earlier workers. But, with the application of Folin's method of quantitative analysis, Klercker (1906) and Folin himself (1906) were unable to obtain any increase in urinary creatinine by means of the administration of creatine. They concluded that, despite the structural similarity between the two, the organism has no power to convert creatine to creatinine, and that the two are independent of each other in metabolism.

But abundant evidence has since been produced that the administration of creatine can cause an increase in urinary creatinine, the most striking, though not the earliest experiments being those of Benedict and Osterberg (1923). Furthermore, Meyers and Fine (1913, 1) demonstrated by direct measurement in eleven adult rabbits that the average daily output of urinary creatinine bears a constant relation to the total creatine of the body, though Chanutin and Kinard (1932-33) deny this.

Additional proof of a direct relation between urinary creatinine and muscle creatine has been obtained from experiments wherein factors, which cause a decrease or increase in urinary creatinine, have been shown to cause a corresponding decrease or increase in muscle creatine. Thus Palladin & Kudrjawzewa (1924, 1,2) showed that a vitamin-free diet produces not only an increase in muscle creatine but also a

/corresponding

corresponding increase in urinary creatinine. Similarly in certain myopathies there is a decrease in muscle creatine and associated with this a decrease in the excretion of creatinine. More recently similar evidence has been obtained by comparing the influence of certain hormones on the muscle creatine and urinary creatinine. Thus Schrire and Zwarenstein (1933) have shown that injection of extracts of the anterior lobe of the pituitary causes an increased elimination of creatinine, and in this thesis it is shown that it produces a similar increase in muscle creatine.

The Significance of Urinary Creatinine: Is it an Index of Endogenous Protein Metabolism ?

Implied or expressly stated in all current text-books of Physiology and in the writings of contemporary workers in the field of creatine-creatinine metabolism, is the view that creatinine is an index of endogenous protein metabolism. Since it has been shown that creatinine is derived from creatine the implication is naturally involved that creatine is likewise a product of the endogenous metabolism of protein.

This view seems to rest on a very unsubstantial foundation. It is based on the well-known work of Folin (1905), in which he showed that creatinine is the only nitrogenous product in the urine which does not undergo diminution when the protein in the

/diet

diet is reduced. From this he concluded that creatinine must be the product of a special type of protein metabolism which proceeds at a uniform rate in all the living protoplasm of the body. This process he termed 'Endogenous Metabolism'.

It is true that the daily output of urinary creatinine is more or less constant and that, therefore, it is in all probability largely produced by an endogenous metabolic process. But Folin's assumption that it was produced by endogenous protein metabolism is not necessarily correct. There is no reason why it should not be the product of the endogenous metabolism of any other nitrogenous compound which occurs in constant amount in the body, such as creatine.

Folin denied the existence of any biological relationship between creatine and creatinine and the fact that he resorted to proteins as the substance whose endogenous metabolism gives rise to urinary creatinine is understandable. But his conclusions, based on a misconception, should not be perpetuated without critical re-examination.

There is no proof whatsoever that urinary creatinine depends on the amount of protein in the muscle, or on the total tissue protein, or that it is related to the extent of the catabolism of protein. Shaffer (1907, 1908,) showed that "subjects of exophthalmic goitres and others in whom the total

/endogenous

endogenous catabolism is probably much increased" may have creatinine coefficients much below normal. This would indicate that creatinine is not derived from tissue protein.

The view that creatine is an intermediary product of protein metabolism, and, therefore, simply a waste product just like creatinine (which represents the terminal stage), is a view which may well have been acceptable some years ago. But it has been made untenable by the discovery that creatine, in the form of phosphocreatine, is a substance with an essential function, which is present in constant amount in a specific type of tissue, namely, muscle.

Since there is no experimental proof for the assumption that creatine is a stage in the endogenous metabolism of protein, a more economical, and therefore scientifically more acceptable, hypothesis is - <u>that muscle creatine is formed in more or less constant amount from the creatine, and probably from certain of the amino acids, of the diet directly and not via the stage of tissue protein.</u> This constant muscle creatine gives rise to a fairly constant amount of creatinine in the urine.

Of each step in this suggested process we have proof. Continued subcutaneous administration of small doses of creatine produced small but definite increases in muscle creatine

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according to Myers and Fine (1913, 3). Similarly, intravenous injection of arginine into rabbits is followed by a rise in muscle creatine (Thompson 1917). The possibility that the administered substances were first built up into protein is not definitely excluded by these experiments, but there is no reason for making such an assumption.

The evidence that creatinine is derived from creatine has already been presented.

Starling (1933) appears to have some such process as that suggested above in mind when he expresses doubt as to whether creatinine "is to be regarded as an invariable end-product of the metabolism of tissue protein, or whether it represents the terminal phase of a metabolic process quite apart".

The Source of Creatine in the Body:

Experiments designed to elucidate this problem have taken the form of administering an adequate supply of the possible precursor, in the hope that, if material of the right sort were provided, the creatine content of the muscle (and, therefore, the output of urinary creatinine) would be increased.

In criticising the rationale of these experiments, Hunter (1928) states that "Creatine in fact, is probably not a waste product, but an essential tissue constituent with a special function. Its rate of production is, therefore, in all

/likelihood

likelihood regulated by an internal demand and it is not to be expected that it should be accelerated by an excessive supply of precursors, any more than the production of adrenaline or thyroxine would be increased by the administration of a dose of tyrosine".

This criticism probably accounts for the universal failure to obtain striking increases on administering possible precursors. But the fact remains that the administration of creatine leads to an increase in urinary creatinine (Rose & Dimmit, 1916; Benedict and Osterberg, 1923; Chanutin, 1926; etc.) and to an increase in muscle creatine (Folin and Denis 1912, 1914; Myers and Fine, 1913).

It is legitimate to expect, therefore, that if the correct precursors of creatine are administered similar effects would result.

Another potentially useful method of approach is that suggested by Rose (1933). viz: to "limit the intake of a suspected precursor below the required level rather than to add excessive quantities to a diet already carrying sufficient to meet the demands of synthesis."

The effects of administering a number of chemical compounds bearing a structural similarity to creatine and creatinine have been investigated. Substances for which some evidence, however inadequate, has been advanced are Arginine, Cystine, Histidine,

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certain purines and purine derivatives, choline and its oxidation product betaine.

More recently Brand and co-workers (1929, 1930, 1932) have adduced evidence in favour of glycine.

In the case of each of the above substances there has always been more negative than confirmatory evidence so that our knowledge has never been unequivocal. But if the recent work of Beard and Barnes (1931) is to be accepted the situation becomes even more confused. They submit the view that a large variety of widely dissimilar amino acids can act as precursors of creatine and creatinine. They consider the increases to be sufficiently large to discount the possibility of the effect being due to the specific dynamic action of the amino acids. A satisfactory explanation of such results is difficult and their findings are undoubtedly in need of re-examination. In this thesis is reported a series of experiments designed to investigate the effects of a number of the amino acids administered by Beard and Barnes.

The Fate of Muscle Creatine:

We have noted that abundant proof now exists that urinary creatinine can be formed from muscle creatine. But creatinine is not necessarily the sole end-product of creatine metabolism.

Benedict and Osterberg showed that when creatine was

/administered

administered to their dogs a certain amount was excreted as such. Of the remainder almost exactly one-third was eliminated as creatinine. To account for the rest of the creatine there are three possibilities:

(1) It might be retained without change in the body.

Folin and Denis (1912; 1914) and Myers and Fine (1913) have shown that an increase of creatine in the muscles does occur following creatine administration, but it does not appear to be sufficient to account for the amount of creatine which has failed to be excreted.

(2) It might be catabolised into end-products other than creatinine.

But Folin (1906) found that creatine ingestion has no effect on the output of urea or ammonia or undetermined nitrogen. Similar results were obtained by Folin and Denis (1912) and by Rose and Dimmit (1916).

(3) It might be utilised anabolically in the synthesis of other chemical compounds.

Thus it has been suggested that in view of its structural similarity to choline it might be used in the synthesis of lecithin. Paton (1919) suggested that through its guanidine nucleus it may take part in the formation of arginine. But there is no direct experimental proof that creatine may function as an anabolite.

So far, therefore, the only change which creatine has been proved to undergo is its conversion into creatinine. Benedict and Osterberg believe, however, that their experiments "seem to demonstrate almost conclusively that creatinine is but one of the end-products of metabolism." Chanutin and Silvette (1929-30) have also advanced evidence for the disappearance of creatine other than by transformation into creatinine, and attribute the loss to destruction by the organism. They are, however, unable to give any indication of the nature of the intermediates or end-products of the process.

THE CHEMICAL STATE AND THE FUNCTION OF CREATINE:

These two problems are intimately connected. That creatine does not exist entirely free in muscle was long suspected. Urano (1906) found that creatine can be rapidly dialysed out of disintegrated muscle but diffuses only very slowly through the sarcolemma of fresh muscle fibres. To explain this he suggested that in living muscle creatine exists in the form of a very unstable non-dialysable colloidal compound forming an integral part of the protoplasm of muscle. Folin and Denis (1914), Myers and Fine (1913, 1,2). Shaffer (1914), Benedict & Osterberg (1923) all expressed the opinion that creatine does not exist as such in muscle. Hunter in his monograph says, "One may suppose that the bulk of the muscle-creatine exists as a highly unstable

/compound

compound of undefined nature". An indication of the nature of this hypothetical compound was indeed given as far back as 1855 by Valenciennes and Fremy, who reported that they had found creatinine in combination with phosphoric acid in muscle.

As far as the function of creatine is concerned, the fact that the quickly contracting pale muscles are richer in creatine than the less active red muscle, that the active hind limbs of frogs contain more than their comparatively inactive forelimbs, and similar correlations between muscular activity and creatine content (Reisser 1922), all indicated that creatine was in some way related to the efficiency of muscular contraction. But the precise nature of this relation remained speculative. Ranke (1865) and Burridge (1910) considered that during contraction creatine is converted to creatinine and that this serves as a stronger base to neutralise the lactic acid produced during contraction. Creatine was, therefore, concerned in promoting the phase of relaxation.

Tiegs (1925) came very near the truth. He agreed with the above view of its function but since he could find no conclusive evidence of the conversion of creatine to creatinine during muscular contraction he postulated that creatine exists in a special form, 'cyclic creatine', in resting muscle, and that during contraction it is converted into a tautomer having the conventional formula and decidedly basic properties. The release

/of

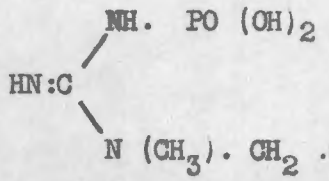
of this basic creatine neutralises the lactic acid produced during contraction and thus brings about relaxation.

The foregoing constitutes a brief summary of the state of knowledge, up to 1927, concerning the chemical state of creatine and its function in muscle.

Phosphocreatine:

In April 1927 Fiske and Subbarow announced that skeletal muscle contains an unstable compound of creatine and phosphoric acid which is broken down to creatine and inorganic phosphate during contraction, and is resynthesised during recovery. They concluded also, as Tieg's work had suggested, that its breakdown affects the buffering power of the tissue. They named this substance 'phosphocreatine'. At about the same time, Eggleton and Eggleton (1927.) demonstrated the presence of a very labile form of organic phosphate which they named 'phosphagen', and which they claimed played an important part in the chemistry of muscular contraction.

Subsequent papers by Fiske and Subbarow (1928, 1929) and by Eggleton and Eggleton (1928, 1929) announced the identity of phosphocreatine and phosphagen and established the chemical structure of the compound. Phosphocreatine is made up of one molecule of creatine and one of phosphoric acid. Its constitution is :-



Probably not all the creatine is present as phosphocreatine. Eggleton (1930) working on frog muscle found that about 20% is in the free state during rest and that stimulation to fatigue trebles the amount. According to White (1931) only about 10% of the total muscle creatine is present as phosphocreatine in the dogfish (Squalus sucklii).

The discovery of phosphocreatine in skeletal muscle was followed by the demonstration of its presence in cardiac muscle (Vollmer, 1929), in smooth muscle, testis and spleen of mammals and birds (Ferdman and Feinschmidt, 1928. and Zanghi, 1930), and in nervous tissue (Gerard and Tupikow, 1931). Its presence in all classes of vertebrates was established by the work of Clark Eggleton and Eggleton (1932), Duliere (1929), Ochoa Grande and Peraita (1932), Tagami (1930), and others. Kutscher and Ackermann (1931) and Lundsgaard (1931) have demonstrated that in invertebrates it is replaced by phosphoarginine.

Apparently phosphocreatine is present wherever creatine is, and represents the effective chemical state of the latter.

Thus was the chemical state of creatine established. The question now arose as to the exact significance of the breakdown of phosphocreatine during contraction. This was elucidated in an

/admirably

admirably executed series of experiments by Lundsgaard of Copenhagen (1930, 1931). He showed that muscle poisoned with iodoacetic acid may contract apparently perfectly well for a time with the breakdown of phosphocreatine, but without any lactic acid formation. Moreover he showed that if lactic acid cannot be produced phosphagen cannot be synthesised. He thus concluded that "Phosphagen is the substance directly supplying the energy for contraction, while lactic acid formation in the normal muscle continually provides the energy for its resynthesis".

Cameron (1933) states - "It is now possible to put forward a reasonable hypothesis for the mechanism of creatinine transformation. It can only be formed in muscle when creatine itself is free. This only occurs during muscular work. Even under neutral conditions the equilibrium Creatine ⇌ Creatinine is in the direction of creatinine formation. Any increase of acidity increases the rate of creatinine production. Every time a muscle contracts, therefore, a little creatine will be transformed into creatinine. This cannot be re-transformed to creatine but will be excreted from the muscle to form part of the urinary creatinine".

There would appear to be certain difficulties in accepting this simple conception in toto. Thus if transformation into creatinine only occurs during muscular work; then the daily excretion of creatinine ought to be far greater in a person

/doing

doing active exercise than in a person at rest. This is not the case. During and immediately after exercise there is an increased output of creatinine in urine but there is a corresponding drop subsequently, so that the daily excretion is but little affected. Therefore, while muscular exercise does appear to temporarily promote the formation of creatinine, another mechanism may be postulated whereby creatinine is continually being formed independently of exercise. The compensatory drop in creatinine following exercise may be explicable on the basis of an inhibition of this mechanism. A certain portion of the creatine in muscle appears to be present as free creatine, and this may be sufficient to account for the creatinine which is being continuously produced.

Buffering Action:

The hydrolysis of phosphocreatine liberates considerable base (Fiske and Sabbarow (1929); Mackler Olmsted and Simpson (1930); Meyerhof and Lipman (1930);) It thus has a buffering action as a result of which very little change in reaction occurs during the contraction and recovery phases.

Sugar Metabolism:

There is some evidence of a relation between creatine and sugar metabolism. Hill and Mattison (1929), and Peabody and Hill (1929) have shown that creatine administration produces hypoglycaemia in fasting dogs. Kopolowitz (1929-30,1,2)

/reports

reports that administration of a single big dose of creatine to diabetics, whose fasting level is 200 mgms. or more, reduces the blood sugar. Continued feeding with creatine ultimately results in a pronounced increase in blood sugar. Mystkowski (1932) has demonstrated that creatine retards and creatinine accelerates the enzymatic hydrolysis of soluble starch and glycogen.

Possible Effect on Circulatory System during Exercise:

Since phosphocreatine is broken down with the liberation of creatine during muscular contraction, it occurred to the writer that creatine might play a part in bringing about certain changes known to occur as a result of exercise; for example, local vasodilation in the muscles, and even perhaps more remote effects such as the increase in activity of the heart. The indications for an investigation of this problem are still further strengthened by the fact that up to the present these functions have been tentatively assigned to lactic acid. It has been shown, however, that during exercise lactic acid is liberated in quantities quite inadequate to produce the effects ascribed to it.

An investigation of the problem was thus undertaken and is reported in this thesis.

It is remarkable that creatine remained for so long a substance without any apparent function. The discovery of its important role in the organism lends a new and added

significance to all work on creatine.

THE ENDOCRINE CONTROL OF CREATINE-CREATININE METABOLISM:

It is only during the last few years that the relation of the internal secretions to the metabolism of creatine and creatinine has been actively investigated. Even as recently as the appearance of Hunter's monograph (1928) there was insufficient data available to warrant a separate section dealing with the influence of the endocrine organs. And no review of the literature on the subject appears to have been written as yet.

In view of these facts, and since investigations into the influence of certain endocrine organs on muscle creatine are reported in this thesis, a brief survey of our knowledge in regard to the relation of the glands of internal secretion to creatine-creatinine metabolism follows.

The effects of injection experiments are reported with a full sense of the limitation of the conclusions to be drawn therefrom. Swale Vincent (1927) says, "It is just as necessary to-day as it was some years ago to lay great stress on the fact that many, if not most, of the results obtained by the study of the action of tissue extracts are of purely pharmacodynamical interest". The action of an extract may be due to the mechanical and chemical manipulations employed in preparing the extract, and the experiment should, therefore,

/be

be rigidly controlled to show that similar extracts of other tissues do not produce the same effects. If the effect of such an extract is opposite to that brought by removal of the gland in question it may be considered to afford evidence of an endocrine relationship. Conclusive proof of a role in the economy of the organism would require a demonstration of the active substance in the blood or lymph leaving the gland.

The Adrenals:

The adrenal bodies have a more marked effect on creatine-creatinine metabolism than any of the other glands so far investigated. The discovery of the importance of phospho-creatine as a source of energy in muscular contraction naturally led to the suggestion that the myasthenia, which is one of the chief symptoms of Addison's disease and of adrenal in-sufficiency, may be due to interference with the phosphagen cycle. As a result, almost all the work relating to the effect of the adrenals on creatine-creatinine metabolism has dealt with their influence on muscle phosphagen. Muscle creatine and urinary creatinine have been relatively neglected.

(1) Adrenalectomy:

Lang (1931) found that after adrenalectomy in cats the phosphocreatine content of their muscles fell to about 33% of normal. Creatine and creatinine content showed no change.

The resynthesis of phosphagen is impaired as a result of the operation.

Ochoa and Grande (1932) working on guinea-pigs confirmed the decrease of phosphagen after adrenalectomy. Ochoa (1932) showed that muscles removed from amphibians a few days after destruction of the suprarenals were able to perform less work than those of control animals, and that this was associated with a decreased rate of phosphagen breakdown.

The decreased rate of phosphagen breakdown, and the diminished capacity of muscle to resynthesise phosphagen after adrenalectomy, have been recently confirmed by Cope Corkhill Marks and Ochoa (1934), and the diminution of phosphagen in adrenalectomised frogs by Maschini (1934,1.)

On the other hand Lundsgaard and Wilson (1934) were unable to obtain any significant alteration in the phosphagen content of cats' muscle after bilateral adrenalectomy, despite the fact that gross symptoms of muscular weakness were present. Their findings would seem to be outweighed, however, by the positive evidence of other workers.

There is no evidence as to whether the cortex, or medulla, or both, are responsible for the effects described.

No investigation into the effect of adrenalectomy on the excretion of creatine and creatinine appears to have been undertaken as yet.

(2) Injection of Adrenal Extracts and related Preparations:

(a) Effect on Phosphocreatine. - Contradictory results have been obtained with epinephrine injections. Feinschmidt and Ferdman (1929) found that the breakdown of phosphagen is increased. This would tally with the decrease in phosphagen breakdown obtained by Ochoa and co-workers after adrenalectomy, i.e., if it be assumed that the effect of extirpation on phosphagen is due to the deficiency of medulla rather than of cortex - such a mechanism is postulated by Akatsuka of Tokyo (1927).

Cori (1930) found that intravenous infusion of adrenaline produced no significant change in phosphagen content in rats. Despite the failure to demonstrate any increase in phosphagen on administering adrenaline, the possibility that it does do so is favoured by the fact that many cases of myasthenia gravis improve considerably on treatment with ephedrine.

The effect of an extract of the cortex of the gland has recently been investigated by Moschini (1934, 2.) He used Eucortone prepared by the firm of Allen & Hanbury according to the method of Swingle and Pfiffner (1930). Unfortunately the value of his experiments was greatly minimised by the fact that the majority of his frogs developed an infection. In the case of those which resisted infection, he obtained a rise of phosphocreatine of the order of 15% on injection into normal

/frogs.

frogs. Injection into suprarenalectomised frogs was not effective either in raising the phosphagen content of the muscle or in keeping the frogs alive.

(b) Effect on the Excretion of Creatinine - Tsuji (1915), Palladin and Tichwinskaja (1925) and Abderhalden and Buadze (1130) have shown that repeated injection of big doses of adrenaline results in a creatinuria and an increased output of creatinine. This increase in creatinine may indicate an increase in muscle creatine.

The influence of cortical extracts on creatinine excretion does not appear to have been investigated as yet but it is now being undertaken in this laboratory. Pugsley Anderson and Collys (1934) could not obtain any effect on the creatinine excretion in normal rats after the administration of the adrenotropic hormone.

To summarise: The available evidence indicates that absence of the adrenal decreases the phosphocreatine content of muscle. Whether this is due to the cortex or medulla or both is uncertain. There is some direct proof that Eucortone and some indirect proof that ephedrine may increase the creatine store in muscle. This would suggest that both the cortex and medulla play a part, but the evidence at the moment is far too scanty and unconfirmed to draw any conclusions.

The Thyroid.

Mainly because of the creatinuria which is known to occur in exopthalmic goitre (Shaffer 1907, 1908; Denis 1917, 1,2), the thyroid gland was one of the first endocrine organs whose relation to creatine-creatinine metabolism was investigated.

(1) Thyroidectomy.

Frontali (1913) found that total thyroidectomy is followed by marked creatinuria in dogs. This creatinuria appeared even before the animals refused food and was much more marked than that caused by fasting alone. Hunter (1914) independently made similar observations in sheep. The creatinuria is associated with, and probably due to, a diminution in the creatine content of the muscles.

(2) Experimental Hypothyroidism; and Exopthalmic Goitre:

It has been noted that there is a creatinuria associated with exopthalmic goitre (Shaffer 1907, Denis 1917). A similar creatinuria has been repeatedly induced experimentally by feeding of thyroid preparations (Krause and Cramer 1912; Cramer and Krause 1913; Beumer and Iseke 1920; Gross and Steenbock 1921; Eimer 1931).

