PITUITARY – GONADAL CONTROL

OF

CREATININE METABOLISM.

THESIS

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R. W. S. CHEETHAM. M.A.

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INTRODUCTION.

INTRODUCTION.

The endocrines in general for a considerable period have been known to exert a humoral action on the body, controlling its functions to a large extent. These actions have been closely investigated and a clear correlation between the ductless glands has been shown. The interrelation and dependency of the one upon the other, either by inhibition or by stimulation, contributes towards the finer control of the physiology of the body. The studies recorded here are comprised mainly of the dependency of one gland upon another, the relation the normal gland has to protein metabolism, and the alteration in this protein metabolism brought about by alteration in the normal function of one or other of the ductless glands. These features are demonstrated by observing changes in the elimination of creatinine in urine, for this urinary creatinine is regarded as an index of endogenous protein metabolism, and the amount excreted is remarkably constant from day to day.

CREATINE AND CREATININE METABOLISM.

Urine has as its most important nitrogenous constituent urea. The excretion of urea is, however, extremely variable, and even if exogenous urea is controlled, endogenous urea is still subject to marked variations. Therefore, if an index of the endogenous protein metabolism of the body is required, urea must be neglected and with it the total nitrogenous protein output. A more accurate measure would be to obtain a substance which is subject to the least possible variations. Such a substance is urinary creatinine, which fulfils certain roles in the body. Its significance is discussed below. Many investigations have shown the clear metabolic relation existing between creatine and creatinine, and it is, therefore, advisable to discuss the significance of both in this introduction, especially as creatine is known to exist as such and in different combinations, whereas creatinine and its site and origin are still the subject of much controversy.

HISTORICAL:

1832: Chevreul (French Academy of Sciences) postulates the presence of a new organic substance in meat which he called "creatine" from κρεάς κρέατος meaning meat. Fifteen years later Leibig established the empirical formula $C_4 H_9 O_2 N_3 . H_2O$ and found that on heating with mineral acids a derivative which he called creatinine, with the formula $C_4 H_7 ON_3$, was formed. In 1844, Heinz and Pettenkofer had shown the presence of a hitherto unknown nitrogenous constituent of normal urine. This Leibig later proved to be identical with his creatinine. These were the earliest workers to establish the existence of creatinine in urine.

DISTRIBUTION OF CREATINE AND CREATININE:

Hunter (28), Myers (32), Rose (33 & 35) and Kayser (34), reviewed the metabolism of creatine and creatinine, and it is on their admirable reviews and other current literature that this introduction is based.

Normal Distribution:

Muscle: Creatine is found mainly as a constant component of the skeletal muscles of mammals, birds, reptiles, amphibia, and fish, and of certain organisms of a lower order. It is not, however, found in crabs, where it is largely replaced by arginine. Red muscle contains less than pale, and Seecol, Linegar and Myers have shown the left ventricle to contain more than the right.

Generally the concentration in cardiac muscle, however, is considerably less than in striated muscle of of the same species. There is no convincing evidence as to its isolation from smooth muscle, but this does seem probable. Creatine has also been isolated in the brain, kidneys and testes of vertebrates. Creatinine, however, has not been so definitely isolated, the difficulty being that the creatine present is very easily dehydrated and creatinine formed.

Blood: The occurrence of both substances in blood has been established but there is considerable controversy as to the amounts as the methods used are open to several discrepancies.

The existing values in human blood accepted by many workers are: Creatine 4 mg/100 ml., creatinine 0.8-1.8 mg/100 ml., but according to Bohn and Hahn the true creatine is 0.5-0.8 mg/100 ml., as they state that chromogenic substances are present and these interfere with the estimations. Gaebler is definite that creatinine does not exist in the blood but is derived from a precursor other than creatine, and this yields creatinine in isolation experiments. But Kacl points out that ox and horse corpuscle adsorb creatinine and this is affected by the addition of acids and alkalis, and is diminished according to the degree of acidity or alkalinity.

Linneweh, Hayman, Johnston and Bender all show creatine in blood or serum, while Ferro-Luzzi states that practically all the chromogenic substance in blood in a Jaffe reaction is creatinine; and it is suggested that the Somogi method for blood-creatinine is probably truer than the Folin-Wu by Ferro-Luzzi, Saladino and Santamaura.

Titone, Hagi-Parashiv, Cimino-Beranger et. al. worked on alterations in creatine in blood. Intestinal obstruction (Rigo and Frey) increases creatine in blood, while high sodium chloride diet and poor nitrogen diet and insulin and adrenalin excess give a lowered blood creatine. Linneweh shows that interference with the Folin method of chromogenic substances which are present in urine does not occur in estimations in blood.

In nephritis, however, the apparent creatinine content of the blood may rise considerably and is more readily estimated. The rise depends on the extent of the renal lesion. Stacey finds that following the administration of creatine the creatine content rises and the organic phosphate of the plasma fall: this relationship is not quantitative.

In contrast to their wide distribution in vertebrates creatine and creatinine apparently are not components of invertebrate tissues.

Urine: In urine both occur under certain circumstances. Creatine is not found in the normal mammalian adult male urine on a creatine-free diet, but it does occur physiologically in urine of children, pregnant women and, intermittently, in the urine of non-pregnant adult females.

Creatinine occurs regularly in mammals and in a considerable quantity. As such it is important and its quantity clearly indicates a waste product. Its place is taken by creatine in birds and reptiles.

Factors influencing Creatine Distribution:

Age, exogenous feeding, fasting and disease. The adult level is only reached some time after birth. Chanutin, experimenting on rats, found this to be between the 30th and 40th day.

ORIGIN OF CREATINE.

In '28 Hunter described creatine as probably an essential tissue constituent with a special function, its rate of production being regulated by internal demand. Arginine, cystine, histidine, purine and purine derivatives, choline and oxidation products were suggested as precursors. Numerous theories exist as to the origin of creatine even now. It is not even decided if it is an anabolic product, the formation of which is determined by the requirements of the organism, or a catabolic substance, the production of which is determined by the intake of one or more exogenous precursors.

Arginine:

Takahashi, Kumon, Shapiro and Zwarenstein show that the administration of arginine by injection or parental administration led to an increase in urinary creatinine. The administration of carnatine or γ-amino-β- hydroxybutyric acid also led to an increase, as show Takahashi and Kumon, who suggest a mechanism based on methylation or demethylation and subsequent combination with guanidine. Hyde and Rose, Grant, Christman and Lewis deny this. But the data of both do not include the possibility that creatine and creatinine may be manufactured through arginine as an intermediary, inasmuch as this can be synthesised in the rat, and the production of total creatinine in the rat is not dependent on the consumption of preformed arginine in the food (Scull and Rose).

33. Meyer and Rose: Arginine intake could only account for 2/3 of total creatine and creatinine formed and Hongo in 1935 stated that arginine is only partly converted to creatine in the stomach mucosa.

N.B. that Broude shows arginine to take the place of creatine in muscle of crab Astacus Fluviatilis.

In 1936 Bodansky stated that guanidoacetic acid can form creatine but glycine cannot. The same year Beard and Boggess administered 100 mgm. of creatine, glycoamine, histidine, glycine, alanine, serine and valine; if injected intraperitoneally all produced an increase in muscular creatine from 6 to 30%. A larger dose of arginine produced a greater response, but a smaller response followed with a larger dose of glycine.

<u>Nuclear Material:</u>

Abderhalden and Buadze; Chrometzka - administration of nucleosides, purines or uric acid increases the daily output of total creatinine. This is denied by Schumon but Abderhalden and Buadze show where his discrepancies are probable.

<u>Glycine:</u>

Zwarenstein, Christman and Mosier observed no influence on feeding glycine to normal men on the hourly output of preformed creatinine. But many workers on patients suffering from various types of myopathy show a relation between glycine administered and the creatine and creatinine output.

In certain conditions in which there is marked atrophy of the muscular system creatinine output is generally lowered and creatine appears in the urine in excessive quantities. There is also a diminished tolerance for ingested creatine. (Mader, Selter and Schellenberg; Sullivan and Hess; Magee; Ley and Titeca) Milhorat et al. state that glycine administered to these patients induces a greater output of creatine. This results in a lowered creatinine excretion. This increased excretion attains a maximum after two weeks despite continued glycine feeding. It then begins to fall and with this the creatinine excretion increases.

Harris and Brand fail to confirm this. Mader, Selter and Schellenberg also noted the creatinogenic action of glycine but stated that there was no alteration in the creatinine coefficient. This Reinhold et al confirmed. Gelatin (Kismer, West and Key) increases the creatine output and is notably rich in glycine. According to Beard and Tripoli glycine administration to the primary myopathies or myasthenia gravis brings about distinct clinical improvement; at the same time they show the increase and decrease in creatinuria mentioned above. In the secondary muscular atrophy, although the alterations in creatinuria are shown, there is no real improvement. Milhorat even fails to note changes in creatine excretion in these secondary types.

Sullivan, Hess and Irrevere state that urine in a patient with muscular dystrophy contained no guanidine but a simple guanidine complex such as glycoamine which on oxidation yielded guanidine; creatine, creatinine and arginine are unaffected by this treatment. The substance was not present in the urine of three cases of myasthenia gravis, nor in normal urine. In 1936 Ni indicates that if creatine and creatinine are fed to pigs suffering from nutritional muscular dystrophies a smaller percentage of creatine is retained than the controls, but the creatinine coefficient was lower and the creatine coefficient rose. Creatine did not decrease in heart muscle but it did in skeletal muscle which showed histological dystrophy.

It seems, therefore, that glycine exerts a pronounced effect on the creatine-creatinine metabolism in the primary myopathies. As to the formation of creatine from glycine Kostakov and Slanek believe part of the glycine is transformed to creatine, while Tripoli and Beard state that arginine is synthesised from guanidoacetic acid which is methylised and glycine supplies the methyl group. They attempt to show that glutamic acid form creatine better than glycine. This is denied by Thomas, Milhorat and Techner.

Our knowledge is, therefore, very unsatisfactory. Numerous papers have been published with reports of a multitude of amino acids which increase creatinine elimination, also products such as haemoglobin, allantoin, etc., but the results seem rather insufficient at the moment. There are still different schools; one which believes creatine to be an end product of exogenous protein catabolism, and another that creatine is an anabolic product which serves as an indispensable component of the muscle cell. (the latter especially in view of the work on phosphocreatine).

THE CHEMICAL COMPOSITION OF CREATINE AND CREATININE:

C. H. Fiske and Y. Subbarow in 1929 showed that Chevreul's substance creatine existed in the free form in the form of an internal salt: methyl glycocyamine

$$HN = C \begin{array}{l} \diagup NH_3^+ \\ \diagdown \underset{\displaystyle CH_3}{N} - CH_2 \cdot COO^- \end{array}$$

or methyl guanidine acetic acid.

This is easily converted into creatinine by hydrolysis, the latter being the anhydride of creatine formed by the loss of one molecule of H_2O. In neutral or alkaline media creatine ⇌ creatinine is reversible. The change takes place gradually in acid media and is speeded up with heat qualitatively in 15-20 minutes. Creatine - Creatinine equilibrium is very definitely a function of the H-ion concentration. This seems to indicate a possible metabolic relationship.

Creatinine can be graphically represented thus:

$$HN=C\begin{matrix} \diagup NHCO \diagdown \\ \diagdown N(CH_3)-CH_2 \end{matrix} = C_3\,H_7\,ON_3$$

(Methyl glycocyamidine or 2 imido, 5 keto - 3 methyl Tetrahydroimidazole) Mol. Wt. 113. % composition C - 42.5; H - 6.2; O - 1.41; N - 37.2.

THE PHYSIOLOGICAL STATUS AND FUNCTION OF CREATINE:

At first the view was held that creatine was a waste product since it was excreted in the urine and in some cases in quite marked quantities. Its distribution in skeletal muscle was known as far back as Chevreul 1832, but no significance was attached to this. It was merely thought to be an end product of protein metabolism. It was known to have a relation to creatinine excretion but the general opinion was that it was a precursor of this substance.

1855: Valenciennes & Fremy reported that they had found creatinine in combination with phosphoric acid.

1865: Ranke (and later in 1910) regarded creatine as accelerating muscle activity.

In 1907 Spriggs maintained that the greater part of urinary creatinine is derived from muscular tissue and that it is a product of the internal structural metabolism of the muscle and not of its activity; that its quantity is proportional to the mass of normal functionally capable muscle tissue of the body.

1906, Urana suggested that creatine existed in muscle as a very unstable non-dialysable colloid compound forming an integral part of the protoplasm of muscle.

Folin and Denis '14, Myers and Fine '13, Schaffer '14, Benedict and Osterberg '23 - were all of the opinion that creatine did not exist as such in muscle, and in '22 Reisser indicated that the more quickly contracting pale muscle was richer in creatine than the red, and that the more active hind limb muscles were far richer in creatine than the less active forelimbs.

In 1925 Tiegs stated that creatine existed in a special form, i.e., a cyclic one in resting muscle, and during contraction was converted into a tautomer with the conventional formula and decidedly basic properties. The release of this neutralised lactic acid formed during contraction and brought about relaxation.

Phospho-creatine:

In April 27, however, Fiske & Subbarow made the most fundamental contribution to creatine - creatinine metabolism by announcing that voluntary muscle contains an unstable compound of creatine and phosphoric acid which broke down to creatine and inorganic phosphate during contraction and was resynthesised during recovery. They named it phosphocreatine.

At the same time Eggleton & Eggleton demonstrated the presence of a very labile form of organic phosphate, which they labelled phosphagen. (Later showed it to play an important part in contractility). Eventually the two substances were shown to be identical and Fiske & Subbarow pointed out its physical properties. According to them phosphocreatine is made up of one molecule of creatine to one molecule of phosphoric acid. It probably exists in the muscle cell, and as the secondary potassium salt. It is rather stable in alkaline solutions, but on passing to the acid side there occurs a gradual increase in the velocity of hydrolysis. Probably not all creatine is present as such: Eggleton shows that about 20% is in the free state at rest and this is trebled during stimulation and fatigue. Vollner, Ferdman & Feinschmidt; Zanghi; Gerard & Tupikow; Clark: Eggleton & Eggleton; Duliere; Grand & Peraita; Zagami, et al. have shown this to be present in all classes of muscle; cardiac and smooth, testis, spleen of mammals and birds and in all classes of vertebrates. Kutscher & Ackermann;

Lundsgaard et al. demonstrate phosphoarginine in invertebrates yet another factor in the possibility of arginine acting as a precursor of creatine. Phosphocreatine is, therefore, apparently present wherever creatine is and represents the effective chemical state of the latter. In '30 and '31 Lundsgaard and Copenhagen brought about the "revolution" in the chemistry of muscular contraction and showed that phosphagen was the substance which supplied the energy directly for contraction while lactic acid formation in the normal muscle continually provides the energy for resynthesis. Cameron in '33 suggests that when this breakdown of phosphagen into creatine and phosphoric acid occurs, some of the creatine is converted into creatinine, especially as the creatine-creatinine equilibrium is in the direction of creatinine in the neutral solution, and this is increased with an increase in the H-ion concentration. Every time the muscle contracts, therefore, a little creatinine is formed from creatine.

The only drawback to this hypothesis is, that this should give a greater creatinine excretion in people doing active exercise than a person at rest. This is partially borne out by the fact that creatinine excretion rises immediately after exercise, but there is a corresponding drop afterwards, so that the daily creatine excretion is little affected.

There is probably another mechanism for the formation of creatinine independent of exercise. The compensatory drop in excretion possibly indicates an inhibition of this process. A certain proportion of the muscle creatine appears to exist as free creatine and this may be sufficient to account for the creatinine which is constantly produced.

Creatine has a buffering action due to the liberation of base so that there is little change in reaction during contraction and recovery phases: Myerhof & Lefmann, Fiske & Subberow, Olmsted & Simpson, etc.

It is also related to sugar metabolism as shown by the production of hypoglycaemia in fasting dogs on administration it will also reduce the blood sugar level in a diabetic fasting dog in single doses. Continued feeding results in a pronounced increase in the blood sugar. (Hill & Mattison; Peabody & Hill; Kopolowitz, etc.).

<u>THE RELATION OF CREATINE TO CREATININE:</u>

<u>In vitro:</u> Creatine and creatinine are easily interconvertible and under appropriate conditions are readily transformed, one into the other. The direction and extent of this transformation depends on the medium. In neutral solutions the reaction is reversible, while in acid at ordinary temperature creatine → creatinine is gradual and speeded up with heat. At 120°C. under pressure it is brought about quantitatively in 15-20 minutes. In alkaline solutions creatinine is slowly

transformed to creatine. The creatine ⇌ creatinine equilibrium is very definitely one of the H ion concentration. There is thus a possible metabolic relationship.

<u>In vivo:</u> It is not possible to show the above changes and much of the evidence to indicate the relation of creatine to creatinine has been adduced from feeding with creatine and estimating the change in muscle creatine and also the alteration in the creatine and creatinine excretion in the urine. To this end a considerable amount of work has been done on the excretion of creatinine in the urine of mammals and although there have been various workers who deny this, it is definitely shown now that even with a varying diet and N_2 intake, the 24-hourly creatinine output remains remarkably constant. This was first shown by van Hoogenhuyse & Verploegh, '05; Shaffer, '08; Osterberg & Wolf '07; Lefmann on the dog, etc. In 1913 Myers & Fine demonstrated this in the rabbit. But creatine is not found in normal adult male urine.

The creatine creatinine relation is shown, however, in many ways.

On a prolonged creatine-free diet there is eventually a drop in creatinine excretion; this is not found in short period alterations. Ringer & Raiziss '14 showed that a low protein diet for several months produced this lowered daily creatinine excretion which was independent in the alteration of body weight. They were uncertain whether this was due to a low protein diet or low creatine content of the diet, to a change in the mass of muscle or to a fall in the creatine content of the muscle.

Shaffer '07 had shown that where endogenous catabolism is increased, for example exopthalmic goitre, the creatinine coefficient is much below normal. This would, however, indicate that creatinine is not derived from tissue protein.

There are various hypotheses as to the formation of creatine but this is as yet unsettled. Creatine is definitely not a waste product but essential for muscular contraction in the form of phosphocreatine. Continued subcutaneous administration of small doses of creatine leads to a rise in muscle creatine - Myers & Fine, 1913. What is its relation to creatinine and what, therefore, is the original source of urinary creatinine.

SOURCE OF URINARY CREATININE:

It is a waste product as indicated by the amount in the urine. Folin & Klercher in '06 decided it was independent of creatine as ingested creatine produced no increase in urinary creatinine; for given in 5 gm doses it was excreted unchanged. Babin '13 & '14, Myers & Fine (and Ringer & Raizies), Palladin in 1916, showed that there was some relation and indicated a distinct relationship between creatinine coefficient and the % creatine content of the muscle. Work which several workers deny, e.g., Chanutin & Kinard in '32.

The most conclusive and most favourable evidence comes from the feeding experiments of Benedict & Osterberg in 1923, on dogs, and in '26, Chanutin on man.

Benedict & Osterberg kept dogs on a creatine-free diet and then fed them daily for 5 - 10 weeks with creatine hydrate in 0.45 - 0.62 gram doses. The experiments lasted 84 to 128 days, and they showed that during the first week of feeding with creatine, small doses had no effect on the creatine or creatinine excretion - as shown by Folin & Dennis.

But in succeeding weeks there was an excretion of unaltered creatine, and the daily average output of preformed creatinine began a gradual rise. This rise persisted as long as the creatine administration was continued, and was at one time as much as 33% above the normal daily output.

When the creatine was withheld, its elimination in its unaltered form ceased immediately and abruptly, but the creatinine output on the other hand fell as regularly and gradually as it rose. As late as 7 weeks after the last administrated dose of creatine hydrate the creatinine output still exceeded its original value. The authors point out that of the retained creatine (i.e., creatine not eliminated as such) 29.1 to 34.2% reappeared in the urine as extra creatinine. To account for the rest of the creatine there are three possibilities: it may be retained without change, may be catabolised into end products other than creatinine or used anabolically in the synthesis of other chemical compounds. The only change more or less proved is its change to creatinine. They conclude that only one of the end products of creatine metabolism is creatinine, the remaining two thirds of retained material being catabolised by methods which do not yield creatinine.

Rose, Ellis & Helming conducted similar experiments upon two human subjects and the results were in remarkable agreement with Benedict & Osterberg.

These results gave ample evidence for the formation of urinary creatinine from tissue creatine.

It is not, however, necessary that creatinine is the sole end product of creatine catabolism. But it does give a definite origin to urinary creatinine.

McClugage, Booth & Evans; Hodgson & Lewis believe creatinine output to be proportional to the mass of muscle tissue. This is, however, denied by Chanutin & Kinard, Daniels & Hejinian.

Muscular activity also comes into the discussion but this is eliminated by the remarkable constancy in the daily output of creatinine and the fact that although there is an increased creatinine excretion immediately after exercise there is a fall later on which adequately compensates.

But recently many workers believe that creatinine (and creatine) may be either endogenous or exogenous metabolic products.

Terrsine et al. affirm that creatine and creatinine probably arise from entirely different physiological processes. Also Thomas, Milhorat and Techner.

Beard, Tripoli & Andes believe both creatine and creatinine to have an exogenous origin from the amino acids of the diet; while Brand & Harris; Kisner, West and Key believe creatine only is formed from glycine. Terroine, however, does regard the creatinine as endogenous. Loewy, Eysern and Oprisescus show that severe muscular work induces increased excretion of both total and preformed creatinine. They state that creatine only accounts for a very small part of the rise in total creatine.

In this connection the whole of Benedict & Osterberg is recalled, too, where they state other end products may be formed.

Kapeller-Adler & Toda find that a three to sixfold increase in the urinary output of methylamine occurs with creatine feeding. Also that all creatinurias induce an increased methylamine excretion. They believe methylamine as well as creatinine is a product of creatine metabolism.

Weber regards guanidoacetic acid as a decomposition product of creatine.

Creatinine is regarded by Terroine as a product of mineral nitrogen metabolism, while creatine is derived from excess protein disintegration. Thus they are both endogenous.

Additional proof is that various workers, who show that factors causing a decrease or increase in urinary creatinine, have been shown to cause a corresponding decrease or increase in muscle creatine, e.g., Palladin & Kudrjawzewa on a vitamin-free diet produced not only an increase in muscle creatine, but also an increase in urinary creatinine.

Struck & Visscher, however, administered 2 mg of creatine daily to the diet of rats for 4-6 months, but this caused no change in the creatine content of the skeletal muscle, liver or heart.

Boy, however, regards creatinine to be a measure of a certain mineral N_2 catabolism, while creatine is produced if any additional protein be utilised, whether it be endogenous or exogenous. Pariset shows creatine-creatinine synthesis to occur during fasting in rats. But Mendel & Rose showed the synthesis of creatine from endogenous sources during starvation more than 20 years previously.

The general consensus of opinion is, therefore, that creatinine is endogenous in origin, and it is generally held that it is derived from the creatine in the tissues.

Further evidence of this is the lowered daily creatinine output in the myopathies which show a diminished creatine content in the muscles. Also the work on various hormones, for example, Shapiro '35, '36, indicates that injections of extracts of anterior lobe of the pituitary increases muscle creatine, while in 1933 Schrire & Zwarenstein show that similar extracts cause increased elimination of urinary creatinine.

SOURCE AND SITE OF FORMATION OF URINARY CREATININE:

Source: It is more or less obvious that urinary creatinine is a waste product as indicated by the amount in the urine. This is probably due to its relation to creatine which is playing its very significant part in the body, thus accounting for the production of such large amounts of creatinine. (See above).

Site of formation:

The liver was first thought to be the site of formation, the production being due to an enzyme.

Then in 1863 Neubauer regarded its formation as due to the influence of the aequous medium of the blood. Five years later, Voit said it was simply a change in the process of excretion by the acid reactions of the urine. In 1901 Gerard put the responsibility on a kidney ferment and in 1907 Gottlieb and Strangassinger stated that it was formed by an enzyme in every part of the body.

Verplough, van Hoogenhuyse, Lefmann and Mellanby all substantiated this, but Foster & Fisher, and also Taubes and Voegtlin, using Eck-fistula dogs, showed there was no change in the creatinine excretion in mammals. This therefore excluded the liver.

Mellanby 1915, Meyers & Fine, 1922 Russi, disproved the enzyme theory; although conversion of creatine to creatinir can take place in autolysing substances when the concentration of creatine in the digesting mixture is relatively high, but it is not enzymatic as the change in fresh muscle is very much the same as in boiled muscles.

In 1923 Hahn and Meyer concluded the change to be a physiochemical one, as addition of creatine to autolysing blood soon produced conversion to creatinine at the same rate as a phosphate buffer solution of the corresponding H-ion concentration. Therefore creatinine is formed from creatine whenever creatine is found or formed. Since most is stored in the muscle, most of the creatinine will originate in the muscles.

Evidently there is an equilibrium between creatine stores in muscle and the creatinine formed from creatine. Whether this is free to enter into an equilibrium with the creatinine is an objection raised against this theory, but in the opinion of most workers this theory is very acceptable and at present the problem rests upon it.

FATE OF MUSCLE CREATINE:

There is now abundant proof that urinary creatinine can be formed from muscle creatine although it is not the sole end product.

Benedict & Osterberg have shown that one-third of creatine administered is eliminated as creatinine.

Folin & Denis, Myers and Fine, have observed increase in muscle creatine following the creatine administration, but not sufficient to account for all the creatine; denied by Stuck & Visscher; and also Chauntin & Silvettehave stated that the creatine ingested is destroyed.

ORIGIN OF URINARY CREATININE:

Creatinine when formed, as is generally believed, mostly from muscular creatine,evidently is removed by the blood stream and conveyed to the kidney. It was originally thought that the site of origin of urinary creatinine was the kidney. In 1935 Linneweh suggested that creatinine was obtained from normal blood; and the assumption that this was formed in the kidneys was superfluous, urinary excretion being, in all probability, of preformed creatinine. Hemingway proves a fall in creatinine excretion with increase in plasma concentration. In 1936 Itho showed that with a rise of blood pressure the creatinine excretion is normal, while with a lowered blood pressure there is a decrease in creatinine excretion. But Kaplan and Smith assert that creatinine and inulin excretion in urine were equal, regardless of urine flow, or plasma creatinine. They regard the changes considered as really evidence of change in glomerular activity. With increasing concentration of creatinine in plasma, a diminished proportion is removed by the kidney from the blood. 20 mgms. is the absolute amount that the kidney can remove. It may be a pure filtration factor or else a secretion factor in elimination. Both theories are possible (Kay and Shuhan,1933).

