"On the Bases (Organic) in the Juice of Flesh. Part I." By GEORGE STILLINGFLEET JOHNSON, M.R.C.S., F.C.S., F.I.C. Communicated by Professor G. JOHNSON, F.R.S. Received April 28,—Read May 28, 1891.

The object which I have kept in view in the research, the first results of which are described in this paper, has been to ascertain as far as possible what substances are really present in the watery extract of fresh muscle, and which of the substances commonly described as being present in it are really due to changes taking place in the flesh during the processes of analysis—in short which of the substances obtained from flesh are *educts*, and which are mere *products* therefrom.

And, in this investigation, I have been on my guard against two great sources of error, viz.:—

1st. Changes produced in the ingredients of the muscle-substance by chemical agents and chemical or physical forces generally; and

2ndly. Changes brought about by bacterial action.

This latter source of error seems to me of extreme importance, since it is now well known that profound changes are effected in the composition of such susceptible bodies as flesh through the agency of bacteria, long before those grosser effects are produced which lead to the development of fœtid gases, &c., and which are commonly described as "putrefaction."

Accordingly, I shall describe my experiments in the order in which they are carried out, viz.:—

1st. Preliminary experiments, which are especially designed to exclude the first source of error; and

2ndly. Experiments designed so as to exclude, as far as possible, both sources of error.

1*st. Preliminary Experiments.*

It is well known that when kreatinin is kept in a watery solution, whose reaction is alkaline, at the boiling temperature for a length of time, the base is gradually converted into kreatine by assimilating to itself the elements of water.

Now, in Liebig's process for preparing kreatine from flesh, the radical of phosphoric acid is precipitated from the watery extract of the muscle-substance by addition of baryta-water, so long as any precipitate occurs; one result of which treatment is that the liquid becomes strongly alkaline. It has, therefore, been suggested that during the concentration of the alkaline solution any kreatinin origi-

nally present in the flesh extract would undergo conversion into kreatine, and consequently that the kreatine which is ultimately obtained may have resulted either partly or entirely from a conversion of kreatinin into kreatine, in short that the kreatine is a mere *product* from the flesh, not a true *educt*. My first experiment was designed to test this theory.

Experiment I.—70 lb. of fresh butcher's beef was finely cut up by a sausage machine, after being freed as far as possible from fat and bone, and thoroughly incorporated with water by hand-kneading. By means of a specially designed screw-press, which was made for me by Messrs. Farrow and Jackson, the aqueous extract was removed from the fibre. This process was repeated with fresh additions of water, until four extracts were obtained, each extract being separately examined.

The albuminoid substances were separated from all the extracts by heat (about 80°C.), most of the colouring matter being carried down by the coagulated albumen. The filtrates were then concentrated by evaporation over steam, until a scum began to form upon the surface, after which the further concentration was effected *in vacuo* over sulphuric acid, by means of a Carré's freezing machine. No chemical agent was added to any of the extracts at any time, until the concentration was complete.

In each case the residue obtained by concentrating these extracts was partly crystalline and partly gelatinous.

Complete separation of the crystalline from the amorphous matter was readily effected by means of dilute alcohol, which left the crystalline matter undissolved.

Finally, by fractional crystallisation from water, the crystalline matter was resolved into an organic and an inorganic substance.

The organic crystals were kreatine.

The inorganic salt was acid potassium phosphate, KH_2PO_4.

The results of this experiment prove—

1st, that kreatine may be obtained from the watery extract of flesh whose natural acidity has not been chemically neutralised, so that hydrolytic conversion of kreatinin into kreatine is most improbable; and,

2ndly, that the presence of phosphates in solution does not interfere—as has been stated to be the case by some observers—with the crystallisation of kreatine from extract of flesh.

Inasmuch as I am not aware that acid potassium phosphate has been actually obtained in crystals from the watery extract of muscle hitherto, I have thought it advisable to give analytical results which prove the identity of the substance.

The salt is much more soluble in water than the kreatine with which it is associated, so that the separation of the two substances is

easy. It occurs in hard glistening anhydrous prisms usually radiating from a common centre. The aqueous solution of these crystals is acid to litmus and gives a yellow precipitate on addition of silver nitrate solution. This precipitate (Ag_3PO_4) is much increased on carefully neutralising the liquid with ammonia.

When heated the crystals lose water and fuse. The fused mass dissolves slowly in water to a *neutral* solution, which gives a white flocculent precipitate with silver nitrate ($AgPO_3$).