Pugsley Anderson and Collip (1934) have demonstrated an increase in creatine excretion in normal and hypophysectomised rats following administration of thyreotropic hormone.

Abelin and Spichten (1930) found a decrease in muscle

/creatine

creatine following the administration of thyroid to rats.

We thus have the curious anomaly that, no matter whether a state of hyperthyroidism or of hypothyroidism be induced, a decrease in muscle creatine with an associated creatinuria results.

Shorr et al. (1933) have suggested without advancing any material evidence to prove it, that the thyroid hormone is involved in the breakdown and building up of phosphocreatine.

Stuber Russman and Proebsting (1923) claim that injection of glycocyamine into normal rabbits is followed within five minutes by an increase of preformed creatinine in the blood. No such effect is obtained in animals deprived of their thyroid. They conclude that the capacity to methylate glycocyamine is dependent on the activity of the thyroid. The significance of this experiment is dependent upon the very doubtful value of the blood creatinine estimations.

The Parathyroids:

Creatine:

According to Noel Paton and Findlay (1916, 1.2.) and Noel Paton (1919, 1925), the highly toxic substances guanidine and methyl guanidine are normally produced in the body but are converted into inert creatine under the influence of the parathyroids. On removal of the parathyroids the tetany which develops is due to the accumulation of guanidine and methyl guanidine.

This view has not, however, met with general acceptance. The method whereby they demonstrated an increase of methylguanidine in blood and urine during tetany has been severely criticised. Moreover, instead of the predicted decrease in muscle creatine following parathyroidectomy, Henderson (1918) and Palladin and Griliches (1924) have demonstrated an increase.

Phosphocreatine:

The influence of the parathyroid on the metabolism of creatine and phosphoric acid has been recently investigated by Brown and Imrie (1932) and by Imrie and Jenkinson (1932, 1933).

(a) Injection of Parathormone - The administration of creatine to cats is followed by a temporary reduction in the output of phosphates by the kidney. This is probably due to the administered creatine combining with phosphoric acid and being retained in the muscles as creatine phosphate. If parathyroid extract be administered some time before the injection of creatine there is a still more marked decrease in the excretion of phosphates and a more marked increase in phosphagen than when creatine is given alone.

(b). Parathyroidectomy. - Following thyroparathyroidectomy the creatine phosphate in the muscles tends to be low and is raised to normal by treatment with parathormone.

The rate of resynthesis of creatine phosphate following stimulation of the muscles is much slower in thyroparathyroidectomised

/animals.

animals. Administration of parathormone restores the rate of resynthesis to normal.

These experiments indicate that the parathyroid glands are concerned with the metabolism of creatine phosphate in the muscles and that the active principle is contained in parathormone.

It is not clear how the increase in creatine following parathyroidectomy (Henderson, Griliches) is to be interpreted in relation to the slight decrease in phosphocreatine following thyroparathyroidectomy (Imrie and co-workers). It cannot be attributed to the additional removal of the thyroid in the latter case because injection of parathormone causes a return to normal.

The Pancreas:

Krause and Cramer (1910) showed that a creatinuria is present in diabetes mellitus and it has since then been repeatedly confirmed.

Corbia (1928) found that complete extirpation produces a similar marked increase in excretion of creatine. This he believes is due not so much to the absence of insulin as to the absence of the external secretion of the pancreas, which results in almost complete inhibition of absorption of carbohydrates, fats and proteins and, therefore, to increased tissue breakdown. That the creatinuria is in some part due to insulin deficiency, however, is shown by the fact that on injecting insulin into

/pancreatectomise

pancreatectomised animals he found that the excretion of creatine was diminished.

Conversely, Kopolowitz (1930) found that injection of insulin in diabetics produces a fall in the creatine content of whole venous blood.

It thus seems fairly definitely established that a deficiency of insulin produces a creatinuria which can be counteracted by insulin injection. Whether this creatinuria is associated with a decrease in muscle creatine has not yet been established. Contrary to what might be expected, Duliere (1928) obtained a diminution of phosphagen in muscle after injection of insulin; though Moschini (1932) could not observe any change.

The Gonads and the Pituitary:

The Gonads - Krause and Cramer (1911) and Krause (1911) were the first to point out that, in contrast to normal men, normal females on a creatine-free diet excrete intermittently small quantities of creatine. This appears to be associated with the relatively poor development of the female sex. Read (1921) found that in eunuchs castrated before puberty a creatinuria is present and he associates this with the fact that early castration confers on the male the configuration of the female. Castration at an age when the secondary male characters are already fully developed does not result in the

/excretion

excretion of creatine in the urine. During pregnancy the intermittent creatinuria of the normal female becomes a continuous one. After delivery the output of creatine rises above the level even of pregnancy.

The explanation of these phenomena is still obscure, but they have promoted an interest in the possible influence of the gonads on creatine metabolism.

Tsun-Chee Shen (1927) failed to show any effect of castration on creatine metabolism in dogs or albino rats. On the other hand, Schrire and Zwarenstein (1932. 1, 2), in this laboratory, obtained a 16-50% increase in the excretion of creatinine in castrated male and female rabbits. Injection of testicular extract or grafting of testes into castrated males, and injection of ovarian extract into gonadectomised females, caused the urinary creatinine to fall to the normal level.

The Pituitary:

Schrire and Zwarenstein suggest that the increased excretion of creatinine following castration is due to a concomitant hyperactivity of the anterior lobe of the pituitary. In support of this hypothesis they have demonstrated that injections of anterior lobe extracts into normal male and female rabbits produces a transient increase in the excretion of urinary creatinine (1933). This accords with the results of Braier (1931), who showed that hypophysectomised dogs on a complete diet excrete in the urine 29% less creatinine than the controls.

In fasting animals removal of the pituitary led to a 35-40% decrease.

Creatinuria of Pregnancy. - On the basis of these experiments Schrire and Zwarenstein (1934) have advanced an interesting hypothesis to explain the creatinuria of pregnancy. They make the assumption that the transformation of creatine and creatinine in the muscles is controlled by the gonads which inhibit the formation of creatinine. In pregnancy functional hypertrophy of the anterior lobe of the pituitary occurs. This leads (a) to an increased formation of creatine and (b) to stimulation of the gonads. The latter factor increases the inhibitory action of the gonads on the creatine-creatinine change with the result that some of the excess creatine would appear in the urine as such and some would appear in the form of increased creatinine. This accords with the effects obtained with hypertrophy of the anterior lobe in acromegaly. They suggest that in pregnancy the corpus luteum reinforces the inhibitory action of the ovary on the transformation of creatine to creatinine so that all the excess creatine is excreted as such and the creatinine level remains unchanged.

As the authors admit, this theory rests only partly on experimental evidence. It involves, however, at least one serious contradiction with established fact. If the presence of the corpus luteum is a factor in causing the creatinuria then the

excretion of c re atine should be maximal in the early months of pregnancy when the influence of the corpus luteum is greatest. In the latter months of pregnancy, with the involution of the corpus luteum the creatinuria should diminish. Instead von Hoogenhuyze (1915) found that during the first month the creatine formed 5.8% of the total creatinine, during the third month it rose to 18.9% and remained at about this level until delivery. After delivery the output of creatine rises still further and remains high for about two weeks. Of course it is possible that the post partum increase is due to causes other than the endocrine factors postulated to be operating in pregnancy. But even then the maintained creatinuria of the latter months of pregnancy is in conflict with the involution of the corpus luteum during this period.

Despite this objection the interpretation of the creatinuria of pregnancy as due to endocrine factors is of definite interest and is an indication of the lines along which future enquiry may be profitably based.

Failing Sex Function and Creatine Tolerance - Remen (1932, 1) found that intravenous injection of 500 mg. creatine into normal adult men between the ages of 20 and 53 is followed by no greater excretion of creatine in urine than before injection. Old men (7?-90 years of age) with failing sex function, and a eunuch, showed a decreased tolerance typical of infants and children. Remen concluded that the male sex glands have a

/regulatory

regulatory influence on the metabolism of creatine. Shortly after the same observer (1932, 2) demonstrated that women with failing ovarian function also show a decreased tolerance to creatine. Losch (1932) and Buhler (1933) have come to similar conclusions; and Schrire & Zwarenstein (1933) have found that castrated rabbits also show a low tolerance to subcutaneous injections of large amounts of creatine. Moreover, Buhler (1933) has shown that the injection of Proviron into males, and of Progyron into females, counteracted the effects of loss of sexual function in relation to creatine tolerance.

All these experiments reveal fairly conclusive evidence of a relation between the gonads and the excretion of creatine and creatinine. But no investigation into the influence of the gonads on the creatine content of muscles appears to have been undertaken as yet. It was, therefore, decided to investigate the effect of gonadectomy on muscle creatine.

Moreover, the influence of the pituitary on urinary creatinine as indicated by the work of Braier, and of Schrire and Zwarenstein, make it probable that (a) the hypophysis may affect the muscle creatine, and also that (b) any effect on muscle creatine caused by gonadectomy might be an indirect one through the anterior lobe of the pituitary. The influence of the latter gland on muscle creatine was, therefore, investigated at the same time as that of the gonads.

The scope of the work was extended to include also an investigation of the role, if any, of the posterior lobe. No work appears to have been done on the relation of the posterior lobe to muscle creatine. The only reference connecting the posterior lobe with any aspect of creatine-creatinine metabolism is that of Roux and Tallandier (1914), who claim to have noticed an increase of creatinine in the urine of rabbits which were injected subcutaneously with posterior lobe extract.

THE RELATION OF THE GONADS AND THE PITUITARY GLAND

TO MUSCLE CREATINE

IN THE SOUTH AFRICAN CLAWED TOAD.

THE RELATION OF THE GONADS AND THE PITUITARY GLAND TO MUSCLE CREATINE IN THE SOUTH AFRICAN CLAWED TOAD.

The South African Clawed Toad, Xenopus laevis, was chosen as the experimental animal because of its remarkable ability to withstand severe operative procedures.

During recent years it has been the subject of a growing body of physiological inquiries, including Jolly's researches on reflex action, Hogben's work on colour response and the investigations of Shapiro and Zwarenstein on calcium metabolism.

While removal of the pituitary gland in mammals is an operation of great difficulty and is associated with a high mortality rate, in Xenopus laevis it is performed with ease, the mortality is low, and the animals survive the operation for a year and more. Moreover, the characteristic colour response of the animal serves as a useful and clear-cut indication of the completeness of removal of either one or both lobes of the pituitary (Hogben and Slome, 1931).

It was planned to conduct the research in two stages:

1. Excision Experiments.

Removal of (a) Gonads, (b) Anterior lobe of the pituitary, (c) Both lobes of the pituitary. These operations were performed at approximately the same time in order to permit a comparison of the time taken for the effects, if any, to manifest themselves.

2. Injection Experiments.

Where its removal indicated that a gland had any influence on muscle creatine extracts of that gland were injected. If the effect of injection were opposite to that of removal it would provide evidence of an endocrine relationship between the glands concerned and muscle creatine.

(1) THE EFFECT OF CASTRATION AND OF HYPOPHYSECTOMY.

EXPERIMENTAL METHOD:

Some preliminary experiments were performed in the first half of 1933, the results of which indicated that hypophysectomy does affect the muscle creatine. A more thorough investigation was then undertaken.

In January, 1934, several hundred animals were collected and kept in large open air tanks in the laboratory.

Muscle creatine was estimated in animals fresh from the pond in order to determine (a) the normal concentration in males and in females and (b) to ascertain if any difference exists between the creatine content of corresponding muscles of the right and left hind limbs.

These estimations were necessitated by the fact that the creatine content of the muscles of Xenopus laevis has not been investigated previously.

During the same period as the above determinations were being made the anterior lobe of the pituitary was removed from

/about

about fifty animals, a half of them males and a half females. A similar batch was totally hypophysectomised. Another thirty animals were ovariectomised and a similar number had their testes removed. A batch of unoperated animals was kept under the same conditions as the operated animals and served as "captivity controls". It is important to control carefully the effect of captivity because, in the course of another series of experiments conducted in this laboratory, a progressive involution of the ovaries according to the length of captivity has been demonstrated (Zwarenstein and Shapiro, H., 1933). The histological changes associated therewith are described in a paper by Shapiro, B.G., and Shapiro, H.A., (1934), which is submitted together with this thesis, and in which it is suggested that the involution of the ovaries may be associated with a diminution of pituitary function in captive animals.

In addition to the captivity controls the operative procedure itself was controlled. As a control for the hypophysectomy operation a small hole was drilled into the roof of the mouth posterior to the site of operation for the actual removal of the pituitary lobes. Gonadectomy was controlled by performing the operation to be described for castration but excising a portion of muscle from the posterior abdominal wall instead of the gonads. The operative controls for gonadectomy unfortunately escaped from their tank one night. However, the seriousness of this mishap was somewhat mitigated by the fact that

/castration

castration was subsequently found not to have any effect on the muscle creatine.

The muscle creatine was estimated (a) 6 to 10 weeks and (b) 18 to 22 weeks after operation.

Method of Castration:

The animal is anaesthetised by placing it in a closed vessel containing a solution of ether and water of about 1 per cent dilution. When the animal ceases to make any movements it is floated on its back. If not adequately anaesthetised it will attempt to turn onto its ventral aspect. With a satisfactory depth of anaesthesia it will remain completely inert even when lying on its back.

A skin incision of about $\frac{3}{4}$" in length is made in the mid-abdominal region. The abdominal wall is divided by means of a paramedian incision half an inch lateral to the mid-line, and the opening is maintained by means of a small retractor. The ovary, or the testis, is gently drawn out and its mesenteric attachment to the ventral aspect of the kidneys, defined. The organ is severed from its attachment by means of an electric cautery, care being taken not to remove the fat bodies. The muscles are sutured with fine catgut. Cotton serves satisfactorily for the skin sutures. The animals are then placed in a sink kept damp by means of running water. After a few days they are transferred to tanks with a sufficient depth of

/water

water to enable them to swim.

Ovariectomy is associated with a considerable mortality (about 25 per cent). Asepsis is quite unnecessary. In removing the testes and particularly in hypophysectomy the mortality is negligible despite complete absence of aseptic precautions.

Method of Hypophysectomy:

The operation was performed according to the method of Hogben (1923). The anaesthetised toad is placed on its back and its jaws kept open with the aid of a forceps. A small portion of the roof of the mouth, about $\frac{1}{4}$ inch in diameter and situated just anterior to the eustachian orifice, is drilled away by means of a dental burr. A thin plate of bone overlying the hypophysis is revealed. This plate is raised by means of a sharp blade and the pituitary gland exposed. The anterior lobe lies in immediate contact with the bone and the posterior lobe is situated behind and above the anterior lobe. The lobes are sucked off with the aid of a finely pointed cannula attached to a water suction pump. A lesser degree of suction is used for aspirating off the anterior as compared with that required for the removal of both lobes.

The term anterior lobe is here used to indicate both the pars anterior and the pars tuberalis.

The success of the operation is determined by the characteristic colour-response. A totally hypophysectomised animal

/should

should remain pale on a dark background. In animals with the anterior lobe alone removed there should be maximal expansion of the melanophores even on a white background.

The Estimation of Creatine in Muscle:

The toad is pithed. In order to obtain muscle as free of blood as possible the sternum and xiphisternum are removed, the exposed heart is snipped with a scissors and the animal is bled. The skin is stripped from the hind limbs and the hamstring muscles are removed. The nerves and larger blood vessels which lie on the deeper aspect of the muscle mass are carefully dissected away.

The creatine content of the muscle was estimated according to the method of Rose, Helmer and Chanutin (1927). Approximately 1 g. of muscle is cut up as finely as possible and dropped into weighed 50 ml. Erlenmeyer flasks. These are tightly stoppered to prevent evaporation. The flask with contents is weighed to determine the weight of muscle used. 20 ml. of 2 N. sulphuric acid is then added, the flasks are covered with tin-foil and heated for 45 minutes in an autoclave at 15 pounds pressure. In this way the muscle is disintegrated and the creatine converted into creatinine. The solution is then allowed to cool and is transferred to a 100 ml. volumetric flask using 40 - 50 ml. of distilled water. 18 ml. of 2 N sodium hydroxide is added, and the mixture is then clarified by means of 5 ml. of 10 per cent sodium tungstate. Distilled water is added to make up to 100

/ml.

ml. The contents are then shaken, allowed to stand for 5 minutes and filtered. Alkaline picrate is freshly prepared by mixing 25 ml. of a saturated solution of purified picric acid and 5 ml. of 10 per cent sodium hydroxide. 5 ml. of the alkaline picrate is added to 10 ml. of the clear filtrate. At the same time 20 ml. of a standard creatinine solution containing 40 mg. of creatinine per 1000 ml. is treated with 10 ml. of the alkaline picrate. The colour comparison is made by means of a colorimeter, the standard being set at the 20 mm. mark.

Throughout these experiments the standard was set at 20 mm., but the amount of muscle used was varied so as to maintain the reading of the unknown at approximately the same level. For example when, in the course of the work, it became evident that hypophysectomy produced a decrease in muscle creatine, the amount of muscle used was correspondingly increased.

Purification of Picric Acid:

It is essential that the picric acid used for the estimation of muscle creatine should be pure. The method of purification used was the sodium picrate method of Benedict (1929).

Three litres of water are heated to boiling point in a large porcelain dish. 125 g. of anhydrous sodium carbonate is added and as soon as this has dissolved 250 g. of moist picric acid is gradually poured in. The solution is allowed to stand for a few minutes and is then decanted from any dirt at the bottom. It is allowed to stand overnight and in the morning the crystallised

/sodium

sodium picrate is filtered off on a hardened filter in a large Buchner funnel. The picrate is sucked dry on a filter, washed with a litre of 10 per cent sodium chloride solution and again sucked dry. 250 ml. of 20 per cent HCL is added to the precipitate, the mixture thoroughly stirred, and the acid then sucked into a receiving flask. This process is repeated with three more portions of hydrochloric acid, and then with about a litre of cold distilled water. The picric acid is then removed from the filter, dried in an oven at about 90°C. and powdered. The product should read 13.5 to 14 mm. by the Folin Doisy Test (1917). Hunter and Campbell (1916) have drawn attention to the fact that picric acid solutions, if exposed to the action of light, come to contain a substance which gives a deep red colour on treatment with alkali and so is liable to render the estimation of small quantities of creatinine inaccurate. The powdered picric acid and the picric acid solutions were, therefore, kept in amber-coloured bottles placed in the dark.

EXPERIMENTAL RESULTS:

In the following tables the figures for muscle creatine are expressed as mg. total creatinine per 100 g. fresh tissue. Total creatinine includes preformed creatinine which, however, does not exceed a few mgm. in muscle.

(a) Normal Muscle Creatine.

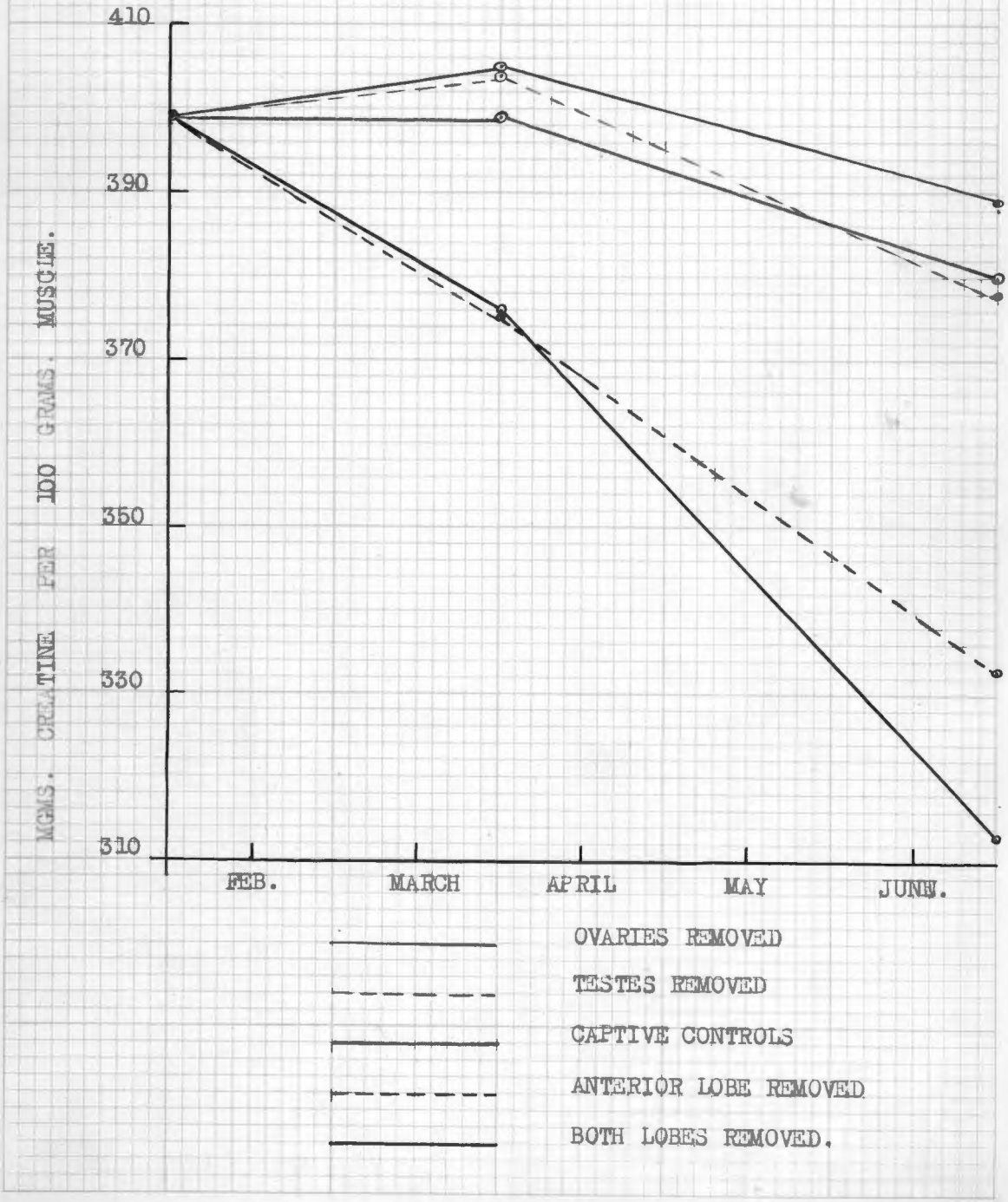
TABLE 1.