Domiquez, Goldblatt and Pomerene introduced creatinine into the blood stream. This was distributed very quickly in a large portion of the volumn of fluid. At first the concentration of creatinine in the plasma diminished rapidly, partly by kidney excretion, and partly by reversible diffusion into the tissue fluid. After equilibrium is reached between the tissues and the plasma the concentration in the plasma depends solely on the kidneys. The rate is proportional to plasma concentration and output of exogenous creatinine is equal to intake of exogenous creatinine. (Cameron: doses of creatinine ingested are eliminated in 24 hours).

Goudsmit, Jnr, kept dogs under amytal with urinary excretion maintained by continuous intravenous saline; and the concentration of chromogenic material ("apparent" creatinine) in arterial and renal venous blood, and in urine was estimated. It was invariably lower in the venous blood returning from the kidney than in arterial blood by 0.08-0.21 mg. per 100 . This decrease averaged 11% of the concentration in aterial blood and is of the same order as the extraction percentage of injected or ingested creatinine.

From the above evidence it seems fairly clear that the kidney merely excretes creatinine, which is brought there as such, and the amount excreted depends on the plasma concentration.

SIGNIFICANCE OF URINARY CREATININE:

In 1905 Folin showed that creatinine is the only nitrogenous product which does not undergo diminution when the protein in the diet is reduced. From this he concluded that creatinine must be the product of a very special type of protein metabolism which proceeds at a unkform rate in all living protoplasm "of the body". He calls this process Endogenous Metabolism and that it is an index of the total normal tissue metabolism! This daily output is more or less constant, but it is in all probability produced largely by endogenous metabolism. Closely related to this metabolism is the constant muscular activity in the body, and it seems reasonable that it may be due to the endogenous metabolism of any other nitrogenous compound in the body such as creatinine.

In 1908 Schaffer et al, refused to accept Folin's views, as he showed in exopthalmic goitre patients that <u>total tissue</u> metabolism has no effect on creatinine; but it was postulated that in disease of the muscles there is a direct parallelism between the creatinine coefficient, (Creatinine in mgs. N2/ Weight in Kgms) of an individual and his muscular development. Schaffer suggested therefore that creatinine is not derived from, nor is an idea of, total endogenous catabolism but of one part of this catabolism - the muscle catabolic process.

This has been especially borne out by the fact that the main source of creatine is the muscle where it is found as phosphocreatine and is continually being broken up into creatine and phosphoric acid.

1907 Spriggs, 1910 Paton, concluded that creatinine is a product of the internal structural metabolism of the muscle, and not of itsactivity.

In 1913 Myers and Fine and in 1916 Palladin, show that a high or low percentage of body creatine is correlated respectively with a low or high creatinine coefficient. This parallelism strongly suggests that there exists a constant relation between the amount of creatine in the body and the rate of creatinine excretion.

There is, however, although the above is accepted, no definite proof that creatine of the muscles gives rise to creatinine of the urine. The work of Benedict & Osterberg leaves no loophole for criticism, and it is universally accepted that creatine can, and does give rise to creatinine in the body. Since by far the major part of the creatine in the body is in the muscles, and since such a large amount of creatinine is excreted daily, and also cases of muscular atrophy, e.g. in the myopathies where a lowered creatinine content of the urine is associated with a lowered creatine content of the muscles, it seems fairly reasonable to assume that the source of creatinine is from muscular creatine.

There are however dissentients, e.g. E.F.Terroine and M.Champagne, who regard creatinine as a product of the universal nitrogen metabolism - i.e. of endogenous protein origin. Therefore, although the actual origin of creatinine is disputed, it is nevertheless universally regarded as endogenous in origin; thereby creatinine metabolism, would act as an index of endogenous protein metabolism since the daily output in the human is more or less constant at about 1.5 gms.per twenty-four hours. This was first shown by Folin in 1906. He stated that on a meat-free diet there was a constant quantity excreted, which varied in different individuals, but was wholly independent of quantitative changes in the total amount of nitrogen excreted. He also showed that changes in urinary volume, too, have no effect on the amount eliminated, and that though the percentage of creatinine might vary over different periods of the day, the 24-hourly output was remarkably constant.

This...

This was confirmed by van Hoogenhuyse & Verplough; Shaffer; Osterberg and Wolf, Lefmann & Shaffer on the dog and man, and Myers & Fine on the rabbit.

But Schulz in 1921 and Zwarenstein in 1926, showed that there were marked variations. The latter's theories have now given way to a definitely established and universally recognised fact that, with a changing diet and nitrogenous intake, the creatine output for days and weeks does not vary by more than 10%.

Urinary creatinine therefore provides an extremely valuable index to endogenous protein metabolism, and alterations in the amount excreted indicates some alteration in endogenous protein metabolism.

THE RELATION OF THE ENDOCRINES TO CREATININE METABOLISM:

The endocrines have, for a considerable period, been known to exert a controlling action of a chemical nature on the functions of the body. As scientific knowledge has progressed, investigation has revealed a close inter-relation between the ductless glands. It is a well established fact that the carbohydrate metabolism is controlled to a very large extent by the actions of the glands of internal secretion; fat metabolism and protein metabolism have not been shown to have such a close connection, yet there is sufficient evidence to indicate that the endocrines do play a part in their metabolism.

These studies recorded here indicate this relationship of the different endocrines to protein metabolism by observing that various endocrine glands alter the normal constant creatinine excretion, and by so doing are altering the endogenous protein metabolism.

THE GONADS AND PROTEIN METABOLISM:

Curatullo and Tarulli in 1895 could find no change in the urinary nitrogen of a bitch three months after castration. In 1898 Perizani indicated a diminution in the nitrogen and urea excreted two to three months after ovariectomising a bitch, but Lüthje four years later was also unable to find any change in the nitrogenous balance of castrated dogs.

In 1913 Kalestinov and also Zuntz showed a post castration decrease after seven weeks, and two years respectively, whereas Korenchevsky in 1925 found a decrease in nitrogenous metabolism in his "fat" castrated dogs, and a slight or no decrease in his "thin" castrated dogs.

These results were all in the nature of gonadectomy, but the first experiments on the effects of injection of testicular tissue were done by Brown-Sequard who, in 1889, when aged 72, injected a very dilute crude aequous extract into himself. He claimed remarkable return of physical and mental endurance and normal intestinal function for four weeks. This was repeated by Pregl in 1896 and Zoth in 1898, with the Brown-Sequard extract plus glycerol. The results are not convincing, and are probably due to suggestion. They have never been confirmed.

Korenchevsky (1921, 1925 and 1928) et al. produced an increased nitrogen excretion with crude emulsions from testes or prostate tissue. He claims that the testis acts as a synergist, and the prostate as the active agent. He limits the protein-metabolism stimulating hormone to a crude lipin fraction from bull's testes and prostate, and suggests that the gonads are involved in regulating body temperature. He suggests two hormonal factors, one from seminiferous tubules and the other from Leydig cells.

In 1932 he regards the high B.M.R. to be due to rapid resorption of these seminiferous cells. He shows obesity in castrated rats, this however is not confirmed by Halt, Keeton and Vennesland in 1936.

This above is suggestive of testis tissue control of protein metabolism, but more specific is the action on creatine - creatinine metabolism which has been investigated recently.

In 1927 Tsun - Chee - Shen could find no change in creatine metabolism after castration in dogs and albino rats. But in 1921 Read worked on the metabolism of eunuchs and found that prepuberal castration induced a continuance into adult life of prepuberal creatinuria but that post-puberal castration did not result in a creatinuria.

Lasch & also Remen indicated, in 1932, the effects of ovarian and testicular function on creatine metabolism, but it was as early as 1911 that Krause and Cramer were the first to point out that in contrast to normal man, normal women, on a creatine-free diet, excrete intermittently small amounts of

creatine...

creatine. This appears to be associated with a relatively poor development of the female sex in so far as muscle is concerned, according to these workers.

During pregnancy the intermittent creatinuria of the normal woman becomes a continuous one, and after delivery the creatine excretion rises even above the pregnancy level.

In 1935 Kochakian and Murlin produced a decrease in protein metabolism with concentrates of male urine.

CREATINE TOLERANCE AND LOSS OF GONDAL CONTROL:

Remen injected 500 mgm. of creatine into normal adult men between the ages of 20 and 50, and no greater creatine excretion followed than before injection. But in older men, aged 70 to 90, with failing sex functions, there is a decreased tolerance as found also in older women, eunuchs, infants and children. Lasch, and also Bühler, confirm these findings. In 1933 Schrire and Zwarenstein report that castrated rabbits also show a low tolerance to subcutaneous injection of large amounts of creatine. Bühler the same year counteracted the effects of loss of sex function in relation to creatine tolerance by injecting "Progynon" and "Proviron" into females and males respectively.

In 1935 Kun and Peczenik indicate a sex specificily in the control of creatine metabolism. They give 0.4 mgm. creatine per os to post-puberally castrated rats, and produce

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a creatinuria which they did not obtain in normal rats. Injection of 25 units of "Proviron" (prepared from adult male urine -- Schering - Kahlbaum) actually increased the creatine excretion, but 30 to 125 units decreased the creatine content.

They show that only in dioestrus is the urine of the female rat free from creatine. After castration the creatine disappeared from the urine, only to reappear if "folliculin" were ingested. Folliculin (theelin or oestrin) increased the creatinuria in male castrates even if no creatine were ingested. They conclude that the male sex hormone regulates the destruction, and the female sex hormone the formation, of creatine.

Kochakian and Murlin report in the same year, that castrated dogs did not excrete creatine if on a creatine-free diet, thus indicating that the castration does not affect endogenous creatine metabolism, but that the castrate may not be able to fix endogenous creatine as well as the normal male.

CREATININE EXCRETION AND LOSS OF GONADAL CONTROL:

In 1933 Schrire and Zwarenstein observed a definite and steady rise in creatinine elimination after castration. This appears after a latent period and not immediately after gonadectomy. They suggested that presumably the testis was secreating some substance into the body which, to a certain extent, controls the elimination of creatinine. This possibly acts on some other endocrine. The post castration rise was from 25

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to 50%. In the female it was 30-40%. It is very interesting to note that the castration effect only appears in nine months in the female, whereas it appears in three months in the male. Injection of ovarian or testicular extracts respectively causes the high urinary creatinine to fall to the precastration levels.

In 1935 Shapiro and Zwarenstein were, however, unable to produce any change in muscle creatine following castration in frogs - Xenopus laevis. They offer the suggestions that there is a species difference in Xenopus laevis and the rabbit, or that the testes and ovaries in the toad have no influence on creatine - creatinine metabolism, or that the postulate that the increase in urinary creatinine after castration is due to alteration in muscle creatine, is incorrect. This latter is, however, hardly tenable in view of the creatine-creatinine relationship mentioned before, and also, as is shewn later, hypophysectomy is followed by a change in muscle creatine. Braier's work has an important bearing on this for he obtained a fall in urinary creatinine following the removal of the pituitary in dogs.

Another suggestion may be offered that there is a decrease in muscle creatine following captivity due to a possible lowered creatine-phosphate content, and that the hypertrophy of the pituitary maintains the creatine level which otherwise would fall.

CREATININE EXCRETION AND IMPLANTATION OF TESTES:

Schrire and Zwarenstein implanted testes into castrated rabbits and produced a fall in the high creatinine excretion down to normal levels. Subsequent removal of the graft led to reappearance of the usual castration effects, that is, an increased creatinine excretion. This also suggested a hormonal relationship between the gonads and creatinine metabolism by action on another gland.

INJECTION OF EXTRACTS AND CREATININE METABOLISM:

In males, saline suspension of testes, and also testicular extracts reduce the high creatinine excretion in animals to the precastration level, and in females, saline suspension of ovaries or ovarian extract will do the same.

There is therefore the very highly suggestive evidence of a gonadal control of creatinine metabolism, and the possibility that creatine varies inversely as creatinine in the urine of normal and castrated female rabbits.

ANTERIOR PITUITARY:

The first evidence of the relation of the pituitary to metabolism were the clinical features in hypopituitarism. In 1901 Frolich described a disease which now bears his name, and which is associated with hypofunction. Before him, in 1894, Tamburini had described what is now known as oversecretion of the eosinophil cells, but he confused it with myxodema.

Paulesco...

Paulesco, in 1907, demonstrated that removal of the pituitary led to muscular weakness, loss of weight and death. This, in 1914, was again described by Simmonds. He reported atrophy of the kidneys, ovaries, pancreas and liver, but today it is realised that the general muscular weakness is associated with atrophy of all the other endocrines, and a resultant generalised wasting and atrophy of the whole body. Therefore, at least indirectly, the pituitary must control creatine and creatinine metabolism, but it seems, according to further evidence and also to the results recorded here, that the pituitary exerts a direct influence on creatinine metabolism.

ANTERIOR PITUITARY - CREATININE METABOLISM:

In 1931 Braier produced the first conclusive experimental proof that the hypophysis has a control over creatinine metabolism as removal led to a marked decrease in urinary creatinine. He showed a loss of 29% in hypophysectomised dogs as compared with controls, and a decrease of 25-40% in fasting dogs on hypophysectomy. Two years later Schrire and Zwarenstein suggested that the increased excretion of creatinine following castration is due to the concomitant hypertrophy of the anterior lobe of the pituitary. To confirm this they injected pituitary extracts into normal male and female rabbits. The creatinine excretion rose. If this extract were injected into a castrated rabbit showing an increased creatinine excretion, the anterior pituitary extracts have no effect. They suggest, therefore, that there is an excess secretion of the anterior pituitary and

that...

that any further extracts will have no effect on an animal whose pituitary is already producing amounts of the hormone in excess of that required.

In 1935 Shapiro and Zwarenstein, working on Xenopus laevis and muscle creatine produce a slow decrease in muscle creatine concentration following hypophysectomy, and show that this is due to loss of the anterior-pituitary alone. This took place over a long period and they suggest a possible anterior pituitary - gonadal relationship. Injections of anterior pituitary extract (Bellerby) in small doses over a prolonged period give rise to an increased muscle creatine,whereas acute doses have no effect.

In 1934 Marenzi obtained a decrease in phosphagen of 33% following anterior pituitary removal, while Shapiro and Zwarenstein's figures were those of a loss of 15% for total creatine in muscle.

From the above it is seen that urinary creatinine and also muscle creatine are increased by anterior pituitary extract, and decreased by removal of the pituitary. This evidence does suggest a control of the creatinine excretion by the pituitary to some extent. As the gonads also appear to be involved in creatinine excretion the possibility of an anterior-pituitary-gonadal control is a very strongly suggested possibility.

TESTIS - ANTERIOR PITUITARY RELATIONSHIP:

There are innumerable clinical and experimental studies to indicate this relationship, especially since Collip published his results in 1935, postulating the action of different hormones produced by the anterior pituitary, that is, the gonadotropic, thyreotropic, parathyrotropic, adrenotropic and the different pancreatotropic hormones, acting on the particular gland whose name they bear. Removal of the anterior lobe of the pituitary brings about atrophy of all the glands mentioned, and each of the specific hormones produces hypertrophy of the gland it controls, if this particular preparation is injected.

On the other hand, gonadectomy leads to an hypophyseal hypertrophy which is associated with characteristic histological changes in the anterior lobe, and with alterations in the physiological activity. This hypertrophy was first shown by Fischera in 1905, but twelve years later, Addison demonstrated the extent of hypertrophy. This is shown to be an increase in size and number of the basophils which later undergo vacuolisation with development of signet ring or so-called "castration" cells (These also have been shown to develop during pregnancy).

Physiological changes involve increased content or secretion of gonadotropic hormones. This can be demonstrated by implantation experiments, high concentration of the gonadotropic substances in the urine after castration, or by parabiosis studies on gonadectomised rats and mice, and observing the changes in the gonads of the normal male and in the accessory organs in both animals. For a considerable time it has been held that the gonads exert an inhibitory effect on the pituitary, and this is generally postulated. In these studies here this inhibitory action of the gonads on the anterior pituitary is also stressed as it has a definite bearing on the results obtained and their interpretation.

(a) Histological changes. These changes can be inhibited in the castrate by injection of the different male sex hormones as shewn by Holweg, Dolern, McCullagh, Walsh, Nelson et al, but the majority find that pure synthetic male sex hormones require five times the dose as those with traces of oestrogenic material. This, and other work, seems to indicate a biological role for the presence of oestrogenic material in the male, the combined action of known androgenic and oestrogenic substance being sufficient to account for natural control of the anterior pituitary (Nelson & Gallagher).

Steyn, Hermann, Steinach, Sand, Moore and Price, Lipschitz, Gallagher & Kock et al shew that the antagonistic effect exerted by the gonads of one sex on the gonads of the other sex is not a direct one, but acts through inhibiting the gonad-stimulating activity of the anterior pituitary of the other. This has been confirmed repeatedly, but will not be quite so simple if the results of Kisaw, Wolfe, Clark, Pfeiffer et al are confirmed as they suggest that the adult male rate anterior pituitary contains the follicle stimulating hormone.

(b) ...

(b) Implantation: Smith, Zondek, Ascheim, Evans, Simpson, Engle, Meyer, Leonard, to name a few workers, establish a definite anterior pituitary - gonadal relationship by implantation experiments. They indicate that the hypophysis of the gonadectomised rat shows greater activity than that of the non-castrate. They also conclude that the female pituitary is more easily depressed by oestrin than the male anterior lobe.

(c) Parabiosis Studies: Matsuyama, Yatsu, Goti, Fels and Kallas, Martin & Rocha; Nelson, McCullagh, Walsh; Witschi, Levine and Hill; Mottram and Cramer, all indicate a control of the anterior lobe by either one gonad or the other or the hormone produced by it. From all these studies the general conclusion arrived at is that known testicular and urinary male sex hormonal preparations can inhibit the hypophyseal-gonad stimulating activity. The dosage however is tremendous and is at least five times for the male as the female.

Oestrogenic substances on the basis of weight are more potent, but the dose for the male is also approximately that for the female.

There is considerable evidence however that another substance is elaborated by the testis. This X-substance of the Laqueur School appears to be active in the immature animal but does not act on the accessory sex organs. In the control of the anterior lobe it appears to be more effective, or else higher concentrations thereof exist in the testis tissue than of the known androgens. The substance seems to disappear from

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the testes with destruction of the seminiferous tubules(Koch).

(d) <u>More involved effects.</u> There are several more involved effects to indicate this anterior pituitary-gonadal relationship, and it is the aim of the studies recorded here to give conclusive evidence of this relationship with regard to creatinine metabolism.

There are known differences in <u>creatine</u> and <u>creatinine</u> excretion in relation to sex and puberty, and naturally suggest a control of creatinine excretion by the male sex hormones and the pituitary. These will be discussed later under the Creatinurias.

Hoskins, in 1911, was the first to suggest the inhibitory control of the pituitary by the gonads, and when this inhibition was removed the pituitary manifests itself by increased activity leading to altered metabolism. This has been proved and the histological picture of pituitary hypertrophy has been discussed shortly. Schrire and Zwarenstein demonstrate an increased creatinine excretion in gonadectomised rabbits both male and female. The period before the increased excretion varies however, in the male it is eight weeks, while in the female it is 9 months. This agrees with the work of Hatai in 1913, who produced pituitary hypertrophy in male rabbits 90 days after castration, whereas 9 months were required before hypertrophy developed in the female (Wolfe 1925).

The observations of Braier (1931) who hypophysectomised dogs and produced a marked fall in creatinine excretion after a short latent period, fits in well with this hypothesis. His was the first conclusive evidence that the pituitary was associated with creatinine metabolism.

Shapiro and Zwarenstein were, however, not able to demonstrate an increased muscle creatine following castration, but the other workers all suggest an anterior-pituitary-gonadal relationship.

Following on this Kun & Peczenik in the same year produced a creatinuria in post-puberally castrated rats if 0.4 mgm. creatine were given per os. This did not happen in normal rats. Large doses of Proviron (Schering-Kahlbaum) decrease the creatine content. During the whole oestrus cycle, with the exception of dioestrus, creatine is present in the urine. This indicates yet another step in the anterior pituitary-gonadal control.

After castration, creatine disappears from the urine only to reappear if folliculin is injected; folliculin also increasing the creatinuria in the male castrate, even if no creatine was ingested. These workers suggest that castrated dogs are not able to fix exogenous creatine as the normal dog does.

SUMMARY:

It has been shown that the anterior-pituitary and the gonads are closely interrelated in that:

(a) the gonads exert an inhibitory function on the pituitary as shown by the hypertrophy of the pituitary following

castration...

castration, and the appearance of castration cells.

(b) the gonads atrophy following hypophysectomy and hypertrophy following hypersecretion of the pituitary.

(c) removal of the anterior-pituitary results in a decreased urinary creatinine and a decrease in muscle creatine.

(d) injection of anterior pituitary extracts produce an increased muscle creatine and an increased urinary creatinine.

(e) Anterior pituitary extracts produce a high creatinine excretion in the normal but not in the castrated animals.

Thus the evidence is indeed suggestive of an anterior pituitary gonadal relationship in the control of creatinine metabolism.

Thyroid.

It is, however, necessary, in an introduction of this nature, to review the actions of the other endocrines as these may also throw light on the control of creatinine metabolism.

Besides the testes and pituitary much work has been done on the control of metabolism in general by the endocrines, but in the studies recorded here only the relation of the endocrines to creatinine metabolism is discussed.

One of the earliest evidences of this relation was the observation of Schaffer in 1907 that a creatinuria occurred in exopthalmic goitre. 1910 Krause and Cramer - creatinuria

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in diabetes mellitus was demonstrated.

From an experimental point of view this was followed up by Frontali who produced a creatinuria following thyroidectomy in dogs. Meanwhile a year previously, 1912, Krause & Cramer had confirmed Schaffer's observation in that an experimental hypothyroidism would also produce a creatinuria, and two years following this Hunter confirmed Frontali's work on sheep.

The adrenals were the next endocrine, and in 1915 Tsuji administered repeated and big doses of adrenalin and produced a creatinuria together with an increased output of creatinine.

To deal with each gland separately:-

Thyroid:

As shown above, Schaffer and later, 1917, Denis, and also 1921 Kepler and Boothby, demonstrated a creatinuria in exopthalmos. Experimentally Frontali and Hunter obtained the same result on removing the thyroid gland and suggested that it was due to a diminution in the creatine content of the muscle. This is contradicted by Poncher, Visscher and Woodward 1934, in that a hypothyroidism in young children induces a decrease in or complete cessation of the creatine. Thyroid extract returns this to normal, and this occurs before the increase in B.M.R.

Krause & Cramer, Beumer & Iseke, Gross & Steenbach, and later Eimer, Kepler and Boothby, Pugsley Anderson & Collip also produce a creatinuria on feeding thyroid gland.

In 1930 Abelin & Spichtän, in 1935 Bodansky, fed thyroid to

rats...

rats and produced a fall in the muscle creatine; the latter, a decrease of as much as 30%. This decrease also occurred in heart muscle. These changes took place not in the acute administration but with long continued feeding. Phosphocreatine fell even more (Pugsley Anderson & Collip 1934).

It seems, however, that muscle creatine is decreased either by hyper- or hypothyroidism, from the above data, and also that the thyrotropic hormone induced a creatinuria in normal or hypophysectomised rats (Anderson & Collip).

Palmer, Carson and Sloan administered iodine to exopthalmic goitre patients; the resultant reduction in the creatine output they attributed to the improvement in the physical condition of the patient. The low tolerance of thyrotoxic patients to creatine is raised by administration of iodine, or by partial thyroidectomy, and the high tolerance of the myxoed group lowered by the administration of Thyroid (Thorn 1936).

Brentano showed a creatinuria with thyroxine feeding, and Hedrick and Eimer independently in severe cases of Basedow's disease a low creatinine excretion universally proportional to the increase in the B.M.R.

The weight of evidence is not however sufficient to indicate a hormonal control.

Parathyroid. Paton & Findlay's views that guanidine and methyl guanidine formed inert creatine in the absence of the parathyroid have been severely criticised. Henderson, Palladin and Grilichee show an increase in muscle-creatine following thyro-parathyroidectomy, but in contrast Imrie and Jenkinson report a decrease with parathyroidectomy only.

Brown and Imrie and Jenkinson have worked mainly on the phosphocreatine and by administering creatine to rats reduce the phosphate output. This is probably due to creatine-phosphate formation. Injection of parathormone some time before the creatine is administered results in a still more marked decrease in phosphate excretion. They also show some relation to creatine-phosphate metabolism by removing the parathyroids & in these animals the muscle phosphagen which is now low can be raised to normal levels by injecting parathormone. The rate of phosphagen resynthesis, which is also low in those animals, is restored to normal.

Pancreas:

Krause and Cramer in 1910 have had their report of creatinuria in diabetes millitus repeatedly confirmed.

Corbia believes that the creatinuria which follows complete extirpation of the pancreas is due to an upset in absorption due to loss of the external secretions.

This, however, is contradicted by the injection of insulin into pancreatectomised dogs and diminishing the creatinuria. Besides this Kopolwitz in 1930 injected insulin into a diabetic and produced a fall in the creatine content of whole blood. (With regard to this, however, one must consider the difficulties in creatine estimation in whole blood).

Is the creatinuria of diabetics associated with a decrease in muscle creatine, for Duliere demonstrated a diminution in phosphagen in muscle after insulin injection, yet four years later, in 1932, Moschini could observe no change?

Associated with this is carbohydrate metabolism and Brentano found a close correlation between creatinuria and glycogen control of skeletal muscle. In all cases where muscle glycogen is decreased there is a creatinuria. But Brentano maintains that it is due to the rate of reduction of muscle glycogen. Liver glycogen is not related to the creatinuria. Brentano; also Querol and Reuter; Steinitz & Steinfeld, indicate that injections of adrenalin fail to increase the blood lactic acid if there is a condition causing creatinuria. In acute creatinuria some alteration in carbohydrate-metabolism can be detected. Rigo and Frey find that injections of adrenalin cause a decrease in the total creatinine, creatine and unorganic phosphate.

It seems, therefore, that (vide Hunter, Rose) adequate carbohydrate is necessary to prevent creatinuria since phosphagen is resynthesised at the expense of energy derived from the transformation of glycogen to lactic acid.

Adrenals:

Clinically we find evidences of the relation of the adrenal to creatine and creatinine in that one of the symptoms of Addison's disease is myasthenia, and the suggestion offered above is that it might be due to an interference with the phosphagen cycle. Following on this a great deal of work was done on creatine-phosphoric acid;and the muscle creatine and urinary creatinine not investigated to any extent.

Adrenalectomy.