1·349 grams of the crystals lost 0·1835 gram of H_2O on ignition, corresponding with 13·602 per cent of the original weight.

According to the equation $KH_2PO_4 = KPO_3 + H_2O$, the crystals would theoretically lose 13·22 per cent.

0·985 gram of the crystals gave 2·999 grams of Ag_3PO_4, corresponding with 0·680 gram PO_4, *i.e.*, 69·03 per cent. PO_4.

The filtrate from the Ag_3PO_4 was freed from silver by HCl, and the K in the filtrate converted into K_2SO_4 by H_2SO_4, evaporation, &c.

The weight of K_2SO_4, obtained from 0·985 gram of the KH_2PO_4, was 0·6652 gram, which corresponds with 0·2986 gram of potassium, or 30·31 per cent. K.

The *hydrogen* in the salt was determined by titration with lime-water of known strength. 0·112 gram of the salt was neutralised by 0·05739285 gram of $Ca(HO)_2$.

According to the equation $3KH_2PO_4 + 3Ca(HO)_2 = Ca_3(PO_4)_2 + K_3PO_4 + 6H_2O$, this result indicates 0·00155 gram of hydrogen, or 1·38 per cent. of hydrogen.

These results agree with the formula KH_2PO_4.

	Required for KH_2PO_4.	Found.
Loss of H_2O on ignition	13·22	13·602
K	28·72	30·31
H	1·47	1·38
PO_4	69·81	69·03
	100·00	100·72

The preparation of pure acid potassium phosphate from the watery extract of flesh is especially interesting as showing how a mere product may be taken for an educt.

It is well known that Liebig has described *potassium chloride* as a constituent of fresh muscle substance; and no doubt many observers who have followed out his process for the preparation of kreatine have obtained cubical crystals of potassium chloride on treating the mother liquors from the kreatine with alcohol. I have myself never

failed to obtain this salt after the use of baryta-water, as directed by Liebig.

But the action of baryta-water on the watery solution of KH_2PO_4 would be represented thus—

$$2KH_2PO_4 + 3Ba(HO)_2 = Ba_3(PO_4)_2 + 2KHO + 4H_2O.$$

And the potassic hydrate thus produced would of course decompose the hydrochlorides of any organic bases present in the solution, forming potassium chloride and liberating the bases. The KCl, therefore, would be a *product*, not *an educt, from the flesh.*

Experiment II.—This was a preliminary experiment made with 50 lb. of butcher's beef, in order to ascertain the action of *mercuric chloride* in aqueous solution. Briefly summed up, the results were as follows:—

The addition of a sufficient quantity of mercuric chloride solution to the watery extract of fresh beef causes complete and instantaneous precipitation of the albuminoid constituents and of the whole of the colouring matter.

The filtrate from the coloured albuminous precipitate deposits, on standing, a spherical mercury salt, isomorphous with the spherical mercury salt of urinary kreatinin described by me in the 'Roy. Soc. Proc.,' vol. 43, pp. 493—534.

This spherical mercury salt yields a kreatinin isomorphous with the tabular kreatinin of urine, when subjected to similar treatment to that described in my paper quoted above.

Encouraged by these results, I determined to apply the mercuric chloride method, which I had formerly made use of in examining the kreatinin of urine, to the examination of the bases in the juice of flesh.

2*nd. Experiments Designed to Exclude Errors due to Bacterial Action, as well as those due to Chemical and Physical Action.*

Before describing these experiments, I will briefly indicate some of the special advantages possessed by what I have called the "mercuric chloride method" for the examination of organic bases.

The method itself, as applied to the examination of urine, is fully described in my paper quoted above. It enabled me to isolate from human urine what I believe to be the excrementitious kreatinin which has always been secreted by the human kidney, but whose properties I was the first to describe.

Perhaps the claims of my method to excellence in preparing an *educt* will be best appreciated by comparison with other methods, as in the following table.

Methods for Isolation of Kreatinin from Urine.