MUSCLE CREATINE IN NORMAL POND ANIMALS (January, 1934).

MALES			FEMALES.		
Right Leg	Left Leg	Av.	Right Leg	Left Leg	Av.
418	410	414	435	435	435
420	407	413	415	391	403
409	387	398	421	407	414
343	355	349	391	404	397
415	409	412	388	408	398
404	394	399	415	402	408
428	453	440	401	381	391
420	422	421	407	409	408
394	413	404	398	402	400
380	378	379	408	433	420
424	406	415	371	397	384
378	385	381	385	366	376
432	420	426	359	363	361
382	391	386	358	374	366
409	426	417	389	368	378
376	396	386	396	396	396
402±24	403±22		396±21	396±22	

Reference to Table I. shows that on averaging the values obtained for the right and left legs there is no significant difference between the two sides.

GRAPH SHOWING THE CREATINE CONTENT OF MUSCLE AFTER CASTRATION AND HYPOPHYSECTOMY.



The other two explanations depend on the possibility that the difference between the results in rabbits and in Xenopus is due to the species difference.

Thus in Xenopus the effect of castration may take longer than 22 weeks to manifest itself. In the case of female rabbits nine months did actually elapse before Schrire and Zwarenstein noted any increase in the excretion of creatinine, although in male rabbits the latent period was only one to three months. The experiments on the toads was not prolonged beyond 5½ months because the castrated animals retrogressed very severely after that period.

A third possibility is that the testes and ovaries in the South African Clawed toad, unlike the rabbit, may have no influence at all on creatine-creatinine metabolism. In view of the relation shown in this thesis to exist between the pituitary and muscle creatine this would imply that castration in Xenopus laevis does not influence the pituitary, or, more correctly, the factor in the pituitary which is related to creatine metabolism - despite the fact that evidence for such a mechanism in the rabbit has been advanced by Schrire and Zwarenstein. Hypertrophy of the anterior lobe after castration in either sex is a well-established fact in mammals. But no similar effect has been demonstrated in Xenopus as yet. The great dissimilarity between the histological structure of the ovary in the rabbit

/and

The difference obtained between the two legs of any individual toad indicates the range of experimental error, the main source of which lies in the difficulty of reading the colorimeter to a greater degree of accuracy.

The average of right and of left legs taken together is 402 mg. creatinine per 100 g. muscle in males, and in females is 396 mg. per 100 g., so that there is no significant sexual difference.

It was also noted that the concentration of creatine in the muscle bears no relation to the size of the animal even where the weight of one toad was two or three times that of another.

The average, inclusive of right and left legs of both sexes is 399 $\pm$ 21. mg. creatinine per 100 g. muscle. This figure, based on sixty-four determinations, represents the average normal creatine content of the hamstring muscles of Xenopus laevis.

Taking the average of the right and the left leg of any individual toad as the true value for that animal, the muscle creatine of the toads was found to range between 349 and 440 mg. per 100 g. About 80 per cent of the toads gave values ranging between 375 and 425 mg. per 100 g.

(b) Hypophysectomy and Castration:

During 1933 the muscle creatine was estimated in a number of toads which had been hypophysectomised 6 months previously. This preliminary experiment showed that hypophysectomy produced

/a

and in Xenopus laevis (see appended reprint Shapiro and Shapiro, 1934) makes the possibility of some difference in function not at all surprising. When the muscle creatine of toads fresh from the pond was being investigated the ovaries were examined in every case. It was noted that in a small percentage of animals there was marked regression of the ovaries. Despite this fact their muscle creatine was normal. This would afford support for the view that there is no relation between the ovaries and muscle creatine in Xenopus. It would be interesting to investigate whether castration produces any change in the muscle creatine in the rabbit, since the effect of castration on urinary creatinine in that animal makes it very probable that the muscle creatine is influenced too. But to the best of the author's knowledge no other worker has yet investigated the effect of castration on muscle creatine either in the amphibian or in mammals.

Hypophysectomy.

An examination of Table III. reveals the fact that the lowest value obtained for removal of both lobes is no lower than for removal of anterior lobe alone. This suggests that the effect of total hypophysectomy can be explained as solely due to the loss of the anterior lobe. On the other hand, the upper limit of the anterior lobe animals shows a tendency to be higher than is the case when both lobes are removed, though the figures are too few to enable one to state whether this tendency is significant.

It may be due to the fact that some regeneration is known to occur when the anterior lobe alone is removed. It has been mentioned that the term "anterior lobe" is employed in this thesis to include both the pars anterior and the pars tuberalis. In Xenopus the pars tuberalis is attached to the superior margin of the pars anterior and is inserted by a tapering process into the cleft extremity of the tuber cinereum. It is usually removed more or less completely with the anterior lobe, but sometimes a part of the pars tuberalis may remain attached to the tuber cinereum and may regenerate (Hogben and Slome, 1931; Rimer 1932).

The significance of the long latent period which elapses before the effect of hypophysectomy becomes manifest will be discussed at a later stage.

While the above experiments were in progress a report appeared of some investigations, conducted by Marenzi in the Argentine, on the phosphagen content of muscles in bufo arenarum after hypophysectomy. The Revista de la Sociedad Argentina de Biologica, in which the report appeared, is not obtainable in South African libraries and it has been necessary to rely on the Physiological Abstracts (1934). Marenzi obtained a decrease in phosphocreatine of 33 per cent following removal of the anterior lobe, as compared with a 15% decrease in creatine here reported. This may be accounted for by the difference in species and by the

/fact

that in these experiments the total creatine was estimated, whereas Marenzi only investigated the phosphacreatine fraction.

The next step in establishing a relationship between the pituitary and muscle creatine was to investigate the effect of injecting extracts of the gland. By injecting anterior lobe extracts and posterior lobe extracts, the suggestion that the decrease in muscle creatine following hypophysectomy is probably due to the removal of the anterior lobe, and that the posterior lobe plays no part, could be tested. If this be true extracts of anterior lobe but not of posterior lobe should elevate the muscle creatine.

In view of the failure to obtain a change after castration the injection of gonadal extracts was not undertaken.

II. THE INJECTION OF (a) ANTERIOR LOBE EXTRACT AND (b) PITUITRIN.

PITUITARY EXTRACTS USED:

The extract of anterior lobe used was prepared according to the method of Bellerby (1933). Oviposition is induced in the South African Clawed Toad by the preparation, and this was used as an indication that an active extract had been injected. Thus, in these experiments ready criteria were available as to

the efficacy of the procedures adopted for total and partial hypophysectomy (colour response), and for injection of anterior lobe extracts (oviposition).

The Bellerby anterior lobe extract was prepared as follows:

The heads of freshly slaughtered sheep were brought from the abattoirs and the whole pituitary gland removed. The anterior lobe is then carefully dissected out and weighed. It is then chopped up finely and ground with an equal volume of sand which has been previously moistened and drained. Extraction is carried out by means of 1 per cent acetic acid, the amount added being equivalent to 1½ times the original weight of the tissue. This is left for 24 hours at room temperature. The mixture is then centrifuged, the supernatant fluid poured off and neutralised with 40 per cent NaOH until salmon pink to phenol red indicator. After neutralisation the fluid is centrifuged again to remove the slight precipitate which comes down at the neutral point. The supernatant fluid is stored in the refrigerator.

Control extracts of brain tissue were prepared in the same way.

By this method 1 ml. of brain or anterior lobe extract is equivalent to 640 mg. of fresh tissue.

"Pituitrin", prepared by Parke, Davis & Co., was used as the posterior lobe extract because the small size of this lobe in

/sheep

sheep made it impracticable to collect a sufficient amount for extraction by the method used for anterior lobe.

EXPERIMENTAL DATA:

Animals for injection experiments were collected in January, 1935.

Determinations were made in a series of normal animals and the results coincided with the figures obtained in January of the previous year, the average of right and left legs being 395 mg. per 100 g. muscle.

TABLE IV.

MUSCLE CREATINE IN NORMAL POND ANIMALS (Females). January, 1935.

Right Leg	Left Leg.
427	436
409	395
376	352
400	414
386	380
390	384
367	357
406	400
430	425
395	398
374	368
435	430
400	394
377	383
390	392
AVERAGE 397	394

The figures in Table IV. were obtained by estimating the muscle creatine in a normal toad on every occasion on which an injection experiment was performed. They thus served as a control in addition to the animals injected with brain extract. In interpreting Tables V., VI., VII. and VIII., the figures are to be compared with those of Table IV.

Injection of Anterior Lobe Extract into Normal Animals.

Only those animals which oviposited after injection with posterior lobe extract were used for muscle creatine determinations.

(a) Acute Series:

It was decided to first see if any effect was obtained with a single intraperitoneal injection of a large dose of extract, viz: 1 ml. 1 ml. of brain extract was injected as a control.

Twelve animals were injected with anterior lobe extract and a similar number with brain extract. Two animals from each set were killed off at intervals after injection and the muscle creatine determined. The muscles of the right and left legs were estimated in each animal. Each figure in the Table refers to the average value for the four hind limbs of two animals.

/TABLE V.

TABLE V.

Muscle Creatine after Injection of Anterior Lobe and Brain Extracts into Normal Animals. Acute Series.

TIME AFTER INJECTION.	BRAIN EXTRACT (1 ml.)	ANT. LOBE EXTRACT (1 ml.)
20 minutes	395	392
8 hours	372	383
16 "	410	422
18 "	412	436
21 "	415	408
48 "	350	368

All the figures fall within the normal range (c.f. Table IV.) so that acute injections have no effect. It was, therefore, decided to investigate the effect of chronic injections.

(b) Chronic Series:

(1) Animals were injected daily with 0.2 ml. each of anterior lobe extract and killed at intervals from 1 day up to 3 weeks after injection. A parallel series of brain extract injections (0.2 ml. each daily) served as controls. Each figure in Table VI. represents the muscle creatine value in a single animal.

/TABLE VI.

TABLE VI.

Muscle Creatine after Injection of Anterior Lobe and Brain Extracts into Normal Animals. Chronic Series, (1)

NUMBER OF DAYS AFTER INJECTION	BRAIN EXTRACT (0.2 ml.)	ANTERIOR LOBE EXTRACT (0.2 ml.)
1	396	425
3	364	408, 368
6	382	392
8	391	430, 425, 463
14	395	468,
21	411	496, 515.

Brain extracts had no effect (c.f. Table IV). Eight days after injection one toad gave a value of 463 mg. per 100 g., which is definitely above the upper limit of the normal range. Similarly, 14 days after injection. 21 days after injection both toads showed a definite rise; the one 27 per cent, the other 32 per cent, above the average value for the brain controls.

(2) The experiment was repeated, injection being continued up to 6 weeks on this occasion.

/TABLE VII.

TABLE VII.

Muscle Creatine after Injection of Anterior Lobe and Brain Extracts into Normal Animals. Chronic Series, (2).

NUMBER OF DAYS AFTER INJECTION	BRAIN EXTRACT (0.2 ml.)	ANTERIOR LOBE EXTRACT (0.2 ml.)
3	435, 412	397, 403.
8	380, 392	434, 405.
16	416, 385, 365	463, 473, 458.
25	427, 416	485, 458, 448.
32	397, 400.	529, 506.
39	403	463, 498.

Again brain extracts had no effect. 16 days after injection of anterior lobe extract an increase was manifest. The highest values occurred 32 days after injection, increases of 26 per cent and 31 per cent above the brain controls being obtained.

Injection of Anterior Lobe Extracts into Hypophysectomised Animals.

An experiment to investigate the effect of chronic injections of anterior pituitary extract into animals which had been totally hypophysectomised for 11 months was planned. Only a limited number of animals were available. 12 were injected with 0.2 ml. of brain extract daily and a similar number with 0.2 ml. anterior

/pituitary

pituitary extract.

Unfortunately a number of animals escaped, and in addition the hypophysectomised toads did not tolerate the injections well and several died. Of those injected with brain seven survived and were killed at varying intervals up to 32 days after injection commenced. Their muscle creatine averaged 326 mg. per cent. Of those injected with anterior pituitary extract five survived. The muscle creatine of two of these was estimated during the first eight days and showed no effect. The remaining three were estimated from 25 - 32 days after injections commenced. They gave values of 352, 360 and 386 mg. per cent, which values were all above the upper limit of the values obtained for brain injections.

These results indicate that anterior pituitary extract causes an increase in muscle creatine in hypophysectomised animals, but the experiment requires repetition on a larger scale before definite conclusions can be drawn.

Injection of Pituitrin.

28 normal animals were injected intraperitoneally with 1 ml. Parke Davis pituitrin. Animals were killed in batches of four at intervals from 1 - 30 hours after injection. Each figure in the table represents the average muscle creatine of four animals.

/TABLE VIII.

TABLE VIII.

Muscle Creatine after Injection of Parke, Davis Pituitrin.

NUMBER OF HOURS AFTER INJECTION	NORMAL CONTROLS	PITUITRIN (1 ml.)
1	371	363
3	394	402
5	397	388
10	393	343
16	392	341
24	403	341
30	400	324.

Pituitrin thus leads to a decrease in muscle creatine. This effect comes on between 5 and 10 hours after injection. 30 hours after injection the muscle creatine in 4 toads averaged 324 mg. per cent, i.e., 18 per cent below the normal level.

This result is not in harmony with the effects of hypophysectomy.

It has been noted that the effect of removing both lobes is not significantly different from that of removing the anterior lobe alone, suggesting that the effect of

/hypophysectomy

hypophysectomy is probably due solely to the removal of the anterior lobe. If this be the case injection of posterior lobe extract should have no influence on the muscle creatine.

But even supposing the suggestion that the higher value for removal of the anterior lobe is due to regeneration be dismissed, and the figures be taken to mean that the additional removal of posterior lobe causes a greater depression of the muscle creatine than when the anterior lobe alone is removed, it would then be expected that the injection of posterior lobe extract would cause a rise in muscle creatine.

Thus either no effect or the remote possibility of rise might have been predicted on injection of a posterior lobe extract. The decrease in muscle creatine after pituitrin injection would only have been compatible with a hormonal function if total hypophysectomy had resulted in a higher creatine value than removal of anterior lobe alone. Thus the fall obtained with pituitrin is in direct conflict with the results of hypophysectomy and, therefore, no conclusions can be drawn regarding the role of the posterior lobe. The result obtained is possibly an extraneous effect due to the method of extraction used in preparing the pituitrin. It was not possible to control this owing to the fact that it is a proprietary preparation and the exact method of extraction is not known to us.

Mechanism of the Decrease in Muscle Creatine following Injection of Pituitrin.

It was noticed that the injection of pituitrin was followed by an oedema in the subcutaneous spaces. It is thus possible that the fall in concentration of muscle creatine may be not an absolute but a relative fall, owing to the absorption of water from the surrounding medium giving rise to an increase in weight of the muscle.

An experiment was conducted along the lines described by Steggerda and Essex (1934) to investigate whether, in addition to the subcutaneous spaces, the body tissues take up water as a result of the injection of pituitrin.

Drainage of the subcutaneous spaces along the sides, back and legs of 12 toads was effected by incisions in the skin. Six animals received intraperitoneal injections of pituitrin and six controls received intraperitoneal injection of frog Ringer Solution. The toads were weighed at intervals after injection. Since the subcutaneous spaces were drained any increase in weight would be due to fluid actually taken up by the body tissues.

The results obtained were as follows:

(The figures represent the weight in grams of toads at various intervals after injection).

/TABLE IX.

TABLE IX.

WEIGHT OF TOADS AFTER INJECTION WITH PARKE DAVIS PITUITRIN.

(1) Injected with Pituitrin (1 ml.)

No. of Frog	10.30 am. (Initial Weight).	11.30 am. (1 Hr.)	1.30 pm. (3 Hrs).	3.30 pm. (5 Hrs.)	5.30 pm. (7 Hrs.)	8.30 pm. (10 Hrs.)
1	39.7	41.2	43.2	44.5	46.0	46.7
2	46.8	47.2	48.7	48.7	50.2	50.2
3	36.0	37.2	36.8	36.0	36.0	36.6
4	39.1	38.2	39.4	39.7	39.0	39.0
5	76.0	78.7	79.0	78.7	78.0	79.6
6	58.6	59.7	60.5	60.2	60.7	62.6
AVERAGE WEIGHT	49.3	50.4	51.3	51.3	51.6	52.5
PERCENTAGE VARIATION FROM INITIAL WEIGHT		+ 2.2%	+ 4%	+ 4%	+ 4.6%	+ 6.4%

(2) Controls injected with Saline (1 ml.)

No. of Frog	10.30 am.	11.30 am.	1.30 pm.	3.30 pm.	5.30 pm.	8.30 pm.
1	53.6	53.5	53.1	52.5	52.2	52.0
2	48.5	49.5	49.8	49.5	49.3	50.1
3	34.0	34.4	34.6	34.2	33.0	33.9
4	50.5	50.5	50.1	49.0	48.5	49.9
5	46.7	46.7	47.4	47.0	46.2	46.5
6	52.5	52.2	52.6	52.3	51.5	52.5
AVERAGE WEIGHT	47.6	47.8	47.9	47.4	46.8	47.5
PERCENTAGE VARIATION FROM INITIAL WEIGHT		+ 0.4%	+ 0.6%	-0.4%	- 1.6%	- 0.2%
% INC. IN WEIGHT OF TOADS INJECTED WITH PITUITRIN AS COMPARED WITH 'SALINE CONTROLS'		1.8%	3.4%	4.4%	6.2%	6.2%

It will be seen that an hour after injection of pituitrin there was an average increase of 1.8 per cent in body weight as compared with the control series. Seven hours after injection the increase had reached 6.2 per cent. Since a large portion of the body weight is comprised of material which cannot absorb fluid to any degree such as bone and cartilage, most of the fluid is absorbed by muscle, so that the percentage increase in weight of muscle is much greater than indicated by the above figures.

Since the muscle does evidently absorb water following injection of pituitrin the decrease in the concentration of muscle creatine must be due, at least partly, to this absorption of fluid. It will be noticed that the absorption of fluid commences almost immediately after injection whereas the fall in muscle creatine can only be definitely recognised between 5 and 10 hours after injection. This may be to some extent due to the fact that owing to the large individual variations in the muscle creatine of toads, and to the fairly considerable experimental error in reading the colorimeter, it requires a very definite fall in the concentration of muscle creatine to be recognised.

DISCUSSION:

Role of the Anterior Lobe.

The removal of the anterior lobe influences the concentration

of creatine in muscle but very slowly. 1½ to 2½ months after operation no significant change had yet appeared. Furthermore, acute injection of a large dose (1 ml.) did not affect the muscle creatine, whereas chronic injections of a smaller dose (0.2 ml.) produced a rise in creatine 8 - 16 days after injection.

These facts are suggestive of an endocrine relationship between the anterior lobe of the pituitary and muscle creatine; and the long latent period intervening before the effects of hypophysectomy and injection appear, indicates that the relationship is probably an indirect one through some other endocrine organ. The existence of endocrine inter-relationships has now been firmly established. There is, however, a tendency to let the imagination run riot when writing on this subject, giving the impression that every endocrine organ can affect every other one. This assumption should not be made and only those inter-relationships should be accepted which have been demonstrated experimentally or clinically to exist. The pituitary body, however, has been shown to have a profound effect on most of the other glands of internal secretion. Thus recent work, ably summarised by Collip (1935) has revealed the existence of gonadotropic, insulotropic, thyrotropic, parathyrotropic and adrenotropic hormones in the anterior pituitary so that, in suggesting that the effect of the anterior lobe on muscle

/creatine

creatine is an indirect one through some other organ, all the above-mentioned glands must be considered.

The possibility that the anterior lobe affects muscle creatine through the gonads is discounted by the fact that in Xenopus laevis no relation between the gonads and muscle creatine was demonstrable. In considering other glands it is not possible to exclude dogmatically any particular one, especially as the scantiness of the data at present available makes it necessary to utilise results obtained on one species of animal when considering the significance of effects obtained in a different species. This is not satisfactory but is unavoidable since no work on creatine metabolism in Xenopus has been done previously. An attempt is merely made to consider which endocrine organs are more likely and which less likely to be the gland or glands concerned.

The insulotropic, thyrotropic, parathyrotropic and adrenotropic hormones have each been demonstrated to produce hypertrophy in the gland indicated by its respective name. There is evidence to show that this hypertrophy is associated with hypersecretion. Now removal of the anterior lobe produces a decrease in muscle creatine (and according to Braier a decrease in urinary creatinine in the dog). Injection of anterior lobe extract increases the muscle creatine (and according to Schrire and Zwarenstein it also increases the

/urinary

urinary creatinine of the rabbit). Therefore, if the action of the anterior lobe is an indirect one through one or other of these glands the removal of the gland concerned should produce a fall in muscle creatine and urinary creatinine, and its injection should cause a rise in both. The glands which may be concerned will now be examined according to these criteria.

As far as the pancreas is concerned the available evidence indicates that insulin does not increase muscle creatine and produces either no effect on or a decrease in phosphagen. Similarly after extirpation of the pancreas no decrease in the creatinine in the urine, only a creatinuria, has been demonstrated. Thus both the effects of extirpation and of injection make it unlikely that the pancreas is the gland through which the anterior lobe of the pituitary acts.

The thyroid appears unlikely to be concerned since administration of thyroid produces not an increase but a decrease in muscle creatine (Abelin and Spichtin, 1930).

Similarly the parathyroid must be excluded on the grounds that its removal does not produce a fall but a rise in muscle creatine.

There is both clinical and experimental evidence that the anterior pituitary influences the adrenal cortex. (No definite effect on the adrenal medulla is described following hypophysectomy, injections or implants). Adrenalectomy produces a marked

/decrease

decrease in phosphocreatine in muscle, though nothing definite can be said of the muscle creatine. Injection of Eucortone has been demonstrated by Moschini (1934) to produce an increase in phosphocreatine.