Although Lang's findings of a fall of 33% in phosphocreatine in cats following adrenalectomy were confirmed by Ochoa and Grande, Cope Corkhill, Marks & Ochoa, who also report a decreased rate of phosphagen breakdown, and that less work is done ,Lundsgaard and Wilson failed to demonstrate this in bilaterally adrenalectomised cats. Lang indicates that creatine and creatinine are not changed.

Injection of Extracts on a) Phosphagen:

Akatsuka describes the above effects as due to medulla insufficiency rather than cortical. This statement is borne out by the work of Feinschmidt & Ferdman, who obtained an increased rate of phosphagen breakdown on injecting epinepherine. But in 1930 Cori was unable to alter the phosphagen with intravenous adrenalin. Four years later Moschini, using Eucortone (Allen & Hanbury's preparation of the adrenal cortex) produced a rise of 15% in muscle phosphagen if injected into normal frogs. He was not able to keep adrenalectomised frogs alive with this preparation. Nor could he raise the phosphagen content in these frogs.

Indovina and Baena lengthen the time of exhaustion in rats at work by injecting cortical extract. The decrease in muscle glycogen and increase in non-protein N is smaller than in non injected animals.

(b) <u>Creatinine excretion</u>: Tsuji, Palladin & Tichwinskaja, Abderbalden and Buadze produced a creatinuria and increased creatinine output with continuous adrenalin injections. Rigo & Frey ,however, produce a decrease in total creatine and creatinine and inorganic phosphorus in blood, but the preformed creatinine & total acid phosphorus is not affected. Lucia and Stolfi found that the nitrogenous constituents of urine are not altered by injection of adrenalin. But Brentano and also Querol and Reuter show that if the blood lactic acid does not rise appreciably following adrenalin administration, the glycogen content of the muscle is low and creatinuria occurs. They also add that in acute creatinuria some alteration in carbohydrate metabolism may be almost always detected.

PugsleyAnderson & Collys also were unable to produce any effect on creatinine with adenotropic hormone.

Hales, Haslerud and Ingle adrenalectomised rats which 36 hours afterwards developed fatigue, after performing normally at first. They called this adrenalin shock. Adrenal cortex extracts could prevent or reverse this failure. They also experimented with grafts and concluded that work-capacity does not depend on mobilisation of adrenalin from the medulla. The cortical tissue in the transplants did not degenerate as the medulla tissue did, and therefore the cortex seems to be implicated in the control of fatigue.

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The information is therefore rather inconclusive but there is evidence of a relation between muscle work and the adrenal cortex. There is also work to show the relation of the medulla to creatine and creatinine excretion. This however has yet to be substantiated.

CREATINURIAS

In conclusion of the studies on the relation of the gonads and the pituitary on creatinine metabolism, a summary of the creatinurias occurring would add to the evidence of a pituitary-gonadal control and also aid the suggestion that various other glands are also involved.

Creatinuria of children:

From birth to puberty creatine is a constant but variable component of the urine of children of both sexes. After four to seven months it constitutes about 10% of the total creatinine output. Wang & Kaucher; Daniels & Hejinian, regard the creatinine produced as an end product of muscle metabolism but the creatinuria is due to precursors in protein, and these bear no relation to urinary creatinine. Terroine, Champagne, Danmanville et all, however, do not confirm this. Beumer and Fasold express the opinion that sexual maturity is a significant factor in bringing about the destruction of creatine. Light and Warren state that the percentage of boys shewing a creatinuria at yearly intervals remains fairly constant at the ages of fourteen to seventeen, and btween 17 and 18 manifests a perceptible drop. But Buadze finds boys excrete creatine until six to ten years of age, and girls manifest a creatinuria till puberty; after which they develop a cyclic creatinuria. The author suggests that the male sex hormone inhibits the output of creatine and the female sex hormone promotesits appearance in urine. This, however, has yet to be substantiated.

Creatinuria of Women:

Wang, Hawks, Huddleston, regard the creatine as directly proportional to the protein content of the diet, while Hunter indicates an entirely exogenous source of creatine. Rose, Ellis and Helming suggest that women are less efficient than men in storing or metabolising the portion of creatine which does not yield creatinine. Mühlboek & Kaufmann do not confirm this diminished creatine tolerance in normal women.

Creatinuria of pregnancy:

Krause & Cramer (1910 & 1911) and thereafter many workers noticed a continuous excretion of creatine in pregnant women. The generally intermittent creatinuria of normal women becomes a continuous one. There is also a marked increase above this level immediately after delivery, giving rise to the term post-partum creatinuria. (First observed by Schaffer (1908) in the human). It may even be as high as 1.5 gramsper day. The output remains high for two weeks, reaching its height somewhere between the third and the eighth day. There have been various theories but none however have proved absolutely satisfactory.

Krause...

(Krause 1911, Schaffer & Murlin, Beker; Schrire and Zwarenstein 1934).

The cretinuria of pregnancy increases as the pregnancy develops, and then at parturition becomes even more marked; and indicates, therefore, some relation of the control by the gonads, and possibly the pituitary.

Creatinuria and the Sex Glands:

This has been dealt with in the above discussion, in which the work of Remen, Lasch on the human, Schrire and Zwarenstein, on rabbits, Kun and Peczenik on rats, and the sex specificity of Creatine - creatinine metabolism has been fully discussed.

Creatinuria and the Thyroid:

This has also been considered, the work of Schaffer, Denis, Hunter et al showing a creatinuria associated with exopthalmic goitre. Feeding of thyroid also produces this creatinuria.

Creatinuria and Carbohydrate Metabolism:

Brentano gives evidence to associate a creatinuria with all conditions which induce a decrease in muscle glycogen. The creatinuria depends on the extent and rapidity of reduction of the muscle glycogen. Recovery from an induced creatinuria is generally associated with an increase in muscle glycogen. Brentano, Querol and Reuter, Steinitz & Steinfeld, Morgulis, Torroine; Wolff et al have all shown a close relationship

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between diminished muscle glycogen and a creatinuria, and it is therefore feasible that carbohydrate metabolism may acquire a new significance since resynthesis ofphosphcreatine from its components is accomplished ordinarily at the expense of energy derived directly or indirectly from the transformation of glycogen into lactic acid.

Helditch & Thompson induced creatinuria by ingestion of glucose and fructose, and by exercise.

Protein Metabolism and Creatinurias:

Have been very fully discussed: vide supra.

SUMMARY:

From the view point of a relationship between the other endocrines and the pituitary on creatinine metabolism we find:

Pancreas: Insulin injection does not increase or decrease muscular creatine. There is no decrease in creatine, only a creatinuria after entirpation. This is unlikely to have an effect, but there is some slight carbohydrate - creatinine metabolism relationship which is probably an indirect one.

Thyroid: The evidence at the moment is somewhat contradictory, there is evidence to show that a decrease of muscular creatine either with hypo-or hyper-thyroidism. On the other hand, thyroid feeding, injection of thyroxine and other procedures producing a hyperthyroidism do produce a creatinuria, and so indicate that some definite relationship exists between the thyroid and creatine-creatinine metabolism.

<u>Parathyroids</u>: There is some evidence of a relation to phosphagen metabolism.

<u>Adrenals</u>: The adrenals are involved in the normal control of the work of muscle. The cortex, however, produces a hormone which is known to be androgenic. This is distinct from corticosterone a synthetic product which resembles the action of the cortex.

Large doses of adrenalin have been reported to cause an increased creatine-creatinine excretion. This may be an indirect effect on carbohydrate metabolism, but these results have not yet been confirmed.

Recently Verzar et al have brought to light some remarkable facts with regard to the adrenal cortex. Adrenalectomised rats on a vitamin B. free diet cannot be kept alive with cortin.

They suggest that the fuction of cortin is to convert pro-vitamin B_2(Flavin) to Vitamin B_2(Flavin Phosphate),and they show that the mortality of adrenalectomised animals is materially reduced by injections of Flavin phosphate. Flavin itself cannot prevent any symptoms of adrenal insufficiency whereas flavine phosphate can. (This is altered by the addition of cortin as suggested above). Carbohydrates and fats require phosphorylation before they can be absorbed from the intestinal tract and it is a suggestion offered here that, as Verza has shown monoiodoacetic acid-poisoned rats show the same signs and symptoms ,and exactly resemble adrenalectomised rats, it is possible that the cortin controls the phosphorylation of creatine to form phosphagen, as monoiodocetica acid interferes with phosphorylation. If this is so the cortex will play a certain role in the creatine-creatinine metabolism.

In addition, injections of testosterone and other allied androgens cause a return to normal of the adrenals in castrated rats. The X-zone disappears and the increase in weight disappears.

Anterior Pituitary:

A definite direct effect on the creatinine elimination is postulated in this thesis.

Posterior Pituitary:

No conclusive evidence.

Gonads:

The evidence of control of creatinine metabolism is fairly conclusive and indicates an indirect action, probably through the pituitary as submitted in these studies,and by the work of other experimenters.

THE MALE SEX HORMONES:

Excellent reviews by Butenandt (1936); Fieser (1936), Dannenbaum (1936), Koch (1937) have appeared on the chemical structure, properties, precursors,actions, etc., of the male sex hormones, but in the following short summary based on these articles only the relevant points have been emphasised.

In...

In conducting this work, besides using testicular and urinary extracts, synthetic products were also used, and it was noticed that each had a somewhat individual action. For this reason it is necessary to give a brief description of each substance. Thus it does not purport to review the whole of this subject, but rather discuss in short the origin, structure, and function of each of the male sex hormones, which have been isolated either from testis tissue or from adult male urine. The word "so-called" is sometimes used for, as will be indicated in this study, these hormones also possess oestrogenic properties.

Many workers indeed have shewn the testes to affect metabolism, accessory sex organs, secondary sex characteristics such as comb growth, spur growth etc., that they are closely related to other endocrines and many other functions. All these actions are produced by injections of various testis tissue extracts, implants of testis, castration etc., in which the testis itself comes into play. However, just as with the pituitary, as was shown by Zondek, the active principles of the testis can also be found in the urine of the adult male. Four principles have been isolated, two from testis tissue and from from adult male urine.

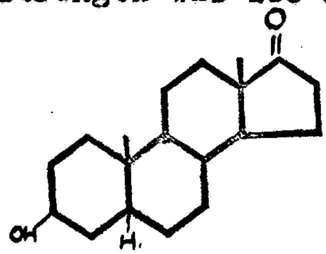
Androsterone:

In 1931, 1932, Butenandt isolated a substance from adult male urine to which he assigned the formula $C_{16}H_{26}O_2$, with a

strength...

strength of 1 capon unit per 1-28 (The capon unit being regarded as the International Standard). This however has never been confirmed.

Later analysis led to the formula $C_{19}H_{20}O_2$ or $C_{18}H_{28}O_2$. On the basis of the former empirical formula he suggested the structure now adopted for androsterone. This he confirmed with Tscherning in 1934, but the strength was 150-250y for 1 capon unit.



Androsterone.
3-epi-Hydroxyaetio allocholanone-17.

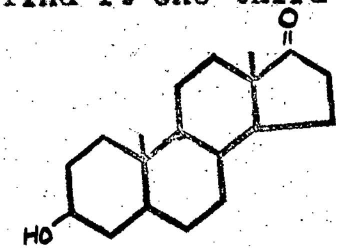
Its structure is basically of an oxyphenanthrene ring and is closely related to the sterols. It is a derivation of cholesterol and is prepared synthetically from epidihydro-cholesterol (Ruzicka, Goldberg, Meyer, Butenandt, Hanisch, Dannenbaum, Kudszus et al).

Dehydroandrosterone:

In 1934 Butenandt and Dannenbaum separated a second male sex hormone fraction from male urine and gave it the formula of $C_{19}H_{27}OCl$ - an ester chloride derivation of dehydroisoandros-terone, but this is probably an artefact of dehydroandrosterone. Many workers have prepared it from cholesterol, eg. Ruzicka and Wettstein, Wallis & Fernholz, Schiller Serina & Gebrke, Butenandt, Dannenbaum, Hanisch & Kudszus et al. It has also been prepared from many related sterols.

These...

These two substances occur naturally in urine. Dehydro-androsterone is one-seventh to one-tenth as active as androsterone using the Tschopp method of assay with the capon unit. Butehandt et al. find it one-third as active.



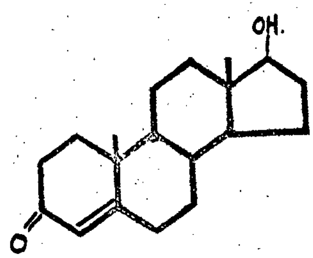
Dehydroandrosterone.

Testosterone:

In 1930, 1931, David, Dingemanse, Freud, Kober, Laqueur, and Mündi reported the isolation of a hormone from the testicle of the bull, while in 1929 and 1934 Gallagh & Koch, and in 1927 McGee had carried the activity of the concentrate from bull testis to approximately ten times that of urine. Thus it was ten times as potent as androsterone. In 1935 David et al. announced the separation of the crystals with the activity of 7-10 times that of androsterone. These were the only crystals separated from testis tissue. This has been confirmed by Frattini and Manio, Ogata & Hirano.

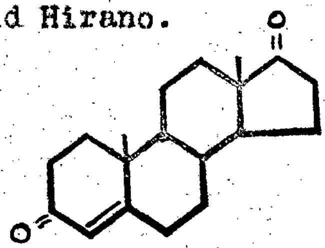
Testosterone.

Androstenol - 17-one-3.



Androstenedione.

or Δ^4 androstenol - 17-one-3 was suggested by Ruzicka and Wettstein in 1935 as the active principle of the testis. Wallis and Fernholz, Butenandt, Hanisch & Tshopp came to the same conclusion; Ruzicka, Butenandt and Hanisch prepared it from dehydroandrosterone and finally it was isolated from boar testes by Ogata and Hirano.



Androstenedione.

Thus we have two substances which occur naturally in urine, and two in testis tissue. Each can be separated and the activities tested by means of the capon unit.

PHYSIOLOGICAL PROPERTIES.

Although these hormones have been called male sex hormones this name has originated from the site of origin of these products, namely the testes and adult male urine. It had long been recognised that oestrin, or one of female sex hormones, was to be found in male urine, and in large quantities in the urine of the stallion.

The so-called male hormones were at first credited with purely male qualities in that they had an effect on the secondary sex characteristics, and accessory sex organs of the male, but Korenchevsky, Dennison, Eldridge and Brown, in a series of papers, have shown various members of the male sex hormone group to exert a powerful effect on the organs of ovariectomised rats.

Similarly, various so-called female sex hormones exert a powerful effect on castrated males.

On this basis Korenchevsky et al have decided that...

that a hormone which is defined as a male or female sex hormone must have an effect ONLY upon the male or ONLY upon the female sex organs. Up to the present the only hormone which can be defined as a pure sex hormone is progesterone, which has no definite effect on male rats, but only on females, and therefore can be classified as a pure female sex hormone.

The rest of the hormones which occur naturally and which have been isolated from tissues or urine have bisexual actions. Some of these are chiefly male and some chiefly female, whereas others are truly bisexual.

To summarise the findings of many workers we find that the hormones act on different accessory organs in the male, some indicating a selective action, e.g. either on growth in the capon, the seminal vesicle, or the prostate in the castrated animal, and therefore any standardisation on this basis must be accepted with reservation, as one hormone may have a definite action on one organ, and little effect on another; whereas the opposite is true for another hromone.

On the female castrate, too, these substances have different effects and shew the bisexual action which Korenchevsky alludes to.

<u>SUMMARY:</u>

<u>Androsterone:</u> This hormone has a more selective action on the prostate; it does cause some return towards the normal of the seminal vesicle, and is only 1/5-1/6th as active as testosterone on the capon comb; only in massive doses will it cornify

the...

the vagina in ovariectomised rats. It is also inactive on vaginal opening, and growth of uterus on infantile non-castrated rats. It restores the hypertrophied "castration" adrenals to normal. No effect on plumage in the Sebright cock.

Moore & Price claim it to provide a complete substitution for the hormone secreted by the testes, and that testes damage by androsterone is an indirect one due to its action on the anterior pituitary. (Work supported by Callow & Parkes). It therefore seems to come just within the group of bisexual hormones, and is known as partially bisexual.

Dehyroisoandrosterone: This is even less potent than androsterone. It has the same action on the seminal vesicle but it can reproduce the action of oestrin by premature opening of the vagina, oestrus, and uterine growth in the immature rat. Generally it causes a return towards normal of all the atrophied sexual organs, and approximately to the same degree, in both male and female gonadectomised rats. Thus it belongs to the group of bisexual hormones.

Testosterone: Has an effect two to five times as great on the prostate in the castrated rat than androsterone, but an effect ten times as great on the rat seminal vesicle, and is also ten times as active as androsterone in the capon test.

In the female it produces vaginal opening, oestrus and uterine growth in the immature rat, but no vaginal cornification. It will also cause disappearance of the X-zone in adrenal of the castrated mouse. It feminises the plumage of the Sebright cock.

On this basis it will fall into the bisexual group.

Androstenedione: It bears a very close relationship to testosterone in all its activities and although some workers regard it as having more male than female properties, it's close relationship to testosterone indicates that it is really a bisexual hormone.

Thus we see in the naturally occurring male hormones it is only androsterone which belongs to the partially bisexual group, producing a greater degree of recovery in the atrophied sexual organs of the male castrated rats than in the female. The other three cause a return towards normal of all atrophied sexual organs either in the male or female gonadectomised animals.

ARTIFICIAL PREPARATIONS:

Deanesly and Parkes, Miescher, Wettstein & Tschopp, Butenandt & Kudszus, et al, have experimented with various esters of testosterone, e.g. testosterone acetate, benzoate and proprionate. Deanesly in the Lancet (1938) indicates that testosterone proprionate and acetate are many times more effective than the free hormone, and their action is so prolonged that injection can be restricted to twice a week without loss of effectiveness. Of the two compounds the proprionate has the more intense and more prolonged action than the acetate. It seems that in this form the hormone becomes slowly, but continuously available to the animal, so that the loss by excretion and destruction is very much less than when testosterone as the free hormone is administered.

Prolonged injection into castrated male rats produces a complete recovery to normal of the atrophied sexual organs, and a decrease in the weight of castration adrenals. The changes produced are, to some extent, (e.g. sex organs) or completely, (e.g. adrenals) maintained 9 days after the last injection.

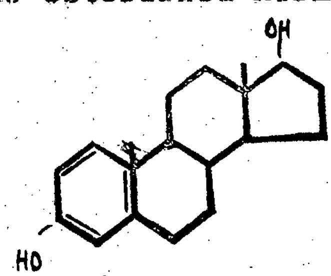
Following a single injection of testosterone proprionate the prostate and seminal vesicle continue to grow strongly for ten days or more, by which time they reach a size similar to that produced by the same dose of free testosterone split up into 20. 12-hourly injections, and where the response to a single injection of testosterone is negligible.

FEMALE SEX HORMONES.

In my work on creatinine metabolism little consideration has been paid to the action of the female sex hormones, and it is only with a view to correlating the effects of themale sex hormones with the work of Korenchevsky et al. on the oestrogenie properties, that injections of oestradiol benzoate in the form of Progynon (Schering Kahlbaum) were injected.

Schrire and Zwarenstein experimenting on female fabbits, found that ovariectomy produces an increased creatinine elimination in the urine; injections of ovarian extract into normal female animals produced a slight fall. In the studies, here, however, the injections of female hormones have been into male animals. Oestradiol, according to the above work of Korenchevsky et al., is a hormone with chiefly female properties in that it produces female sex changes typical of oestrus in the ovariectomised rat; it is not able to stimulate all of the atrophied organs in the male castrate to return to normal, but does exert some slight effect in that it does produce a definite increase in the weight of the seminal vesicles with specific histological changes in this gland, and the prostate. There is little evidence of an increase of weight in the prostate and there is no action on the other atrophied accessory sex organs.

Oestradiol is more active than oestrone from which it was first prepared. Doisy has isolated it from ovaries. The artificial preparation, oestradiol benzoate, has been used to delay excretion and allow a prolonged action, as in the case of testosterone proprionate, the ester of testosterone, which is more effective and has more prolonged action that testosterone itself. This action is attributed to the slower absorption and delayed excretion of the esterified hormone.



OESTRADIOL.
$C_{18}H_{24}O_2$.

EXPERIMENTAL TECHNIQUE.

EXPERIMENTAL TECHNIQUE.

The experiments were performed on adult male and female rabbits. These rabbits were kept in special cages for the collection of the urine and the necessity for catheterisation in obtaining accurate 24-hourly collections of urine was obviated. Each cage was of a comfortable size with a floor consisting of fine wire netting. Thus only urine could leave the cage as food or faeces were caught up on the netting. The cages fitted into special holders with funnel-shaped sides converging together and ending in a spout. The spout of the holder opened above a wide-mouthed glass flask with a glass funnel in which the urine was collected. A fine wire-mesh sieve in this funnel prevented any foreign material from entering the urine flask without affecting the collection of urine in any way.

At the termination of the 24-hour period the sides of the funnel-shaped holder were washed down with water and the washings added to the urine. The urine was filtered and used, for the estimation of creatinine and creatine by the methods indicated below.

The animals remained in the cages for different periods varying from a week onwards to a period of three weeks, or, in some cases, a month. While in these cages their diet consisted of cabbage leaves and carrots. In the earlier experiments it was usual to transfer the rabbits from the cage to a large run in the open air outside the animal house. In the later experiments the rabbits were transferred to somewhat larger cages. Here they were kept isolated from one another and not allowed to mix together in the run. This method of isolation seemed to maintain the rabbit in a good condition for a longer period.

The special cages also consisted of wirenetting but the urine and faeces were allowed to pass through the wide meshed floor and were collected in trays, filled with saw-dust, placed about an inch below the floor of the cage. They were also placed in a large sunny room with good ventilation. In the winter a heater was kept burning so that the room was always at a mild temperature.

The diet in the run and in the cages not used for collecting urine consisted of vegetables, bran and oats.

The animals used were always in a good condition.

METHODS FOR THE DETERMINATION OF CREATINE AND CREATININE:

The volume of each 24-hour collection of urine is measured, filtered and the filtrate is used for the colorimetric estimation of creatinine and creatine by the micro-method of Folin 1914.

The urine is diluted with tap water so that 10 ml of the diluted urine will give a reading on the unknown side of the colorimeter not more than 5 mm above or below the standard reading. The standard is always set at 15 mm.

<u>Preformed Creatinine is estimated as follows:-</u>

10 ml. of the filtered urine is placed in a 10 ml. volumetric flash with 20 ml. saturated picric acid which had previously been purified. To this is added 3 ml. of 10% sodium hydrate. (Folin's method indicates that only 1.5 ml. of NaOH should be used. However, after a long series of readings in the Department of Physiology it was found that in rabbits' urine the colour was more intense with 3 ml. than 1.5 ml. of sodium hydrate, and this enabled more accurate readings to be made).

The volumetric flask is well shaken and allowed to stand for 10 minutes, in which time the colour develops. The colour is orange, and Folin's method is based upon the development of a reddish orange colour with sodium hydrate, picric acid and creatinine. After 10 minutes standing distilled water is added up to the 100 ml. mark of the flask. The flask is well shaken and the contents filtered into the colorimeter cup ready for reading.

Estimation of Creatine:

This is also carried out according to Folin's method and consists of the transformation of creatine to creatinine and the determination of this total creatinine. By subtracting the preformed from the total creatinine, creatine expressed in terms of creatinine is obtained. 10 ml. of the urine with 120 ml. of water and 20 ml. of picric acid are placed in an Erlenmeyer flask and brought to the boiling point. This is left boiling gently for one hour and is then rapidly boiled till about 25 ml. of fluid remains in the flask. Weighed flasks are used and the weight of the fluid in the flask is determined fairly accurately on a spring balance. The flask is cooled, 3 ml. of 10% NaOH are added and left for 10 minutes. The colour develops during this period and the fluid is rapidly transferred to a 100 ml. volumetric flask diluted to 100 ml. filtered into the colorimeter cup and is ready for reading.

Preparation of the standard:

A solution of 1 mgm. creatinine per 1 ml. is required. 1.602 mgs. of creatinine zinc chloride is dissolved in 100 ml. $^{N}/_{10}$ HCL. This forms a perfectly stable standard solution. 1 ml. of the standard solution is placed in a

100 ml. volumetric flask together with 20 ml. of saturated picric acid, 3 ml. 10% NaOH and 9 ml. distilled water. The water is added so as to ensure the volume in the flask during the development of the colour is the same as that when using urine; 10 ml. of urine being used. The flask and its contents stand for 10 minutes, dilution to the 100 ml mark is made and it is ready for use. The known or standard side of the colorimeter is always set at 15 mm.

The determination of the amount of creatinine present is worked out as follows:

$$\frac{15}{\text{Unknown Reading}} \times \frac{\text{Vol. of urine (total)}}{\text{Vol. of urine used}} = \text{mgm. creatinine/24 hrs.}$$

<u>Purification of Picric Acid:</u>

Pure picric acid is essential for the Folin method. This is prepared by the sodium method of Beneditt (1929).

3,000 ml. water are heated to boiling in a porcelain dish and 125 mgs. anhydrous $Na_2\ CO_3$ are added. This stands for a few minutes and is decanted into a large beaker and left overnight at room temperature. Crystals form and are filtered off in a Buchner funnel and sucked dry. The crystals are washed in 1,000 ml. of 10% sodium chloride and sucked dry once more. Suction is stopped and 250 ml. of 20% HCL poured on the mixture, stirred and sucked dry. This is repeated three times. The mixture is washed with 2,000 ml. cold water and again aspirated until the crystals are dry. The crystals are then dried in an oven at 90°C., placed in an amber-coloured bottle and kept in the dark.

Part of this stock of purified picric acid is put into a glass jar, distilled water is added and well shaken. Enough picric acid is added to ensure a sediment in the jar and the fluid is thus always kept saturated. The jar of saturated picric acid, when not in use, is kept in the dark as Hunter & Campbell (1916) found that such a solution when exposed to light came to contain in steadily increasing quantities a substance which gives a deep red colour when treated with alkali.

The picric acid was tested for purity according to the method of Folin & Doisy (1917).

OPERATIVE TECHNIQUE.

Method of Castration:

The animals were not fed for twelve hours before operation. They were anaesthatised with ether and the testes were removed with strict surgical asepsis. Bismuth-iodoform-paraffin paste was applied to the sutured wounds. The wounds in every case healed without difficulty. There was no obvious sepsis. The animals were then returned to the animal house until recovery was complete or else transferred directly to the cages (vide infra).

Controls were always used.