I. Heintz and Pettenkofer.	II. Liebig.	III. Maly.	IV. The Author.
Fresh urine neutralised with sodium carbonate, evaporated to a syrup. Syrup exhausted with alcohol, and alcoholic zinc chloride added.	Fresh urine neutralised with milk of lime. Calcium chloride added to complete precipitation. Filtrate evaporated till the salts crystallise out. Zinc chloride added to the liquor. Kreatinin zinc chloride dissolved in boiling water and treated with lead hydrate at the boiling temperature. Filtrate evaporated.	Urine evaporated to $\frac{1}{3}$rd of its original bulk. Lead acetate added. Filtrate freed from lead by H_2S. Filtrate neutralised by sodic carbonate and precipitated by mercuric chloride. Precipitate suspended in water, and decomposed by H_2S. Filtrate evaporated. Residue recrystallised from alcohol.	Fresh urine + $\frac{1}{20}$th of its volume of cold saturated solution of sodic acetate, + $\frac{1}{4}$th of its volume of cold saturated solution of mercuric chloride. Filter immediately. Collect the precipitate which forms in the filtrate in 48 hours. Decompose the Hg salt by H_2S under water. Treat filtrate with $Pb(HO)_2$ at ordinary temperature. Evaporate filtrate *in vacuo* over H_2SO_4.
	Product, kreatinin mixed with kreatine.	Product, kreatinin hydrochloride.	Product, efflorescent urinary kreatinin.

The following is a brief summary of the advantages possessed by the mercuric chloride method, which render it peculiarly applicable to the case of such an easily changed substance as fresh muscle:—

1. The germicidal action of mercuric chloride is so powerful that, if added in sufficient quantity, bacterial action is rendered impossible.
2. It removes from solution *at once* the more putrescible constituents of the liquid (albuminoid matters).
3. It precipitates kreatinin *gradually*, so as to allow of separation of that base as mercury salt, and of subsequent isolation of the base itself, without any application of heat.
4. By removing from solution the putrescible substances, it favours the isolation of any bases, &c., which it does not precipitate, inasmuch as these substances are protected by it from any danger of alteration by bacterial action.

Having thus indicated my reasons for adopting the mercuric

chloride method in examining the bases in the juice of flesh, I will now describe the details of my experiments, in which both bacterial action, and change due to chemical agents have been avoided as far as possible.

The two experiments described above were made with ordinary butcher's meat; and, although the substance was not of course grossly putrid, I had no precise knowledge of the date of the death of the animals which supplied it, and consequently no knowledge of the duration of exposure of the flesh to aërial, *i.e.*, to bacterial, influences.

I was enabled to overcome this difficulty, and to avoid this uncertainty, by the kind help of Professor G. T. Brown, who volunteered to obtain for me a healthy animal at the Royal Veterinary College, and to allow me to commence my experiments in the chemical laboratory at that institution.

Experiment, commenced at the Royal Veterinary College.

A healthy cow was killed at 10.45 A.M. on Thursday, January 3, 1889.

I am indebted to Professor G. T. Brown for the following account of the mode in which the animal was killed:—"The cow was killed by an expert slaughterman from the Metropolitan Abattoirs at Islington. As your object was to free the system from blood as quickly and completely as possible, the animal was rendered unconscious by a single blow from the poleaxe, and instantly the large vessels emerging from the front of the chest were divided. The death of the cow was almost instantaneous."

Professor Brown examined the internal organs, and assured me that he had found no trace of organic disease in any of them.

The flesh was removed at once from the carcass, and brought to me whilst still warm.

After chopping some of it finely, I endeavoured to express juice from it with my specially constructed screw press, but not a drop could be obtained. It, therefore, became necessary to add water to the macerated muscle.

30 lb. (= 13·62 kilograms) of the finely-chopped flesh was minced thoroughly by kneading with the fingers with 5 litres (5 kilograms) of water. This was done at 12 noon January 3. At 3 P.M. on the afternoon of the same day the expression of the juice was commenced, and was completed at 5.30 P.M., *i.e.*, rather *less than seven hours after the death of the animal.* In all 2500 c.c. of a red liquid was squeezed out, mixed with 3750 c.c. of a solution of mercuric chloride, saturated at 15° C., and filtered immediately. Both precipitate and filtrate were of course preserved. From this time onwards the substances obtained

from these 30 lbs. of beef were free from all danger of change by bacterial action; and the source from which the extract was made had been exposed to bacterial action for only seven hours. This extract will be spoken of as "portion A."

The muscle fibre from which the above extract was taken was left in a porcelain bath all night (the weather was very cold at the time), and no more water was added to it.

On Friday, January 4, this fibre was again put through the press, and, although as much as possible had been squeezed out of it on the previous day, and no more water had been added, an additional 3750 c.c. of a red liquid was obtained.