It is, therefore, possible that the removal of the pituitary exerts its effect on muscle creatine by inhibiting the action of the adrenal cortex, and injection of anterior lobe extract by producing hypersecretion on the part of the adrenal cortex.

This is merely a suggestion based on admittedly scanty evidence. It is being tested in this laboratory at present.

It is interesting to note that although in one set of experiments frogs were injected with anterior pituitary extract for 3 weeks, and in another set of experiments for over 5 weeks, no antihormonic effect was obtained. Collip and Anderson (1935) have shown that continued injection of anterior pituitary extract containing thyrotropic hormone results in the formation of an anti-hormone which antagonises the effect of the thyrotropic hormone within 2 - 3 weeks. There is also evidence of an anti-parathyrotropic hormone. The fact that no antihormone effect on the muscle creatine was manifest even after 5 weeks affords additional evidence that the action of the anterior pituitary extract on muscle creatine is probably not through the thyroid or parathyroid.

Role of the Posterior Lobe.

The results obtained do not suggest that the posterior lobe has any influence on the muscle creatine. The effect of injecting pituitrin is not opposite to that of hypophysectomy and, therefore, in all probability does not represent a function of the secretion of the posterior lobe. The results obtained with pituitrin demonstrate the importance of not accepting injection experiments as indicative of a hormonal function, unless excision experiments give opposite results. Moreover, the fact that pituitrin, unlike the injection of anterior lobe extract, gave results after a single injection, does not fit in well with the long latent period which elapses before hypophysectomy produces any effect.

Bearing on Relation between Muscle Creatine and Urinary Creatinine.

An increase in urinary creatinine such as has been shown to follow injection of anterior lobe, is interpretable in two ways. It might conceivably be associated with a decrease in the creatine content of the muscle, the increased excretion of creatinine being due simply to an increased breakdown of muscle creatine. Or it may be associated with an increase in muscle creatine on the assumption that a constant proportion of the creatine in muscle is converted to creatinine. Chanutin and Kinard (1933) have adduced evidence against this latter view. But the fact that injection of anterior lobe causes an increase in muscle creatine

/and

and also in urinary creatinine favours the latter alternative.

III. Application to Treatment of Myasthenia Gravis.

Myasthenia gravis is associated with a decreased excretion of creatinine and a creatinuria. A decrease in the amount of creatine in the muscle has been demonstrated by Williams and Dyke (1922).

The muscles in this disease tire rapidly but after an interval they recover. If the decrease in creatine can be assumed to be associated with a decrease in phosphagen, the rapid tiring may be explained as due to the deficient store of phosphocreatine. On resting, resynthesis of phosphate and creatine occurs and so muscular power returns. Injection of anterior lobe extract might, therefore, have an ameliorating effect by increasing the creatine content of the muscle and coincidentally, it is assumed, the phosphocreatine. It is not suggested that the decrease in muscle creatine is the primary cause of the disease. Available evidence tends to indicate that some neuromuscular defect is the primary event.

While the above treatment is suggested on the basis of some as yet unproved assumptions, it was thought worthy of a trial in view of the lack of satisfactory treatment of the disease. Previous to 1930 no specific treatment of any sort was known. Harriet Edgeworth (1930), who suffered from the disease herself, found that large doses of ephedrine ($\frac{3}{8}$ grain twice daily) had

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a beneficial effect. But improvement is not always constant, and Boothby (1934) finds that excessive doses may cease to produce any improvement and may even aggravate the weakness. It may also have the effect of producing intense nervousness. In 1932, glycine , because of its effect on creatine metabolism, was introduced in the treatment of this condition. Encouraging results were obtained. While many cases were greatly benefited, in many others recovery was incomplete or entirely absent.

Myasthenia gravis is a rare disease, and cases are few in a population so small as that of the Cape Peninsula. A case, however, was found in the New Somerset Hospital, Cape Town. The patient was a married European female aged 26. The disease commenced 5 years ago with ptosis of the eyelids. It was not diagnosed until 9 months ago, when the limbs, muscles of expression, mastication, etc. had become involved. Glycine had been tried without any effect on this patient. Ephedrine produced slight and very transient alleviation of the symptoms. In view of the failure to effect a cure by the above methods it was decided, on the suggestion of the author, to give injections of anterior pituitary extract. Antuitrin, an acid aqueous extract of the anterior lobe prepared by Parke, Davis and Co. was used. Daily subcutaneous injections of 1 ml. of antuitrin were commenced. Injections were continued for 10 days without any evident improvement. It is interesting, therefore, to note that a report by H. Simon

/has

has since appeared in the J.A.M.A. (June 8th, 1935) of excellent results obtained in two cases of myasthenia gravis on injecting anterior lobe extract. He does not give the reason which prompted him to use this substance. He used the same preparation as the author, namely, Parke, Davis' antuitrin and gave daily subcutaneous injections of 1 ml. as did the author. In both of Simon's cases the response to antuitrin was prompt and complete and a relapse followed when the injections were omitted. On resumption of treatment the symptoms again promptly disappeared. Muscular discomfort disappeared after the second day in both cases. By the tenth day no symptoms remained in the one patient. The other was able to walk at least half a mile without undue fatigue.

In the light of Simon's results it is difficult to understand why there was no response whatsoever in the author's case. The identical preparation was used and was administered in exactly the same way for a period longer than that taken by Simon's patients to show improvement. The treatment will be tried out on other cases as soon as they are available.

IV. SUMMARY.

I. The Normal Toad.

(a) In a normal toad the creatine content of the 'hamstring' muscles is the same for the right and left legs.

(b) There is no difference in the muscle creatine level in the two sexes.

(c) The average value is 399 mg. creatine per 100 g. muscle.

(d) The highest value obtained for a normal toad was 440 mg. and the lowest 349 mg. per 100 g.

(e) The creatine content bore no relation to the size of the animal.

2. Captivity.

The muscle creatine falls slightly with captivity.

3. The Gonads.

(a) Castration of males and females does not affect the muscle creatine as long as 4½ - 5½ months after the operation.

(b) There was no difference in the concentration of creatine in the muscles of toads with normal ovaries or with ovaries which had markedly regressed.

4. Hypophysectomy.

(a) There is a decrease in muscle creatine after removal of anterior lobe alone and after total hypophysectomy, 5½ - 6½ months after operation.

(b) The decrease after total hypophysectomy is not significantly greater than after removal of anterior lobe alone.

(c) The average decrease after hypophysectomy is about 15 per cent.

5. Injections of Anterior Lobe Extract.

(a) Acute injections of 1 ml. of a Bellerby extract of anterior lobe does not affect the muscle creatine of normal toads.

(b) Chronic injections of 0.2 ml. of Bellerby extract of anterior lobe results in a rise of muscle creatine in normal toads. This increase commenced 8 - 16 days after injections were started.

The maximal increases obtained were in the region of 30% above control animals injected with brain extract.

(c) No antihormonic effect on the muscle creatine was observed.

(d) Some evidence was obtained that the muscle creatine of toads which had been hypophysectomised 11 months previously, could be raised by chronic injections of anterior lobe extracts.

Insufficient animals were used, however, to permit making a definite statement.

6. Injections of Pituitrin.

(a) Acute injections of 1 ml. Parke, Davis Pituitrin produced a decrease in muscle creatine within 5 to 10 hours after injection. 30 hours after injection an average fall of 18% was observed.

(b) Evidence is submitted to show that this fall is at least partly due to the absorption of water from the surrounding medium by the muscles.

(c) Reasons are given why it is considered that this decrease in muscle creatine is not considered to be a function of the posterior lobe.

(d) There is no evidence that the gonads influence the muscle creatine in Xenopus laevis.

7. Endocrine Relationships.

It is concluded that

(a) the evidence presented is suggestive of an endocrine relationship between the anterior lobe and muscle creatine;

(b) the relation is probably an indirect one via some other endocrine organ, possibly the suprarenal;

(c) there is no evidence that the posterior lobe has any influence on muscle creatine;

(d) there is no evidence that the gonads influence the muscle creatine in Xenopus laevis.

8. Relation of Urinary Creatinine to Muscle Creatine.

The findings in regard to the anterior lobe, taken in conjunction with the work of other investigators, afford additional evidence for the view that not only is there a metabolic relation between creatine and creatinine, but that an increase in urinary creatinine is indicative of an increase in muscle creatine.

9. Myasthenia Gravis.

It is suggested that injection of an extract of the anterior lobe of the pituitary may be of use in the treatment of myasthenia gravis.

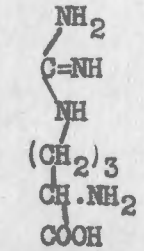
THE PRECURSORS OF CREATINE AND CREATININE.

THE PRECURSORS OF CREATINE AND CREATININE.

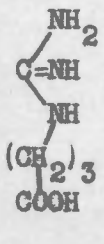
The fact that the body can maintain its supply of muscle creatine at a more or less constant level even on a creatine-free diet, indicates that the organism is capable of synthesising creatine. The tissues of herbivorous animals are as rich in creatine as the tissues of carnivorous animals.

Numerous attempts have been made to determine from what substances the organism can synthesise creatine, and the last few years especially have witnessed a renewed activity in connection with investigations into this problem. Hunter (1928) has discussed the literature up to 1926, and Rose (1933) has ably reviewed the work from that date to 1932. Evidence has been advanced at various times in favour of a number of chemical compounds bearing a structural similarity to creatine and creatinine. Creatine may be represented as methyl-glycocyamine or methyl guanidine acetic acid; creatinine as methyl glycocyamidine or 2-imido-5 keto-3 methyl tetrahydro-imidazole.

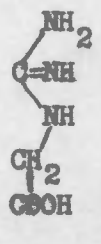
Arginine has been suggested as a possible precursor in virtue of its guanidine radical.



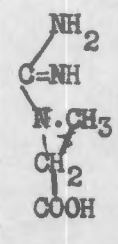
Arginine



γ-Guanidino butyric acid

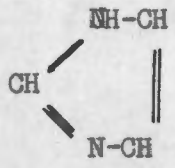


Guanidino acetic acid (Glycocyamine)

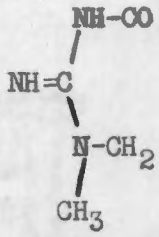


Methylguanidino-acetic acid (creatine)

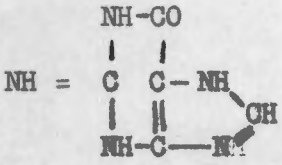
Guanine, uric acid and histidine have been suggested in virtue of their imidazole nucleus.



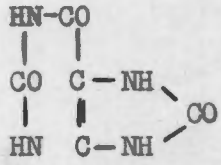
Imidazole



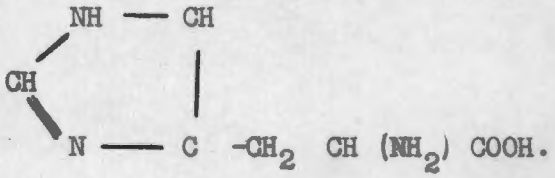
Creatinine



Guanine



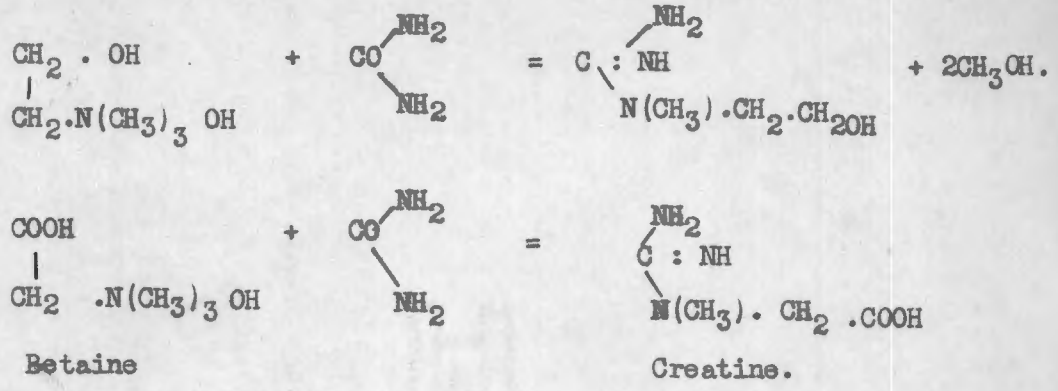
Uric Acid



Histidine.

Harding and Young (1920) have suggested that creatine may be derived from cystine "through the intermediate stages of taurine and amino-ethyl alcohol, followed by methylation, combination with urea and oxidation."

Koch (1905) and Reisser (1913) have pointed out that creatine might arise from choline or its oxidation product betaine by condensation with urea.



Betaine Creatine.

More recently, the production of creatine from glycine has been noted upon oral administration of glycine to patients with progressive muscular dystrophy and pseudohypertrophic muscular atrophy by Brand and co-workers (1929, 1930, 1932). This work has been confirmed by Thomas, Milthorat and Techner (1932). The production of creatine from glycine has not been shown to occur in the normal individual, however. (Christman and Mosier, 1929; Zwarenstein, 1928).

Compared with the few investigators who have obtained increases in muscle creatine or urinary creatinine following the administration of one or other of the above-mentioned chemical compounds, there is a far greater number of workers who have obtained negative results with these substances. As a result it has never been possible to assert with conviction which substances constitute the precursors of creatine and creatinine. But, to quote Rose in his review of the subject (1933), "If one is to accept seriously the recent paper of Beard and Barnes, (1931) the situation becomes chaotic." They report an increase

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in urinary creatinine and muscle creatine after feeding a large variety of proteins and amino acids. They investigated the effect of these substances on muscle creatine and urinary creatinine in adult rats and on creatinine elimination in man. Muscle creatine was increased by feeding the amino acids argininemonochloride, histidine, valine; by the proteins casein and edestin, and also by choline and glycocyamine. The urinary creatinine was augmented by all the proteins and amino acids studied, the increases ranging from 14.2% for aspartic acid to 35.9% for glycine.

Beard and Barnes believe that their results cannot be ascribed to the effect of specific dynamic action, and come to the conclusion that it is due either to the fact that (1) all the proteins and amino acids fed are definite precursors of creatine and creatinine or (2) these substances stimulated creatine-creatinine metabolism in some way other than by specific dynamic action. They favour the former view. It seems unlikely, however, that so large a variety of substances can be normal precursors of a substance to which many of them bear little or no chemical relation. A really satisfactory explanation of their results is difficult, and the findings of Beard and Barnes are in obvious need of re-examination. In the work described below, arginine, histidine, glycine and alanine were injected, and tyrosine, cystine and glutamic acid administered per os.

The effect of these substances upon the excretion of creatine and creatinine in the urine was investigated. In addition to these amino-acids the effect of the ingestion of glycocyamine was also noted.

The presence of enzymes such as arginase and histidinase in the liver has no doubt militated against any positive results in the many negative experiments reported in the literature. It was, therefore, decided to administer the substances subcutaneously wherever it was possible to prepare them in a form suitable for injection. Large amounts were administered in order further to lessen the risk of total destruction by enzymes.

EXPERIMENTAL METHOD:

The experiments were carried out on adult male rabbits which were found to be very suitable animals because of the fact that, on the usual diet of carrots, cabbages and bran, their daily creatinine output remains remarkably constant. For the purpose of collecting the urine the animals were kept in special metabolism cages which enabled accurate 24-hourly collections of urine to be obtained without the necessity for catheterisation. The floor of each cage consisted of fine wire netting. Each was fitted with a large collecting funnel, the converging sides of which ended in a spout. The urine passed through the sieve-like floor, and down the funnel into a glass flask in which the urine was collected. The mesh of the floor was so fine as to

/prevent

prevent any food or faeces coming through. As a double precaution the urine was made to pass through a glass funnel with a fine wire filter before entering the receiving flask.

At the end of each 24-hour period the sides of the funnel-shaped collecting portion were washed down with water and the washings added to the urine. Creatine and creatinine were estimated by the method of Folin (1914). The 24-hour collection of urine is diluted with water so that the reading of the unknown on the colorimeter will be not more than 5 mm. above or below that of the standard. The standard was always set at 15 mm.

Estimation of Preformed Creatinine.

10 ml. of filtered urine is placed in a 100 ml. volumetric flask with 20 ml. of a saturated solution of purified picric acid. 3 ml. of 10 per cent. sodium hydroxide is added. (This is a slight modification of Folin's method. He used only 1.5 ml. of NaOH. But it has been observed in this Department that with rabbits' urine a more intense colour is obtained with 3 ml. than with 1.5 ml. NaOH, and this permits of more accurate readings).

The volumetric flask is well shaken and allowed to stand for 10 minutes during which time the characteristic orange colour develops. After 10 minutes, distilled water is added to the 100 ml. mark, the flask is well shaken, and the contents filtered into the colorimeter cup. The colour is then compared with that of the standard.

Preparation of the Standard.

1.602 g. creatinine zinc chloride is dissolved in 1000 ml. $\frac{N}{10}$ HCL, which is equivalent to a solution of 1 mg. creatinine per 1 ml. This solution is perfectly stable. 1 ml. of the standard solution is placed in a 100 ml. volumetric flask. To this is added 20 ml. picric acid, 3 ml. 10% NaOH, and 9 ml. distilled water. The flask and its contents are allowed to stand for 10 minutes and distilled water is then added to the 100 ml. mark. The standard was always set at 15 mm.

Estimation of Creatine.

The amount of creatine in the urine is determined by converting the creatine to creatinine, estimating the total creatinine and subtracting from this the preformed creatinine.

10 ml. of the filtered urine is run into an Erlenmeyer flask, and 120 ml. water and 20 ml. picric acid are added. The contents of the flask are brought to boiling point, allowed to boil gently for one hour and then rapidly boiled until about 25 mcc. of fluid remains. The flask is allowed to cool and the creatinine estimated as described above.

EXPERIMENTAL DATA:

For the first month (referred to as the pre-period), the animals were kept in their cages for 4 or 5 days each week, and their normal daily output of creatine and creatinine determined. After their stay in the cages they were released into a large run

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in the animal house which communicated with a similar run in the open air outside the house. Throughout the period of investigation the animals remained in good health.

The average normal daily output of each animal for 18 occasions was first obtained, and then the investigation of the effect of the various amino acids was proceeded with. The animals were put in their cages the day before injection and their output for the 24 hours determined in order to see that the output was at the normal level. As will be seen from the tables the animals were generally injected for three successive days. In the case of arginine and histidine, the effect of one-day injections was also investigated.

The histidine hydrochloride, arginine, glycine and alanine were dissolved in Locke's solution (30 - 35 ml.) and the solutions of the first two were neutralised with dilute NaOH or HCl before injection so as to leave the reaction of the blood unaffected. Cystine, tyrosine, glutamic acid and glycocyamine were fed mixed with bran. Only in the case of cystine did the animals fail to ingest all of the mixture so that the amounts for cystine in the table below are actually rather less than stated.

Eight animals were used in all. Every animal in its turn served as a normal control in the various experiments. In the feeding experiments the control animal received the normal diet

while the other animals had the substance to be investigated added to the bran. In the injection experiments the control animal received an injection of Locke's solution. Injections of 35 ml. of Locke's solution daily for three days had in all animals no effect on the excretion of creatinine. For the sake of convenience the data in the following Tables are arranged in a slightly different sequence from that in which they were actually obtained.

The precaution was taken to investigate the effect of the addition of the various amino-acids to the urine upon the colour development in the Folin method of estimating creatinine. It was found that even if all the substances administered were excreted quantitatively, the colour development would not be significantly interfered with.

The figures in the tables below, unless otherwise stated, refer to milligrams creatinine excreted in 24 hours. The figures in brackets refer to the amount of the substance in grams fed or injected.

RESULTS:

TABLE I.

24-HOURLY EXCRETION OF CREATININE IN NORMAL RABBITS.

Rabbit No.	I	II	III	IV	V	VI	VII	VIII.
	95	96	93	100	66	72	79	91
	94	95	93	95	72	71	61	69
	96	95	95	85	71	77	73	70
	94	90	93	93	75	69	75	75
	96	90	99	89	80	74	81	81
	96	100	96	96	70	74	67	83
	96	107	91	88	70	73	69	73
	96	100	90	100	75	70	72	75
	93	107	93	93	73	75	70	73
	96	100	90	89	72	71	80	80
	100	96	85	89	72	70	72	74
	97	104	85	85	77	69	79	76
	96	97	83	85	75	77	77	79
	90	100	80	100	64	65	65	81
	98	100	98	94	62	70	75	73
	97	100	82	97	77	73	75	70
	96	-	81	94	73	71	74	69
	95	-	90	-	70	75	69	70
Average	96±2	98±5	90±6	92±5	72±4	72±3	73±5	76±6.

Creatine: Variable amounts of creatine (0-27 mg.) were found in the urine of these rabbits.

TABLE II.

EFFECT OF INJECTING HISTIDINE AND ARGININE ON THE 24-HOURLY EXCRETION OF CREATININE IN RABBITS.

Rabbit No:	I	II	III	IV.
Weight (kilos.)	2.46	2.52	2.18	2.03
A. PRE-PERIOD. Mean daily output (Average of 18 days.)	96±2	98±5	90±6	92±5
B. ARGININE. (1) Preceding day	100	101	90	95
Injection Period (1 day)	109 (5g)	136 (5g)	106 (5g)	89 (2.5g)
	103	101	103	99
	107	93	93	103
	102	110	80	99
(2) Preceding day				91
Injection Periods (3 days)				91 (2.5g)
				114 (3.0g)
				114 (5.0g)
C. HISTIDINE. (1) Preceding day	99	106	84	92
Injection Period (1 day)	94 (3g)	138 (5g)	104 (3.5g)	109 (3.5g)
	100	126	96	96
	110	109	85	80
	103	113		
(2) Preceding day	104		93	
Injection Periods (3 days)	125 (2.5g)		93 (2.5g)	
	96 (3.5g)			
	117 (4.0g)		96 (3.5g)	
			120 (5.0g)	

Variable amounts of creatinine (0-36 mg.) were found in the urine after injection of arginine and histidine but similar amounts were excreted during the normal pre-periods and also after injection of Locke's solution. Consequently the occurrence of creatine after injection of the amino-acids cannot be regarded as significant.