Weights of animals were taken at regular intervals.

<u>Method of Grafting:</u>

The animal is prepared for operation in the usual surgical manner; asepsis again being strictly observed. Fresh testes were removed aseptically immediately before the main operation; healthy adult donors were used and the testes, when removed, are kept in aseptic dishes on ice until ready for grafting, which takes place within 10 minutes.

An incision is made in the midline of the abdomen of the animal to receive the graft; on each side of the mid-line beyond the rectus abdominis muscle sheath incisions are made in the muscle and the external abdominal oblique muscle separated from the internal abdominal oblique on both sides. The surface of the external oblique muscle is scarified with the point of the knife; one of the testes is split carefully in half and each half is sewn into the prepared areas. The external oblique muscle is sewn over the grafts and the skin sutured with silkworm gut, treated with "Bipp" and covered with gauze-soaked in colloidin. The grafts are made 1½ - 2 inches above the symphysis pubis, In all cases the animals recovered without any trouble or sepsis. After complete recovery they were returned to the cages and urine collected once more.

Injections:

These were made with a glass-walled syringe with a metal plunger and metal fittings. A large serum needle was always used although the size of the syringe varied with the volume of extract to be injected.

The site of injection - the flank in all cases except one - was cleaned either with iodine or ether and the needle inserted through the skin into the subcutaneous tissue. Care was taken to separate the fur away so that thevneedle came into contact with skin only. Also the needle was not inserted into muscle but was always freely movable just below the skin surface. Where large volumes of fluid were to be injected the needle was moved around and in some cases the volume was divided, one half being injected into one flank and the other half into the other flank.

Aseptic technique was observed as strictly as possible, the syringes and needles being boiled before use and any glass container used for extracts being thoroughly sterilized.

EXTRACTS.

Testicular, urinary and adrenal cortex extracts were prepared. In addition, control extracts of lipoid material were prepared from brain, liver and kidney. For the extraction of these tissues the same method as that employed for testicular extract was used.

Testicular Extract:

Fresh bulls' testes are stripped of the Epididymies. The testicular tissue is then stripped of tunics, minced and placed in 4 volumes by weight of alcohol (95%) at room temperature for 48-72 hours. This is shaken at various times. At the end of the specified period the alcohol is strained off through muslin, the residue is pressed out and the total yield of alcoholic extract is concentrated to a sludge on a water-bath at about 60°C. and under diminished pressure. To this warm sludge an equal volume of benzine is added, this is well shaken and allowed to stand for a few hours. The benzine extract is syphoned off and concentrated in a water-bath (below 60°C). to a small volume. (The benzine has to be completely removed, for if not complete the yield of solids in acetone varies considerably and the complete removal of activity from the lipid precipitate is is not obtained). Seven to eight volumes of cold acetone are added and allowed to stand in the refrigerator for 25 hours. The acetone extract is then filtered off and concentrated on a water-bath (at B.P. of acetone) to a final syrupy mass and all the acetone removed. This acetone-free extract is then weighed and the necessary quantity of olive oil (or nut oil) added so that 1 ml. of the final product is equivalent to 300 mgm. of lipoid material.

Urine Extracts:

At least ten litres of urine was collected from young adult men, small amounts of thymol being used as a preservative. The urine was acidified with hydrochloric acid Two litres of chloroform are addedto every ten litres of urine and the mixture heated under reflux for about eight hours. When the boiling point of the chloroform is reached the particles stream through the urine layer and a second extraction with chloroform is not necessary. The product is cooled and separates into a watery layer and a chloroform layer. The watery layer, which consists mostly of urine is carefully drawn off. The residue is transferred to a separatory funnel, stirred and allowed to stand, and the chloroform layer drawn off.

The emulsion in between the chloroform layer and the residual urine is now drawn off, filtered (using a Buchner, with gentle suction) and washed with a little chloroform. The filtrate is again separated in a separatory funnel, the chloroform fraction drawn off and added to the main chloroform portion. The combined chloroform extracts are evaporated in a distilling flask using a waterbath. Using the same flask the residue is next steam distilled and this is continued until no more oil comes over.

Enough NaOH is added to make the residue alkaline to phenolpthalein. The mixture is warmed and filtered in the cold; the pH of the filtrate is adjusted to 7.4 and made up to 50 ml. with water. This gives 1 ml. of the final product equivalent to 200 ml. of the original urine.

Anterior Pituitary Extract.

The method employed is that described by Bellerby in 1933.

Heads of freshly slaughtered sheep are brought to the laboratory, split open and the whole pituitary removed. The anterior lobe is carefully dissected out, weighed, chopped up finely and ground with an equal volume of sand which has been previously thoroughly cleaned, moistened and drained. Extraction is carried out by means of 1% acetic acid, the amount added being 1½ times the original weight of tissue. This is left for 24 hours at room temperature, the mixture is centrifuged, the supernatant fluid is poured off and neutralised with 40% NaOH until salmon-pink to phenol red indicator. After neutralising the fluid, it is again centrifuged to remove the slight precipitate which comes down at the neutral point. The supernatant fluid is stored in the refrigerator until required for use.

This method results in 1 ml. of the extract being equivalent to 640 mgm. of fresh tissue.

Oviposition is induced in the South African Clawed Toad by this extract and this is used as an indication that an active extract had been prepared with each series of extracts made. Control preparations of brain are made using the same method.

Adrenal Cortical Hormone.

The method employed in preparing the active lipid fraction is that of Swingle and Pfiffner (31, 34).

Fresh sheep adrenals were brought to the laboratory within an hour and a half of collection. After removing extraneous fat and connective tissue the glands were cut lengthwise and dissected as free as possible from medullary tissue. The ground cortical tissue is then extracted with 2.5 volumes of 95% ethyl alcohol for 24 to 72 hours; the material being occasionally stirred. The extraction alcohol is removed by straining through muslin and filtering, the gland tissue pressed as dry as possible, ground and extracted with two volumes (calculated from the weight of fresh cortex) of 80 per cent ethyl alcohol for 24 to 72 hours. The extraction alcohol is removed in the same manner as in the first extraction.

The alcoholic filtrates are concentrated individually in partial vacuo to one-fifteenth to one-twentieth of their original volume, mixed and extracted five times with an equal volume of benzine. The benzine washings are combined and the benzine removed in partial vacuo at a temperature of 45-50°C. The benzine soluble fraction is then extracted twice with acetone which is removed and the fraction partitioned twice between 70% alcohol and petroleum ether. The 70% alcohol layer is removed, washed three times with petroleum ether and filtered through permutit. The filtrate is concentrated and water added. The final water soluble fraction is injected. 1ml. was equivalent to 40 mgm. of the cortical tissue). The permutit removes all except a very small trace of adrenalin.

Control injections of Lipoid Material:

To eliminate the possibility of the lipoid material of the testes being the cause of alteration in creatinine metabolism when lipoid testicular extracts were injected, lipoid extracts of brain, liver and kidney tissue were also prepared.

The method of preparation of the extract is the same as that used for testicular extracts - that of Gallagher & Koch, 1929. A complete series of experiments was performed and the results embodied in a separate section.

EXPERIMENTAL DATA.

ELIMINATION OF CREATINE AND CREATININE IN NORMAL AND CASTRATED ADULT RABBITS.

Normal Male.

Creatinine: During this series of experiments at least 50 male rabbits were used. In each of these the minimum period of time in which normal urinary creatinine was estimated was one month. The creatinine excretion is remarkably constant from day to day, showing a variation of 10% at the maximum. The protocols of each individual rabbit are appended at the end of the thesis.

Creatine: Occasionally small amounts of creatine were found in the urine of the adult male rabbits. On the few days upon which creatine was present the creatinine excretion remained at the normal level.

Normal. Female.

Creatinine: Excretion was also remarkably constant.

Creatine: This was present from day to day in varied quantities. Only six female rabbits were used.

Castrated Male Rabbits.

At least 15 rabbits were castrated during the experiments. These all show a post-castration rise as indicated by Schrire & Zwarenstein ('32). In some cases the rabbits, after castration, were removed to the animal-house until they had completely recovered from the operation. In other cases a more acute experiment was performed and the rabbits were placed in the metabolism cages immediately after operation.

The results are indicated below of two typical cases of the chronic and two of the acute experiments:-

TABLE I.

CHRONIC				ACUTE.			
Date	No.T4	No.T5	No.T6	Date	M2.	M3.	M5.
31. 6.35	66	70	64	1.2. 37	52	46	56
1. 7.35	60	75	56	2.2. 37	48	54	53
2. 7.35	56	64	63	3.2. 37	42	58	48
3. 7.35	60	68 x	61x	4.2. 37	44	47	56
				5." "	47	40 x	19 x
9. 7.35	60	70	60	6." "	46	45	38
10. 7.35	60	70	64	7." "	48	50	45
11. 7.35	56	68	60	8." "	42	48	54

x T5 and T6 castrated on 3.7.35

M3 and M5 " " 5.2.37.

Inspection of the tables show that the creatinine excretion is only impaired for two days after operation, and although the animals do not recover for some time there is an initial fall in creatinine excretion for two days, this probably being due to the effects of the anaesthetic, shock, etc. This creatinine excretion remains at a constant level for some time and then begins to increase in amount; the increased excretion occurring between the second and third or the third and fourth months. The following tables are those of average daily creatinine excretion over several days prior to the date given. The number of 24-hour periods which are used for the determination of the figures for that particular date are given in the last column. The tables are those of the creatinine excretion just prior to castration, and one, two three, four and six months after castration.

TABLE II.

AT CASTRATION AND 1 MONTH AFTER:					
Rabbit No.	Date	Creatinine in mgs %	Date	Creatinine in mg %	No. of Hour Periods.
3	2.8. 34	89	5. 9.34	91	5
8	2.8. 34	70	5. 9.34	70	5
12	18.10.34	101	20.11.34	101	5
E	14. 2.35	60	10. 3.35	63	6
T_2	28. 5.35	50	1. 7.35	50	6
T_5	2. 6.35	69	9. 7.35	70	6
T_6	2. 6.35	60	9. 7.35	60	6
K	29. 5.36	62	25. 6.36	61	5
L	29. 5.36	55	25. 6.36	52	5
O	29. 5.36	58	25. 6.36	57	5
M_3	4. 2.37	52	6. 3.37	52	8
M_5	4. 2.37	50	6. 3.37	54	8

Castration took place on the day following that date given in Column 2.

TABLE III.

2 AND 3 MONTHS AFTER CASTRATION.					
Rabbit No.	Date	Creatin.	Date	Creatin.	No. of Hr. Periods.
3	6.10. 34	90	15.11.34	98	4
8	6.10. 34	74	15.11.34	80	4
12	19.12. 34	102	20. 1.35	113	4
E	10. 4. 35	58	10. 5.35	63	3
T_2	21. 7.35	51	3. 8.35	52	4
T_5	2. 8. 35	71	29. 8.35	75	4
T_6	2. 8. 35	64	29. 8.35	78	4
K	20. 7. 36	65	22. 8.36	72	6
L	20. 7. 36	57	19. 8.36	63	6
O	20. 7. 36	56	19. 8.36	71	6
M_3	3. 4. 37	56	3. 5.37	63	4
M_5	3. 4. 37	54	3. 5.37	65	4

TABLE IV.

4th AND 6th MONTH AFTER CASTRATION.					
Rabbit No.	Date	Creatin.	Date	Creatin.	No. of Hr. Periods.
3	12.12. 34	102	20. 2.35	101	10
8	12.12. 34	82	20. 2.35	81	10
12	22. 1. 34	113	15. 3.35	112	10
E	14. 6. 35	69	14. 8.35	70	5
T_2	15. 9. 35	58	28.11.35	59	6
T_5	29. 9. 35	81	2.12.35	-	6
T_6	29. 9. 35	81	2.12.35	77	6
K	29. 9. 36	84	29.10.36 (5 mths)	78	10
L	29. 9. 36	72	29.10.36 (5 mths)	75	10
O	28. 9. 36	72	29.10.36 (5 mths)	71	10
M_3	12. 6. 37	65	-		10
M5	12. 6. 37	67	-		10

Creatin. = Creatinine in mgm %.

TABLE V.

No. of Rabbit	At Castration Kgms	2 months post castration Kgms	4 months post castration. Kgms
3	2.31	2.41	2.46
8	1.77	2.00	2.10
12	2.53	2.52	2.50
E	2.38	2.40	2.40
T2	1.72	1.68	1.69
T5	2.30	2.25	2.35
T6	2.30	2.32	2.40
K	2.10	2.30	2.48
L	1.97	2.05	2.10
O	2.10	2.30	2.19

Control rabbits increase in weight slightly, on an average 1.95 to 2.25 kgms. yet the creatinine excretion remains constant.

If we analyse each result separately:

Rabbit No.3 produces 14% more creatinine in the urine in a period of four months after castration. This does not occur immediately but after a latent period of two to three months; during the interval of the third to the fourth month there is an increased elimination of creatinine. His figures over this period swing wildly and are not constant, 95, 100, 90, 84, 92, 102, being daily readings at the beginning of November until he eventually settled down at the higher level, which remained constant. Figures cannot be given at weekly or fortnightly intervals as the rabbits were used for injection purposes (see later).

Rabbit No.8. In this case there is a 16% increase in creatinine elimination, with the change to the higher level occurring between the second, third and fourth months.

Rabbit No.12. 13% increase, the change again taking place between the second and third months.

Nos. E. M_3 and M_5. show increased elimination of 24%, 30% and 15% respectively, the higher level being reached 3 months after castration and, therefore, occurring between the second and third month.

T2 increases its elimination between the third and fourth month to the extent of 14%.

T5, K and L show a gradual increase from the second month after castration onwards until they reach a maximum excretion of 17%, 22% and 31% respectively. This is maintained and is present six months after castration.

T6 eliminates more creatinine between the second and the third month after castration but reaches a still higher level of 31% at the fourth month.

Rabbit O reaches his peak at the third month after castration and this is 24% above his normal pre-castration level.

Discussion.

These figures indicate that:

(a) just as shown by Schrire & Zwarenstein there is a definite and steady increase in creatinine elimination after castration;

(b) there is a latent period before the increased excretion appears, which varies from two to four months after castration;

(c) this increased excretion persists and is maintained It may be a gradual increase or else sudden in onset.

The Increased Creatinine Excretion:

The reason for this increased creatinine excretion may be due to several factors which should be considered. It might be due to a simple increase in body weight. However, although the body weight actually is related to the amount of creatinine excreted it is the muscle weight of the body which determines the amount of creatinine. Korenchevsky produced both "thin" and "fat" castrates and showed the increase in weight after castration to be due mainly to the alteration in fat metabolism with an increased deposition of fat in the body. In Korenchevsky's work the results indicate a big increase in the nitrogen elimination in the "thin" castrate.

In Table V, E, T2 and T5 hardly alter in weight and yet there is an increase of 24%, 14% and 17% respectively after castration. T2 actually lost 0.03 kilograms after castration.

It is, therefore, obvious that although creatinine excretion is related to body weight, the marked post-castration rises in the post-castration periods are not dependant on alteration in body weight.

<u>The latent period:</u>

This is a very significant feature. Removal of the testis results in atrophy of the prostate within 48 hours of the operation (Moore, Price & Gallagher; Vatna; Heller) changes in the accessory sex organs and the formation of the "X-zone" in the adrenals and increase in weight.

But these can have little or no bearing on the increased creatinine elimination for the mechanism must be an indirect one, as shown by the latent period. If it were a direct one there would be no latent period or one which is very short.

This latent period rather suggests a slow and gradual change in another endocrine organ. Schrire & Zwarenstein suggest the possibility of the pituitary, and this seems a reliable hypothesis, especially as Hatai & Wolfe, separately, show that the pituitary hypertrophies three months after castration in males and 9 months after ovariectomy in females; and the two former workers produce an increased urinary creatinine 3 months after gonadal removal in males and only 9 months after removal in females.

In the introduction to these studies the suggestion of a relationship between the anterior pituitary and the gonads was stressed. In view of our later results it has been decided to regard those animals which have not yet shown a rise in urinary creatinine after castration as animals whose pituitary has not yet hypertrophied to the critical level and, therefore, who are not producing any increased gonadotropic hormone. These have been called Recent castrates as distinct from the Longstanding Castrates, which are regarded as rabbits whose pituitary shows a definite hypertrophy. The former show no increase, while the latter show the usual post-castration rise. The relationship between the increased creatinine elimination and the pituitary hypertrophy with reference to the Recent and Longstanding Castrates will be discussed more fully later; this is a significant feature as they react differently to different extracts and serve to show the anterior-pituitary-gonadal relationship.

Creatine:

In six of the castrates creatine estimations were made. Occasionally creatine was found but this was very intermittent and closely resembled the occasional output found in normal males. It, therefore, confirms Read's observation that postpuberally castrated males did not produce creatinuria.

Summary.

1. The constancy of creatinine eliminated in the urine of adult male and female animals is corroborated.

2. The absence of creatine in the urine of males castrated after puberty is confirmed.

3. After castration of adult male rabbits there is 13-31% increase in creatinine eliminated in the urine.

4. The increased urinary creatinine elimination is evident after a latent period of two to four months.

5. Those rabbits which have not yet shown an increased urinary creatinine after castration are designated Recent castrates.

6. Those rabbits which show a definite rise in urinary creatinine and which are regarded as having an hypertrophied anterior pituitary are called Longstanding Castrates.

THE EFFECT ON URINARY CREATININE OF GRAFTING TESTES INTO ADULT MALE RABBITS.

There is a constant and steady increase in urinary creatinine after castration in male rabbits. This indicates some relation to the internal secretion of the testes. The proof of this hypothesis is effected by transplantation of testes and the results noted.

Grafting Experiments:

Nine animals were used. Of these only two gave any results. In one, No.4., the graft on removal was very vascular and the histology was fairly normal. The graft of No. T4 was also vascular and the vessels to the graft had to be ligatured; the histology was indefinite. In the other four animals the graft was found to be fibrosed and avascular, and in one case partly calcified.

The figures for No.4 and No.T4 and controls are, therefore, the only ones given.

The controls were non-operated normal animals, and also rabbits which had been grafted, but in which the graft had not taken. (This was shown by the avascularity of the graft and confirmed histologically).

TABLE I.

Date	No.4.	No.12.	Hr.Periods&
Preperiod	85	98	8
3.8.34	Operation	Operation	
17.8.34	83	100	4
5. 9.34	64	99	4
14.9.34	60	100	4
19.9.34	65	103	4
	(Graft removed	(Graft removed and rabbit castrated	
24.11.34	83	100	4
4.12.34	87	101	4
26. 2.35	90	113 (Castration effect)	4

TABLE II.

Date	No.T1	No. T3	No.T4	Hr.Periods
Preperiod	52	60	60	4
3. 6.35	Grafted	Control	Grafted	
9. 7.35	50	63	60	4
21.7.35	52	64	56	4
9.9.35	51	61	49	6
	Graft removed	-	Graft removed	
10,12.35	Died	66	58	5

From these two cases we note a certain fall in creatinine excretion. 23% in the one case and 30% in the other. (This fall does not occur in the controls).

In animal No.4. this fall in excretion comes on within a month of castration and is sudden in onset. This is contrasted with that of animal No.T4, who only shows a gradually lowered urinary creatinine which reaches its lowest level three months after operation.

After removal of the graft No.4 shows a sudden return to normal after two months, while No.T4 on the other hand three months later is only gradually increasing his creatinine output to the normal level.

Discussion.

Schrire & Zwarenstein grafted testes into castrated animals which are showing an increased creatinine excretion and produced a fall down to the normal precastration level. The removal of the grafts led to the reappearance of the usual castration effects, viz: an increased creatinine excretion. They also show a latent period of two to three weeks before the graft has any effect, and they suggest that it may be due to vascularisation of the grafts or else a period of time is necessary for the secretions of the graft to antagonise the effects of castration. This latter is the more feasible, especially in view of the relation between the gonads and anterior pituitary discussed in the introduction. (Also it is noted that their grafts were not examined histologically, vascularisation being considered sufficient evidence of a functioning graft).

In these two cases mentioned here the fall in urinary creatinine where testes are grafted into normalmales rather corroborates the finding of these two co-workers. Also return to the normal creatinine elimination on removal of the graft and the definite latent period which occurs affords strong evidence in favour of the existence of an endocrine relationship between the testes and creatinine metabolism, and as will be fully discussed later, the direct relationship of the anterior lobe of the pituitary gland, and creatinihe metabolism.

Summary.

1. Implantation of testes into normal adult male rabbits causes a fall in the normal creatinine excretion to a lower level - 23% in one case and 18% in the other.

2. Removal of the graft leads to the reappearance of the normal creatinine elimination.

3. In both cases there is a definite latent period.

4. This augments the suggestion of a testes-anterior pituitary control of creatinine metabolism.

INJECTION OF TESTICULAR EXTRACTS INTO NORMAL ADULT MALE RABBITS.

Castration has been shown to affect the creatinine level; it is suggested that this is due to removal of the inhibitory action on the anterior lobe of the pituitary.

Grafting of testes into castrated animals and the injection of different testicular extracts produces a fall to the precastration level of urinary creatinine. Again the suggestion offered is that of restoring the inhibitory action of the testes on the pituitary which has hypertrophied. What is the action of testicular extract on the normal animal however; does it act by inhibiting the pituitary, or has it an inhibitory action on the testes themselves by acting via the pituitary as is shown by Moore & Price ?

For this experiment normal adult male rabbits were used. The amount of urinary creatinine of a preperiod of at least one month was determined before injections were commenced. This enabled the urinary creatinine level to be definitely established.

Testicular extracts were prepared as described earlier and injected subcutaneously in the flank. The estimations were continued for at least a week afterwards, or if a sustained rise were observed the determinations were continued until the level of creatinine excretion returned to normal and remained thus for several days.

Control injections of lipoid material from brain, liver and kidney were also used. The combined figures of control injections will be given separately later. The tables of injections of testicular extract only contain figures of the normal controls and the animal injected with testicular extract, as control injections with lipoid liver extracts, etc. did not affect the creatinine elimination. (see later).

TABLE I.

Date	No.1.	No.3.	No.4.	No.8.	No.9.	No.12.
Preperiod	96	80	78	80	83	98
22.4.34	90	75	84	74	80	95
23.4.34x	135x	94x	107x	82	90x	105x
24.4.34	104	45(32)	87	77	82	100
25.4.34x	104x	77	93	83	80	97
26.4.34x	129x	82	90	76	78	92
27.4.34	98	-	-	76	-	96
28.4.34	96	-	-	-	-	-

Injections of testicular extract; 1 ml. being equivalent to 300 mgms. of lipoid material.

23.4.34	4ml	3ml	5ml	Control	1 ml	2 ml
26.4.34	4ml	-	-	-	-	-

These results all indicate a definite increase in the urinary creatinine. No.3. shows a fall on the second day after injection, but the creatine eliminated was also estimated and this was found to be extremely high, as under normal circumstances there is no creatine present. Creatine estimations in the other rabbits failed to indicate any creatinuria. In several of the later experiments the creatine output was determined but this yielded negative results and was, therefore, discontinued.

In this table above, No.9. (injected with 1 ml) does show a rise of 8%, but this is within the normal variation. No.12, too, has a slight increase, but this is also within normal limits. Amounts of 3 mls. to 5 mls. all produce a definite and marked increase in creatinine elimination.

/TABLE II.

TABLE II.

Date	No.3.	No.4.	No.8.	No.9.	No.12.
Preperiod	80	84	63	82	98
29.6.34	82	86	62	81	97
30.6.34	97x	102x	79x	82	123x
1.7.34	85	88	64	81	97
2.7.34	83	87	62	75	96
3.7.34	84	90	65	80	100
4.7.34	108x	8 6	89x	75	133x
5.7.34	87	88	66	76	102
10.7.34	84	87	60	78	96
11.7.34	82x	Urine lostx	65x	81	94x
12.7.34	79	84	60	76	92
13.7.34	103x	87x	80x	70	120x
14.7.34	87	85	68	80	98
15.7.34	82	80	65	72	95

Table of Injections in which a) 1 ml = 300 gm. of fresh testes.

30.6.34	2 ml	2 ml	2 ml	Control	2 mls.
4.7.34	3 "	Control	3 "	"	3 mls.

b) 1 ml = 300 mgm lipoid material.

11.7.34	1 ml	1 ml	1 ml	Control	1ml.
13.7.34	4 "	1.5 "	4 "	"	4 mls.

Injections of one to one and a half ml. of testes extract dissolved in olive oil have no effect on the elimination of creatinine. In Table II. two mls. of the extract does cause an increased creatinine excretion. As compared with Table I, however, one ml. of the extract in Table II. is equivalent to 300 mgm. of fresh testes, whereas in the former table one ml. of extract is equivalent to 300 mgm. of lipoid material. In all, the later experiments a standard of one ml. being equivalent to 200 or 300 mgm. of <u>lipoid material</u> was adopted and the relation to a certain weight of fresh testes discarded.

TABLE III.

Date	No. 9.	No. 14.	No. 12.
Preperiod	60	73	101
12.9.34	54	73	100
x 13.9.34	77 x	70	123 x
14.9.34	69	69	108
15.9.34	65	73	105
19.9.34	56	75	100
x 20.9.34	84 x	70	129 x
21.9.34	70	69	108
22.9.34	63	77	103.

x (Both No.9 and No.12 were injected with 4 mls. of testes extract. No.14. was the control on the 13th.

(On the 20th Nos. 9 and 12 were injected with 5 mls. of testes extract

In this case 1 ml. of testes extract was equivalent to 200 mgm. of lipoid material. Again a significant rise is shown and as in the preceding tables this rise is acute in onset occurring on the day of injection.

TABLE IV.

Date	No.9.	No.12.	No.14.
Preperiod	63	102	70
27.11.34	70	110	67
28.11.34	87x	103	90 x
29.11.34	80	108	77
30.11.34	74	108	64
1.12.34	82x	91	61
2.12.34	64	93	54
3.12.34	73	103	60
4.12.34	67	100	61

No.9. and No.14. received 3 ml. of T.E. on the 28th and No.9. 2.5 mls. on the 1st. 1 ml. of Testes Extract in this instance is equivalent to 300 mgm. lipoid material.

Here 2.5 and 3 mls. cause an increased creatinine elimination in the urine with the same characteristics as previously shown.

TABLE V.

Date	No.A.	No.C.	No.D.	No.E.
Preperiod	62	49	45	36
3.4.36	53	50	50	36
4.4.36	60	46	45	35
5.4.36	59	50	46	40
6.4.36	84 x	75 x	67 x	39
7.4.36	68	64	60	32
8.4.36	64	57	54	35

Nos. A, C and D were injected with 4 mls. of Testicular extract on the 6th, April. 1ml = 300 mgm lipoid material.