The total volume of liquid obtained from these 30 lbs. of flesh amounted, therefore, to 6250 c.c., whilst the volume of water added was 5000 c.c. Therefore, 1250 c.c. of actual juice must have been expressed from 13·62 kilograms of flesh, *i.e.*, the juice obtained was about $\frac{1}{11}$th of the weight of the flesh taken.

This second portion of juice was not mixed with portion A, but 2250 c.c. of the mercuric chloride solution was added to it at 1 P.M. on Friday afternoon, and it was filtered at once. The precipitate and filtrate were preserved and labelled "Portion B." The utmost exposure to bacterial action undergone by portion B was twenty-six hours.

So far there is evidence that one result of bacterial action is to render the muscle substance more fluid.

The following four portions of flesh from the same cow were extracted with water, and the extracts mixed together in one large vessel with mercuric chloride solution:—

(1.) 22 lb. 8 oz. + 4 litres of water at 4 P.M. on Thursday January 3, and left all night.

Expressed 5220 c.c. of red liquid (of which 1220 c.c., or about $\frac{1}{10}$th of the flesh, must have been juice) at 3 P.M. on Friday, and added 2000 c.c. of HgCl solution.

(2.) 18 lb. 12 oz. (= 8·576 kilograms) of flesh + 3 litres of water at 1.30 P.M. on Friday, January 4.

Expressed 3700 c.c. of red liquid (of which 700 c.c., or $\frac{1}{12}$th of weight of the flesh, must have been juice) at 6 P.M. on Friday, January 4, and added 2000 c.c. of $HgCl_2$ solution.

(3.) 22 lb. 8 oz. (= 10·215 kilograms of flesh) + 3 litres of water at 4 P.M. Friday.

3000 c.c. of red liquid squeezed out at 8 P.M. Friday, and 2000 c.c. of $HgCl_2$ added.

(4.) 22 lb. 8 oz. of flesh + 3300 c.c. of water.

Expressed 3100 c.c. of red liquid at 9 P.M. on Friday, January 4, and mixed with 2000 c.c. of $HgCl_2$ solution.

The juice from these last four portions was mixed with the mer-

curic chloride in one vessel, and this mixed extract will be described as "portion C." The entire weight of flesh contributing to portion C amounted to 86 lb. 4 oz. (= 39·22 kilograms), and the duration of exposure to bacterial action varied from 26 to 34 hours. After standing a week, the turbid liquid was filtered. Filtrate and precipitate both preserved, and labelled "Portion C." Altogether, then, I succeeded in working up 116 lb. 4 oz., or 52·84 kilos., of lean muscle-fibre from the cow.

In this paper I shall describe only the results obtained from the examination of the three *filtrates* after addition of mercuric chloride, viz., portions A (exposed 7 hours to air), B (exposed 26 hours to air), and C (exposed 34 hours at most).

My endeavour has been to treat these three filtrates in exactly the same way as far as possible, so as to avoid introducing any change in one of them by an agency to whose influence the others were not exposed. By these means I should feel justified in attributing any difference between the products obtained to bacterial action upon the flesh before the addition of the antiseptic mercuric chloride solution. One change took place in all the three solutions, viz., the very gradual separation of a white precipitate, which appeared granular macroscopically, and which consisted of minute spheres of perfect transparency when examined microscopically. In short, all the filtrates gradually deposited a spherical mercury salt isomorphous with the mercury salt of the kreatinin of urine.

These precipitates were not finally separated by filtration until the liquid had in each case ceased to deposit the spherical compound.

The spherical precipitate was separated from portion A in April, 1890, washed, dried at the ordinary temperature, and weighed. Its weight was 44·16 grams.

The spherical compound was separated from portion B in February, 1891. Its weight was 28·01 grams.

The spherical compound was separated from portion C in January, 1890. Its weight was 76·5 grams.

The three filtrates from these precipitates all remained perfectly clear on standing for not less than a week in each case, showing that the mercuric chloride still in solution had no further power to cause formation of insoluble compounds.

It is remarkable that the precipitates from A and B, which contain the extracts from 30 lb. of flesh, weigh together nearly as much as the entire precipitate from portion C, which is derived from 86 lb. 4 oz. of flesh. This lesser proportional weight of Hg precipitate from the portion C cannot be accounted for by the delay of a week in the filtration of the first precipitate by the mercuric chloride, because the separation of the spherical precipitate is so extremely slow in the case of the watery extract of flesh. The ex-

planation of this diminution in the weight of spherical mercury salt obtained from portion C must therefore be sought in the more prolonged exposure of the flesh from which this extract was obtained to bacterial influences.