TABLE III.

EFFECT OF ADMINISTERING GLYCINE ALANINE AND CYSTINE ON THE 24-HOURLY EXCRETION OF CREATININE IN RABBITS.

Rabbit No.	III	IV.	V.	VI.
Weight in kilos	2.18	2.03	1.80	1.77
A. PRE-PERIOD.				
Mean daily output (Average of 18 days	90±6	92±5	72±4	72±3
B. GLYCINE.				
Preceding day	92	90	75	70
Injection Periods (3 days)	91 (4.5g) 96 (3.5g) 91 (3.5g)	85 (4.5g) 96 (3.5g) 92 (3.5g)	65 (5g.) 78 (5g) 70 (5g)	70 (5g) 73 (5g) 69 (3g)
C. ALANINE.				
Preceding day	90	93	72	80
Injection Periods (3 days)	92 (4.5g) 80 (3.5g) 90 (3.5g)	87 (4.5g) 93 (3.5g) 80 (3.5g)	67 (4 g) 72 (4g) 65 (3g)	70 (4g) 75 (4g) 72 (3.5g)
D. CYSTINE.				
Preceding day	82	90		80
Feeding Periods (2 - 3 days)	83 (5g) 80 (1g) 56 (4g)	90 (3.5g) 90 (4g)		70 (3.5g) 69 (4g) 65 (3.5g)

TABLE IV.

EFFECT OF ADMINISTERING TYROSINE AND GLUTAMIC ACID ON THE 24-HOURLY EXCRETION OF CREATININE IN RABBITS.

Rabbit No:	III	IV	VII	VIII.
Weight in Kilos	2.18	2.03	1.80	1.99
A. PRE-PERIOD.				
Mean daily output (Aver.of 18 days)	90±6	92±5	73±5	76±6
B. TYROSINE.				
Preceding day	85	91	71	73
Feeding periods (2 - 3 days)	90 (5g) 93 (5g) 85 (3g)	89 (4.5g) 94 (10g)	76 (5g) 70 (5g) 73 (3g)	70 (5g) 75 (5g) 80 (3g)
C. GLUTAMIC ACID.				
Preceding day	96	92	69	73
Feeding periods	90 (5.5g) 98 (3.0g) 85 (4.0g)	89 (7g) 93 (5.5g) 87	70 (5.5g) 69 (3.0g) 69 (4.0g)	73 (7.0g) 77 (5.5g) 76

TABLE V.

EFFECT OF ADMINISTERING GLYCOCYAMINE ON THE 24-HOURLY EXCRETION OF CREATINE & CREATININE.

GLYCOCYAMINE.

Rabbit No.	II		III	
	Preformed Creatinine	Creatine	Preformed Creatinine	Creatine
Preceding day	80	0	84	0
Feeding Periods (2 - 3 days)	92 95 90 94	39 (3g) 47 (4g) 107 (3g) 161	91 68	179 (3g) 269 (3g)

Table I. reveals the great constancy of the daily output of creatinine. This makes it easy to recognise any small increase or decrease in creatinine which might result from injection or other experimental procedures.

The results in Table II. showed that the injection of arginine and histidine caused increases in urinary creatinine of the order of 10 - 40%. The effects were definite in all the animals injected, and although the reaction to arginine and histidine in any one animal was very similar, individual variations occurred in different rabbits. Single day injections into Animal II. gave increases of about 40%; in Animal II. the increases were about 14%. Injections on successive days caused these increases to persist; in some cases further rises occurred. There was, however, no significant increase in urinary creatinine. These effects are in striking contrast to those obtained with other amino-acids.

In Tables III. and IV. it is seen that none of the amino-acids glycine, alanine, cystine, tyrosine and glutamic acid had any effect on the excretion of creatinine or creatine, whether administered subcutaneously or per os.

The large increases in creatine excretion as a result of feeding glycocyamine (Table V.) confirm the results of previous investigators. The preformed creatinine except for a drop on the second day in Animal III., remained unaffected.

DISCUSSION:

It is highly significant that with arginine and histidine which have structural affinities to creatine and creatinine there are prompt and definite increases in creatinine, while with all the other amino-acids, which have no such direct relationship, there is no effect. Glycocyamine increases creatine excretion but has no effect on creatinine. An analysis of Table I. indicates that arginine and histidine are identical in their effects upon urinary creatinine. From this it may be concluded that the iminazole nucleus in histidine and the guanidine nucleus in arginine undergo transformation into creatinine, probably through the intermediate stage of creatine. On the other hand, when the guanidine nucleus is introduced in the form of glycocyamine it is transformed directly into creatine which is excreted as such.

This difference in effect may be due to the fact that arginine and histidine were injected and glycocyamine was administered per os, that is, that the locus of transformation in the former case was muscle and in the latter the liver. Reviewing the evidence with regard to the site of formation of creatinine from creatine, Hunter (1928) comes to the conclusion that the liver plays no part in this change and that the muscles are most probably the chief site of creatinine formation. This does not exclude the possibility of creatine formation

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from glycocyamine in the liver; consequently the liver probably can convert glycocyamine into creatine but cannot convert creatine into creatinine. The creatine is formed from glycocyamine rapidly and in large amounts and is excreted as such. The point raised in regard to the different effects obtained with parenteral or oral administration of creatine-creatinine precursors requires further detailed investigation and may lead to an explanation of the contradictory results encountered in the literature.

The evidence presented above, although not constituting conclusive proof, indicates that arginine and histidine are normal precursors of creatine and creatinine. The fact that the other amino-acids were without effect whether injected or administered per os, shows that the increase of creatinine in the urine obtained with arginine and histidine could not have been due to specific dynamic action. The results obtained do thus not support the view of Beard and Barnes that a large variety of unrelated amino-acids can give rise to creatine and creatinine. Rather would it appear that only those substances with close direct chemical relationships to creatine and creatinine can be taken into account in any attempt to elucidate the still rather obscure problem of the significant biochemical process involved in creatine and creatinine metabolism and in their final inter-relationships.

The absence of any effect on administering glycine is of interest in view of the fact that it gave the maximum effect in the experiments of Beard & Barnes and in view of the effect it is said to have in cases of muscular dystrophy. The results here obtained are in agreement with the conclusions of Zwarenstein (1928) and Christman & Mosier (1929) that glycine has no effect on creatinine excretion in the normal organism.

It is also interesting to note that the creatine of the vertebrate is replaced by arginine in most invertebrates (although in some invertebrates it has not been possible to find either creatine or arginine. All the available evidence tends to indicate that creatine and arginine mutually exclude each other in their distribution and possess the same function in animal economy. Thus on phylogenetic grounds the most probable precursor of creatine would seem to be arginine. The fact that histidine was also found to increase the creatinine in the urine suggests the possibility that an investigation of the distribution of histidine in the animal kingdom might reveal that where creatine and arginine have not been found histidine may take their place.

SUMMARY:

1. The findings of Beard and Barnes are not supported.
2. The injection of arginine and histidine into adult male rabbits gives rise to a 10 - 40% increase in the elimination

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elimination of urinary creatinine.

3. Feeding with glycocyamine has no effect on creatinine excretion but leads to a large output of creatine.

4. The amino-acids, glycine and alanine (injected), and tyrosine cystine and glutamic acid (ingested), have no effect on creatinine excretion.

5. The fact that five out of the seven amino-acids administered were without effect indicates that the increases obtained with histidine and arginine are not due to specific dynamic action.

6. The fact that glycine was without effect is of interest in view of the effect it is said to have in muscular dystrophies. The conclusions of Zwarenstein (1928), and of Christman & Mosier (1929), that glycine does not influence the creatinine excretion in the normal animal is corroborated, and the more recent work of Beard & Barnes (1931) is not confirmed.

7. It is suggested that the transformation of arginine and histidine into creatinine takes place in the muscles, probably via creatine, and that the glycocyamine-creatine change is a direct one taking place in the liver.

THE ACTION OF CREATINE AND CREATININE

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CIRCULATORY SYSTEM.

I. THE ACTION OF CREATINE AND CREATININE ON THE CIRCULATORY SYSTEM DURING MUSCULAR EXERCISE.

The exact means by which the circulatory changes accompanying exercise are brought about, such as increased cardiac activity and local vasodilatation in the active muscle, have not yet been fully elucidated.

In addition to nervous mechanisms, increased attention has been drawn in recent years to the possible role of hormones (e.g., adrenaline) and of metabolites. Attention has also been called to various vasodepressor substances obtainable from tissue extracts, e.g. histamine, acetyl choline, adenylic acid, cytidylic acid. Investigation has not shown that any of these latter substances causes local vasodilatation during muscular activity.

The present work is concerned with the role of metabolites in producing the circulatory changes; it has long been assumed that chemical products liberated in the active muscle produce these changes, but the nature of these chemical products has not been satisfactorily demonstrated.

Samson Wright (1931) quoting Gaskell states, "the nature of these metabolites is not known for certain but may be CO_2 or lactic acid." While it is true that the addition of dilute acids to perfusion fluids produces vasodilation, Fleisch (1921) has shown that the H-ion concentration generally employed to

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prove this has been one hundred to several thousand times more acid than can ever obtain in the blood. Fleisch himself showed that vasodilation could be produced by altering the pH within physiological limits, but the increases obtained by him are too small to account for the increase in blood flow through active organs (Krogh, 1922).

These doubts regarding the action of lactic acid and CO_2 led us to consider the possibility of other metabolites producing these changes. Until 1926 no other metabolite of importance was known to be produced during muscular activity. Subsequently the work of the Eggletons (1927, 1928, 1929) and of Fiske and Subbarow (1927, 1928, 1929) revealed the breakdown of phosphagen into phosphate and free creatine during muscular contraction. On the assumption that, before being resynthesised into phosphagen this creatine might exert a local action on the bloodvessels of the muscle, or that some might escape into the general circulation and produce more remote effects, it was decided to investigate the effects of creatine on the circulatory system.

According to the views of Hahn and Meyer (1922, 1923) creatinine will arise from creatine wherever the latter substance is found, the change being a purely physico-chemical process dependent upon temperature and reaction of the tissues. Therefore, since most of the creatine of the body is found in the muscles, most of the creatinine will be formed there.

Because of this intimate relationship between the two compounds, and because a temporarily increased production of creatinine during exercise is accepted by most workers, it was decided to investigate the action of creatinine as well as of creatine.

EXPERIMENTAL DATA:

The effect of creatine and creatinine was investigated on the heart and bloodvessels. Solutions of the drug in various concentrations were made, using frog or mammalian Ringer's solution according to the tissue investigated.

The creatine solutions were neutral to litmus, and creatinine solutions feebly alkaline.

ACTION ON HEART:

(a) Xenopus laevis: Ringer's solution from a Mariotte bottle was perfused through the isolated heart at a pressure of 2 cms. using the Greene perfusion cannula tied into the inferior vena cava, fluid escaping from a divided branch of the aorta; movements were recorded from the apex of the ventricle. In some cases the Clark-Hartung method was used.

The Ringer's solution was replaced by the solution of the drug flowing at identical pressure from another Mariotte bottle.

With creatine in concentrations of 1 in 300 or better 1 in 150, there was an increase in the amplitude of the heart beat

systole being increased by some 15 - 20%; the heart rate only increased slightly.

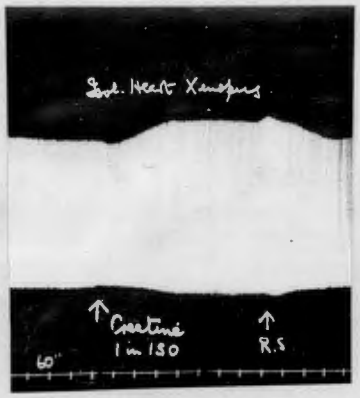

Fig.1. Perfusion of heart of Xenopus laevis with creatine 1 in 150.
R.S. = Ringer's Solution.

The increased amplitude of the beat was preceded by a short period during which the beats were smaller than the normal (Fig.1.); this occurred immediately after the entrance of creatine solution into the heart. These smaller beats gradually increased in size to reach an amplitude greater than normal. On the other hand when the creatine solution was replaced by Ringer's solution a further increase in the size of the beats occurred before they returned to the normal amplitude; this

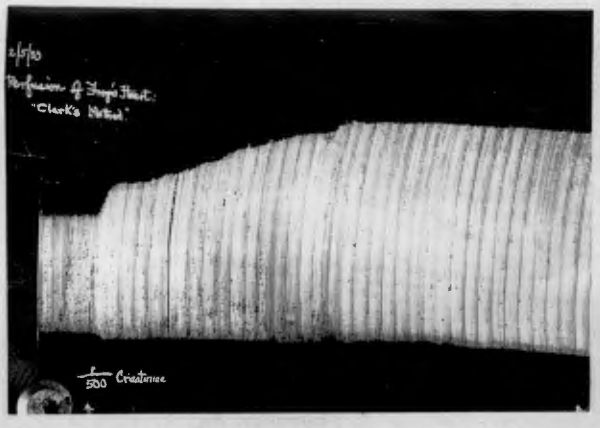

Fig.2. Perfusion of heart of Xenopus laevis with creatinine 1 in 500.

most peculiar tracing will be discussed more fully later.

Creatinine produced a greater increase in amplitude, solutions of 1 in 500 showed improvement of the beat, systole increasing up to 30% in some experiments (Fig.2.) No preliminary decrease in the size of the beats was observed, and no further increase when the drug was replaced by Ringer's solution.

(b) Rabbit, Cat: Langendorff's method of perfusion of the coronary arteries with warm (37°C) oxygenated Locke's solution was employed. Cushny's myocardiograph was also used to study the effects on the heart in situ.

Creatine produced similar changes to those observed in the amphibian heart with solutions of 1 in 300, though the preliminary diminution in cardiac systole was not apparent. Similar results were obtained by Backman (1908) on the dog's heart.

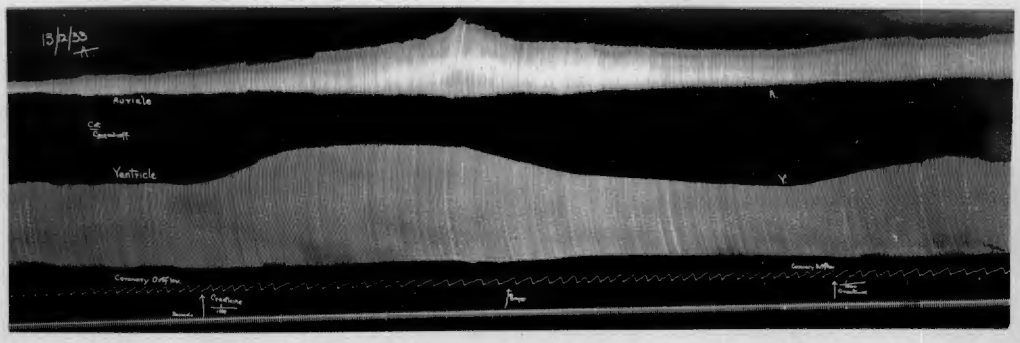

Fig.3. Perfusion of cat heart with creatinine, 1 in 1000.

Creatinine 1 in 500 produced even more marked increase in the size of the beat than did creatine, and with solutions 1 in 1000 a systolic increase up to 30% was obtained. (Fig.3.).

BLOOD PRESSURE:

Intravenous injections of either creatine or creatinine (e.g. 30 - 40 mg. per Kgm.) into cats produced slight increases in blood pressure, as compared with injection of Locke's solution, probably secondary to the increased cardiac activity. Backman (1912) came to the same conclusions using creatine.

BLOOD VESSELS:

Perfusion of the vessels of a pithed frog. Though this method is not well suited for exhibiting the action of vasodilator drugs, a tendency to vasodilator action was observed, the outflow of perfusing fluid being increased some 10% - 20% with both creatine and creatinine.

CONCLUSIONS:

An appreciable increase in the amplitude of the mammalian and frog heart was obtained with high concentrations of creatine and creatinine, as well as some evidence of vasodilation and a slight increase of blood pressure, but the concentrations used were greater than would occur in vivo. Thus, while it is possible that creatine and creatinine, like lactic acid and CO_2, may to some extent assist in bringing about the cardiovascular

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changes accompanying muscular activity it is unlikely that these substances are of any great importance.

It is interesting to note that in various forms of kidney disease, e.g., chronic nephritis, a marked increase in blood creatinine is said to occur. It is not impossible that such a rise in blood creatinine, persisting over a long period may be at least in part responsible for the cardiac hypertrophy which occurs in these conditions.

SUMMARY:

1. Creatine and Creatinine produce an increase in amplitude of the beat of the perfused isolated heart of the South African clawed toad, the rabbit and the cat.

2. Creatinine produces a greater increase in amplitude of the heart beat than corresponding concentrations of creatine. A 30% increase is produced by a 1:500 solution of creatinine in toads and by a 1:1000 solution in rabbits and cats.

3. Both creatine and creatinine produce only a slight increase in blood pressure in the cat, and a slight dilatation of the vessels on perfusing pithed toads.

4. The concentration required to produce the above

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effects is too great to suggest that creatine and creatinine are agents of any importance in assisting in the production of the cardiovascular changes which accompany muscular exercise.

II. AN UNUSUAL ACTION OF THE TOAD'S HEART SHOWN BY ITS RESPONSE TO CREATINE.

The unusual nature of the response of the isolated heart of Xenopus laevis to creatine - a response which was never obtained with creatinine - warrants further investigation. This effect of creatine has not been reported before. Its resemblance to the tracings obtained by Burridge with other drugs (1923, 1934), makes it of special interest.

EXPERIMENTAL METHOD:

Pithed toads (Xenopus laevis) were used. A Greene perfusion cannula was tied into the inferior vena cava, and one branch of the aorta tied to a glass rod, so placed as to keep the isolated heart suspended between these two points, the perfusing fluid escaping from untied branches of the conusarteriosus. The apex of the ventricle was connected by a thread over a pulley to a light recording lever.

Ringer's solution was perfused through the heart from a Mariotte bottle at a pressure of 2 cms. and the drug solution was perfused at a similar pressure from an identical Mariotte bottle. The two solutions, therefore, entered the heart at the same rate and pressure, and at room temperature (17°C - 19°C).

The Ringer's solution had the formula 0.7% NaCl, 0.03%KCl, 0.02% $CaCl_2$. Creatine solution consisted of creatine

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dissolved in this Ringer's solution and was neutral to litmus.

RESULTS:

Perfusion of the heart with solutions of creatine of a concentration varying in different experiments from 1 in 100 to 1 in 300 produced always a similar type of change.

The sequence of events is as follows: A diminution in the height of cardiac systole occurs (Fig.I.) then a gradual increase in the height of the beat until an increase of 15 - 20% above the normal was obtained, this increased amplitude being maintained for apparently as long as the drug was perfused through

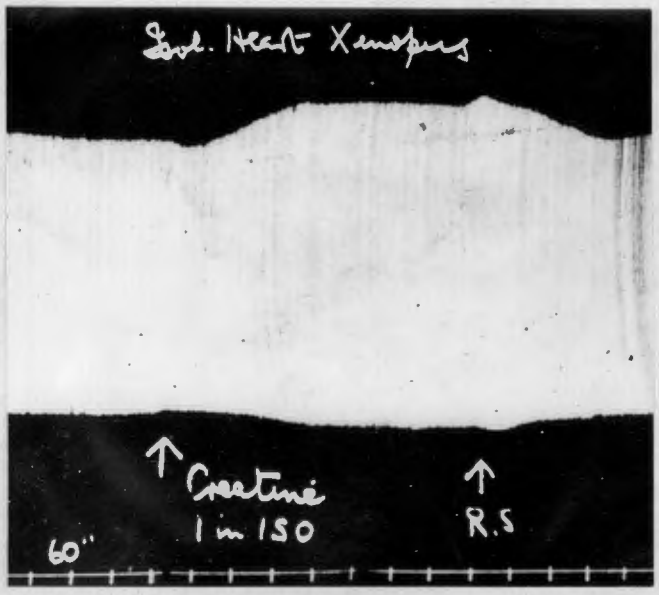

Fig.2. Perfusion of heart of Xenopus laevis with creatine 1 in 150.

the heart. On replacing the creatine solution by Ringer's solution there was a still further increase in systole, followed by a reduction in the height of the ventricular contraction until the normal systole was obtained (Fig.2.)

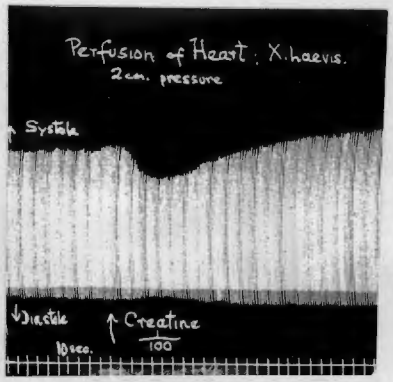

Fig.1: Perfusion of heart of Xenopus laevis with creatine 1 in 100. (Note initial depression of amplitude).

Creatinine, which is the internal anhydride of creatine and serves as an admirable control, produces merely an augmentation of the heart beat (Fig.3).

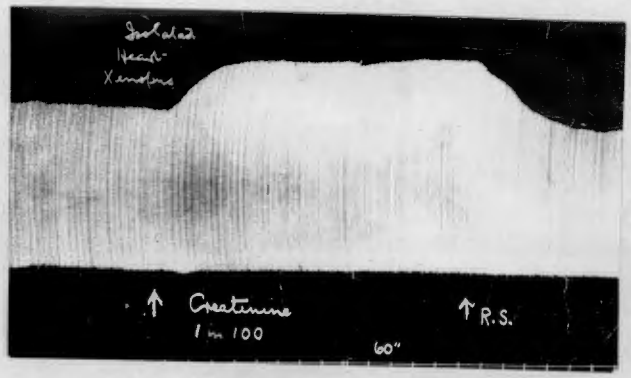

Fig.3: Perfusion of heart of Xenopus laevis with creatinine, 1 in 100.