TABLE VI.

Date	No.A.	No.B.	No.C.	No.D.	No.E.	No.F.
Preperiod	59	41	51	46	31	57
17.5.36	54	36	49	41	31	52
18.5.36	62	42	55	47	33	62
19.5.36	63	40	51	46	31	63
20.5.36	61	43	54	42	30	60
21.5.36	75 x	46	66 x	60 x	46 x	75 x
22.5.36	67	45	47	49	42	62
Table of Injections:						
21.5.36	3ml	Control	3ml	3ml	3ml	3ml

Where 1 ml. is equivalent to 300 mgm. of lipoid material.

In both Tables V and VI. we observe a significant rise on the day of injection. The extent of the increased creatinine elimination will be discussed later. The increase is notably more than any possible variation from the normal. On analysing these results the conclusion can be drawn that there is a marked increase in urinary creatinine following the injection of a sufficiently large dose of testicular extract.

On closer examination a quantitative response is observed. Although not absolutely definite, larger doses of the testicular extract provoked larger amounts of creatinine elimination and an attempt was made to obtain results which would lead to a method of assay of the hormone based on the creatinine eliminated. Accordingly, in a new set of experiments in which every method of technique was as accurate as possible, the dose of testicular extract per kilogram of body weight was plotted against the percentage increase in creatinine excretion per kilogram of body weight. The results were very convincing.

Below are tables which include the figures for daily creatinine estimations at the time of injection. These figures show the actual rise in mgms. Below these figures is a detailed table of the dose of lipoid material in mgms.

per kilogram of body weight, obtained from the weight of the animal and the dose in millilitres.

Control injections and normal controls are omitted as being irrelevant and confusing.

TABLE VII.

Date	Rabbit 7	Rabbit 8	Rabbit 9.	
Preperiod	78	63	80	
13. 6. 35	85	62	84	
x 14. 7. 35	91	72	93	
15. 7. 35	87	74	90	
Dose	2 ml	2 ml	2 ml	1ml =300 mgms. Lipoid material
Weight	2.37kgm	1.78kgm	2.34kgm	
Dose/Kgm Body Weight	253mgm	332mgm	260mgm	
% rise/kgm Body Weight	7%	8%	7%	

/TABLE VIII.

TABLE VIII.

Date	1.	2.	3.	4.	5.
Preperiod	59	51	46	31	57
19. 4.36	61	54	42	30	60
x 20. 4.36	75	66	60	46	75
21. 4.36	67	47	49	42	62
Dosage	3ml	3ml	3ml	3ml	3ml
Weight	2.15Kgm	1.90Kgm	1.65Kgm	1.57Kgm	2.11Kgm
Dose/Kgm B.W.	418mgm	475mgm	545mgm	550mgm	426mgm
% rise/Kgm B.W	12.6%	18.3%	20.2%	22.2%	12.8%

TABLE IX.

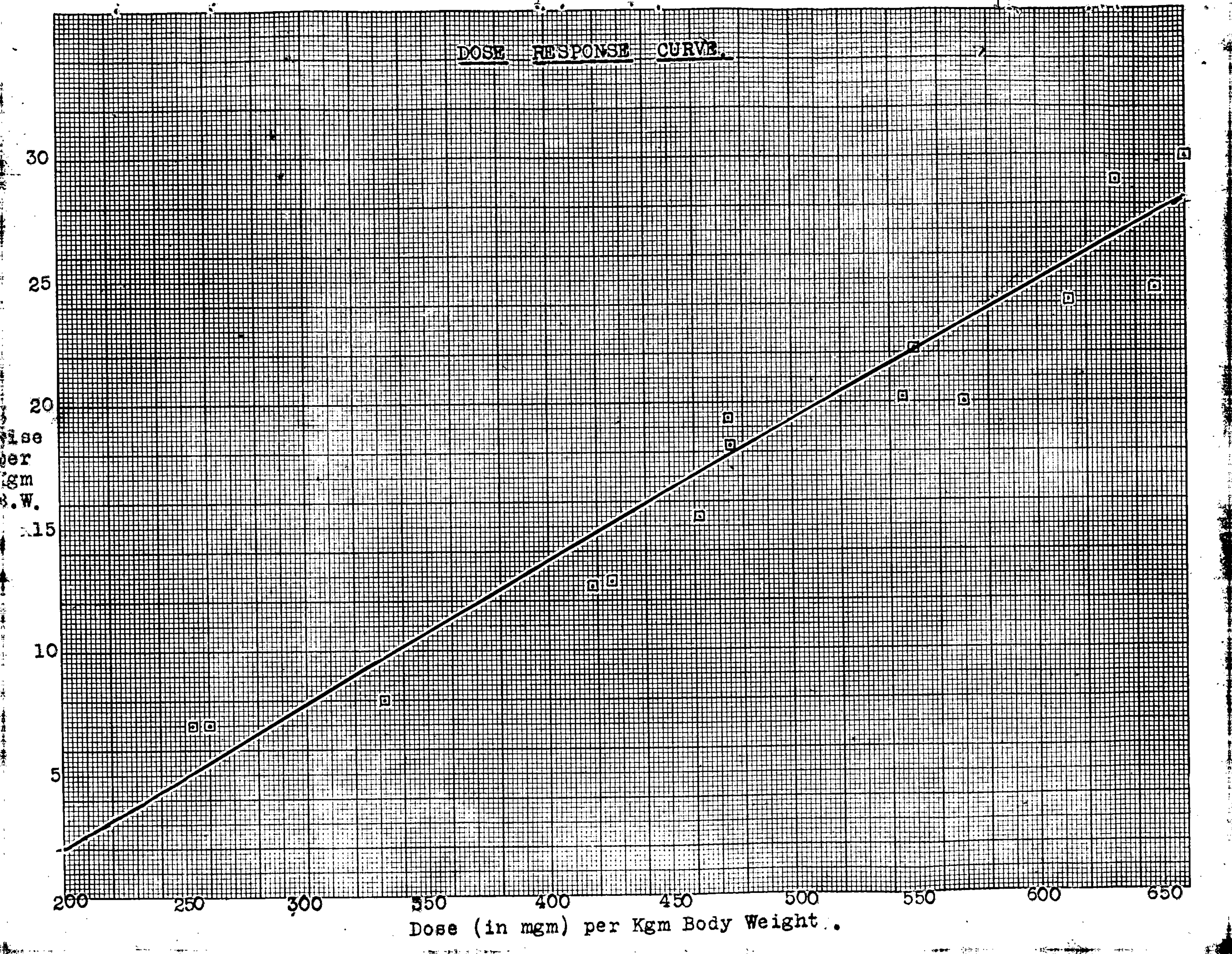
Date	2	3.
Preperiod	51	46
14.7.36	55	50
x15.	65	63
16.	58	55.
Dose	3ml	3ml
Weight	195 Kgm	1.90Kgm
Dose/Kgm B.W.	462 mgm	474 mgm
% rise/Kgm B.W	15.4%	19.4 %

TABLE X.

Date	1.	2.	3.
Preperiod	59	51	46
5.4.36	59	50	46
x6.4.36	84	75	67
7.4.36	68	64	60
Dose	4mls	4mls	4mls
Weight	2.10kgm	1.95 kgm	1.85kgm
Dose/Kgm B.W	571mgm	615 mgm	648mgm
% rise/Kgm BW	20%	24.1%	24.6%

TABLE XI.

Date	2	3	4
Preperiod	51	47	37
20.6.36	52	48	36
21.6.36	77	69	59
	72	60	45
Dose	4ml	4ml	4ml
Weight	1.90Kgm	1.80Kgm	1.60Kgm
Dose/Kgm B.W	631mgm	660mgm	750mgm
% rise/Kgm BW	29%	30%	39.3%


DOSE RESPONSE CURVE.
30
25
20
15
10
5
Rise per Kgm B.W.
200
250
300
350
400
450
500
550
600
650
Dose (in mgm) per Kgm Body Weight.

Thus we see that not only does the injection of testicular extract produce an increase in the creatinine excretion, but this is a quantitative one dependent upon the amount of extract injected.

Discussion:

The rise occurs on the day of injection. This stimulation of the formation of urinary creatinine if a direct effect of the testes on creatinine would result in an alteration in the daily amount of creatinine eliminated soon after castration, whereas actually there is a long latent period prior to the post castration increase. The indirect effect, therefore, is probably one via another endocrine. The increased creatinine output gradually returns to the normal level, which is reached within three days.

The response varies according to the dose and in the series of experiments in which careful attention was paid to response, dose and weight of the animal; if the response per kilogram of body weight is plotted against the amount of testicular extract injected per kilogram of body weight, the graph represents a straight line. This suggests that there exists a definite quantitative response which would form the basis of a method of assay. These later results also serve to confirm the action of testicular extract upon creatinine metabolism, producing an increased elimination in the urine.

Summary.

1. Testicular extract into normal animals produces an increased elimination of creatinine in the urine.

2. This response takes place on the day of injection

3. The response is a quantitative one, varying with the amount of testicular extract injected.

4. A method of assay of testicular extract is suggested.

INJECTION OF TESTICULAR EXTRACT INTO RECENT CASTRATES. (MALE).

The effect of castration on the elimination of creatinine has already been discussed. The conclusion arrived at was that the creatinine does not increase in amount until the pituitary has reached its critical hypertrophied stage, when there is a rise in the creatinine level. Until this new level was reached the animal was regarded as one in which the pituitary had not hypertrophied to the level required to affect creatinine excretion, and as such was termed a recent castrate. As soon as the urinary creatinine had definitely risen the animal, now called a "long standing castrate", was regarded as possessing a hypertrophied pituitary which produced an increased amount of the hormone controlling creatinine metabolism and causing this increased output.

In the following series of experiments testicular extract is injected into these recent castrates and the results obtained commented upon. In the Tables are included normal controls (uninjected) and also normal controls injected with testicular extract. The extract was regarded as active if there was an increase in creatinine elimination in the normal control animal on the day of injection. (1ml of the testes extract was equivalent to 300 mgm of lipoid material.

TABLE I.

Date	No.3.	No.4.	No.8.	No.9.
Preperiod	90	78	71	58
21.8.34	87	80	75	55
x 22.8.34	106x	96x	86x	63
23.8.34	90	76	84	61
24.8.34	87	75	82	56
Injections) 22.8.34)	3mls	3mls	3mls	Control
Animal	R.C.	C.I.	R.C.	Control.

In the above Table "R.C." signifies Recent Castrate
"C.I." " Control injection into normal.

The animals were castrated on the 3rd of the same month.

TABLE II.

Date	No.3.	No.8.	No.9.	No.14.
Preperiod	90	71	58	73
12.9.34	90	73	54	70
x13.9.34	99x	79x	74x	60
14.9.34	93	75	69	73
15.9.34	89	70	66	71.
Injections 13th	2.5ml	2.5ml	3ml	Control
Animal	R.U.	R.C.	C.T.	Control

TABLE III.

Date	No.3.	No.8.	No.9.	No.14.
Preperiod	90	78	58	73
19.9.34	96	87	56	75
x 20.9.34	96x	80x	88x	70
21.9.34	93	75	70	69
22.9.34	96	82	63	77
Injections 20th.	3.3ml	3.3ml.	3.3ml	Control
Abimal	R.C.	R.C.	C.I.	Control

TABLE IV.

Date	No.3.	No.8.	No.9.	No.12.
27.11.34	92	80	70	110
x 28.11.34	96x	80x	87x	103
29.11.34	96	84	80	108
30.11.34	98	84	74	108
Injections 28th	3ml	3 ml	3ml	Control
Animal	R.C.	R.C.	C.I.	Control

From this first set of experiments it will be noted that injections of testicular extract within the first four to six weeks of castration produce an increase in creatinine elimination.

After this period, however, there is a tendency towards an increased daily urinary creatinine and an inclination towards the increased creatinine excretion of castration. Injection of testicular extracts produces little or no response and the creatinine level remains at a normal level.

When the daily level is established at a constant level injection of testicular extract does produce an effect, but this is dealt with in the next section on long standing castrates.

Rabbit No.3.

There is a significant rise to within six weeks after injection. This increase, however, in the urinary creatinine does not occur after this period, and by the time the creatinine excretion is beginning to reach a higher level the injection of testicular extract produces no response in the form of an altered creatinine output on the day of injection.

Another feature is the quantitative response to the dose, which is less than in the normal animal.

It can be argued, therefore, that a bigger dose is necessary in the recent castrate than in the normal to produce the same percentage increase in creatinine excretion per kilogram of body weight.

No.8. presents similar features to No.3. Up to six weeks after the operation injections of testicular extract produce an increase in creatinine elimination. This does not occur after two months, when the daily creatinine level is beginning to find a higher level.

No.9., the control animal, shows a quantitative response to different amounts of the extract injected.

If these results are compared on a quantitative basis, the results (e.g.ofTable II) are:

TABLE V.

	No.3.	No.8.	No.9.
Weight	2.20	1.77	2.20
% rise/Kgm B.W.	4.8%	6.2%	14.0%
Dose/Kgm B.W.	364mgm	452mgm	409mgm

Thus the injection of testicular extract into recent castrates will produce an increase in urinary creatinine, but the response is not equal to that in the normal animal.

Details of further experiments are given in the next series of tables.

TABLE VI.

These rabbits M3 and M5 were castrated on 5th February.

Date	No. M3.	M5.	M2.	M4.
Preperiod	52	50	46	54
25.2.37	52	52	44	55
x26.2.37	71 x	70x	48	72x
27.2.37	59	62	53	67
28.2.37	53	54	50	59
Injections:26th	5ml	5ml	Control	4ml
Animal	R.C.	R.C.	Control	C.I.
% rise/Kgm B.W.	22.5%	24.0%	-	20%
Dose/Kgm B.W.	660mgm	654mgm		452mgm.

TABLE VII.

Date	M3	M5	M2	M7.
Preperiod	52	50	46	52
14.3.37	45	50	47	47
x15.3.37	70x	63x	46	75x
16.3.37	61	52	53	52
17.3.37	58	50	-	50
Injections:15th	5ml	5ml	Control	4ml
Animal	R.C.	R.C.	Control	C.I.

These two animals still give a response to testicular extract in that there is an increase in creatinine elimination, but two months after castration they fail to reproduce this rise in the creatinine excretion, as seen in Table VIII. It will also be noted that they are beginning to reach a higher daily level.

TABLE VIII.

Date	M3	M5	M2	M4
31. 4.37	58	59	45	52
1. 4.37	60	57	50	55
x 2. 4.37	58x	52x	52	69x
3. 4.37	58	55	57	57
4. 4.37	64	52	48	56
Injections:2nd	4ml	4ml	Control	3ml
Animal	R.C.	R.C.	Control	C.I.

Here no response is obtained with the exception of the control injection and the animals remain at the slightly higher daily creatinine level which they have suddenly reached.

Besides these two complete series of experiments, there are several more acute ones in which the injections have been made into castrates two months after the operation, e.g., in the following table in which no alteration in the amount of urinary creatinine from the daily level followed:-

TABLE IX.

Date.	T1	T2	T3
19.8.37	53	70	65
x20.8.37	56 x	72 x	61 x
21.8.37	50	76	62
22.8.37	51	74	68
23.8.37	56	70	58
24.8.37	49	71	60

An injection of 3 mls. of extract was given on the 20th. T1 was the control and T5 and T6 the animals which were castrated on the 3.6.37. Their normal daily creatinine had not yet reached a higher level.

TABLE X.

Date	No.4.	No.12.
Preperiod	86	100
x 26.11.34	84	104 x
27.11.34	87	112
28.11.34	90	103

No.12. received 3 mls. of testicular extract and was castrated on October 19th. No.4 was the control. There is a slight increase on the second day, but this may not be due to the injection but to the animal finding a higher level.

Discussion:

The conclusions drawn from this data are that, if testicular extract in a sufficiently large dose is injected into an adult male rabbit within six weeks of castration there will be an increased creatinine elimination.

This suggests that some gradual change is taking place in the animal, especially as the response to an injection of testicular extract is considerably less than the response of the normal animal to the same amount of the extract. This increase in creatinine excretion also takes place on the day of the injection.

Injections into these same animals, two to four months after castration, however, fail to reproduce the effect obtained, and the urinary creatinine is not altered by the injection. It (urinary creatinine) is in the transition period between the high castration level of the long standing castrate and the lower level of the recent castrate (which approximates normal). The creatinine level is now rather inconstant and shows many individual variations, but injections of testicular extract do not appear to have any effect and seem to have lost the stimulatory effect on the production of urinary creatinine.

If the action of the testes on creatinine metabolism is an indirect one through another endocrine, these results indicate a gradual change in this endocrine, which is stimulated at first by the testes to produce an increased urinary creatinine. As the transition following castration takes place, this stimulatory action is gradually diminished. Eventually the gland is not acted upon at all by the testicular extract and the creatinine metabolism remains unaffected by this addition of the testicular preparation. until the next phase - that of long-standing castrates - appears. This will be discussed in the next sextion.

Summary.

1. Injection of testicular extract within six weeks of castration in sufficiently large amounts produces an increase in urinary creatinine.

2. The response in the recent castrate is less than that of the normal to the same dose.

3. Injection of testicular extracts two to four months after castration has no effect on creatinine elimination.

4. The results suggest a transition stage between a normal and a high post-castration creatinine level.

5. This transition stage is probably due to a gradual change probably in the pituitary.

INJECTIONS OF TESTICULAR EXTRACT INTO LONG-STANDING MALE CASTRATES.

In the previous section attention was drawn to the recent castrates, in which the creatinine level had not yet reached the high level which follows within four months of castration, and in which the pituitary is not regarded as having hypertrophied to the "critical" stage. In this section the long-standing castrate is considered an animal which shows the high post-castration level and which is regarded as possessing a definitely hypertrophied pituitary.

The new level was accurately determined and an average for the period previous to injections of testicular extract estimated. Control injections of testicular extract into normal animals were made at the same time to ensure active preparations.

No complete series of experiments, as with the two stages of recent castrates, could be performed, as castrated animals did not appear to thrive too successfully and the results obtained are only those of isolated injections into healthy long-standing castrates whose preperiod of creatinine excretion did not vary and remained very constant as in the normal animal.

TABLE I.

Date	No.M7	No.A.	No.M2.
Preperiod	52	98	46
14.3.37	47	97	47
x15.3.37	75 x	96 x	46
16.3.37	62	73	53
17.3.37	60	72	52
18.3.37	56	82	-
19.3.37	53	87	-

Injections: 15th. 5 mls. of testicular extract into A and 4 mls into M7.
(1 ml is equivalent to 300 mgm lipoid materia

Rabbit A. is the long-standing castrate and M7 is the control injection. 5 ml of testes extract was injected into A and 4 ml into M7. There is a significant drop in the long-standing castrate on the second day after injection. In the previous experiments the increased creatinine elimination in the normal animal takes place on the day of injection.

TABLE II.

Date	No.M4	No.A.	No.M6.
31.3.37	52	95	68
1.4.37	55	90	73
x 2.4.37	69 x	95 x	72
3.4.37	57	70	63
4.4.37	53	76	67

Injection 2.4.37: No.A. and No.M4 received 3 ml of Testis Extract in which 1 ml is equivalent to 300 mgm lipoid material. A. is the long-standing castrate, M4 the normal control injection and M6 the normal control.

For the next ten days the figures for A are 76, 75, 79, 82, 70, 77, 62, 86,82, 91 and, finally, 97, on the 15th of the month. Just as in Table 1. the fall was a prolonged one. This fall was followed up and daily estimations prove that the duration of lowered creatinine excretion is twelve days. As Rabbit A had a level previous to castration of about 67 to 70 ~~and~~ in this case the fall of the creatinine excretion is to the precastration level.

TABLE III.

Date	T1	T3	12.
19.7.35	53	64	115
20.7.35	56	66	120
x 21.7.35	50	77x	91x
22.7.35	51	75	89
23.7.35	56	66	93
24.7.35	49	68	113
25.7.35	51	64	115
Injection 21st	Control	3ml	3ml
Animal	Control	C.I.	L.S.C.

No.12. here, again, a long-standing castrate gives a decreased creatinine elimination which, although it is not sustained, does diminish to the precastration level of ± 93. It is noteworthy in this case that the fall occurs on the day of injection and not the following day, as in the previous tables.

TABLE IV.

Date	T3.	12.
9.IX.35	60	118
x10.IX.35	64	115 x
11.IX.35	58	96
12.IX.35	62	99
13.IX.35	60	110

No.12., a long-standing castrate, was injected with about three mls. of the standard testicular extract used in all these experiments. The fall in the creatinine level takes place on the second day after injection and is not sustained.

TABLE V.

Date	8	A	B
Preperiod	78	70	58
27.2.35	75	72	56
x 28. 2.35	70 x	70 x	64
1.3.35	64	86	62
2.3.35	69	79	60
3.3.35	63	71	56
4.3.35	80		
Injection 28th	3ml	3ml	Control
Animal	L.S.C	C.I.	"

The above table again indicates a significant fall. Abimal No.8., which was castrated on the 3rd August about six months previously had an original level of about 63 mgm., so that the fall in creatinine, although sustained for only three days, fell to the precastration level. The decrease in creatinine elimination occurred on the second day after injection.

TABLE VI.

Date	T5	T6	F	Al.
Preperiod	80	78	64	62
2.12.35	79	75	63	61
3.12.35	74	70	66	60
x4.12.35	82 ↗	80 ↗	82 ↗	63
5.12.35	68	60	78	64
6.12.35	65	63	69	64
7.12.35	63	68	70	60
8.12.35	69	75	65	56
9.12.35	70	72	60	64
Injections, condition of rabbit & precastration creatinine level.				
4.12.35	3ml	3ml	3ml	Control
Abimal	L.S.C.	L.S.C.	C.I.	Control
Precastration	65	60	-	-

The injection is followed by a fall in creatinine level in the long-standing castrates and the precastration level, while in the normal control animal the creatinine elimination increases.

All the above results show injection of testicular extracts into the long-standing castrate reduces the high post-castration to the normal precastration level. The duration of the decreased creatinine is dependent upon the amount of extract. With moderate doses the effect is transitory and with large doses is greatly prolonged.

Discussion:

These long-standing castrates have a high urinary creatinine level, which is regarded as probably due to a hypertrophied pituitary.

Injections of testicular extract stimulated the pituitary in the normal animal to increase the amount of creatinine eliminated. In the recent castrate the pituitary was stimulated at first, but this stimulation of the testicular extract gradually diminished until eventually it produced no effect on the pituitary until the definite phase of the long-standing castrate was reached four to six months later. At this stage the testicular extract produced an inhibitory effect on the non-hypertrophied pituitary with its altered physiological status and a lowered creatinine excretion resulted.

The effect of testicular extract on the urinary creatinine, on normal, recent and long-standing castrates is one of both stimulation and inhibition, the stimulation being on the normal gland and the inhibition on a gland which has definite different physiological properties from the normal. The recent castrate, in which the pituitary is probably in a state of transition, is unaffected.

Summary.

1. Injection of testicular extract into long-standing castrates leads to a fall to a precastration level.

2. This is probably due to an inhibition of the hypertrophied pituitary.

GENERAL SUMMARY.

1. Injection of testicular extract into normal animals produces an increase in urinary creatinine.

2. Injection of testicular extract into recent castrates first produces a rise, and 3 months later has no effect on the elimination of creatinine.

3. Injection of testicular extract in long-standing castrates produces a fall in the creatinine excretion to the pre-castration level.

4. These results are explained on the basis of inhibition or stimulation of the pituitary, which, after castration, gradually undergoes hypertrophy, and alters its physiological functions.

THE EFFECT OF ADMINISTRATION OF SALINE SUSPENSION OF TESTES ON CREATININE ELIMINATION.

INJECTION OF SALINE SUSPENSION OF TESTES INTO NORMAL ADULT MALE RABBITS.

Bulls' testis tissue was finely divided up with a scalpel, the macerated liquid mass suspended in Locke's Solution and injected subcutaneously by means of a large veterinary syringe and needle.

The creatinine level of the rabbits used in these experiments wasdetermined for at least a month before and the average daily excretion estimated. The results obtained following injection of the suspension are detailed below:-

TABLE I.

Date	No.2.	No.5.	No.6.	No.7.	No.10.	No.11
Preperiod	99	78	96	98	93	89
29.4.34	105	75	93	90	90	87
30.4.34	111	73	90	84	90	93
x 1.5.34	110 x	77 x	90	93 x	91	90
2.5.34	109	77	90	91	90	93
3.5.34	104	45	85	75	78	82
4.5.34	92	60	88	73	76	78
5.5.34	81	69	89	77	70	67
Table of Injections:						
1.5.34	10ml	20ml	C.I.	10ml	10ml	10ml.

Control injection = 10ml saline. 1ml = 0.3 mg fresh tissue.

In each of the animals injected there is a significant decrease in the creatinine eliminated.

Animal No.2. has a decreased urinary creatinine on the fourth day following injection, as is found in No.11.

In animals No.7.and 10, which also receive 10mls.of the suspension, diminished output occurs on the third day after injection. The fall varies between 13% and 24% in these four animals. The creatinine of animal No.5, however, which received 20 ml. of the suspension, fell to 45 mgs., which represents a 42% and occurred in the third day after injection.

The control animal received 10 mls.of saline. This had no effect on the creatinine level.

TABLE II

	Date.	No.1.	No.3.	No.4	No.8.	No.9.	No.12.
	Preperiod.	96	80	78	60	43	98
	13.5.34.	100	90	82	68	82	102
x	14.5.34.	97x	84	83x	Incomplete x	Incom. x	100
	15.5.34.	104	85	86	113	145	98
	16.5.34.	79	87	70	66	78	85
	17.5.34.	75	84	69	63	64	72
	18.5.34.	67	83	60	55	58	75
	19.5.34.	48	82	67	40	69	66
	20.5.34.	84	80	67	23	71	87
	21.5.34.	89	84	73	67	74	95
	22.5.34.	88	85	79	74	82	93
	Injections; 14.5.34.	20ml.	C.I.	20ml.	20ml.	15ml.	

Control injection was 15 ml. of saline.

1ml. is equivalent to 0.2 grm.fresh testes.

The urine from No.8 and No.9 each measured less than 85 mls. on the 14th. On the following day the amount of urine was not increased above the average normal output, but the creatinine excretion was extremely high. It is suggested that the figures on the fifteenth represent almost the total creatinine eliminated for the two days.