The spherical mercury salts obtained as above were decomposed by H_2S under water, and the acid filtrates evaporated *in vacuo* over H_2SO_4 at the ordinary temperature. In each case crystals were obtained isomorphous with the hydrochloride of urinary kreatinin. These crystals when dissolved in water yielded acid solutions which became strongly alkaline when digested with pure lead hydrate at the ordinary temperature. On evaporating the alkaline filtrates *in vacuo* over sulphuric acid, the sarcous kreatinin formed in each case anhydrous crystals isomorphous with the tabular kreatinin of urine.

This sarcous kreatinin, therefore, differs from the urinary kreatinin in yielding anhydrous tables instead of efflorescent prisms when prepared without application of heat.

It appears, however, that the sarcous kreatinin may be rendered efflorescent by similar treatment to that which changes tabular urinary kreatinin into the efflorescent base, for the washings from the precipitate by lead hydrate in solution of hydrochloride of kreatinin from portion C were evaporated at 60° C., instead of at the ordinary temperature, and the acid solution was treated with $Pb(HO)_2$, filtered, and the alkaline filtrate also evaporated at 60° C. A number of long transparent needles formed, isomorphous with the efflorescent kreatinin of urine, and these needles became opaque when dry. On redissolving the efflorescent base in water and again evaporating, tabular kreatinin separated out.

A further comparison of the properties of this sarcous kreatinin reveals additional differences between this base and the kreatinin of urine.

In the table on p. 528, vol. 43, of the 'Proceedings,' I have laid stress upon the following points in comparing different kreatinins:—

Solubility in water and alcohol, properties of platinum and gold salts, and reduction of CuO, compared with glucose.

Accordingly I have especially examined the sarcous kreatinin, obtained as above described, with relation to these particulars.

(1.) *Solubility in Water of Sarcous Kreatinin.*

4·1995 grams of solution of sarcous kreatinin in water, saturated at 13·7° C., left, on evaporation, 0·3575 gram of kreatinin.

Therefore, 3·8420 grams of water dissolved 0·3575 gram of kreatinin.

Hence, 1 part by weight of kreatinin is dissolved by 10·74 parts by weight of water at 13·7° C.

(2.) *Solubility in Alcohol of Sarcous Kreatinin.*

14·9815 grams of solution of sarcous kreatinin in alcohol of sp. gr. 0·800, saturated at 13·7° C., left, on evaporation, 0·0305 gram of kreatinin.

Therefore, 14·9510 grams of alcohol dissolved 0·0305 gram of sarcous kreatinin at 13·7° C.

Hence, 1 part of kreatinin dissolves in 490·2 parts of alcohol at 13·7° C.

(3.) *Properties of the Platinum Salt of Sarcous Kreatinin.*

When sarcous kreatinin is dissolved in dilute hydrochloric acid, and a solution of platinic chloride added, an orange-coloured platinum salt separates out in crystals on evaporation over sulphuric acid. This platinum salt resembles that of urinary kreatinin in containing 2 mols. of water of crystallisation, which are expelled at 100° C., leaving the anhydrous salt as a lemon-yellow mass.

2·8577 grams of air-dried platinum salt of sarcous kreatinin lost 0·1567 gram of water at 100° C., becoming 2·7010 grams of anhydrous platinum salt.

These numbers correspond with a loss of 5·47 per cent. H_2O.

Required for $2(C_4H_7N_3O.HCl).PtCl_4.2H_2O$.	Found.
5·34 per cent. H_2O.	5·47 per cent. H_2O.

In calculating the percentage composition of the platinum salt of sarcous kreatinin, I have adopted the following atomic weights:—

C = 12, H = 1, N = 14, O = 16, Cl = 35·5, Pt = 194·4.

0·5288 gram of platinum salt of sarcous kreatinin, dried at 100° C., left, after ignition, 0·1628 gram of platinum.

According to these numbers, the dry platinum salt contains 30·78 per cent. of platinum.

Required for $2(C_4H_7N_3O.HCl).PtCl_4$.	Found.
30·59 per cent. Pt.	30·78 per cent. Pt.

Determination of the Solubility in Water of the Platinum Salt of Sarcous Kreatinin.

3·942 grams of solution of the platinum salt of sarcous kreatinin in water, saturated at 15° C., left, on evaporation at 100° C., 0·167 gram of dry platinum salt.