DISCUSSION:

In order to explain the tracing obtained with creatine it is necessary to postulate two mechanisms: (1) a depressor mechanism causing the initial depression and (2) an augmentor mechanism causing the subsequent increase in amplitude to the level C D (Fig.4).

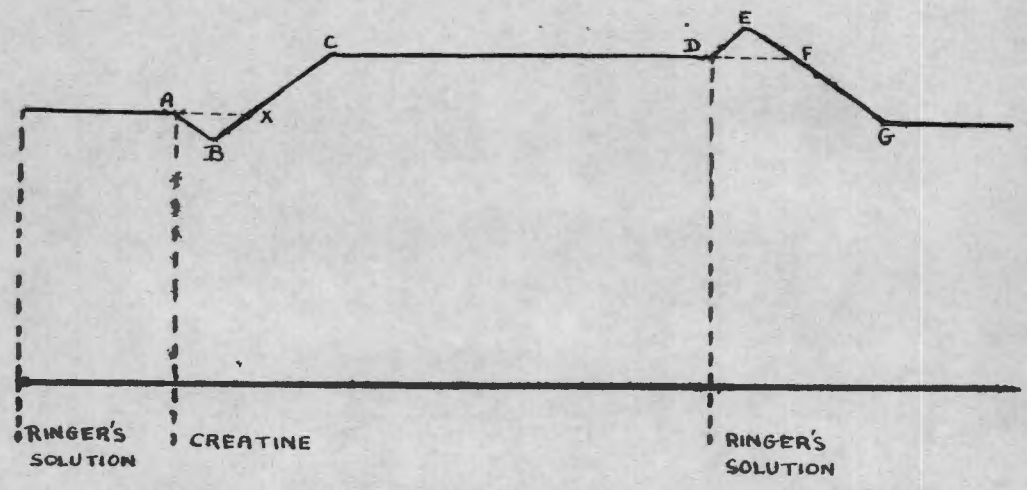

Fig. 4.

The terminal augmentation on reperfusing with Ringer's solution is explicable on the basis of a removal of the depressor effect. This means that the depressor effect must have been in action all the time and C D is the resultant of the augmentor and the depressor effects.

Since the resultant, C D, of the augmentor and depressor effects is augmentation it is evident that the augmentor effect

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of the drug is greater than its depressor action. Yet the first manifestation of the action of the drug is depression, which means that the depressor mechanism must come into play more rapidly than the augmentor effect else it would be masked by the more powerful augmentor action.

Again on re-perfusing with Ringer's solution the depressor effect must be removed more rapidly than the augmentor effect of the drug. (If the augmentor effect were removed more rapidly, or as rapidly, a continuous decline would occur without any terminal rise.

These facts fit in very well with the assumption that creatine exerts its depressor action on the cell surface and its augmentatory action on the interior of the cell.

Thus on substituting creatine solution for Ringer's solution (at A) the creatine first encounters the cell 'membrane' where it exerts its depressor effect and only later diffuses into the interior of the cell where it exerts its augmentor effect. When equilibrium has been attained between the creatine inside and outside the cell the two opposing mechanisms are in equilibrium; namely, depression due to the action of creatine on the surface and stimulation due to the action of creatine on the interior of the cell. C D represents the resultant of these two mechanisms in a state of equilibrium, and the magnitude of systole is, therefore, constant.

On re-perfusing with Ringer's solution at D, the creatine is first diluted at the cell surface and the removal of its inhibitory action there results in the terminal rise to height E. Only later does the Ringer solution dilute the creatine in the interior of the cell, causing a removal of the augmentor effect and so a decline of systole.

Detailed Analysis of the Record:

In the annotated diagram (Fig.4) the letters A-G refer to points along systole.

At A.frog's Ringer's solution is replaced by a solution of creatine in Ringer's solution. The creatine diffuses into the cell limiting layers, causing depression. As its concentration there increases, so the depression increases. Meanwhile creatine gradually reaches the interior of the cell where it commences to exert an excitatory action.

B represents the point at which depressor effect on surface exceeds stimulant effect in the interior of the cell by the maximum. The augmentor effect then begins to overtake the depressor effect.

At X the augmentor effect just balances the depressor effect. The predominance of the augmentor effect still further increases till the level C has been attained.

At C. equilibrium has been reached between the perfusing fluid in the interior, in the periphery, and outside the cell.

CD is, therefore, at a constant height.

At D the creatine perfusing solution is replaced by Ringer's solution. As the passage of the drug from cell interior to perfusing medium takes place the concentration of creatine is highest within the cell, less in the cell surface and least in the surrounding Ringer's solution. Thus the concentration of creatine is reduced first and to the greater extent in the cell "membrane", producing a removal of inhibitory effect which allows a further augmentation of systole, from D to E. At E the excess of removal of inhibitory effect over removal of augmentor effect is maximal.

From what is known of diffusion processes it must be assumed that with the dilution of creatine in the membrane which occurs at D there is a concurrent though lesser dilution of creatine in the cytoplasm, i.e., a removal of augmentor effect begins at or very soon after D. Hence at E some considerable augmentor effect has been lost, so that, if it were possible to obtain the augmentor effect of the drug uncounteracted by any depressor effect, systole would be somewhat higher than E.

At F the removal of inhibitory and augmentor effects is such that the resulting systole is at level CD. Beyond this point the removal of augmentor effect overtakes that of the inhibitory effect, the final traces of creatine affecting the size of contraction to an inconsiderable extent, until at G.

/all

all the creatine has been washed out.

Burridge (1923, 1934) obtained similar tracings by the action of alcohol on the frog's heart (Rana temporaria) and by increasing the salts, e.g., Na-ions, in the perfusing solutions. To explain his results he, too, postulated that the drugs exerted "simultaneous actions of exaltation and depression". (1934). In an earlier paper he says, "Excitability is mediated by two independent mechanisms (through each of which it may be increased or decreased). An augmentation mediated through one mechanism may exist side by side with a depression mediated through the other (and vice versa). (1923)." Burridge considers this a revolutionary conception. He maintains that according to the theory hitherto current "a drug either raised or lowered the various properties of a muscle. On this basis only one mechanism was available for exalting or depressing cardiac activity, and so any augmentation which followed a depression was necessarily believed to have removed the depression." But in interpreting tracings such as that recorded above, the necessity for postulating two mechanisms appears to be unavoidable, as Burridge also concluded.

Thus these observations on the effect of creatine on the heart of Xenopus laevis, lend support to the findings of Burridge working with other drugs. In addition, it is suggested that the probable site of action of the one mechanism is on the cell surface and of the other on the cell interior.

SUMMARY:

1. Attention is drawn to the unusual nature of the response of the heart of the South African Clawed Toad to creatine. It resembles the response of the heart of Rana temporaria to alcohol and to an increase of Na-ions in the perfusing fluid, as reported by Burridge (1923, 1934).

2. In order to explain the tracings obtained, two mechanisms are postulated. (1) A depressor mechanism due to the action of creatine on the cell surface, and (2) an augmentor mechanism due to the action of creatine on the interior of the cell.

BIBLIOGRAPHY.

Abderhalden, E., and Buadze, S.,(1930). Ztschr. f. physiol. Chemie, 189, 65.

Abelin, I., and Spichtin, W., (1930). Biochem. Ztschr. 228, 250-256.

Akatsuka, H., (1927). J. Biochem. Tokyo, 8, 57.

Backman, E.L., Scand. Archiv. f. Phys. 20, 49, 1908.

Backman, E.L., Zentr. Physiol. 26, 168, 1912.

Beard, H.H., and Barnes, B.O., (1932). J. Biol. Chem., 94, 49.

Behre, J.A., and Benedict, S.R. (1922). J. Biol. Chem. 52, 11-33.

Bellerby, C.W., (1933). Biochem. J. 27, 615.

Benedict, S.R., (1929). Biochem. J. 82, 2.

Beneddict, S.R., and Osterberg, E. (1923). J.Biol. Chem., 56, 229-252.

Beumer, H., and Iseke, C. (1920). Berlin klin. Wochschr.57, 178-181.

Boothby, W.M. (1934). Proc. Staff. Meet., Mayo Clin. 9. 593-597.

Braier, B., (1931). Rev. Soc. Arg. Biol. 7, 340.

Brand, E., Harris, M.M., Sandberg, M., and Ringer, A.I., (1929). Amer. J.Physiol. 90, 296.

Brand, E., Harris, M.M., Sandberg, M., and Lasker, M.M., (1930). J.Biol.Chem., 87. IX.

Brand, E., Harris, M.M., (1932). J. Biol. Chem. 92 lIX.

Brown, M., and Imrie, C.G., (1932). J. Physiol. 75 366-373.

Buhler, F., (1933). Z. ges. exp. Med. 86, 638-649.

Burger, M., (1919). Z. ges. exp. Med. 9, 262-284; 361-399.

Burridge, W., (1910). J. Physiol. 41, 285-307.

Cameron, A.T., (1933). A textbook of Biochemistry. 4th Edition.

Chanutin, A., (1926). J. Biol. Chem., 67, 29-41.

Chanutin, A., and Kinard, F.W., (1932-1933). J.Biol.Chem. 99, 125.

Chanutin, A., and Silvette, (1929-1930). J.Biol.Chem. 85, 179.

Chevreul (1932). J.pharm., 21, 231-242 and J. prakt.Chem., 6, 120-130.

Christman, A.A., and Mosier, E.C., (1929). J.Biol.Chem. 83, 11.

Clark, A.J., Eggleton, M.G., and Eggleton, P., (1932). J.Physiol. 75, 332 (1932).

Collip, J.B., (1935). Journ.Amer.Med.Assoc. 104, 11, 916-921.

Collip, J.B., & Anderson, E.M., (1935). Journ.Amer.Med.Assoc. 104, 1, 2, 965-969.

Cope, O., Corkill, A.B., Marks, H.P., and Ochoa, S., (1934). J.Physio 82, 305-320.

Corbia, A., (1928). Clin. pediat. 10, 195-212.

Cori, G.T., (1930). Amer.J.Physiol., 94, 557-63.

Cramer, W., and Krause, R.A., (1913). Proc. Roy. Soc. 86, 550-560.

Denis, W., (1917) 1, J. Biol. Chem., 29, 447-451.

Denis, W., (1917) 2, J. Biol. Chem., 30, 47-51.

Duliere, W., (1928). Comptes Rend.Soc. de Biol. 98, 1252.

Duliere, W., (1929). Biochem. J., 23, 921.

Edgeworth, H., (1930). Journ. Am. Med. Assoc., 94, 1136.

Eggleton, P., (1930). J. Physiol., 70, 294.

Eggleton, P. and Eggleton, G.P., (1927). Biochem.J., 21, 190; J.Physiol. 63, 155.

Eggleton, P. and Eggleton, G.P., (1928) J.Physiol. 65, 15.

Eggleton, P. and Eggleton, G.P., (1929) J. Physiol. 68, 193.

Eggleton, P. and Eggleton, M.G., (1927). J.Soc.Chem.Ind., 46, 485.

Eimer, K., (1931). Ztschr. f.d., ges. exper. Med., 77, 455-475.

Ferdman, D., and Feinschmidt., O., (1928). Z. physiol.Chem. 178, 173.

Feinschmidt, O., and Ferdmann, D., (1929). Biochem. Ztschr. 205, 325-328.

Fiske and Subbarow (1927). Science, 65, 401.

Fiske and Subbarow (1928). Science, 67, 169.

Fiske and Subbarow (1929). J. Biol. Chem., 81, 629.

Fleisch, A., (1921). Zeitschr. f. allg. Physiol. 19, 269

Folin, O., (1904). Z. physiol. Chem., 41, 223-242.

Folin, O., (1905). Am. J. Physiol., 13, 117-138.

Folin, O., (1906). Festschr., f. Olof Hammarsten, 111., Upsala.

Folin, O., (1914). Journ. Biol. Chem., 17, 469.

Folin, O., and Denis, W. (1912). J. Biol. Chem. 12, 141-162.

Folin, O., and Denis, W. (1914). J. Biol. Chem., 17, 493-502.

Folin, O., and Doisy, E.A., (1917). J. Biol. Chem., 28, 349.

Frontali, G., (1913). Arch. intern. de. Physiol., XIII. 431-463.

Gaebler, O.H., (1930). J. Biol. Chem., 89, 451.

Gerard, R.W., and Tupikow, N., (1931). Amer. J. Physiol., 97, 523.

Gross, E.G., and Steenbock, H., (1921). J.Biol.Chem., 47, 33-43.

Hahn, A., and Meyer, G. (1922, 1923). Z. Biol., 76, 247-256; 78, 91-11

Harding, V.J., and Young, E.G., (1920). J.Biol.Chem., 41.

Heintz, W., (1844). Pooggendorff's Ann. Phys. Chem., 62, 602-606.

Henderson, P.S., (1918). J. Physiol. 52, 1-5.

Hill, R.M., and Mattison, I.H., (1929). J.Biol.Chem., 82 679.

Hogben, L.T., (1923). Quart.Journ.Exptl.Physiol., 13, 177.

Hogben, L.T., and Slome, D., (1931) Proc.Roy.Soc.B., 108, 10.

Hunter, A., (1914). Quart.J.Exptl.Physiol., VIII 23-32.

Hunter, A. (1928). Creatine and Creatinine, London.

Hunter, A., and Campbell, W.R. (1916). J.Biol.Chem., 28, 335-348.

Imrie, C.G., and Jenkinson, C.N., (1932). J.Physiol. 75, 373-379.

Imrie, C.G., and Jenkinson, C.N., (1933). J.Physiol. 79, 218-225.

Klercker, K.O., (1906). Beitr. chem. Physiol. Path., 8, 59-61.

Kopolowitz, E., (1929-30). 1, Z.klin.Med., 112, 150.

Kopolowitz, E., (1929-30). 2, Z.klin.Med., 112, 584.

Kopolowitz, E., (1930). Z.klin.Med., 113, 605.

Krause, R.A., (1911). Quart.J.Exp.Physiol., 3, 289-296.

Krause, R.A., and Cramer, W., (1910). J.Physiol., 40, Proc.Physiol. Soc.lxi.

Krause, R.A., and Cramer, W., (1911). J.Physiol., 42, Proc.Physiol. Soc.XXXIV.

Krause, R.A., and Cramer, W., (1912). J.Physiol., 44, Proc.Physiol. Soc.XXIII.

Krogh, A., (1922). Anat. and Physiol. of the Capillaries. P.127.

Kutscher, F., and Ackermann, D. (1931). Z.physiol.Chem.,199, 266.

Larg, K., (1931). Pflugers Arch. 229, 60.

Lasch, F., (1932). Z. ges. exp. Med., 81, 314.

Liebig, J., (1847, 1) C.R.Acad.Sci., 24, 69-73; 195-198;

Liebig, J., (1847, 2) J.prakt.Chem., 40, 288-292.

Liebig, J., (1847, 3) Ann. Chem. Pharm., 62, 257-369.

Lundsgaard (1930, 1) Biochem. Z., 217, 162.

Lundsgaard (1930, 2) Biochem. Z., 227, 51.

Lundsgaard (1931) Biochem. Z., 230, 10.

Lundsgaard, E., and Wilson, A.T., (1934). J.Physiol. 80, 4, 29P.

Mackler, H., Olmsted, J M.D., and Simpson, W.W., (1930). Am.J. Physiol. 94, 626.

Marenzi, A.D. Physiol.Abstracts 1934. (& summarised version of report in Rev.Soc.Argent.Biol. 7, 43, 1933.

Meyerhof, O., and Lipmann, F., (1930). Naturwissenschaften, 18, 330.

Moschini, A., (1932). Arch.internat.de pharmacodyn.et de therapie 42, 328-333.

Moschini, A., (1934), 1. Compt.rend.Soc.de biol. 115, 215-218.

Moschini, A., (1934), 2. Compt.rend.Soc.de biol. 115, 218-221.

Myers, V.C. and Fine, M.S., (1913, 1) J.Biol.Chem. 14, 9-26.

Myers, V.C. and Fine, M.S., (1913, 2) J.Biol.Chem. 15, 283-304.

Myers, V.C. and Fine, M.S., (1913, 3) J.Bidl.Chem. 16, 169-186.

Mystkowski, E.M., (1932). Biochem. J., 26, 910.

Ochoa, S., (1932). Pflugers Arch., 231, 222-33.

Ochoa, S., and Grande, F., (1932). Pflugers Arch. 231, 220-1.

Ochoa, S., Grande, F., and Peraita, M., (1932). Biochem.Z. 253, 112.

Palladin, A., and Griliches, L., (1924). Biochem.Z., 146, 458-466.

Palladin, A., and Kudrjawzewa, A., (1924, 1) Biochem.Z. 152, 373-387.

Palladin, A., and Kudrjawzewa, A., (1924, 2) Biochem.Z. 152, 104-124.

Palladin, A., and Tichwinskaja, W., (1925). Arch.ges.Physiol., 210, 436-441.

Paton, D.N., (1919). Rept.Brit.Ass.Adv.Sci., 294-307.

Paton, D.N., (1925). Glasgow Med.J.

Paton, D.N., and Findlay, L., (1916), 1. Quart.J.Exp.Physiol., 10, 315-343;

Paton, D.N., and Findlay, L., (1916), 2. Quart.J.Exp.Physiol., 10, 377-382.

Peabody, W.A., and Hill, R.M., (1929). J.Biol.Chem. 82, 687.

Pettenkofer, M., (1844)., Ann.Chem.Pharm., 52, 97-100.

Pritchard, E.A.B., and Walker M.B., (1935)., Journ.Physiol. 84, 2, 35P.

Pugsley, L.I., Anderson, E.M., & Collip, J.B., (1934)., Biochem. J.28, 1135-1140.

Ranke, J., (1865). Leipzig: cited by Hunter from von Furth (1903).

Read, B.E., (1921). J.Biol.Chem., 46, 281-283.

Riesser, O., (1922). Z.physiol.Chem., 120, 189-206.

Remen (1932) (1) Z.ges.exp.Med., 80, 238.

Remen (1932) (2) Z.ges.exp.Med., 81, 223.

Rimer, G.E.G., (1932). PhD. Thesis University Cape Town

Rose, W.C., (1933). Ann.Rev.Biochem.II.

Rose, W.C., and Dimmit, F.W., (1916). J.Biol.Chem., 26, 345-353.

Rose, W.C., Helmer, O.M., and Chanutin, A., (1927). J.Biol.Chem., 75, 543-548.

Roux, and Tallandier (1914). Intern.Beitr.Z.Path.d.Ernahrungsstor., V

Schrire, I. and Zwarenstein, H., (1932) (1) Biochem.Journ.XXVI. 1. 118-121.

Schrire, I. and Zwarenstein, H., (1932) (2) Biochem.Journ.XXVI. 6. 1886-1889.

Schrire, I. and Zwarenstein, H., (1933). Biochem.J.XXVII. 5. 1337-1341.

Schrire, I. and Zwarenstein, H., (1934). Biochem.J.XXVIII. 1. 356,359.

Shaffer, P.A., (1907). J.Biol.Chem.,3. Proc.Am.Soc.Biol.Chem., XIII.

Shaffer, P.A., (1908). Am.J.Physiol., 23, 1-22.

Shaffer, P.A., (1914).

Shen, T.C., (1927) Chinese J.Physiol. 1, 363.

Shorr, E., Richardson, A.B., and Wolff, H.G., (1933)., Proc.Soc.Exp. Biol.Med. 31,207

Starling, (1933). Starling's Principles of Human Physiology, 6th Edition revised by Lovatt Evans)

Steggerda, F.R. and Essex, H.E., (1934). Proc.Soc.Exp.Biol.and Med. 32, 3. 425-428.

Stuber, B., Russman, A., and Proebsting, E.A. (1923). Biochem.Z., 143, 221-235.

Swale, Vincent (1927). Physiol.Reviews. 7.

Thomas, K., Milhorat, A.T., and Techner, F. (1932). Proc.Soc. Systl.Biol.Med. 29, 609.

Thompson, W.H. (1917) J.Physiol. 51, 111-153; 347-376.

Tiegs., O.W., (1925). Austr.J.Exp.Biol.Med.Sci., 2, 1-18.

Tsuji, K., (1915). Biochem.J., 9, 449-455.

Urano, F., (1906). Beitr.chem.Physiol.Pathol., 9, 104-115.

Vollmer, H., (1929). Z.ges.exptl.Med., 65, 522.

Vollmer, H., (1929). Ber.ges.Physiol.exptl.Pharmakol, 51, 505.

White, F.D., (1931). Contrib.Can.Biol.Fisheries, 6, 341.

Williams, B.W., and Dyke, C.S. (1922). Quart.J.Med. 15, 269-278.

Wright, S., (1931). Applied Physiology. Oxford Univ.Press. P.30

Zagami, V., (1930). Chem.Abstr., 24, 2781.

Zanghi, G., (1930). Arch.fisiol., 28, 372.

Zwarenstein, H., (1928). Biochem.Journ. 20, 307.

Zwarenstein, H., and Shapiro, H.A., (1933). Journ.Exp.Biol. 10, 372.

AS COLLATERAL TESTIMONY OF WORK UNDERTAKEN.

THE TOPOGRAPHY AND HISTOLOGY OF THE PARA-THYROID GLANDULES IN *XENOPUS LAEVIS*

BY

B. G. SHAPIRO

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THE TOPOGRAPHY AND HISTOLOGY OF THE PARATHYROID GLANDULES IN *XENOPUS LAEVIS*

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During the past few years the exceptional suitability of the South African clawed toad, *Xenopus laevis*, for physiological investigations has been increasingly appreciated. Especially useful is its ability to withstand the severest of operative procedures, and also the power of the living animal or of its isolated organs to resist great variations in the environment.

Of particular interest to the experimental physiologist is the endocrine system of *Xenopus* which has only been carefully investigated during the last two years. Rimer (1) published accounts of the pituitary and thyroid glands, while Zwarenstein and Schrire (2) and Epstein, Gunn, Epstein and Rimer (3) elucidated the distribution and structure of the adrenal glands. The parathyroids have not hitherto been located. The need to do so has recently become more pressing in view of the large amount of work done during the last few years on the part played by the pituitary and ovaries in the calcium metabolism of *Xenopus*. The fact that the situation of the parathyroids in this animal is unknown prevented the further elucidation of the problem of the factors controlling the blood calcium, and impressed the need for investigating the whereabout of the glandules.