In the other animals the drop in the urinary creatinine occurs on the third day after the injection. This fall gradually increases until it reaches its lowest level on the fifth day in Nos.9 and 4, and on the sixth day after injection in Nos.1, 8 and 12.

The percentage drop varies. The lowest figures on their respective days indicate that there is a decrease of 23% in rabbit No.4., 30% in No.9, and 33% in Nos.8 & 12. Animal No.1, however, on the nineteenth reaches a creatinine level which is 50% less than normal.

The control animal only shows variations within normal limits.

DISCUSSION:

These above results indicate that there is probably an inhibitory factor acting on the pituitary, thus producing the fall in the creatinine output. The point is again emphasised that it is not a direct effect of the testes. The latent period suggests the possibility of slow absorption and excretion of the

substance...

substance or possibly a relatively low activity of the suspension.

Summary.

1. Injection of saline suspension into normal adult male rabbits produces a fall in urinary creatinine.

2. This decreased urinary creatinine may be as low as 50% of the normal daily amount.

3. There is a latent period of at least two days before the creatinine level commences to fall.

INJECTION OF SALINE SUSPENSION OF TESTES INTO RECENT CASTRATES. (MALE).

As with Testicular extracts, "recent castrate" experiments are carried out on two sets of animals,

(a) those that have been castrated up to six weeks previously, and

(b) those that have been castrated for two to four months but which do not already show the post-castration increase in urinary creatinine.

/TABLE I.

TABLE 1.

Date.	T2	T3	T5	T6	T1
20.6.35.	49	63	68	53	50
21.6.35.	53	60	72	64	45
x 22.6.35.	50x	68 x	70 x	60x	53
23.6.35.	56	66	75	52	56
24.6.35.	51	62	60	40	46
25.6.35.	42	58	57	40	53
26.6.35.	38	47	55	59	52
27.6.35.	39	54	65	58	45

Table of Injections & type of animal.

22.6.35.	15ml.	15ml.	15ml.	15ml.	Control.
Animal.	R.C.	Normal. C.I.	R.C.	R.C.	Normal.

1 ml. is equivalent to 0.3 gm. fresh testes.

This above table consists of the results obtained from injecting suspension of testes into rabbits which were castrated on the third of the month, that is nineteen days previously. In each instance there is a fall in the creatinine output; this drop also occurs in the normal animal injected with the same amount of testes suspension. The fall lies within the limits of 20 to 23%. This is almost the same as that of the normal animal which shows a 27% decrease.

TABLE II.

Date.	No. 3.	No. 8.	No. 14.
Preperiod	89	73	72
19.10.34.	90	82	77
x 20.10.34.	82x	75x	73x
21.10.34.	96	80	70
22.10.34.	93	68	67
23.10.34.	79	67	51
24.10.34.	72	64	53
25.10.34.	77	80	65

Table of injections:

20.10.34.	19ml.	20ml.	19ml.
Animal	R.C.	R.C.	Normal. C.I.

1ml ≡ 0.2 gm.fresh testes tissue.

This series of experiments was performed on animals which had been castrated on the third of August, two and a half months previously, but whose creatinine level remained within the normal precastration limits.

Again there is a fall in the urinary creatinine.

Animal No.3. has a diminished creatinine output of 22%, and No.8. of 20%. The creatinine of the normal animal No.14. decreases by 28%.

DISCUSSION:

Injections of a saline suspension of testes into both types of recent castrate produces a lowered creatinine elimination.

This again indicates an inhibition of the pituitary. At the same time it suggests an alteration in the function of the gland, for the fall in the creatinine output in the recent - castrate is not quite as great as that in the normal animal which received the same dose. In both cases there is a latent period before the effects of the injection are evident. This latent period, as in the normal adult male rabbit, varies from three to four days.

The effect of testicular suspension on recent castrates is different from that obtained with testicular extract, for this saline suspension is capable of acting on the pituitary and altering the daily urinary creatinine level in all stages of castration.

SUMMARY:

1) Injection of saline suspension of testes into both types of recent castrates is followed by a fall in urinary creatinine.

2) This fall is not as pronounced as that obtained with the same dose in the normal animal.

3) There is a latent period after the injection and before the creatinine excretion diminishes.

INJECTION OF SALINE SUSPENSION OF TESTES INTO LONG STANDING CASTRATES (MALE)

The rabbits used clearly showed the post-castration increase in urinary creatinine, and this level was carefully determined prior to injection. In the tables below the pre-periods are given, and these indicate the level of the increased creatinine elimination.

TABLE I.

Date.	No.3.	No.8.	No.9.
Preperiod	96	86	69
11.12.34.	103	90	75
12.12.34.	100	90	70
x 13.12.34.	103x	87x	65x
14.12.34.	81	69	68
15.12.34.	83	70	48
16.12.34.	95	78	69
17.12.34.	-	90	75
Injection:			
13.12.34.	20ml.	20ml.	20ml.
Animal	L.S.C.	L.S.C.	Normal. C.I.

Animal No.3. had a precastration average level of 80 mgs. creatinine, and No.8. a level of 63. Thus the above results show a fall to the precastration level in both cases. The latent period here is only a day and is therefore less than in the two previous sets of experiments on normal and recent castrates.

TABLE II.

Date.	No.K.	No.L.	No.M.
Preperiod	78	75	75
2.11.36.	82	79	70
3.11.36.	75	80	80
x 4.11.36.	77x	77 x	78
5.11.36.	69	62	74
6.11.36.	60	58	78
7.11.36.	64	59	70
8.11.36.	66	64	72
9.11.36.	69	74	78
10.11.36.	72	73	79
Injections:			
4.11.36. Animal	20ml. L.S.C.	18ml. L.S.C.	Control. Normal.
Precastration level	62mgms	55mgms	-

1ml. of the suspension was equivalent to 0.33 gm. of testes tissue. The control animal received 15 ml. of saline.

The long standing castrates respond to the injection of saline suspension with a fall in the creatinine excretion to the precastration level. The animals K.& L. were castrated six months previously, and prior to injection the high pre-castration levels were quite definite and well maintained.

The...

The creatinine output gradually returns to normal after the lowest point has been reached,as the fall to the precastration level does not persist.

DISCUSSION:

Injection of saline suspension of testes into long standing castrates produced a decrease in the creatinine in urine to the precastration level. This fall persists for three to four days when it gradually returns to normal. The long latent period observed in the normal animals, and recent castrates, is diminished, and the response to the injection occurs on the day following the injection.

The effect is probably one of an inhibition of the pituitary, thus restoring the creatinine output to its original level. In considering the results of the whole of the series it is seen that the saline suspension exerts an inhibitory action on the pituitary in the normal animal, and through all the stages following castration.

The response to the injection in the normal animal and in the different castrates varies as far as the quantitative response, and the latent period are concerned. This altered reaction is reasonably explained on the basis of the altered physiological properties of the pituitary, due to its hypertrophy following castration, and thus the removal of the inhibition of the testes.

SUMMARY:

1) Injection of saline suspension of testes into long standing castrates produces a fall to the precastration levels.

2) The latent period before the creatinine falls in response to the injection is only one day.

3) The results suggest an inhibition of the hypertrophied pituitary.

GENERAL SUMMARY

1) Injections of saline suspension into normal adult male rabbits is followed by a fall in the daily urinary creatinine.

2) Injection into recent castrates results in a decreased creatinine output.

3) Injections into long standing castrates leads to the high post-castration creatinine level, diminishing to a lower precastration level.

4) These results strongly suggest an inhibition of the anterior pituitary hormone, which controls creatinine metabolism in the normal, the recent castrate, and the long standing castrate.

INJECTION EXPERIMENTS ON CREATININE METABOLISM WITH EXTRACTS OF NORMAL ADULT MALE URINE.

INJECTIONS OF EXTRACTS OF URINE INTO NORMAL ADULT MALE RABBITS.

The extracts were prepared according to the methods described earlier. The animals used were normal adult males which had been kept in metabolism cages for at least twenty one days, so that an accurate estimation of the average daily creatinine output could be determined. The mean of these figures are included in the following tables.

In addition to the extracts prepared in the laboratory, preparations of Proviron were obtained from Messrs Schering-Kahlbaum. The results obtained, both with these and with our own preparations, are detailed below and the different responses analysed:

TABLE I.

	Date	No.2.	No. 5.	No. 6.	No.11.
	Preperiod	97	78	96	81
	4.5.34	92	68	88	78
x	5.5.34	96 x	69	64 x	84x
	6.5.34	45	82	54	67
	7.5.34	125	75	129	102
	8.5.34	92	75	90	84
	9.5.34	90	69	91	77
	Injections:				
	5.5.34	7ml	Control	12ml	4ml

1 ml of the urine extract is equivalent to 200 mls of urine.

Note: Only adult male urine is extracted.

On analysing these figures:

Rabbit No.2 shows a fall in the creatinine output of 50%, on the day following injection. The next day the creatinine level has suddenly risen 25% higher than the normal level. This increased urinary creatinine then drops back to normal in the next twenty four hours.

Rabbit No.6. The Creatinine falls on the day of injection, and still farther the following day, when it reached a level of 43% less than normal. In the next twenty four hours the creatinine excretion had risen to 30% above the normal, and then returned to its normal figure the next day.

Rabbit No.11. which received a smaller dose, gives a drop of 17% the day after injection of the urine extract and an increase above the normal of 24% the next day. All this data, therefore, indicates a biphasic reaction which suggests, first, an inhibition, followed by a stimulation of the pituitary.

/TABLE II.

TABLE II.

Date	No.1.	No.3.	No.4.	No.12.
Preperiod	96	80	78	98
21.5.34	89	84	73	95
22.5.34	88	85	79	105
x 23.5.34	96	67x	72x	100x
24.5.34	95	61	65	80
25.5.34	95	102	104	121
26.5.34	90	89	86	96
27.5.34	94	82	84	92
Injections:				
23.5.34	Non-injected Control	14ml	10ml	10ml

1 ml of the urine extract is equivalent to 200 ml. urine.

The creatinine elimination in No.3. falls on the day of injection and is lower on the second day. This is followed by a rise of 25% above the normal level.

Rabbit No.4. shows a diminished urinary creatinine on the day following injection (to an extent of 17%) and a 27% increase above normal on the third day of injection.

No.12. confirms the above results by a drop of 19%, followed by an increase of 22%.

These results clearly indicate that injections of adult male urine extract produce a biphasic effect, and the next series of experiments are the results obtained using Proviron (a preparation of Messrs Schering-Kahlbaum which was obtained from the urine of adult males).

TABLE III.

Date	4.	A.
Preperiod	93	76
26.2.35	90	72
27.2.35	87	70
x 28.3.35	93 x	84
1.3.35	70	79
2.3.35	80	72
3.3.35	77	76
4.3.35	111	78
5.3.35	87	75
6.3.35	89	79

No.4. was injected on the 21st with 1 ml. of Proviron. No.A. was the non-injected.control.

Here the fall (25%) in the amount of creatinine excreted occurs on the day following injection, but this is maintained for three days and then the sudden increase of 20% on the fourth day is succeeded by a return to the normal level.

TABLE IV.

Date	B	E	F
Preperiod	61	63	74
21.6.35	-	63	67
x 22.6.35	61 x	66	78 x
23.6.35	50	70	61
24.6.35	64	64	74
25.6.35	60	66	90
26.6.35	74	56	82
27.6.35	69	67	69
28.6.35	65	69	68.

Nos. F. and B were injected with 1.5 ml. of Proviron each on the 22nd of June. No.E. was the control animal. In both cases of injection with Proviron the effect obtained with urine extract is confirmed, in that there is a diphasic response. With urine extracts the rise followed within 24 hours of the fall but with Proviron the rise occurred several days later.

/TABLE V.

TABLE V.

Date	A1	B1	F
10.2. 35	63	61	75
x11.2.35	67 x	65 x	80
12.2.35	51	52	80
13.2.35	68	62	78
14.2.35	65	75	74
15.2.35	89	87	72
16.2.35	66	79	75
17.2.35	70	65	75
11.2.35	2ml Pro-viron	2ml Pro-viron	Control (Non-injected)

In Aland B1 the effect is repeated in which there is a diphasic response to the Proviron injection. All the above results with "Proviron" were obtained from the first supply of Proviron from Schering-Kahlbaum. This was prepared from adult male urine. A second supply, some time later, was also labelled Proviron. This, however, was not prepared from adult male urine but was pure androsterone benzoate. It failed to reproduce the biphasic results obtained in the first instance with the extract of adult male urine.

Discussion:

Injections of extracts of adult male urine, or proprietary preparations from adult male urine produced a biphasic effect on creatinine excretion. This biphasic result suggests that two substances are present in the urine of adult males, one which inhibits, and the other which stimulates the pituitary. Thus saline suspension of testes contained the one substance as it caused a decrease in urinary creatinine; and lipoid testicular extracts contain the other substance which stimulated the production of creatinine and its elimination in the urine.

Summary.

1. Injections of extract from urine of adult males produce a biphasic effect on creatinine excretion.
2. This biphasic result consists of a fall in urinary creatinine followed by a rise the next day.
3. Proprietary preparations from urine reproduce a similar effect, which is, however, slightly modified.
4. The results suggest the presence of two substances in the urine which act by (a) depressing, or (b) stimulating anterior pituitary.

In the introduction to this work a short discussion appears on the male sex hormones, which occur naturally and which have been isolated either from testis tissue, or from adult male urine. Their structure was accurately determined and their relation to cholesterol ascertained, which formed a basis for the preparation of these hormones synthetically. In addition to the naturally occurring substances, which have been termed androgenic, many related androgens have also been prepared.

Synthetic preparations are in pure crystalline form and the results on creatinine metabolism are, therefore, those of the true hormone. The possibility of a modified effect, due to combination with some other hormone or substance, is, therefore, automatically eliminated, whereas impurities may be present in laboratory preparations of testicular or urinary extracts.

Several of these crystalline preparations were obtained and injected in different amounts into normal adult male rabbits under the same conditions as those employed in the testicular extracts. An accurate preperiod creatinine level was determined and those rabbits which showed the least daily variations were used for these experiments.

A totally new batch was employed in the majority of the experiments, so that any previous injections should have no effect whatsoever.

The crystalline products was accurately weighed out and the required amount of nut oil added. This was heated on a sand bath.

Besides these crystalline products, the proprietary product of androsterone benzoate (Schering-Kahlbaum) was injected.

INJECTION OF TESTOSTERONE INTO ADULT MALE RABBITS.

Injections of testosterone at first gave no response whatsoever in the amounts injected, as will be seen from the first table.

TABLE I.

Date	A	B
Preperiod	95	57
18.1.37	100	57
19.1.37	96	68
20.1.37	x 96	62
21.1.37	87	46
22.1.37	90	56
23.1.37	x 92	45
24.1.37	92	59
25.1.37	x 88	41
26.1.37	94	57
27.1.37	90	64
28.1.37	90	48

Injections:	20.1.37	A	5mg.
	22.1.37	A	6.5mg
	25.1.37	A	10 mg.

Animal B was the normal control.

These results show that testosterone in small doses had no effect on the creatinine metabolism. The figures were confirmed by a further series of injections in which small amounts of testosterone had no effect. Larger doses of testerone did, however, have some effect as shown by the following table.

TABLE II.

Date	M_3	M_7
Preperiod	67	53
8.6.37	62	50
9.6.37	67	49
x10.6.37	72	69 x
11.6.37	63	60
12.6.37	69	61
13.6.37	64	60
14.6.37	73	58
15.6.37	77	55
10.6.37	-	22mgm

M3. was the control animal. 22 mgs. of testosterone (dissolved in nut oil) were injected into M7 on the 10th. This resulted in an increase of 30%, on the day of injection, which fell almost immediately to within normal limits. The effect, therefore, of this large injection was a transient rise without any prolonged action on the creatinine metabolism.

The next step was to take a series of animals and inject different amounts of the crystalline preparation into different animals.

TABLE III.

Date	M4	M6	M7	M10	M11
Preperiod		70	53	95	71
16. 7.35	41	64	59	100	75
x 17. 7.35	36x	84x	62x	94	77x
18. 7.35	44	78	66	102	74
19. 7.35	38	58	68	96	70
20. 7.35	43	70	62	95	65
21. 7.35	45	59	56	98	62
22. 7.35	39	66	50	94	67
Injections:					
17. 7.35	5mgm	20mgm	20mgm	Control no injection	15mgm

The crystals of testosterone are dissolved in nut oil as before.

On analysing the results we find that injection of doses of testosterone up to 15 mgs. produce no alteration in the amount of creatinine excreted.

Of two animals injected with 20 mgms., one, M6., responds by excreting an increased amount (20%) on the day of injection. This falls to within normal limits the following day. In the other, M7, there is a slight rise on the day following injection and then a return to the normal level. This effect is not as marked as in the previous one.

Discussion.

If 20 mgm. of testosterone or more are administered there is an increase in the amount of creatinine eliminated on the day of injection. This rise is not sustained as the creatinine eliminated returns to normal immediately. Testosterone will, therefore, stimulate the pituitary and, as a result, there is an increased elimination of creatinine, but, as pointed out by Parkes et al., excretion of the hormone is very rapid and this probably accounts for the very brief action, even in large doses.

Small amounts of testosterone do not cause any alteration in creatinine metabolism, probably because the excretion is too rapid to allow any stimulation of the pituitary.

Summary.

1. Injections of testosterone in small doses (1-15 mgs) are not followed by any alteration in creatinine metabolism.

2. Injection of 20 mgm of testosterone (or more) result in a rise in the urinary creatinine.

3. These results suggest a rapid excretion of testosterone.

4. Testosterone stimulates the anterior pituitary to increase the output of creatinine.

INJECTIONS OF TESTOSTERONE PROPRIONATE INTO ADULT MALE RABBITS.

Parkes has shown (see introduction) that the effectiveness and action of testosterone is greatly increased and prolonged if it is administered in the form of an ester. This is due to its slower absorption and excretion. Accordingly crystals of testosterone proprionate dissolved in nut oil were injected into normal adult male rabbits and the results compared with those obtained with the pure unesterified hormone. "Testoviron" (S chering-Kahlbaum) a proprietary preparation of testosterone proprionate was also used.

TABLE I.

Date	M4	M5	M2
Preperiod	54	51	46
14. 3.37	47	50	47
x 15. 3.37	70x	63x	46
16. 3.37	62	52	53
17. 3.37	60	50	52
18. 3.37	56	53	-
Injections:			
15. 3.37	5mgm	5mgm	Non-injected Control.

M4 is the normal and M5 is the recent castrate. It was, however, castrated less than five weeks previously. M4 gives an increase of 28%, while M5 gives a rise of 22%. In these results 5 mgm. of testosterone proprionate increases the elimination of creatinine on the day of injection only, in a normal adult male rabbit, and to a lesser extent in a recent castrate in which the pituitary has not yet hypertrophied to the critical stage.

TABLE II.

Date	M2	M3	M5
9.4.37	55	54	67
x 10.4.37	55x	60x	61
11.4.37	43	51	63
12.4.37	78	58	59
13.4.37	58	58	66
14.4.37	58	60	69
15.4.37	58	60	70
x 16.4.37	56x	60x	68
17.4.37	53	56	-
18.4.37	48	62	69
19.4.37	54	68	70
20.4.37	61	65	68
21.4.37	52	58	66
22.4.37	54	62	60
23.4.37	53	60	68

Injections:

10.4.37 16.4.37	5mgm testos-terone proprion-ate	5mgm testos-terone proprion-ate.	Control non-in-jected.

The first injections were given in the neck, while of the injections on the 16th, M2 was injected on the flank and M3 in the gluteal muscles.

M2 was a normal animal, but M3 was an adult male which had been castrated two and a half months previously. M3 gives no response at all to the injection as far as creatinine metabolism is concerned, but M2 does show a slight increase in creatinine elimination. The output on the eleventh is somewhat low, but the increase the following day is rather too much to be compensatory.

With the second injection there is also a slight increase on the fourth day after injection, and the level is somewhat raised. It is, however, hardly a significant effect.

From these above results little conclusion can be drawn, with the exception that 5 mgm of testosterone proprionate has no effect whatsoever on the elimination of creatinine in the adult male castrate of two and a half months, whereas it does appear to have a stimulatory action on the normal animal.

/TABLE III.

TABLE III.

Date	M2	M6
Preperiod	46	68
27.4.37	50	72
x 28.4.37	59 x	72
29.4.37	58	68
30.4.37	57	66
1.5.37	61	73
2.5.37	54	70

On the 28th M2 was injected with 10 mgm. testosterone proprionate.

The increase in creatinine output on the day of injection is 29%, but this persists for three days, and on the third day has reached a level of 32% above the normal. 10 mgm., of testosterone proprionate therefore, has increased the elimination of creatinine on the day of injection and maintained this increase for three days.

M6., the control animal, does not show any alteration in creatinine elimination.

/TABLE IV.

TABLE IV.

Date	M6	Y
Preperiod	68	95
15.6.37	72	94
x 16.6.37	87x	90
17.6.37	82	96
18.6.37	78	95
19.6.37	82	100
20.6.37	65	89
21.6.37	78	96
22.6.37	67	100
23.6.37	67	100
16.6.37	15 mgm	N.I. Control

M6 received 15 mgm of testosterone proprionate and this resulted in an increase of 26% on the day of injection. This increase is maintained for at least four days. On the 21st, that is six days after injection, the creatinine elimination is again high, thus the effect of testosterone proprionate is greatly prolonged.

/TABLE V.

TABLE V.

Date	M3	M4	M6
Preperiod	67	42	68
8.7.37	62	39	61
x 9.7.37	67	39 x	68 x
10.7.37	72	51	80
11.7.37	63	49	75
12.7.37	69	53	83
13.7.37	64	48	78
14.7.37	73	38	66
15.7.37	74	37	64
Injections:			
9.7.37	Non-injected Control	15 mgm	10 mgm

Again, with the injection of the testosterone proprionate, there is an increased excretion of creatinine, which persists for four to five days before the creatinine falls to the normal level. It is notable that in this experiment the creatinine output did not rise on the day of injection, but on the following day, and that this rise persists, so that five days after injection the creatinine level is still high.

TABLE VI.

Date	M6	M12
Preperiod	68	65
2.6.37	74	60
x3.6.37	85x	70
4.6.37	85	-
5.6.37	82	69
6.6.37	76	68
7.6.37	70	65
8.6.37	61	72
3.6.37	20mgm Testosterone Proprionate	Non-injected Control.

20 mgm. of testosterone proprionate injected into M6 did not cause any greater increase than did 15 mgs. (see Table Iv), but, once again, the increase is on the day of injection, and this persists for four days.

Discussion:

Testosterone proprionate, in a dose of five mgms., causes an increased output of creatinine on the day of injection in one rabbit and in two other normal animals on the third day of injection. In the recent castrate of less than five weeks standing it also caused an increased elimination of creatinine. This, however, was not apparent in the recent castrate of two and a half months standing, in which the pituitary is regarded as having reached the transition stage.

In doses of 10 mgms. or more injected into normal rabbits, the increased elimination of creatinine occurs on the day of injection in the majority of experiments, and this rise persists for four to five days. The creatinine excretion then returns to normal.

If the results obtained with testosterone proprionate are compared with those of testosterone, it is at once apparent that not only smaller doses of the ester are more effective than the pure hormone, but this effectiveness is greatly increased and prolonged. Just as Parkes et al. have shown that the slower absorption and excretion of testosterone proprionate have a greater and more prolonged action on the growth of accessory sex organs, it seems, too, that the stimulatory action of testosterone on the pituitary to produce an increased creatinine excretion, is augmented and of longer duration if it is administered in the esterified form.

Summary.

1. Injections of testosterone proprionate in doses of 5 to 20 mgm. result in an increased elimination of creatinine in the urine.

2. The increase takes place on the day of injection.

3. It is prolonged for three to five days if doses of 10-20 mgms are administered.

4. The effectiveness of testosterone on the pituitary is greatly increased and prolonged if administered in the form of testosterone proprionate.

INJECTIONS OF ANDROSTERONE INTO NORMAL ADULT MALE RABBITS.

Crystals of Androsterone are prepared from adult male urine. (These are referred to in the introduction). Androsterone is less active than testosterone on the accessory sex organs of the rat and the capon comb. The crystals were dissolved in olive or nut oil and injected into adult male rabbits, the same methods being adopted as used in the previous experiments.

TABLE. I.

Date	A	B	C	F	D
Preperiod	83	40	50	74	45
11.10.36	80	46	45	73	44
x12.10.36	90	43x	55 x	81x	46x
13.10.36	84	40	53	75	41
14.10.36	85	36	62	75	41
15.10.36	83	45	50	70	48
16.10.36	85	43	52	76	49
Injection:					
12.10.36	Non-injected Control	5mgm	14mgm	5mgm	10mgm

The above data shew that administration of 5 or 10 mgm of androsterone will not affect the creatinine excretion. 14 mgm, however, does result in an increased excretion of 21% on the third day after injection. This effect is very transitory and on the following day the creatinine elimination is again at the average daily level. Thus the effect, which is very short-lived, requires two days before the pituitary is stimulated to increase the creatinine level.

TABLE II.

Date	M2	A
Preperiod	46	90
17.2.37	45	91
x18.2.37	47 x	86
19.2.37	50	86
20.2.37	70	89
21.2.37	62	95
22.2.37	56	91
23.2.37	50	87

M2 injected with 15 mgm androsterone on the 18th.
A was the control animal which was injected with 5 ml saline.

The results indicate that injection of 15 mgm of androsterone result in a 40% rise on the third day after injection. The following day the creatinine excretion is still above the average but falls within the next 24 hours to normal.

/TABLE III.

TABLE III.

Date	M3	M11
Preperiod	67	71
8.7.37	62	70
x9.7.37	67	79 x
10.7.37	72	63
11.7.37	63	95
12.7.37	69	83
13.7.37	64	68
14.7.37	13	66
15.7.37	74	69
Injections:		
9.7.37	Non-injected Control	20mgm.

Injections of 20mgm produce a 34 % rise on the third day after injection. This rise persists for two days; the increase on the second day being only 15%. The following day it returns to normal.

Discussion.

From the above results we see that amounts of androsterone from 11 mgm to 20 mgm will cause an increase in creatinine elimination. Only on the third day after injection does the increase in the urinary creatinine become apparent, and this increase is not sustained for more than two days.

Doses of 5 and 10 mgms have no effect.

Summary.

1. Injections of 14-20 mgm of androsterone produce an increased output of creatinine on the third day after injection.

2. This increase is only of two days duration at a maximum.

3. Administration of 10 mgm and 5 mgm of androsterone has no effect on creatinine metabolism.

INJECTION OF ANDROSTERONE BENZOATE INTO NORMAL ADULT MALE RABBITS.