According to these numbers, 22·6 parts by weight of water dissolve 1 part by weight of the platinum salt.

4. *Properties of the Gold Salt of Sarcous Kreatinin.*

When sarcous tabular kreatinin is dissolved in diluted hydrochloric acid, and the solution mixed with one of auric chloride, a splendid gold salt crystallises out, on evaporation over sulphuric acid, in glistening yellow plates, which are permanent in the air and lose no weight at 100° C.

0·1060 gram of the gold salt of sarcous kreatinin, having been dried at 100° C., left on ignition 0·0465 gram of gold, equivalent to 43·86 per cent. of Au.

Required for $C_4H_7N_3O.HCl.AuCl_3$.	Found.
43·46 per cent. Au.	43·86 per cent. Au.

In the above calculation, the following atomic weights were employed:—

H = 1, C = 12, N = 14, O = 16, Cl = 35·5, Au = 196·8.

The gold salt of sarcous kreatinin differs from the gold salts of all the urinary kreatinins, described by me in June, 1887, in that it is completely dissolved by ether.

However, during evaporation of its ethereal solution, even at the ordinary temperature, the salt undergoes decomposition, and a mixture of auric chloride and the hydrochloride of sarcous kreatinin crystallises out.

5. *Reduction of Cupric Oxide in Boiling Alkaline Solution by Sarcous Kreatinin, compared with that of Glucose.*

0·1 gram of sarcous kreatinin was dissolved in 50 c.c. of water.

14·2 c.c. of this solution decolorised 40 c.c. of Pavy-Fehling solution, *i.e.*, 0·0284 gram of kreatinin reduces as much cupric oxide as 0·02 gram of glucose. Therefore 10 grams of $C_6H_{12}O_6$ are equivalent in reducing power to 14·2 grams of $C_4H_7N_3O$. Therefore 4 mols. of glucose = 9 mols. of sarcous kreatinin,

for $10 : 14{\cdot}2 :: \underset{= C_6H_{12}O_6}{180} : 255{\cdot}6$

and $\underset{= 4\times(C_6H_{12}O_6)180}{720} : \underset{= 9\times113(C_4H_7N_3O)}{1017} :: 180 : 254{\cdot}25.$

The differences between the properties of the sarcous and urinary tabular kreatinins will be apparent at a glance when arranged in a table, as follows:—

Comparison between Sarcous and Urinary Tabular Kreatinins.

	Tabular kreatinin of urine.	Tabular kreatinin from urinary kreatine.	Sarcous kreatinin.
Solubility in water	1 in 10·78 at 17° C.	1 in 10·68 at 16·5° C.	1 in 10·74 at 13·7° C.
Solubility in alcohol	1 in 362 at 17° C.	1 in 324 at 18·5° C.	1 in 490·2 at 13·7° C.
Properties of platinum salt	Contains 2 mols. H_2O. Solubility 1 in 14·1 at 15° C.	Contains 2 mols. H_2O. Solubility 1 in 24·4 at 15° C.	Contains 2 mols. H_2O. Solubility 1 in 22·6 at 15° C.
Properties of gold salt	Unchanged by ether.	Decomposed by ether.	Soluble in ether. Decomposed on evaporation.
Reduction of CuO, compared with that of glucose	4 mols. $C_4H_7N_3O$ = 2 mols. glucose.	5 mols. $C_4H_7N_3O$ = 2 mols. glucose.	9 mols. $C_4H_7N_3O$ = 4 mols. glucose.

Besides the differences apparent in the above table, it will be remembered that sarcous kreatinin appears in the efflorescent form only after its solution has been kept at 60° C. for some time; whereas the natural kreatinin of urine, when prepared most carefully without heat, is always efflorescent ($C_4H_7N_3O.2H_2O$).

Also the tabular crystals formed by sarcous kreatinin are not so large as those formed by the tabular kreatinin of urine.

Having thus isolated and examined a sarcous kreatinin by the mercuric chloride method, my attention was turned in the next place to the examination of the filtered solutions from which the spherical mercury salts of the sarcous kreatinin had been separated. These filtrates were three in number, viz., from portions A, B, and C. In each case, the filtrate was allowed to stand for at least a week, in order to ensure that it remained perfectly clear. This having been ascertained, my next endeavour was to separate the mercuric chloride from the solutions, if possible, without adding any reagent to them which would be likely to alter the organic constituents during subsequent evaporation.