Amphibia are the lowest animals in which parathyroids have been found. Toldt (4) referred to the parathyroid bodies in Amphibia as "Nebenschilddrusen," a term which led to their being confused with true accessory thyroids. Maurer (5), however, recognised that they were not accessory thyroids, but homologous with the parathyroid glandules of higher vertebrates.

The following is an account of the anatomical relations and histological structure of the parathyroid glandules in *Xenopus*. In order to locate them serial sections of the head and thorax as far down as the apex of the heart were cut.

TOPOGRAPHY

The glandules lie very deeply, and in order to expose them the skin, pectoral muscles and sternum must be removed, and the heart with its large blood vessels revealed. The glandules are most easily located by reference to their relations to these blood vessels which should be clearly defined.

The sinus venosus is joined by a single inferior vena cava and right and left superior venae cavae. Each superior vena cava is seen to receive (1) the

external jugular vein passing down from the region of the ventral wall of the mouth; (2) the vena anonyma formed by the junction of the internal jugular vein and a smaller vein, the subscapularis, from the muscles of the scapula. The vena anonyma then runs downwards parallel with the musculus petrohyoideus. In this connection the nomenclature of Grobbelaar (6) has been preferred to that of Gilchrist and von Bonde (7) on both phylogenetic and embryological grounds. The superior vena cava also receives (3) the subclavian vein formed by a branch from the abdominal muscles, a branch from the skin and by the brachial vein from the arm.

The conus arteriosus is seen to divide into two branches, each of which subdivides into three arches. (1) The carotid arch, which passes laterally and is characterised by a bulbous enlargement—the carotid body. From the region of this body two arteries pass forwards. The more medial is the muscular artery (referred to as the hyothyroid artery by Rimer) which supplies the mylohyoid muscle and the thyroid gland, the lateral is the lingual artery. They sometimes have a common stem of origin which may be considered to represent the external carotid of *Rana*. Beyond the carotid body the arch continues as the internal carotid artery. (2) The systemic arch, which passes laterally and backwards on each side, and unites with its fellow of the opposite side to form the dorsal aorta. It is important to note, in connection with the precise relations of the parathyroids to be described below, that the systemic arch curves round the lateral aspect of the distal end of the processus thyroideus of the hyoid apparatus, and that as it does so it is pronouncedly kinked. (3) The pulmocutaneous arch which curves just below the systemic arch and divides into a pulmonary and a cutaneous artery.

Once the blood vessels of the region have been clearly defined localisation of the parathyroids is easy and accurate despite the fact that they are so small as to be invisible to the naked eye.

They were found to lie remarkably far down and, as in other Amphibia, at a very considerable distance from the thyroid gland.

There are two glandules on either side situated in a triangular area bounded by the carotid arch anteriorly, the systemic arch posteriorly, and the hypoglossal nerve, as it passes upward to supply the muscles of the ventral wall of the mouth, laterally. They usually lie in the lateral part of this area in a line with the carotid body anteriorly and the systemic arch at the point where it is kinked as mentioned above, posteriorly (fig. 1). The glandules are often found attached by fibrous connective tissue to either of the two last-mentioned structures.

In *Rana*, on the other hand, the parathyroids occupy a somewhat different position. They lie on the lateral side of the external jugular vein in the sinus sternalis and medial to the external carotid artery near its origin (fig. 2). It must be explained that external carotid is here used in conformity with the nomenclature of Gaupp (8) and corresponds to what most English text-books less advisedly call the lingual artery.

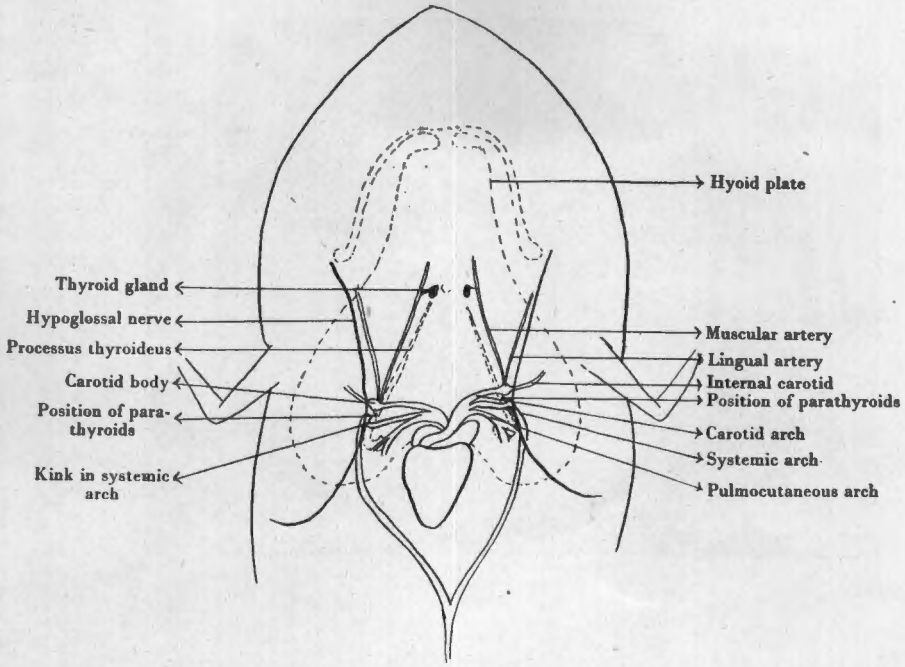

Fig. 1. Diagram showing relations of parathyroid glandules in *Xenopus laevis*.

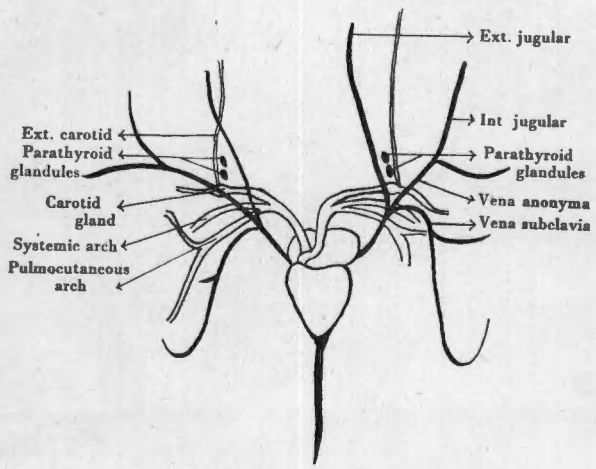

Fig. 2. Diagram of position of parathyroids in *Rana*.

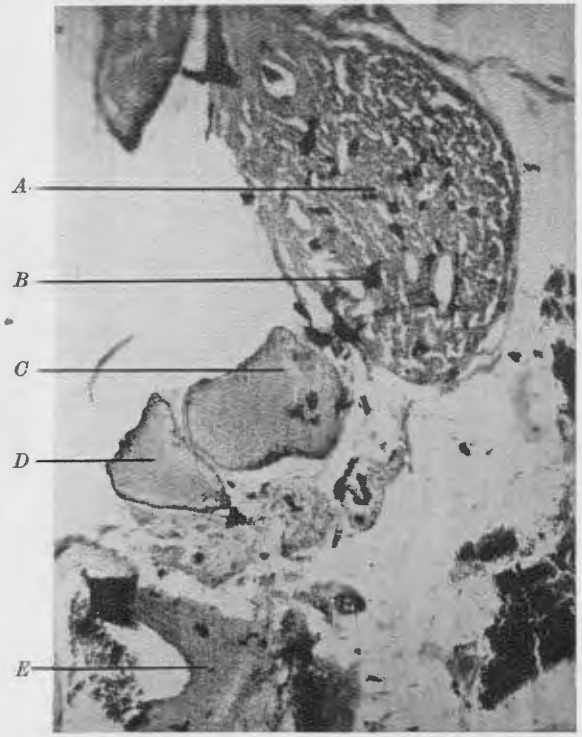

Fig. 3. Photomicrograph of sagittal section through parathyroid region in *Xenopus laevis*. × 50.
A. Carotid body; *B*. Pigment in carotid body; *C*. Antero-dorsal glandule; *D*. Postero-ventral glandule; *E*. Systemic artery.

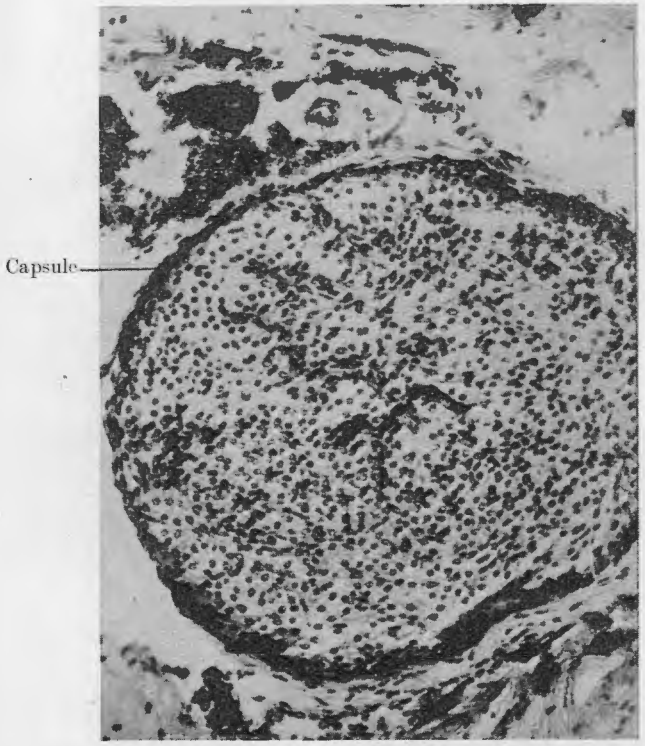

Fig. 4. High power view of a parathyroid glandule. × 200.

In *Xenopus* it was found that one glandule is generally dorsal, anterior and somewhat medial to the other, the adjacent surfaces being separated by fibrous tissue. They are both irregular bodies, the antero-dorsal glandule tending to be spheroidal and the postero-ventral one pyramidal in shape (fig. 3). Each glandule is of the order of 0·3–0·4 mm. along its greatest axis as compared with 1 mm. in other Anura generally. Occasionally only three glandules could be found, two on one side and one on the other.

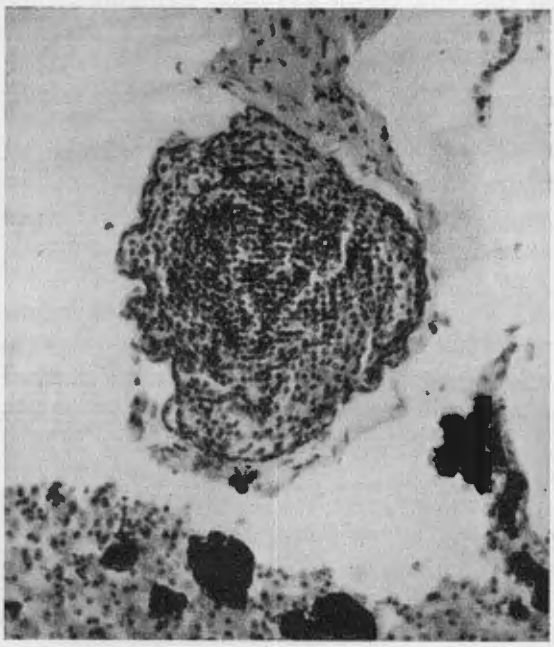

Fig. 5. Photomicrograph of a glandule which showed a whorl arrangement. × 200.

HISTOLOGY

The tissues were fixed in formalin, and some were stained with haematoxylin and eosin and others with van Gieson's stain.

Each glandule is invested in a strong compact fibrous tissue capsule which is surrounded by looser fibrous tissue continuous with the adventitia of the neighbouring blood vessels.

The structure of the glandules (fig. 4) is identical with that described for other Anura in that the interior of the body is compact and consists of epithelial cells which are closely packed especially towards the centre. Some of the cells are round, others elongated, and they contain round, spindle-shaped or elongated nuclei which stain very deeply. Some of the round cells tend to be vacuolated.

On the other hand it must be remarked that, whereas the cells of the parathyroids of other Anura are characterised by a whorl arrangement of the cells as if the body had been subjected to a process of torsion, in only a very small percentage of the parathyroids of *Xenopus* was there any suggestion of such a disposition of the cells. Fig. 5 is a photomicrograph of one such case. In the fact that it generally lacks this characteristic and in the smallness of its dimensions the parathyroids of *Xenopus* resembles more that of the Urodeles than of the Anura. In this connection it is interesting to note that Rimer finds that the pituitary of *Xenopus* essentially conforms to the salamandrine type in the incomplete separation of the pars tuberalis from the pars anterior, and that Zwarenstein and Schrire noted that the adrenal gland of *Xenopus* is essentially similar in distribution and structure to the adrenal of Urodeles.

I wish to thank Mr C. A. du Toit for helpful criticism of the manuscript, and Mr B. McManus for the photomicrographs.

REFERENCES

(1) Rimer, G. E. G. (1931). *Trans. Roy. Soc. S.A.* vol. xix.
(2) Zwarenstein and Schrire (1932). *Proc. Roy. Soc. Edin.* vol. lii, Part 3.
(3) Epstein, Gunn, Epstein and Rimer (1932). *J. Exp. Biol.* vol. ix.
(4) Toldt, C. (1868). *Sitzungsber. d. k. Akad. d. Wissensch. Wien, Mathem.-Naturw. Cl.* Abthl. ii, Bd. 58.
(5) Maurer (1899). *Semon, Zoolog. Forschungsreisen*, Bd. 3.
(6) Grobbelaar (1924). *S. A. J. Science*, vol. xxi.
(7) Gilchrist and von Bonde (1922). *Practical Zoology.*
(8) Gaupp. *Anatomie des Frosches.*

SUBMITTED AS COLLATERAL TESTIMONY OF WORK UNDERTAKEN.

HISTOLOGICAL CHANGES IN THE OVARIES AND OVARIAN BLOOD VESSELS OF *XENOPUS LAEVIS* ASSOCIATED WITH HYPOPHYSECTOMY, CAPTIVITY AND THE NORMAL REPRODUCTIVE CYCLE

By B. G. SHAPIRO and H. A. SHAPIRO

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HISTOLOGICAL CHANGES IN THE OVARIES AND OVARIAN BLOOD VESSELS OF *XENOPUS LAEVIS* ASSOCIATED WITH HYPOPHYSECTOMY, CAPTIVITY AND THE NORMAL REPRODUCTIVE CYCLE

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(From the Department of Physiology, University of Capetown.)

(*Received 2nd May*, 1933.)

(With One Plate and One Text-figure.)

INTRODUCTION.

Hogben (1930) and Hogben, Charles and Slome (1931) have shown that hypophysectomy results in involution of the ovaries in *Xenopus*. In the course of a different series of experiments in this laboratory (Zwarenstein and Shapiro, 1933) it was observed that "animals which had been kept in the laboratory for several months showed a progressive involution of the ovaries according to the length of captivity," and that the involution was not as marked as that observed after hypophysectomy in Hogben's experiments or in the experiments of Shapiro and Zwarenstein (1933).

In order to unmask this captivity effect on the ovaries, it was necessary to compare captive toads with fresh animals taken from the ponds (called "vleis" in South Africa) so as to take account of possible variations under natural conditions.

In this paper it is proposed to investigate also, for the first time, the detailed histological changes in the ovaries of *Xenopus laevis*, following hypophysectomy and captivity; and the significance, if any, of the time factor involved.

EXPERIMENTAL.

All the toads used throughout the period of these experiments were obtained from the same pond in the Cape Peninsula. Some 500 animals were collected in January, 1932, and transferred to a large open-air tank, to investigate the effect of captivity. Another batch of toads was hypophysectomised by the method of Hogben, one half of the batch having their anterior lobes, and the other half of the batch having both lobes removed. These animals were also placed in open-air tanks. The water in the tanks was changed three times a week, and the animals were fed on meat twice weekly. On each occasion when vlei material was collected and killed for examination, corresponding samples of captive and hypophysectomised material

were investigated in the same way. The animals were pithed and then immediately weighed, after which the ovaries were removed and also weighed.

A useful numerical index of the condition of the ovaries is obtained from the ratio of ovary weight/body weight.

Typical samples of the ovaries were prepared for histological examination.

RESULTS.

The gonad ratio.

The figures in Table I represent mean values of the ovary weight/body weight ratios of toads killed in batches of ten at intervals throughout the year. Figures recording the body weights of the corresponding batches of animals are also included.

In the pond. An analysis of Table I will show, in the first instance, the important fact that there are seasonal variations in the condition of the ovaries of *Xenopus laevis*. These changes have not been described before, nor has the breeding season

Table I. *The figures represent the body weights of the animals as well as the mass of the ovaries relative to their body weight*, i.e. *the gonad ratio.*

	(1) Vlei toads		(2) Captive toads	
	Body weight	Gonad ratio	Body weight	Gonad ratio
Jan.	46 ± 1·5	0·075 ± 0·006	46 ± 1·5	0·075 ± 0·006
Feb.	43 ± 3·0	0·064 ± 0·014	59 ± 1·0	0·070 ± 0·005
March	—	—	56 ± 5·8	0·062 ± 0·007
July	37 ± 6·3	0·113 ± 0·024	30 ± 6·6	0·043 ± 0·008
Sept.	39 ± 6·1	0·105 ± 0·025	32 ± 5·1	0·035 ± 0·005
Dec.	45 ± 8·1	0·052 ± 0·020	19 ± 2·4	0·030 ± 0·007

	(3) Anterior lobe hypophysectomy		(4) Total hypophysectomy	
	Body weight	Gonad ratio	Body weight	Gonad ratio
Jan.	46 ± 1·5	0·075 ± 0·006	46 ± 1·5	0·075 ± 0·006
Feb.	Animals hypophysectomised			
March	—	0·058 ± 0·012	—	0·055 ± 0·010
July	41 ± 5·3	0·013 ± 0·006	42 ± 6·1	0·011 ± 0·006
Sept.	37 ± 7·0	0·008 ± 0·004	51 ± 9·5	0·010 ± 0·004
Dec.	—	—	—	—

of *Xenopus laevis* been clearly established. It must therefore be reported that copulating couples were observed in the material collected from the vlei in July and September, thus indicating the probable breeding season, which would correspond to mid-winter and early spring in South Africa and to the rainy season in the Cape Province. In conformity with this tadpoles undergoing metamorphosis, *i.e.* 3–4 months old, were observed in the pond material collected in December. Further, the ratio of the mass of the ovaries to their body weight, *i.e.* their relative

mass, is highest in the late winter and early spring months. This coincides with and is substantiated by the fact that during these months the ovaries are full of large mature eggs ready for extrusion, whereas in the summer months the ovaries are smaller and lighter.

The effect of captivity. The ratios of the captive material show a steady diminution with increasing duration of captivity. This is correlated with a steady increase in the degree and number of involuted ovaries in the samples examined. This phenomenon probably explains the fact that *Xenopus* cannot be induced to breed in captivity. It must be recorded that occasional toads, about one out of every ten, did not show the ovarian regression so characteristic of captivity animals after 5 or 6 months. In spite of regular feeding and change of water, a marked degree of emaciation was observed in the females in the later months of captivity, whereas all the male toads appeared to be quite well and in good condition.

Anterior lobe hypophysectomy. Removal of the anterior lobe of the pituitary results in an ovarian regression qualitatively similar to that of captivity only much more rapid. The ovaries regress from a healthy state to a gelatinous mass in which individual ova cannot be distinguished macroscopically. This is clearly demonstrated by comparing the gonad ratios of captive animals in column 2 with gonad ratios of anterior lobe hypophysectomised animals in column 3 in Table I.

Total hypophysectomy. The gonad ratios for the first 6 months following total hypophysectomy are slightly, but not significantly, lower than the gonad ratios for anterior lobe removal over the same length of time. By the seventh month after total hypophysectomy, however, the gonad ratios for totally hypophysectomised animals were slightly but not significantly higher than the ratios for animals which had had only their anterior lobes removed.

Hogben and co-workers were able to show significant differences between the ratios of animals which had been deprived of both lobes of the pituitary as compared with animals which had had only their anterior lobes removed. The present authors were able to report a tendency in this direction, but the differences cannot, in the experiments above, be called significant differences.

All the above results are summarised in Text-fig. 1.

Histology.

Numerous sections of the ovaries of different animals were prepared and stained. Haemotoxylin and eosin, Mallory's stain, and van Gieson's stain were employed in all the preparations made.

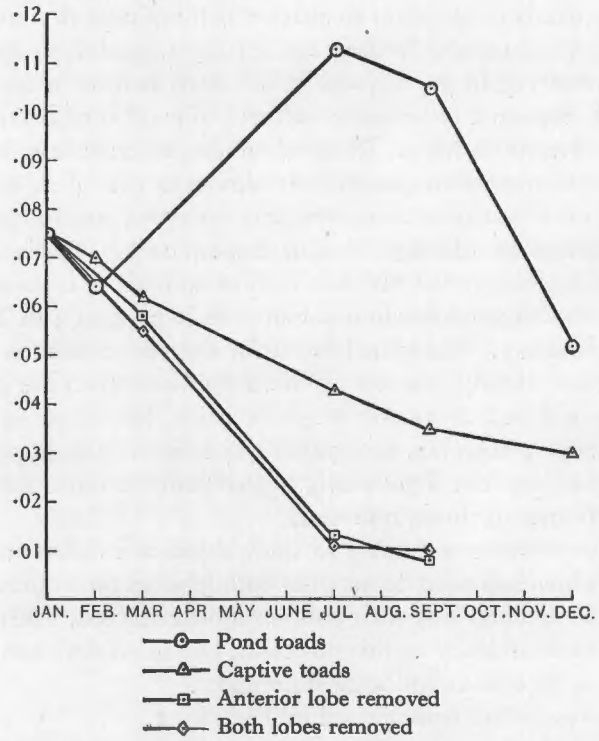
The normal ovary. For a typical example of the normal ovary of *Xenopus laevis*, see Fig. 1, which is a photomicrograph of a normal ovary stained with Mallory's stain. The ovary was removed from a copulating female.