Androsterone benzoate in the form of crystals was not obtainable but Proviron (Schering Kahlbaum) is a proprietary preparation of Androsterone benzoate. In the tables the actual weight of androsterone benzoate is given.

Testosterone proprionate was more effective than testosterone and, similarly, an ester of androsterone is shown below to have an altered effect on the creatinine excretion.

TABLE I.

Date	A	F
Preperiod	73	70
14.7.36	65	66
x15.7.36	82x	80x
16.7.36	103	105
17.7.36	88	86
18.7.36	75	58
Injections:		
15.7.36	10 mgm	10 mgm

There is a slight increase on the day of injection. This is greater on the following day and the creatinine excretion remains above the average level on the third day, although the increase is not quite as pronounced as on the second day after injection.

TABLE II.

Date	A	C	F
Preperiod	74	50	72
18.8.36	68	56	75
x19.8.36	77x	56	74x
20.8.36	90	49	90
21.8.36	98	44	90
22.8.36	91	50	66
23.8.36	67	51	-
19.8.36	14.5mg	Non-injected Control	15.0mg

In this experiment 14.5 and 15 mgm. of androsterone produce an increase in creatinine elimination on the second day after injection. This raised level of the creatinine output persists for three days before it returns to normal. The rise, however, is not as marked as with the smaller dose of 10 milligrams (Table I).

TABLE III.

Date	A	C	F.
Preperiod	82	49	71
15. 9.36	83	48	72
x16. 9.36	80x	47	66x
17. 9.36	Urine lost	56	82
18. 9.36	98	48	86
19. 9.36	79	49	77
Injections 16.9.36	7.5mgm	Control	7.5mgm

Unfortunately the urine from A was lost on the day following injection, but both A and F exhibit no alteration in the creatinine level on the day of injection. On the following day the output of creatinine has risen and on the third day both A and F show an increased elimination of creatinine.

Discussion.

Injections of androsterone benzoate in amounts of 7.5 - 20 mgm result in an increased amount of creatinine in the urine, either on the day of injection or the day following. This rise is maintained for two to three days. If these results are compared with those obtained with the pure hormone, it is obvious that the ester is more effective and has an action which is more prolonged; for if androsterone be injected the increase which only occurs on the third day after injection is not sustained. As with testosterone proprionate, the augmented action of androsterone, if administered in the form of an ester, is probably due to slower absorption and excretion.

Thus, from the whole series of experiments, we see that the ester has a greater effectiveness than the pure hormone, and this effectiveness is not only increased but greatly prolonged.

Summary.

1. Injection of 7.5 to 20 mgms of androsterone benzoate causes an increase in the amount of creatinine in the urine.

2. This increase persists for two to three days.

3. Androsterone benzoate stimulates the pituitary to a greater extent and for a longer period than androsterone, as shown by the different creatinine eliminations in both cases.

GENERAL SUMMARY.

1. Testosterone and androsterone in sufficiently large amounts stimulate the anterior pituitary to produce an increased amount of creatinine in the urine.

2. Their effectiveness is increased and greatly prolonged if administered in the form an ester.

3. This increased and prolonged effectiveness is probably due to slower absorption and excretion.

FEMALE SEX HORMONES.

INJECTION OF OESTRADIOL BENZOATE INTO NORMAL ADULT MALE RABBITS.

In view of the work by Korenchevsky et al. on the duplicity in function of the male and female sex hormones, Progynon B O leosum forte (Schering Kahlbaum) i.e., oestradiol benzoate, was injected into normal adult male rabbits and the creatinine output following administration estimated. An attempt is thus made to correlate this work on creatinine with the results obtained on the accessory sex organs by administration of the female sex hormones. In addition, all the hormones used in this series have come under the bisenual or partially bisenual group of hormones in which the chief action has been either a male or female one, or both.
In view of this the alterations in creatinine metabolism, if either female or male sex hormones are injected, should bear some relation to one another.

TABLE I.

Date	M8	M9	M12
Preperiod	58	59	65
2.7.37	58	55	60
x3.7.37	48x	57x	70
4.7.37	72	69	-
5.7.37	43	43	69
6.7.37	68	75	68
7.7.37	53	77	65
8.7.37	56	54	72
Injections: 3.7.37	5mgm	7.5mgm	N.I.C.

N.I.C - non-injected animal.

These results indicate, firstly, an increase in the elimination of creatinine on the day following the injection. The next day there is a lowered urinary creatinine which, in turn, gives place to another increase in the creatinine output. In each case the difference is significant and does not lie within the limits of a normal daily variation. There is, therefore, first a rise above normal, then a fall below normal and, finally, a rise again above normal in the output of urinary creatinine before the normal level is again reached.

TABLE II.

Date	M3	M8	M9	M10	M11
Preperiod	67	58	54	95	71
8.7.37	62	56	54	91	70
x9.7.37	67	62 x	60 x	92 x	79 x
10.7.37	72	46	46	70	60
11.7.37	65	60	61	105	95
12.7.37	69	83	74	110	83
13.7.37	64	58	64	104	68
14.7.37	73	57	57	88	69
Injections:					
9.7.37	N.I.C	15mg	15mg	10mg	10mg.

In this series of experiments there is only a biphasic alteration. No significant rise precedes the initial drop, but this fall in creatinine excretion in each of the four rabbits, but that of the control, is followed by an increase in the amount of urinary creatinine. There is, therefore, a distinct diphasic reaction in which the creatinine output first diminishes in quantity and then increases.

Conclusion.

The main results give a diphasic effect. Here a large dose is used, but with injection of smaller doses of 5 and 7.5 mgm a triphasic result was obtained. These, however, only represent two of the results, and although the chief figures do indicate a diphasic reaction, more data is really necessary.

This diphasic reaction was not obtained if testosterone or androsterone or their esters were administered.
These diphasic effects are very similar to those obtained when extracts of adult male urine or Proviron were used.
It is difficult at this stage to suggest an explanation for this. The difficulty is enhanced by the fact that in the one case a pure crystalline product of known composition was used, whereas in the other a crude extract of urine was injected. In both cases, however, it is probable that the pituitary is first inhibited and then stimulated.

In all these experiments with synthetic preparations confirmatory experiments are really necessary. Work in this laboratory was somewhat limited owing to the small amounts of these pure crystalline products available.

Summary.

1. 10 - 15 mgm. doses of oestradiol benzoate result in a fall in creatinine excretion below the normal level, followed by a rise above the normal level two days later.

2. Smaller doses (5-7.5mgm) suggest a triphasic effect; a rise, followed by a fall below normal, and, finally, a rise above the average level.

3. The main effect of oestradiol benzoate on the excretion of creatinine in the urine is a definite diphasic one.

4. These results show a similarity between the effect obtained by oestradiol benzoate and adult male urine extracts.

THE ANTERIOR PITUITARY

AND

CREATININE METABOLISM.

INJECTION OF ANTERIOR PITUITARY EXTRACTS INTO NORMAL ADULT MALE RABBITS.

In the introduction, the work of several investigators on the pituitary and creatinine metabolism has been discussed. Perhaps the first conclusive work is that of Braier (1931), who removed the anterior pituitary and obtained a decrease in the amount of creatinine eliminated in the urine. This associated the anterior lobe of the hypophysis directly with creatinine metabolism.

Extracts of the anterior lobe were prepared as described previously and injections were made into normal adult male rabbits, of which the normal average daily urinary creatinine had been carefully determined.
In some experiments Antuitrin was used, and in others Ambinon. The majority of experiments, however, were performed with the extracts of anterior pituitary produced, by Bellerby's method, in this laboratory. Antuitrin is a preparation of the anterior lobe produced by Parke,-Davis, and Ambinon an anterior pituitary extract prepared by Organon.

/TABLE I.

TABLE I.

Date	B	E	F
Preperiod	61	63	74
2. 7.35	59	52	66
x3. 7.35	58x	61x	75x
4. 7.35	69	69	77
5. 7.35	70	70	65
6. 7.35	91	90	102
7. 7.35	65	61	88
8. 7.35	61	60	85
9. 7.35	57	63	68
3. 1.35	B.E. and F injected with 2 mls, 3 mls and 3 mls respectively in which 1 ml is equivalent to .650 mgm of tissue.		

Injections of anterior pituitary extract are followed in every case by a definite increase in the urinary creatinine. This is confirmed in several later sets of experiments.

TABLE II.

Date	A	B	4	12
Preperiod	68	56	89	110
13. 2.35	70	58	80	108
x14. 2.35	75x	58x	84x	113
15. 2.35	97	69	101	109
16. 2.35	85	68	79	114
17. 2.35	74	48	79	109
14.2.35	3ml	3ml	2ml	N.I.C.

1 ml is equivalent to 762 mgm of anterior lobe tissue.

The above results confirm those obtained in the previous table in that there is a distinct increase in the amount of creatinine eliminated in the urine on the day following injection. In the three rabbits injected each show this rise. This varies:-

Animal A showed an increase of 42% in response to the injection of 3 mls of extract.

Animal B, which also received 3 mls, gives an increased output of 24%.

In Animal 4, which was injected with 2 mls of the extract, there was a rise of 14% on the day following injection.

These high levels return to normal within one or two days.

TABLE III.

Date	No.4	No.A	No.B.
13. 3.35	80	70	58
x14. 3.35	87	75x	46x
15. 3.35	84	97	68
16. 3.35	75	75	56
17. 3.35	76	72	48
14. 3.35	N.I.C.	2 ml	2 ml

(1 ml = 712 mgm of tissue).

With the dose of 2 mls there is again an increased excretion of creatinine in the urine. This result is confirmed later, where extracts of anterior pituitary are injected into normal animals which act as controls.

when the effect of the anterior lobe extracts on castrated animal is being investigated. An extract is regarded as active if an injection of 2 mls will increase the output of creatinine in the urine of normal adult male rabbits.

Besides the anterior pituitary extract, preparations of Ambinon and Antuitrin have been administered in the form of subcutaneous injections.

TABLE IV.

Date	M7	M12
Preperiod	52	65
2. 7.37	51	60
x3. 7.37	55x	70
4. 7.37	69	-
5. 7.37	67	69
6. 7.37	57	68
7. 7.37	56	65

M7 was injected with 4 mls Ambinon on the 3rd July. This resulted in an increased urinary creatinine on the two days following injection.

This result was repeated using castrates and will be discussed later. Injections of Antuitrin were also used.

TABLE V.

Date	M2	M6
Preperiod	48	68
16. 3.37	53	71
x17. 3.37	55 x	62 x
18. 3.37	69	84
19. 3.37	59	74
20. 3.37	60	76
21. 3.37	45	69
17. 3.37	2 mls	2 mls

On March 17th M2 and M6 were each injected with 2 mls of Antuitrin, which resulted in an increase in the output of creatinine on the day following the injection, and on the two succeeding days the creatinine level is still above the normal average level.

TABLE VI.

Date	M4	M6	A
17. 2.37	53	66	91
x18. 2.37	50 x	69 x	86
19. 2.37	70	82	86
20. 2.37	65	80	89
21. 2.37	54	74	95
22. 2.37	52	63	91
23. 2.37	53	68	87

February 18th - Nos. M4 and M6 were each injected with 3 mls antuitrin. A injected with 3 mls brain extract.

In both cases the rise occurs on the day following injection, persisted for another 24 hours and rapidly returned to normal. A showed no alteration in creatinine output.

Discussion.

If normal adult male rabbits are injected with anterior pituitary extracts prepared in the laboratory, or proprietary preparations containing the active substance of the gland, creatinine excretion rises above the normal level.

Castration causes an increased creatinine excretion, but this effect comes on very gradually, being retarded, as it is dependent upon the hypertrophy of the pituitary. Relatively quicker is the decrease in urinary creatinine produced by hypophysectomy, thus indicating that the anterior lobe has direct control over the elimination of creatinine. Both hypertrophy (due to castration) and injection of anterior pituitary extracts increase the amount of creatinine in the urine. The effect of hypertrophy of the pituitary on various organs has been shown to be due to increased production and secretion. This evidently occurs here as well, the increased secretion bringing about an increased creatinine excretion, as shewn by the increased elimination with injections of anterior lobe extracts.

The action, however, of these extracts upon the recent castrate in both stages should throw more light on the subject.

S u m m a r y.

1. Injection of anterior pituitary extracts, antuitrin or ambinon, leads to an increased elimination of urinary creatinine in normal adult male rabbits.

2. This points to a direct control of creatinine metabolism by the anterior lobe.

INJECTION OF ANTERIOR PITUITARY EXTRACTS INTO RECENT CASTRATES. (MALE)

As mentioned before, recent castrates are divided up into two types - (a) those which have been castrated for six weeks or less, and in which the pituitary is not regarded as having hypertrophied and (b) those in which the pituitary is regarded as having reached the transition stage although there is no increased urinary creatinine.

The first series of experiments deal with those animals which have not been castrated for more than six weeks. The precastration level of creatinine excretion is still maintained. The extracts were prepared as discussed before and control injections were made into normal animals to determine whether the extracts were active or not. If an increase in creatinine excretion did not follow injection into the normal animal, the extract was regarded as inactive. (Actually, there was never any occasion to discard results for this reason in the whole series of experiments).

TABLE I.

Date	K	L	M	N	O
Preperiod	60	52	65	73	60
12. 6.36	58	58	66	76	63
x13. 6.36	62x	50x	65x	72	53
14. 6.36	73	68	83	77	55
15. 6.36	64	62	67	80	60
16. 6.36	64	64	65	74	58
17. 6.36	65	56	60	75	55
18. 6.36	67	56	61	75	62
Table of Injections:					
13. 6.36	5ml A.P.E	5ml A.P.E	4ml A.P.E	N.I.C.	5ml Lockes Solution

Animals	K	L	M	N	O
	R.C.	R.C.	Normal	Normal	R.C.

1 ml = 750 mgm of tissue).

In the control animal injected with anterior pituitary extract there is an increase of urinary creatinine on the day following injection. The two recent castrates M and L, which each received 5 mls of A.P.E., also showed an increase on the day following injection; but the urinary creatinine of both the control animal - one a non-injected rabbit and the other, which received 5 mls. of Locke's Solution - did not alter from the normal daily level.

TABLE II.

Date	K	L	M	N	O
Preperiod	60	52	65	73	60
6. 7.36	64	57	70	70	59
x7. 7.36	73x	64x	78x	71	59
8. 7.36	73	62	67	74	60
9. 7.36	68	57	63	64	64
10. 7.36	67	50	67	60	65
Table of Injections:					
7. 7.36	5ml A.P.E	4ml A.P.E	4ml A.P.E	N.I.C.	5ml Brain Extract

1 ml = 750 mgm fresh anterior lobe or brain tissue.

Animal	R.C	R.C.	Normal	Normal	R.C.

These rabbits were castrated on the 13th May; therefore, they were not yet recent castrates of six weeks standing at the time of injection.

Again, these recent castrates showed an increased urinary creatinine on injection of anterior pituitary extract. The normal animal, too, has an increased creatinine elimination, whereas there is no alteration in the creatinine level of non-injected animals.

TABLE III.

Date	E	F	Y1
Preperiod	62	72	96
27. 2.35	70	77	101
x28. 2.35	68x	75x	90
1. 3.35	86	88	92
2. 3.35	70	79	98
3. 3.35	68	75	96
4. 3.35	58	80	100
5. 3.35	57	71	97
Injections:			
28. 2.35	3ml A.P.E.	3ml A.P.E	N.I.C.
Animal	R.C	C.I.	Normal

(1 ml of extract is equivalent to 712 mgm of tissue).

E was castrated on 14th February.

On the 13th March E and F were again injected with 3 mls of Anterior Pituitary Extract and, as in Table III, there was an increase in urinary creatinine on the day following injection, a rise which was sustained for two days before the creatinine excretion again fell to within normal limits.

Antuitrin injections were next given and the results are shown below:-

TABLE IV.

Date	E	F	A1
Preperiod	62	72	62
3. 4.35	56	70	56
x4. 4.35	56x	78x	50
5. 4.35	80	96	59
6. 4.35	60	70	56
7. 4.35	58	70	53
8. 4.35	58	70	60
4. 4.35	3ml Antuitrin	3ml Antuit-rin	N.I.C.

E, the recent castrate, and F, the normal animal, were each injected with Antuitrin. Just as with extracts of the anterior pituitary, there is an increased output of urinary creatinine on the day following injection. In the case of antuitrin, injections, this increase is not sustained, but rapidly returns to normal on the day after the rise.

The second series of experiments was performed on animals that had been castrated for two to four months, and in which the pituitary is regarded as having reached a transition or refractory stage, although there is, as yet, no post castration increase in urinary creatinine. Injections of testicular extract failed to produce any alteration in the creatinine excretion in these rabbits.

In these animals the average daily creatinine elimination is estimated for a week before injection, and only those animals which do not show a post-castration increase are used, provided they have been castrated for two and a half months.

TABLE V.

Date	T2	T6	F	A1
Preperiod	53	81	63	64
12. 9.35	58	82	66	63
x13. 9.35	61x	80x	62x	64
14. 9.35	69	77	79	60
15. 9.35	58	95	76	56
16. 9.35	56	74	70	64
17. 9.35	58	78	68	60

Table of Injections:

13. 9.35	3ml A?P.E	3ml A.P.E	3ml A.P.E.	N.I.C
Animal	R.C	R.C	Control normal. inject.	
Castrated	29.5.35	2.6. 35	-	-

1ml = 774mg tissue.

Thus the creatinine excretion increases, although the animals have been castrated for three and a half months. Using testicular extract, the pituitary, in its state of transition, was not affected. Here, however, the anterior pituitary extract appears to have a direct effect on the creatinine metabolism. Animals T2 and T6 both show an increased excretion of creatinine, as does the normal animal. This increase occurs both in T2 and F. on the day following injection, but it is not apparent in T6, the other castrate, until the second day after administration of the extract.

TABLE VI.

Date	T2	T6	A
Preperiod	53	81	64
22.8.35	50	75	71
x23.8.35	58x	80	66 x
24. 8.35	64	84	82
25. 8.35	57	88	70
26. 8.35	48	82	69
27. 8.35	51	80	63

T2, a recent castrate, and A, a normal animal, each were injected with 3 ml of anterior pituitary extract (1 ml = 680 mgm of tissue). Both show a rise in the urinary creatinine on the day following injection. This rise is sustained in neither case and falls to the average normal level the next day.

TABLE VII.

Date	E	F	A
Preperiods	62	72	62
19. 4.35	60	70	59
x20. 4.35	61x	68x	56
21. 4.35	78	90	60
22. 4.35	66	79	62
23. 4.35	60	72	50
24. 4.35	64	71	51

Injections:
20.4.35
E & F 3mls
A. N.I.C.

On the 20th April E and F were each injected with 3 mls Anterior lobe extract. El is a castrate of two and a half months' standing, and F is a normal animal. A is the normal control. The injections resulted in an increase in urinary creatinine as found with the other experiments. This rise again occurs on the day following injection, and in the recent castrate it is back to normal the following day, while there is still a slight increase in the creatinine excretion in the normal animal the next day. This is not sustained.

Discussion.

Control injections of anterior pituitary extract into normal animals confirmed the findings discussed earlier in the series, of an increase in urinary creatinine.

Recent castrates of less than two months standing, if injected with these same extracts also showed a rise in the creatinine level. Thus, the extract was not acting through the testes, but directly on creatinine metabolism.

In the normal animal the pituitary can be stimulated by testicular extracts. The animal is then castrated. After castration the pituitary at first can be stimulated by the testicular extracts, but the gland seems to pass gradually into a refractory stage. This persists for about two to four months. The anterior lobe then reaches a stage in which it is inhibited by injections of testicular extract. Thus the crucial experiment here was injection of anterior pituitary extract into animals in which the pituitary is in the refractory or transition phase.

Animals varying from two and a half to four months were used. But injections of the anterior lobe continued to give an increased excretion of creatinine on the day following injection. This result had also been obtained both in the normal animal and in the recent castrate of less than two months' standing.

Thus it seems that the anterior lobe extract acts directly on creatinine metabolism and that the anterior pituitary in the normal animal controls this metabolism directly and not through an action on another endocrine.

These experiments thus provide confirmatory evidence that changes in creatinine elimination due to castration and injections are referable to pituitary activity.

S u m m a r y.

1. Injections of anterior pituitary extracts into recent castrates cause an increase of urinary creatinine.

2. This increase occurs on the day following injection.

3. This suggests a direct control of creatinine metabolism by the pituitary.

4. This rise occurs in any recent castrate not showing a post-castration increased urinary creatinine.

5. These results, therefore, indicate a direct action on creatinine metabolism by the extract and, therefore, also indicate direct control of creatinine metabolism by the anterior lobe of the pituitary.

INJECTIONS OF EXTRACTS OF THE ANTERIOR PITUITARY INTO LONG-STANDING CASTRATES. (MALE)

Long-standing castrates are rabbits which have reached a high creatinine level, considered to be due to the hypertrophy of the pituitary following castration. In the previous set of experiments, the direct action of the pituitary on creatinine metabolism has been indicated, and it remains to determine the result of administering additional anterior pituitary hormone to an animal in which the pituitary, now enlarged, is producing a maximum of the hormone.

This will be interesting in view of the fact that once the increased post-castration level has been reached it remains persistently high.

What, therefore, is the effect of injecting anterior lobe extract into long-standing castrates in which the high level, once reached, remains steady ?

The creatinine level was determined in rabbits which had been castrated and in which the average daily output was definitely higher than the precastration level, and which remained constant at this high level.

The same pituitary extracts were used as in the previous experiments.

/TABLE I.

TABLE I.

Date	E1	A1	T6
Preperiod	71	64	81
22. 8.35	73	71	75
x23. 8.35	75x	66x	80
24. 8.35	73	82	84
25. 8.35	68	70	88
26. 8.35	68	69	82
27. 8.35	70	63	80
Injections:			
23. 8.35	3ml A.P.E.	3ml A.P.E.	N.I.C.
Animal	L.S.C.	N.C.I. x/x	Normal

Here E1, the long-standing castrate, which was castrated on the 15th February and in which the average daily output was 60 mgm. of creatinine per day, shows no alteration at all in creatinine excretion in response to injections of anterior lobe extract.

Rabbit A1, the normal animal, was injected with the same dose of anterior pituitary extract, and this produces an increase of 30% in the urinary creatinine.

T6 was not injected.

In the normal animal the rise again occurs on the day following injection.

x/x Normal Control Injection.

TABLE II.

Date	El	F	Al
Preperiod	71	63	64
12. 9.35	76	66	63
x13. 9.35	72x	62x	64
14. 9.35	67	79	60
15. 9.35	78	76	56
16. 9.35	75	70	64
17. 9.35	80	68	60
13. 9.35	3ml A.P.E	3 ml A.P.E.	N.I.C.

El is the long-standing castrate (used in Table I) and F the normal control injected with 3 mls of anterior lobe extract. The other normal animal, Al, was not injected. The injection of the extract results in an increase in the creatinine output in the normal, but not in the long-standing castrate.

TABLE III.

Date	No.4	No.8.	No.A	No.12
Preperiod	80	79	71	108
13. 3.35	80	78	70	108
x14. 3.35	.87	75x	75x	112x
15. 3.35	84	69	97	115
16. 3.35	75	72	75	107
17. 3.35	76	79	72	111
18. 3.35	82	74	69	113
Table of Injections: 14. 3.35	N.I.C.	2ml APE	2ml APE	2mlAPE.
Animal	Normal	L.S.C.	Control injected	L.S.C.

No.8. was castrated seven months previously and No.12 six months previously, and in each case the high post-castration increase of urinary creatinine remained at a steady level. In each instance injections of anterior pituitary extract, which elicit an increase of creatinine in the normal animal, have no effect on the level of creatinine in these long-standing castrates.

Besides the above experiments, No.12 was injected again on three successive occasions with 3 mls of Anterior pituitary extract, and although there is an increased elimination of creatinine in the urine in the normal animal, no change in the normal daily level takes place in the creatinine eliminated in the long-standing castrate, which remains at its high level.

In addition, "Ambinon" and "Antuitrin" were also tried. The figures obtained are quoted below:

TABLE IV.

Date	M5	M7	M12
Preperiod	67	52	65
2. 8.37	69	51	60
x3. 8.37	59x	55x	70
4. 8.37	69	69	-
5. 8.37	64	67	69
6. 8.37	59	57	68
3. 8.37	4ml Ambinon	4ml Ambinon	Control
Animal	L.S.C.	Control injection	Normal

Ambinon produces an increased creatinine in Rabbit M7 but has no effect on M5, whose precastration level was 50 mgm of creatinine daily.

TABLE V.

Date	K	L	M
Preperiod	86	72	76
15.10.36	78	68	74
16.10.36	84	71	-
x17.10.36	84 x	78	72 x
18.10.36	91	75	92
x19.10.36	81	70 x	84
20.10.36	78	77	79
21.10.36	78	76	78
22.10.36	85	72	74
23.10.36	-	72	72

Table of Injections:

17.10.36	2.75 ml Antuitrin	N.I.C.	2.75 ml Antuitrin
19.10.36	N.I.C.	5 ml. Antuit.	N.I.C.
Animal	L.S.C.	L.S.C.	Normal

In both cases Antuitrin produced no effect at all upon the creatinine level of the long-standing castrates, whereas the same preparation caused an increased amount of creatinine to be eliminated in the urine of the control on the first and second day after injection. Even a dose as large as 5 ml of antuitrin could produce no alteration in the creatinine level in Rabbit No.L.

Discussion:

That the anterior pituitary has a direct control over creatinine metabolism was indicated previously in this particular series of experiments. Castration results in hypertrophy of the pituitary and the increased secretion of the anterior pituitary hormone causes an increased excretion of creatinine. This suggests a continued new formation of creatine and its change to creatinine which is eliminated as such.

On injection of the anterior pituitary extract into long-standing castrates which show the high creatinine level, there is no further increase in urinary creatinine. This suggests that a maximal effect was present and that additional pituitary hormone would not have any further effect. Several workers (Schenk, Evans & Simpson, et al.) have noted that the gonad stimulating activity and cell changes increase progressively up to a certain period, after which there is little change, and a cessation of hypertrophy is reached which acts as a limiting factor. It is possible, therefore, that hypertrophy of the pituitary beyond a certain stage results in excess secretion of the creatinine stimulating hormone and any further administration of the hormone has no effect. This limiting factor will be discussed later.

Summary.

1. Injection of anterior lobe extracts of the pituitary has no effect on the high post-castration urinary creatinine level of long-standing castrates.