Finally, I effected this separation by means of pure lead hydrate, $Pb(HO)_2$. When lead hydrate is added in excess to solution of mercuric chloride, a yellow substance remains undissolved, and, on filtering after a short time, the filtrate is found to be pure water, all traces of lead, mercury, and chlorine remaining in the undissolved matter. Here, then, was a method which removed the $HgCl_2$ with-

out adding anything to the solution. Pure lead hydrate was accordingly added to all three filtrates.

In portion A more time was required to remove all mercuric chloride from the solution than in the case of portions B and C.

Lead hydrate was first added to portion A in April, 1890; and, although more lead hydrate was stirred in from time to time, the solution was not free from mercury until March 23rd, 1891. It was then filtered and evaporated.

The filtrate (portion A was that which was extracted within seven hours of the death of the animal) was evaporated first over steam, then on a hot copper plate at 60° C. No brown colour was developed in the solution during the concentration by heat, but it remained colourless, even when reduced to a syrup. This concentrated liquor was left standing over sulphuric acid.

No kreatine crystals were formed in portion A, but a number of octahedral crystals separated out. These crystals contain potassium, chlorine, and much nitrogenous organic matter. Their solution does not respond to Engel's test for kreatine with mercuric chloride and potassium hydrate.

The aqueous solution of these crystals is neutral to litmus. When heated, the crystals lost 14·7 per cent. of their weight, leaving a black ash consisting of potassium chloride, entangling carbon. I have a large quantity of these octahedral crystals, and I hope soon to submit them to complete analysis and investigation. No PO_4 was found in this part of portion A.

In the case of portion B, the separation of the mercuric chloride from the solution by lead hydrate was completed in three weeks.

The filtrate was evaporated over steam. Only slight darkening of colour took place during concentration. The yellow syrupy liquid deposited crystals on standing. The syrup was mixed with alcohol (in which the crystals did not dissolve) and filtered. The crystalline matter was washed with dilute alcohol, dried, and weighed; its weight was 2·24 grams. Having been weighed, the crystals were redissolved and recrystallised, when they were found to be pure KH_2PO_4. No kreatine crystals could be detected. The alcoholic liquor was, of course, preserved for further examination.

In the case of portion C, six weeks' digestion with lead hydrate removed the mercuric chloride from the filtrate from the spherical mercury salt of the sarcous kreatinin. The liquid was filtered from the mixed lead and mercury precipitate, and concentrated by evaporation over steam and then at 60° C.

Unlike portions A and B, portion C became extremely dark-coloured during evaporation, and the product was a brown jelly, entangling much crystalline matter.

This residue was well stirred with dilute alcohol until only the

crystals remained undissolved. The alcoholic liquor was then filtered. The filtrate was preserved, and the crystals, having been drained and washed with alcohol, were recrystallised from watery solution. The recrystallised product was evidently kreatine, and its weight was 16·63 grams. This kreatine was once more recrystallised from water, and 0·2430 gram of the product (air-dried) was kept at 100° C. till its weight was constant. The previously transparent crystals became opaque during this treatment, and lost 0·0290 gram of H_2O, or 11·93 per cent. of their weight. Kreatine, $C_4H_9N_3O_2.H_2O$, loses 12·08 per cent. at 100° C.

Therefore, the portion of the extract of meat prepared from flesh which had been most exposed to bacterial action became brown during evaporation and deposited crystals of kreatine in abundance, whilst the portions first extracted gave no kreatine, and did not become brown during concentration.

It will be well to summarise these results in tabular form.

Portion A.

Weight of flesh taken.....	30 lb. (13·62 kilograms).
Exposure to air before $HgCl_2$ added....	7 hours.
Volume of cold saturated $HgCl_2$ added..	3750 c.c.
Weight of spherical Hg salt of kreatinin	44·16 grams.
Time required to remove $HgCl_2$ by $Pb(HO)_2$	11 months.
Darkening of colour during evaporation by heat of filtrate after $Pb(HO)_2$	None.
Kreatine obtained	None.
Crystalline product.....	An octahedral compound.

Portion B.

Weight of flesh (second extract from same portion of flesh as A)	30 lb. (13·62 kilograms).
Exposure to air before $HgCl_2$ added....	26 hours.
Volume of $HgCl_2$ solution added	2250 c.c.
Weight of spherical Hg salt of kreatinin	31·68 grams.
Time required to remove $HgCl_2$ by $Pb(HO)_2$	3 weeks.
Darkening of colour during concentration	Very slight.
Kreatine obtained	None.
Crystalline product	KH_2PO_4.