In *Xenopus* during the breeding season the ovaries are large and fill to distension the abdominal cavity. The mass of the ova, which for the most part comprises the ovary, is made up of mature as well as immature eggs, black at one pole and yellow at the opposite pole. The ova are easily visible to the naked eye, while the ovary itself has a mesenteric attachment along the length of the kidney, and is invested

with a very thin connective tissue capsule sending in septa which constitute a framework supporting the ova. The connective tissue capsule of the ovary contains small blood vessels with thin muscular walls.

The ova themselves may be divided into two groups: (1) large central, and (2) small peripheral ova.

The large central eggs are characterised by pale, clear staining nuclei. The cytoplasm consists of ovoid discs, the largest of which are arranged around the nucleus, becoming smaller towards the periphery. A well-marked layer of black



Text-fig. 1. Graph to show the relative mass of the ovaries of the South African clawed toad in the pond, in captivity and after hypophysectomy.

pigment, several granules deep, can be seen lying most peripherally in those eggs which have been cut through the pigmented pole. This granular layer is absent in the yellow hemisphere of the ovum. There appears to be a delicate connective tissue reticulum supporting the ovoid discs of the cytoplasm.

The peripheral ova are small and lie in direct relation to the thin connective tissue capsule which invests the ovary and sends in thin septa. Their nuclei contain a large number of dark red staining granules. The cytoplasm of such ova is hyaline and non-granular in appearance, occasionally staining a deep blue colour with haemotoxylin and eosin. These peripheral ova correspond to the small immature ova which can be seen by the naked eye disposed about the periphery of the ovary.

The ovary in captivity. In captivity the ovaries present a totally different appearance. Fig. 2 is a photomicrograph of the ovary of a toad in captivity for $5\frac{1}{2}$ months, and represents the maximal degree of retrogression which occurs in captivity. To the naked eye the ovary appears as a gelatinous mass in which individual ova cannot be detected. There is also an increase in the number of peripheral ova as compared with the normal ovary.

The blood vessels which lie in the connective tissue capsule which invests the ovary have, in some places, slightly thicker muscular walls than in those of the normal ovarian capsule. The latter itself appears also to have undergone a slight thickening or fibrosis in the captive animals. It is important to note that in animals examined after $4\frac{1}{2}$ months in captivity, the central ova could still be made out, but were smaller than those of the pond animals (Fig. 2 *a*).

The ovary after total hypophysectomy. After removal of both lobes of the pituitary gland the typical result is that seen in Fig. 3, which is a photomicrograph of the ovary of a toad hypophysectomised $4\frac{1}{2}$ months previously. It is to be noted that whereas in $4\frac{1}{2}$ months' captivity animals' central ova could still be made out, these have entirely disappeared from the ovaries of animals hypophysectomised for the same length of time.

In the peripheral ova the same hyaline structure occurs as in captivity and normal ovaries described above. In the case of ovaries from pituitary-less toads, however, there is an increased number of peripheral ova as compared with captive or vlei normal ovaries.

A striking feature is the very marked thickening and fibrosis of the connective tissue capsule of the ovary.

In the blood vessels there is a marked proliferation of the intimal connective tissue, as well as a very definite hypertrophy of the muscular media, to such an extent that the lumen of the vessels is almost completely obliterated. This type of change is similar to that observed in cases of arteriosclerosis in man. The marked degree of arteriosclerosis of the ovarian vessels does not occur at all in normal ovaries and only to a very slight extent, if at all, in captive animals after $5\frac{1}{2}$ months.

In all cases the adventitia of the blood vessels is hardly affected. The veins show a marked connective tissue proliferation. Occasionally several small arterioles are included together in an area of arteriosclerotic change, but the lesion may sometimes affect individual blood vessels only (see Fig. 3 *a*).

The above description refers to animals killed $4\frac{1}{2}$ months after total hypophysectomy. In succeeding months, similar but more advanced arteriosclerotic changes supervene, *e.g.* complete obliteration of the vessel lumen 7 months after hypophysectomy (see Fig. 4).

Ovary after anterior lobe hypophysectomy. In all respects the histological changes in the ovary after removal of the anterior lobe alone are the same as those described for total hypophysectomy in the paragraph above.

In view of the pathological significance and the physiological importance of this possible relationship between the hypophysis and the vascular system as a whole,

further investigations are being undertaken with respect to such organs as the kidneys, spleen, pancreas, heart, aorta, etc.

DISCUSSION.

It is clear from the graph printed above that there is a definite seasonal change in the ovaries, which are largest and filled to distension with ova during the breeding season, *i.e.* late winter and early spring. The ovaries are lightest and smallest in the summer months.

Shapiro and Zwarenstein (1933) have shown that castration in *Xenopus* leads to a persistent fall in the serum calcium, and in this connection it is interesting to observe that the serum calcium of vlei, *i.e.* pond, material is low when the relative mass of the ovary is low, and rises when the ratio rises, except in the post-breeding season in December, when the serum calcium is high but the ovary ratio is low. A complete correlation between the condition of the ovaries and the level of the calcium in the serum cannot therefore be described. A detailed discussion of the factors involved in seasonal ovarian changes and in ovarian retrogression in captivity will be found elsewhere in this *Journal* (Zwarenstein and Shapiro, 1933).

Hypophysectomy and captivity, after a sufficient length of time, result in a disappearance of the central mature ova, while the peripheral ova remain unaffected. That the disappearance of the mature ova is related to pituitary function is clearly established by an analysis of the time relations of this phenomenon in hypophysectomised, as compared with captive, animals.

Sections of ovaries taken from toads kept captive for $4\frac{1}{2}$ months show central ova which are beginning to undergo regression. Ovaries from animals of the same batch, but hypophysectomised $4\frac{1}{2}$ months previously, show only peripheral ova, and no central ova at all. Consequently it would appear that the more rapid regression of the ova in the hypophysectomised animals is due to the removal of the pituitary gland. Thus, as there is the same qualitative change in the appearance of the ovaries of the two cases as compared with pond normals, the true difference being in the degree or intensity of the change, it may be suggested that the degeneration of the central mature ova is dependent on the absence of pituitary function in hypophysectomised and diminution of pituitary function in captive animals. It is possible, however, that the captivity effect may be independent of the pituitary, and depend on some such factor as nutrition.

Since, in addition to the disappearance of the central mature ova, there is at the same time an increase in the number of immature peripheral ova, the tentative suggestion may be made that the pituitary is concerned in the process of maturation of the ova of the South African clawed toad. Such an hypothesis will explain also the fact that all ovaries, whether of captive, hypophysectomised or normal copulating females, contain peripheral ova which are morphologically identical. The regressive changes described in captive and hypophysectomised animals occur only in the fully matured central ova.

There is a marked disparity in the ratios, *i.e.* the mass of the ovaries relative to

their body weight, of vlei animals at the height of the breeding season as compared with the captive animals examined at the same season of the year.

It may be remarked at this stage that, in the experiments of Hogben and co-workers, the ovaries did not regress in captivity. This is probably due to the much greater light ration supplied to their animals. In their experiments, referred to in fuller detail in another contribution in this *Journal* (Zwarenstein and Shapiro), the captive animals were kept in a warm room with the electric light switched on day and night, whereas in the experiments described in this communication the toads were kept in subdued light during the day and in total darkness during the night. That light does affect the mass of the gonads in vertebrates was proved by Bissonnette (1932) who showed that red rays have a stimulating effect on the growth of the testes.

The decrease in the ovary-body weight ratio with captivity appears to be due to the disappearance of the large central ova. With removal of the pituitary there is a still more marked decline in the relative mass of the ovary. (See graph.) This may be correlated with the fact that, in addition to the degeneration of the central ova, severe arteriosclerotic changes supervene in the vascular system of the ovary of the hypophysectomised animal. These changes occur hardly, if at all, in the corresponding captive material. The arteriosclerotic changes would result in a diminished blood supply to the part, thus leading to a general atrophy of the organ concerned. In some cases the sclerotic changes were so severe as to result in a complete obliteration of the vessel lumen 7 months after the operation. The superposed vascular lesion would thus explain the more rapid, as well as the quantitively more severe decline, in the relative mass of the ovary of the hypophysectomised as compared with captivity animals.

Finally, since the histological effects of total or partial removal of the hypophysis are identical, it appears likely that the absence of the anterior lobe alone is the primary factor concerned in initiating the histological changes described.

SUMMARY.

1. Seasonal changes in the ovaries of *Xenopus laevis* (the South African clawed toad) are described.

2. Degenerative changes in the large mature central ova occur more rapidly as a result of hypophysectomy than as a result of captivity.

3. The suggestion is made that the pituitary may be concerned with the maturation of ova in *Xenopus laevis*.

4. Hypophysectomy results in severe arteriosclerotic changes in the ovarian blood supply as early as $4\frac{1}{2}$ months after the operation. Control animals show no such changes.

5. The histological changes observed in the ovaries after hypophysectomy, whether total or partial, are probably due to the removal of the anterior lobe alone.

We are indebted to Mr Bernard McManus for the photomicrographs, and wish to thank Dr Louis Mirvish for helpful criticism of the manuscript.

REFERENCES.

BISSONNETTE, T. H. (1932). *Physiol. Zool.* **5**, 92.
HOGBEN, L. T. (1930). *Proc. Roy. Soc. S. Africa*, March, 1930.
HOGBEN, L. T., CHARLES, E. and SLOME, D. (1931). *Journ. Exp. Biol.* **8**, 345.
SHAPIRO, H. A. and ZWARENSTEIN, H. (1933). *Journ. Exp. Biol.* **10**, 186.
ZWARENSTEIN, H. and SHAPIRO, H. A. (1933). *Journ. Exp. Biol.* **10**, 372.

EXPLANATION OF PLATE I.

Fig. 1. Normal ovary. *A*, connective tissue capsule; *B*, peripheral ova; *C*, central ovum. (×50.)
Fig. 2. $5\frac{1}{2}$ months' captivity. *A*, connective tissue capsule; *B*, peripheral ova. (×50.)
Fig. 2 *a*. $4\frac{1}{2}$ months' captivity. *A*, connective tissue capsule; *B*, peripheral ova; *C*, small central ova. (×50.)
Fig. 3. $4\frac{1}{2}$ months after hypophysectomy. *A*, thickened capsule; *B*, peripheral ova; *D*, arteriosclerotic vessel; *E*, accompanying vein. (×50.)
Fig. 3 *a*. $4\frac{1}{2}$ months after hypophysectomy. *D*, arteriosclerotic vessels; *E*, accompanying vein. (×50.)
Fig. 4. 7 months after pituitary removal. *A*, thickened capsule; *B*, peripheral ova; *D*, arteriosclerotic vessels. (×50.)

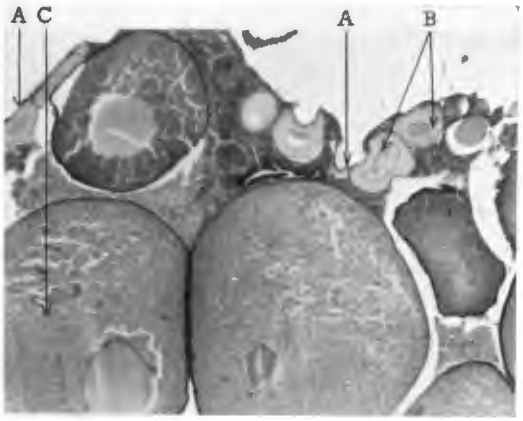


Fig. 1.

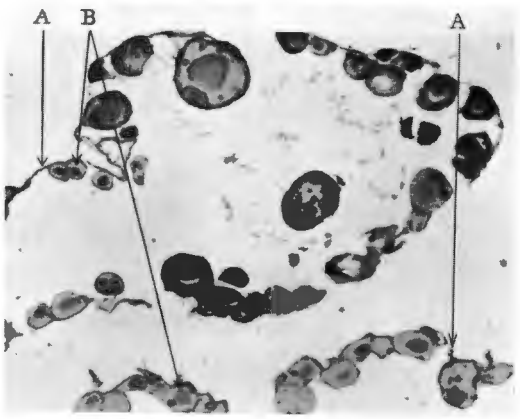


Fig. 2.

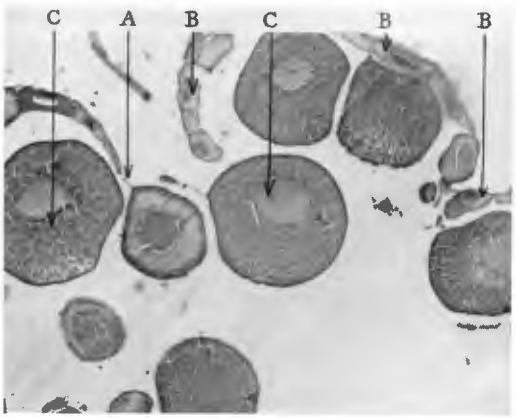


Fig. 2 *a*.

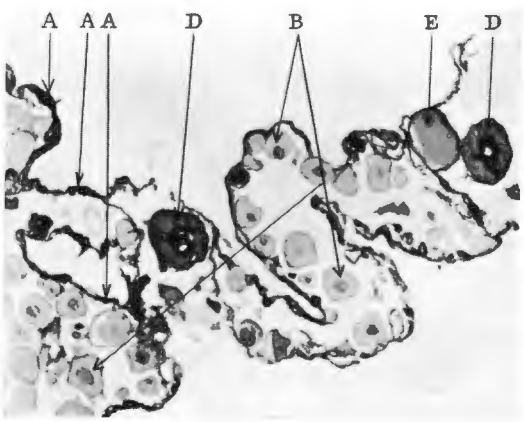


Fig. 3.

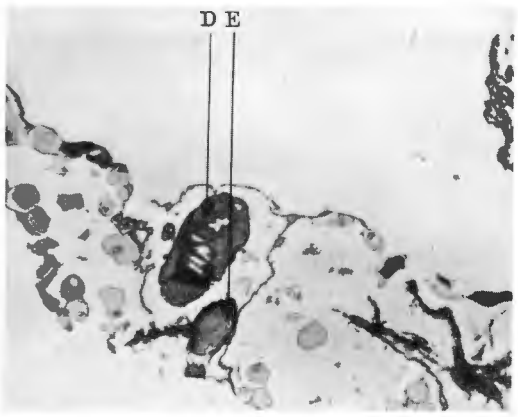


Fig. 3 *a*.

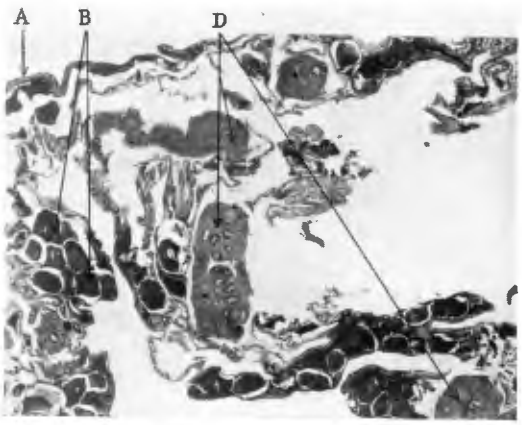


Fig. 4.

SHAPIRO AND SHAPIRO—HISTOLOGICAL CHANGES IN THE OVARIES AND OVARIAN BLOOD VESSELS OF *XENOPUS LAEVIS* ASSOCIATED WITH HYPOPHYSECTOMY, CAPTIVITY AND THE NORMAL REPRODUCTIVE CYCLE (pp. 73—80).

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STUDIES IN THE PHYSIOLOGY OF

CREATINE AND CREATININE.

SUMMARY of THESIS

Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the

DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF CAPE TOWN,

by

B. G. Shapiro, M.A.

1936.

SUMMARY of THESIS.

INTRODUCTION:

The literature up to 1935 is discussed.

Those aspects of the physiology of creatine and creatinine are dealt with, which are necessary in order to obtain a true perspective of the investigations undertaken in relation to the physiology of creatine and creatinine as a whole.

Included in the introduction is a criticism of the widely-held view that creatinine excreted is an index of endogenous protein metabolism. An alternative view is submitted. Various other theories, such as Cameron's hypothesis (1933) for the mechanism of creatinine transformation, and Schrire and Zwarenstein's explanation (1934) of the creatinuria of pregnancy are critically discussed.

EXPERIMENTAL RESULTS:

The investigations undertaken are described in three sections:-

1. The Relation of the Gonads and the Pituitary Gland to Muscle Creatine in the South African Clawed Toad.

The conclusions reached are as follows:

(A) The Normal Toad.

(1) In a normal toad the creatine content of the

/'hamstring'

'hamstring' muscles is the same for the right and left legs.

(2) There is no difference in the muscle creatine level in the two sexes.

(3) The average value is 399 mg. creatine per 100 g. muscle.

(4) The highest value obtained for a normal toad was 440 mg. and the lowest 349 mg. per 100 g.

(5) The creatine content bore no relation to the size of the animal.

B. Captivity.

The muscle creatine falls slightly with captivity.

C. The Gonads.

(1) Castration of males and females does not affect the muscle creatine as long as 4½ - 5½ months after the operation.

(2) There was no difference in the concentration of creatine in the muscles of toads with normal ovaries or with ovaries which had markedly regressed.

D. Hypophysectomy.

(1) There is a decrease in muscle creatine after removal of anterior lobe alone and after total hypophysectomy, 5½ - 6½ months after operation.

(2) The decrease after total hypophysectomy is not significantly greater than after removal of anterior lobe alone.

(3) The average decrease after hypophysectomy is about 15 per cent.

E. Injections of Anterior Lobe Extract.

(1) Acute injections of 1 ml. of a Bellerby extract of anterior lobe does not affect the muscle creatine of normal toads.

(2) Chronic injections of 0.2 ml. of Bellerby extract of anterior lobe results in a rise of muscle creatine in normal toads. This increase commenced 8 - 16 days after injections were started.

The maximal increases obtained were in the region of 30% above control animals injected with brain extract.

(3) No antihormonic effect on the muscle creatine was observed.

(4) Some evidence was obtained that the muscle creatine of toads which had been hypophysectomised 11 months previously, could be raised by chronic injections of anterior lobe extracts. Insufficient animals were used, however, to permit making a definite statement.

F. Injections of Pituitrin.

(1) Acute injections of 1 ml. Parke, Davis Pituitrin produced a decrease in muscle creatine within 5 - 10 hours after injection. 30 hours after injection an average fall of 18% was observed.

(2) Evidence is submitted to show that this fall is at least partly due to the absorption of water from the surrounding medium by the muscles.

(3) Reasons are given why it is considered that this decrease in muscle creatine is not considered to be a function of the posterior lobe.

(4) There is no evidence that the gonads influence the muscle creatine in Xenopus laevis.

G. Endocrine Relationships.

It is concluded that

(1) the evidence presented is suggestive of an endocrine relationship between the anterior lobe and muscle creatine.

(2) The relation is probably an indirect one via some other endocrine organ, possibly the suprarenal.

(3) There is no evidence that the posterior lobe has any influence on muscle creatine.

(4) There is no evidence that the gonads influence the muscle creatine in Xenopus laevis.

H. Relation of Urinary Creatinine to Muscle Creatine.

The findings in regard to the anterior lobe, taken in conjunction with the work of other investigators, afford additional evidence for the view that not only is there a metabolic relation between creatine and creatinine, but that an increase in urinary creatinine is indicative of an increase in muscle creatine.

I. Myasthenia Gravis.

It is suggested that injection of an extract of the anterior lobe of the pituitary may be of use in the treatment of myasthenia gravis.

II. <u>The Precursors of Creatine and Creatinine.</u>

The conclusions reached are as follows:

(a) The findings of Beard and Barnes are not supported.

(b) The injection of arginine and histidine into adult male rabbits gives rise to a 10 - 40% increase in the elimination of urinary creatinine.

(c) Feeding with glycocyamine has no effect on creatinine excretion but leads to a large output of creatine.

(d) The amino-acids, glycine and alanine (injected), and tyrosine cystine and glutamic acid (ingested), have no effect on creatinine excretion.

(e) The fact that five out of the seven amino-acids administered were without effect indicates that the increased obtained with histidine and arginine are not due to specific dynamic action.

(f) The fact that glycine was without effect is of interest in view of the effect it is said to have in muscular dystrophies. The conclusions of Zwarenstein (1928), and of Christman and Mosier (1929), that glycine does not influence the creatinine excretion in the normal animal is corroborated, and the more recent work of Beard and Barnes (1931) is not confirmed.

(g) It is suggested that the transformation of arginine and histidine into creatinine takes place in the muscles, probably via creatine, and that the glycocyamine-creatine change is a direct one taking place in the liver.

III. <u>The Action of Creatine and Creatinine on the Circulatory System.</u>

(A) <u>The Action of Creatine and Creatinine on the Circulatory System during Muscular Exercise.</u>

The conclusions reached are as follows:

(1) Creatine and creatinine produce an increase in amplitude of the beat of the perfused isolated heart of the South African clawed toad, the rabbit and the cat.

(2) Creatinine produces a greater increase in amplitude of the heart beat than corresponding concentrations of creatine. A 30% increase is produced by a 1:500 solution of creatinine in toads and by a 1:1000 solution in rabbits and cats.

(3) Both creatine and creatinine produce only a slight increase in blood pressure in the cat, and a slight dilation of the vessels on perfusing pithed toads.

(4) The concentration required to produce the above effects is too great to suggest that creatine and creatinine are agents of any importance in assisting in the production of the cardiovascular changes which accompany muscular exercise.

(B) <u>An Unusual Action of the Heart of the South African Clawed Toad as shown by its response to Creatine.</u>

(1) Attention is drawn to the unusual nature of the response of the heart of the South African Clawed Toad to creatine. It resembles the response of the heart of Rana temporaria to alcohol and to an increase of Na-ions in the perfusing fluid, as reported by Burridge (1923, 1934).

(2) In order to explain the tracings obtained, two mechanisms are postulated. (1) A depressor mechanism due to the action of creatine on the cell surface, and (2) an augmentor mechanism due to the action of creatine on the interior of the cell,