2. These results suggest that the hypertrophied pituitary produces a maximum amount of the hormone, which stimulates creatinine metabolism and, therefore, added extracts can have no effect on creatinine elimination in the urine.

GENERAL SUMMARY.

1. Injection of anterior lobe extracts of the pituitary leads to an increased excretion of creatinine in normal adult male rabbits.

2. Injection of anterior lobe extracts into ANY recent castrate, not showing a high post-castration creatinine level, causes an increased output of creatinine in the urine.

3. Injection of anterior lobe extracts into long-standing castrates, which have an increased creatinine excretion, due to pituitary hypertrophy, has no effect at all on this high daily creatinine elimination.

4. These results indicate direct control of creatinine excretion by the anterior pituitary and maximal production of the hormone controlling creatinine metabolism following hypertrophy due to castration.

ADMINISTRATION OF

ADRENAL CORTICAL HORMONE.

INJECTIONS OF EXTRACTS OF ADRENAL CORTEX INTO NORMAL ADULT MALE RABBITS.

The introduction to this thesis reviews the more important details with regard to the adrenal cortex control of creatinine metabolism. Previous work indicates that other investigators have not obtained any definite results on the relation of the adrenal cortex to creatinine metabolism. A series of experiments were, therefore, performed with cortical extracts. The extracts used were those prepared in the labotatory according to the method of Swingle & Pfiffer (1931 and 1934) and also Eucortone, a proprietary preparation of Messrs Parke, Davis & Co.

The same procedure with regard to a preperiod estimation was adopted as in the previous series of experiments.

TABLE I.

Date	No.A1	A 2	A4	A3	A5	A6
Preperiod	54	49	51	62	42	60
19.7.35	50	48	50	51	42	63
x20.7.35	47x	55x	52	49x	38x	56x
21.7.35	50	48	47	56	46	60
22.7.35	67	60	54	53	48	54
23.7.35	60	56	45	58	56	57
24.7.35	56	55	50	49	50	62
25.7.35	54	50	48	60	48	60
Injections:						
20.7.35	5ml Adrenal Extract	5ml Adrenal Extract	C.I. Saline Ex	3ml Adrenal Extract	5ml Adrenal Extract	2ml Adrenal Extract

Alshows a slight increase on the second day after injection, but this is not repeated in any of the other animals.

The urinary creatinine level is all the other animals is unaffected by the injection of these extracts, with the exception of slight variations, which are within normal daily limits.

TABLE II.

Date	A1	A2	A4	A5	A6
2.8.35	52	48	55	42	66
x3.8.35	51	47x	46x	39x	60x
4.8.35	60	51	51	39	66
5.8.35	49	51	58	47	57
6.8.35	57	48	49	41	59
7.8.35	54	56	47	50	65
8.8.35	51	53	48	48	60

Table of Injections on 3rd:

A5 & A6 - 3 mls of adrenal cortex extract
A1 - Control 4 mls. saline
A2 - 2 ml Eucortone. A4 - 1.5ml Eucortone.

From these tables it can be seen that extracts of adrenal cortex or Eucortone in the doses administered here have no obvious effect on creatinine administration.

The number of estimations after the injections are quite sufficient to observe any change should the onset of alteration in the creatinine level be gradual.

Summary.

1. In certain strengths injections of extract of adrenal cortex have no effect on the elimination of creatinine.

2. Proprietary preparations of adrenal cortex Eucortone also show no alteration in the normal amount of urinary creatinine.

CONTROL EXPERIMENTS.

CONTROL INJECTIONS.

1. LIPOID EXTRACT FROM LIVER.

As discussed in the introduction, it was necessary to determine whether lipoid material itself was responsible for the altered creatinine metabolism. Lipoid material from liver and brain was accordingly extracted and a series of experiments performed with different animals, using the same methods as those employed for lipoid testicular extract.

The animals used were normal adult males and the creatinine level accurately determined.

The extracts were prepared using the identical method employed for preparing testicular extracts used in the experiments discussed earlier. The final product was of the same strength as the testes, viz: 1 ml of the extract was equivalent to 300 mgm of lipoid material.

TABLE I.

Date	3	4	8	9	12
Preperiod	80	84	63	69	95
24.7.34	80	90	70	72	100
x25.7.35	87x	84x	65x	75	96x
26.7.35	86	85	64	62	100
27.7.34	80	83	66	68	Urine lost
x28.7.34	80x	84x	60x	61	93x
29.7.34	77	81	65	64	100
30.7.34	87	90	66	61	95
31.7.34	82	85	68	69	98
1.8.34	84	90	72	72	94
2.8.34	80	84	64	65	-
Table of Injections:					
25.7.34	2ml	4ml	2ml	NIC	2ml
28.7.34	1ml	2.5ml	3ml	NIC	3ml

From this data it can be concluded that the lipoid material of liver tissue does not alter creatinine metabolism.

2. LIPOID EXTRACT FROM BRAIN.

This was prepared by the same method as that used for testicular extracts and the animals used were normal adult males.

TABLE II.

Date	No.T1	T2	T3	T4	T5	12.
Preperiod	57	55	46	59	56	113
17.4.35	60	50	48	57	60	116
x18.4.35	50x	53x	44x	60x	56x	125
19.4.35	56	48	50	56	55	108
20.4.35	48	50	48	58	50	110
21.4.35	58	50	50	56	50	112
22.4.35	48	55	45	54	48	110
23.4.35	54	48	51	52	56	111
24.4.35	50	53	42	58	47	108
25.4.35	59	59	49	63	50	115
Injections:						
18.4.35	5ml	5ml	5ml	2ml	2ml	N.I.C.

1 ml is equivalent to 300 mgm lipoid material.

These two tables clearly indicate that it is not the effect of the lipoid material itself which produces the alteration in creatinine metabolism.

3. BRAIN TISSUE.

These extracts were prepared by the same method as the Bellerby method for extracting the anterior pituitary. The animals were normal adult male rabbits.

TABLE I.

Date	A1	A2	A3	A5	A6
15.8.35	55	48	57	48	56
x16.8.35	53	47x	64x	52x	70x
17.8.35	58	43	65	48	66
18.8.35	60	50	68	53	57
19.8.35	53	52	64	58	61
20.8.35	58	56	60	48	64
21.8.35	56	50	60	52	60

Table of Injections:

16.8.35	C.I.	5ml	4ml	2ml	3ml

The control injection was normal saline.

The injection of these extracts of brain produced no effect whatsoever on the level of urinary creatinine, and the effect of the extract of the pituitary can, therefore, be regarded as a specific one.

Lipoid substances present in alcohol-acetone extracts of testes are inactive.

Summary.

1. Lipoid extracts of brain and liver tissue had no effect on creatinine elimination.

2. Brain tissue prepared by the same method (Bellerby) as anterior pituitary extracts are prepared had no effect on urinary creatinine.

3. On these results it can be concluded that lipoid matter in the testes does not affect creatinine metabolism.

GENERAL DISCUSSION

AND

S U M M A R Y.

GENERAL DISCUSSION.

The Anterior Pituitary:

That a direct control of creatinine metabolism is exerted by the anterior lobe of the pituitary is indicated by the results of several investigators and the work detailed here.

Although some reports deny the formation of urinary creatinine from muscle creatine and, as such, its endogenous origin, the majority of workers confirm the conclusions of Benedict & Osterberg, who show creatinine to be derived from endogenous protein metabolism. This has been fully discussed in the introduction.

The hypertrophy of the anterior pituitary after castration is a well-established fact. Hatai & Wolfe, however, describe the exact latent period in male and female rabbits between the time of operation and the hypertrophy. Schrire & Zwarenstein found that the increase in creatinine excretion following castration occurs after a latent period identical with that obtained by Hatai & Wolfe.

Injections of anterior pituitary extracts will result in an increased urinary creatinine, as shown here, and these results appear to associate the anterior lobe with the elimination of creatinine in urine.

Is this action a direct one, or an action through another endocrine ?

Braier hypophysectomised dogs and produced a fall in the creatinine level after a relatively short latent period, which seems to indicate that the pituitary acts directly on creatine-creatinine metabolism.

In the experiments in which normal adult male rabbits were injected with lipoid testicular extracts there is a resultant increase in urinary creatinine; if the animal is castrated after two to four months the pituitary undergoes some hypertrophy and reaches a transitional refractory phase, during which injections of testicular extract do not alter the production of urinary creatinine. The anterior pituitary extracts, on the other hand, act directly on the creatinine metabolism and produce an increase in the excretion in the recent castrates in which the pituitary has reached this refractory phase.

Further hypertrophy of the anterior lobe to a maximum leads to an increase in creatinine elimination. The injection of testicular extract now causes a fall in the urinary creatinine to a normal precastration level. (These results also indicate that the testes act on creatinine metabolism through the pituitary). Injection of anterior lobe extracts has no effect on the high level.

This increase in creatinine following castration may be due to an increased transformation in muscle creatine to creatinine, thus lowering the creatine content of the muscle; or it may be due to increased creatine formation in the muscle, which, at the same time, gives rise to increased creatinine in the urine. This latter seems the more feasible if viewed on the lines of Schrire & Zwarenstein, who produce an increase in muscle creatine following chronic injections of small doses of anterior pituitary extract in 8 - 16 days.

After a latent period of four to six months after castration there is a post-castration increase in urinary creatinine. This attains its maximum by the sixth month, and persists, with little alteration as long as the animal lives. What is the reason for this limit in creatinine excretion, and why is the high creatinine level unchanged by injection of anterior pituitary extracts ?

Is the limiting factor referable to pituitary activity or can it be explained on the basis of changesin the muscle creatine metabolism ? Cessation of hypertrophy of the anterior pituitary after six months has been demonstrated by many workers. This can hardly be responsible for the production of a steady maximum yield of creatinine in the urine as injections of an anterior pituitary extract, which produce an increase in urinary creatinine in the normal, has no further effect on the high level of the long-standing castrate.

The following hypothesis, based on the changes in muscle creatine metabolism, is tentatively suggested:

A hormone secreted by the anterior lobe stimulates the formation of creatine from its precursors (see Introduction). Some of this creatine is deposited in the muscles, but some is transformed to creatinine and excreted. It is further suggested that as the creatine content of the muscle rises the increased rate of formation is slowed down and ultimately reaches a steady level - that is, as the creatine content of the muscle rises, more and more creatine is transformed to creatinine and less is stored. The creatine content of the muscles reaches saturation point and this inhibits an increase in the rate of creatine formation, which consequently reaches a maximum. The result is that at this level there is a minimum storage of creatine and a maximum excretion of creatinine. (Since injections of anterior lobe extracts had no effect on long-standing castrated rabbits the anterior lobe hormone does not affect the transformation of creatine to creatinine). The main limiting factor is thus essentially the increased creatine content of the muscle, and this is brought about by gradual hypertrophy and increased activity of the pituitary. Due to the increased muscle content of creatine the formation of creatine from its precursors is slowed down and the increased amounts of anterior lobe hormone can now have no further effect.

In normal animals the creatine content of muscles is comparatively low, and injections of anterior lobe extract stimulate the formation of creatine, some of which is again stored in the muscles, and some of which appears as increased creatinine in the urine.

According to this hypothesis the creatine content of castrates is higher than that of normal animals, and, therefore, castrates should show a low tolerance to injected creatine as compared with normals. This Schrire & Zwarenstein have demonstrated in that there is a considerably lowered tolerance in the castrated animal. The work of Remen, Lasch, et al., as reviewed in the introduction, strongly supports this point. (Although Shapiro was not able to find any change in muscle creatine in the castrated S.A. Clawed Toad, there are various sources of error possible - see introduction).

The increase in creatinine excretion due to castration is, therefore, due to an increase in muscle creatine resultant upon the increased secretion of an anterior pituitary hormone. The persistent high level is not due to the fact that the pituitary hypertrophy has reached a maximum, but to the fact that the creatine content of the muscle has reached a maximum.

Data of similar experiments as these on hypophysectomised animals is necessary to provide final evidence that the gonadal effects on creatinine metabolism are indirect ones through the pituitary. At the moment no such data is available.

Gonads.

Many workers have shown the relation of the gonads to muscle creatine, creatinuria, urinary creatinine, creatine-creatinine metabolism. All these results supply available evidence of a relation between the gonads and creatine-creatinine metabolism if the view that creatinine is endogenous in origin and derived from creatine is held. Therefore, in the light of the results of Buhlr, Schittenhelm, Paschkis & Schwoner, Abderhalden & Buadze, Kun & Peczenik, the experiments of Mühlbock & Kaufmann, who deny the action of the ovary on creatine metabolism, can be discounted.

Gonadal hormones have been shown to act through the pituitary, and in this way influence creatinine metabolism. There has been either an increase or a decrease in the amount of creatinine in the urine following injection of these substances. In two series of experiments both a rise and a fall were produced. Thus there is either stimulation or inhibition of the "creatinine-stimulating" hormone of the anterior pituitary, or both; that is, we have a monophasic (in either direction) or we have a biphasic effect.

It is interesting to correlate this work with that on the effects of gonadal hormones on the gonadotropic activity of the anterior pituitary, but there is no proof that it is the gonadotropic hormone which is acting on creatinine metabolism. On the contrary, the somewhat different results obtained in this work on creatinine stimulation, from those obtained by other workers on the gonadotropic hormones, indicates that the pituitary hormone acting directly on creatinine is different from that which stimulates the gonads. Zondek, too, has indicated a relationship between oestrin and the growth stimulating hormones, in that dwarfism results following injection of oestrin; thus there is an inhibition of the pituitary. There is no definite evidence, therefore, of any connection between the growth-stimulating hormone and the hormone acting upon creatinine metabolism. On the other hand, the creatinuria of children during growth does suggest some relationship.

In some experiments the effect of gonadal hormones on the pitu8tary-creatinine mechanism is directly opposite to that on the gonadatropic action of the pituitary. There is, however, no reason to suppose that if a testicular extract inhibits one function of the pituitary, it must, of necessity, inhibit the other functions. The possibility of a stimulatory action on one or other anterior lobe function must be borne in mind.

If the actions of the extracts be divided up into monophasic and biphasic effects, we find that the synthetic male products as well as testicular extracts and saline suspension of testes result in a monophasic reaction. This monophasic effect on the pituitary is either stimulatory or inhibitory.

On the normal animal, androsterone and testosterone, or their esters, and testicular extracts produce an increased creatinine elimination, while saline suspension of testes decreases the creatinine excretion. This suggested that the results are due to two substances - one which stimulates and the other which inhibits the anterior pituitary.

This suggestion is quite feasible, for referring again to the analogy of the gonadal-stimulating hormones, we find that, although some investigators offer the hypothesis that the gonads inhibit the pituitary, many workers show that the oestrogens can stimulate the pituitary to produce secretion of the luteinising hormone.

In addition, there are the results of Clauberg with Androsterone, and also those of Bokslag, who suggests that androsterone or testosterone in single doses will stimulate the anterior pituitary. (Actually, in one series of experiments Bokslag did obtain stimulation of the male pituitary with androsterone).

Arguing on the lines of this analogy, it does seem possible that the androgens can stimulate the anterior pituitary to produce an increased amount of a hormone which causes increased creatinine elimination in the urine.

Pure hormones act transiently, but, as demonstrated by Parkes, et al., the formation of an ester will delay absorption and excretion, and the effectiveness of the androgens on the accessory sex organs is not only increased but greatly prolonged. It is reasonable, therefore, to explain the increased effectiveness and duration of the esters on the production of the hormone of the anterior lobe, which stimulates creatinine excretion, on this same basis of delayed excretion and absorption.

As far as the effects on urinary creatinine excretion are concerned, saline suspension of testes resulted in a fall in the amount eliminated; lipoid testicular extracts, when injected, produced an increase in the creatinine elimination, as did the pure hormones. These results appear to suggest the presence of two different substances in the testes acting in opposite ways on the pituitary. The difficulty of interpretation is enhanced by the fact that in the case of the saline suspension we are dealing with a very impure product; the testicular extract is rather purer, and the synthetic products absolutely pure. It is thus difficult to correlate the results with any certainty.

These monophasic effects took place in the normal rabbit. Besides this, monophasic effects are also obtained on recent and long-standing castrates.

The pituitary of an animal after castration appears to pass through various stages. Immediately after castration it is still in a normal phase; two to four months later it passes through a refractory stage, and finally reaches a stage of definite hypertrophy, with a resultant hyper-secretion. Thus, as a result of castration, not only is the gland altered histologically, but its physiological properties have also undergone a change.

In the early castrate the anterior lobe is still normal and the testicular extract reproduces the effect obtained in the normal animal; in the "late" recent castrate testicular extract has no effect on the "refractory" anterior lobe; in the long-standing castrate the injections of testicular extract result in an inhibition of the creatinine stimulating hormone, which is evidently (vide infra) being produced in maximal amounts. This is perfectly reasonable physiologically as the reaction of the pituitary to the hormône is merely undergoing a change as a result of the effects of castration.

The injection of salêne suspension continues to depress the anterior lobe in both recent castrates and long-standing castrates, and this also suggests the existence of two substances.

In considering the biphasic effects obtained by injection of extracts of adult male urine, two suggestions are offered:

Firstly, that two substances are present in the urine, one of which is similar in action to that of the lipoid testicular extracts, and the other to that of the saline suspension.

Alternatively, the biphasic effect may be due to a single substance producing a double effect.

The latter suggestion is supported by the results obtained with oestradiol benzoate, a pure preparation which resulted in a biphasic reaction - a single substance causing inhibition followed by stimulation of the pituitary.

Lane, and later Bokslag, suggest a dual effect of Oestrin on the pituitary, first stimulating the production of follicle stimulating hormone, and then inhibiting its production. With oestradiol benzoate the effect on creatinine-stimulating hormone was exactly the opposite; first a depression and then a stimulation, but, nevertheless, a dual action. Again an analogy is used, yet as mentioned earlier there is no reason why a testicular or ovarian substance should act in one way on one function of the anterior lobe, and in another on another.

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GENERAL SUMMARY.

1. The constancy of creatinine elimination in the urine of adult male and female rabbits, and the absence of creatine in the urine of adult males is corroborated.

2. A post-castration increase in creatinine elimination of 13%-31% after a latent period of 2-4 months is shown.

3. Those rabbits which do not yet show an increased urinary creatinine after castration are designated Recent Castrates. Those animals which do show a definite rise in creatinine excretion and which are regarded as having a hypertrophied anterior pituitary are called Long-standing Castrates.

4. Implantation of testes into normal rabbits caused a fall of 23% and 13% in the urinary creatinine. On removal of the graft there is a reappearance of the normal creatinine output. In each case there is a definite latent period before the creatinine excretion is altered.

5. (a) Injections of lipoid testicular extracts into normal adult males and into rabbits up to 5 weeks after castration lead to a rise in urinary creatinine.
(b) Injections into older castrates not yet showing a high post-castration creatinine level have no effect.

(c) In long-standing castrates (several months after castration) a fall to the precastration level of creatinine elimination is produced by injections of lipoid testicular extract.

(d) Lipoid extracts of liver or brain have NO effect on urinary creatinine

6. The Dose-Response curve is a straight line. This relationship suggests a method of assay. The doses necessary to obtain an effect are, however, of the order of 2-4 mls. of testicular extract (1 ml. being equivalent to 300 mgm. of lipoid material.) With the synthetic products the dose was of the order of 5-20 mgm.

7. Injections of saline suspension of testes into normal adult males, recent castrates and long-standing castrates result in a fall in the urinary creatinine.

8. Injections of extracts of adult male urine into normal animals gives a biphasic effect, first a fall and then a rise in the amount of creatinine eliminated.

9. The administration of testosterone and androsterone in sufficiently large doses results in an increased excretion of creatinine in the urine.

10. Their effectiveness is increased and greatly prolonged

if administered in the form an ester. This is probably due to the slow hydrolysis, absorption and excretion of the relatively insoluble ester.

11. Administration of oestradiol benzoate also gives a biphasic effect. (Small doses indicate a triphasic effect). There is a relation between these results and those obtained with urine extracts.

12. (a) Injection of anterior pituitary extracts into normal adult males resulted in a rise in urinary creatinine.
(b) Injection into ANY recent castrate not showing a high post-castration increase in the creatinine level causes an increased output of creatinine in the urine.
(c) Injections into long-standing castrates which have an increased creatinine excretion due to anterior lobe hypertrophy have no effect at all on this high daily creatinine elimination.

13. The results indicate (a) that the pituitary exerts a direct control of creatinine metabolism.

(b) that following castration there is a persistent high level in creatinine due to a persistent maximal secretion of the hormone of the anterior lobe, which controls creatinine metabolism.

14. The results obtained with the different testicular extracts and synthetic hormones are explicable on the hypothesis that two substances occur in the testes:

(a) one which stimulates the formation of the hormone of the anterior pituitary controlling creatinine metabolism.

(b) one which inhibits its formation.

15. The effects obtained with urine extracts suggest either the presence of two substances as above, or one substance giving a biphasic effect. The latter suggestion is supported by the biphasic effect given by oestradiol benzoate.

16. Injections of adrenal cortical extracts were not followed by any alteration in the amount of creatinine eliminated.

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PITUITARY - GONADAL CONTROL

of

CREATININE METABOLISM.

SUMMARY

of

THESIS

Presented for the degree of

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by

R. W. S. CHEETHAM, M.A.,

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INTRODUCTION.

The literature up to 1937 is reviewed.

On discussing the different publications describing the origin of urinary creatinine the conclusion arrived at is that creatinine is endogenous in origin and is directly related to muscle creatine.

This relationship of urinary creatinine to muscle creatine is stressed on account of the important role of creatine in the mechanism of muscular contraction.

The significance of urinary creatinine and its importance as an index of endogenous protein metabolism is indicated.

The literature dealing with the relation of the endocrines to creatinine metabolism is reviewed with special reference to the testes and the pituitary. Experiments to clarify the position are proposed. In addition, a definite relationship is shown to exist between the testes and the anterior pituitary.

Separate discussion on the creatinurias provides further evidence of an endocrine creatinine metabolism interrelation.

The literature on the male sex hormones is briefly considered and indicates that there are four naturally occurring hormones. These have been prepared synthetically. The effectiveness of their action is increased and prolonged if administered in the esterified form. Since this was demonstrated on accessory sex organs, it is proposed here to obtain additional evidence by studying the action of the hormones and their esters on urinary creatinine.

The bisexual properties of the hormones are also considered.

A separate section on experimental technique deals with the estimation of creatine and creatinine, the operative technique employed, the methods of preparation of extracts, and the housing, caging and feeding of the rabbits.

The experimental work consists of a series of experiments so planned as to indicate whether the pituitary exerts a direct control on creatinine metabolism or not and to demonstrate the relation of the gonads to creatinine metabolism.

<u>SUMMARY OF EXPERIMENTAL DATA:</u>

1. <u>The Gonads and Creatinine Metabolism.</u>

   i. The constancy of creatinine elimination in the urine of adult male and female rabbits, and the absence of creatine in the urine of adult males is corroborated.

   ii. A post-castration increase in creatinine elimination of 13-31% after a latent period of 2-4 months is shown.

   iii. Those rabbits which do not yet show an increased urinary creatinine after castration are designated Recent Castrates. Those animals which do show a definite rise in creatinine excretion and which are regarded as having a hypertrophied anterior pituitary are called Long-standing Castrates.

iv. Implantation of testes into normal rabbits caused a fall of 23% and 18% in the urinary creatinine. On removal of the graft there is a reappearance of the normal creatinine output. In each case there is a definite latent period before the creatinine excretion is altered.

v. (a) Injections of lipoid testicular extracts into normal adult males and into rabbits up to 5 weeks after castration lead to a rise in urinary creatinine.

(b) Injections into older castrates not yet showing a high post-castration creatinine level have no effect.

(c) In long-standing castrates (several months after castration) a fall to the precastration level of creatinine elimination is produced by injections of lipoid testicular extract.

(d) Lipoid extracts of liver or brain have NO effect on urinary creatinine.

vi. The Dose-Response curve is a straight line. This relationship suggests a method of assay. The doses necessary to obtain an effect are, however, of the order of 2-4 mls. of testicular extract (1 ml. being equivalent to 300 mgm. of lipoid material.) With the synthetic products the dose was of the order of 5-20 mgm.

vii. Injections of saline suspension of testes into normal adult males, recent castrates and long-standing castrates result in a fall in the urinary creatinine.

viii. Injections of extracts of adult male urine into normal animals gives a biphasic effect; first a fall and then a rise in the amount of creatinine eliminated.

ix. The administration of testosterone and androsterone in sufficiently large doses results in an increased excretion of creatinine in the urine.

x. Their effectiveness is increased and greatly prolonged if administered in the form of an ester.

This is probably due to the slow hydrolysis, absorption and excretion of the relatively insoluble ester.

xi. Administration of oestradiol benzoate also gives a biphasic effect. (Small doses indicate a triphasic effect). There is a relation between these results and those obtained with urine extracts.

II. <u>The Anterior Pituitary and Creatinine Metabolism:</u>

i. Injection of Anterior pituitary extracts into normal adult males resulted in a rise in urinary creatinine.

ii. Injections of Anterior pituitary extracts into ANY recent castrate not showing a high post-castration increase in the creatinine level causes an increased output of creatinine in the urine.

iii. Injections of Anterior pituitary extracts into long-standing castrates which have an increased creatinine excretion, due to anterior lobe hypertrophy, have no effect at all on this high daily creatinine elimination.

<u>THE RESULTS INDICATE:</u>

1.(a) That the pituitary exerts a direct control on creatinine metabolism.

(b) That following castration there is a persistent high level in creatinine elimination, due to a persistent maximal secretion of the hormone of the anterior lobe which controls creatinine metabolism.

2. The results obtained with the different testicular extracts and synthetic hormones are explicable on the hypothesis that two substances occur in the testes:

   (a) one which stimulates the formation of the hormone of the anterior pituitary controlling creatinine metabolism, and

   (b) one which inhibits its formation.

3. The effects obtained with urine extracts suggest either the presence of two substances as above, or one substance giving a biphasic effect. The latter suggestion is supported by the biphasic effect given by oestradiol benzoate.

4. Since injections of adrenal cortical extracts were not followed by any alteration in the amount of creatinine eliminated, it is suggested that the cortex does not affect creatinine excretion in the rabbits. This is supported by the results of several other investigators.

In conclusion it is suggested that although the pituitary acts directly on creatinine metabolism, the persistent high post-castration urinary creatinine level is attributable to the creatine content of the muscle reaching a maximum.

It is further suggested that a series of experiments on these lines be performed on hypophysectomised animals to confirm the results obtained in the course of this work.