Portion C.

Weight of flesh	86 lb. 4 ozs.
Exposure to air before $HgCl_2$	34 hours.
Volume of $HgCl_2$ solution	8000 c.c.
Weight of spherical Hg salt	76·5 grams.
Time required to remove $HgCl_2$	6 weeks.
Darkening of colour during evaporation	Very great.
Kreatine obtained	16·63 grams.

The deductions which I am inclined to draw from these results are :—

1. That kreatine is not present in fresh muscle substance, but that it is a product of bacterial action upon some constituent of the flesh.
2. That the source of kreatine obtained from flesh is either the sarcous kreatinin or some closely-allied substance.
3. That sarcous kreatinin is *probably* a true "*educt*," *i.e.*, is really present in the fresh muscle-substance; but, having regard to the extremely slow separation of its mercury salt, it is just possible that it may result from gradual changes effected in some closely allied substance by the prolonged action of solution of mercuric chloride.

In conclusion, I will briefly record some experiments which I have made to ascertain whether kreatine may be converted into other bases by the prolonged action of mercuric chloride in aqueous solution at the ordinary temperature. It is commonly asserted that aqueous solution of kreatine is not acted upon by mercuric chloride. This statement, however, requires modification. It is true that there is no instantaneous action, but, after standing for 24 hours, a slight cloud forms in a mixed aqueous solution of kreatine and mercuric chloride. This precipitate increases week after week, and month after month, and is the mercury salt of kreatinin (spherical).

0·5 gram of pure kreatine, dissolved in 70 c.c. of water and mixed with 20 c.c. of cold saturated solution of mercuric chloride, deposited in the course of five months 0·7591 gram of spherical mercury salt of kreatinin, from which a tabular kreatinin was obtained in well-formed crystals. This tabular kreatinin formed a beautiful gold salt, which left, on ignition, 43·63 per cent. of gold.

Required for $C_4H_7N_3O.HCl.AuCl_3$, 43·46 per cent. Au.

This gold salt resembled that of the tabular kreatinin obtained from urinary kreatine by Liebig's process, in being *decomposed by ether*, $AuCl_3$ being dissolved and the kreatinin hydrochloride left.

It is certain that this conversion of kreatine into kreatinin does not take place when mercuric chloride is added to the watery extract of flesh—

1. Because abundance of kreatine is obtained after this treatment, as in portion C.
2. Because the kreatinin obtained from flesh differs in properties from the one obtained by action of $HgCl_2$ upon pure kreatine, the gold salt of the former kreatinin being soluble in ether, and decomposed only during evaporation; whilst the gold salt of the latter is insoluble in ether, but is at once decomposed thereby.

"Contributions to the Chemistry of Chlorophyll. No. IV." By Edward Schunck, F.R.S. Received June 16,—Read June 18, 1891.

Action of Alkalis on Phyllocyanin (continuation).—In Parts I and III of this memoir I gave an account of the action of aqueous alkalis on phyllocyanin, and of the products thereby formed.* By the action of caustic alkali in a state of fusion phyllocyanin undergoes a more profound decomposition, leading to the formation of several products, one of which I shall now describe.

When caustic potash lye to which phyllocyanin has been added is boiled down nearly to dryness a green mass is left which still contains phyllocyanin, for on dissolving a little of it in water, adding an excess of acetic acid, and shaking up with ether, a solution is obtained which shows the spectrum of phyllocyanin. On heating the green mass to near the point of fusion its colour suddenly changes to brown, and the phyllocyanin is now completely altered. In order to ensure complete decomposition water is added, and the solution is then boiled down, and the residual mass again heated to near the point of fusion. The mass is then dissolved in water, and to the reddish-brown solution there is added an excess of acetic acid, which gives a voluminous brown precipitate. The whole is now shaken up with ether without any previous filtration. The ether dissolves a portion of the precipitate, acquiring a red colour, and, having been separated in the usual manner, is slowly evaporated. During evaporation the solution deposits a dark-brown mass, which is filtered off and treated with boiling alcohol. The latter acquires a red colour, leaving behind a good deal of impurity, which is filtered off. On adding zinc acetate to the filtrate a brown precipitate falls, while the liquid acquires a bright purple colour. The latter after filtration is

* 'Roy. Soc. Proc.,' vol. 39, p. 355, and vol. 44, pp. 448—454.