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Thesis for the Degree of
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Subject.

HEPATIC INSUFFICIENCY AND CREATINURIA IN THEIR RELATIONSHIP TO CARBOHYDRATE METABOLISM - AN EXPERIMENTAL STUDY.

by

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In general there is too little constancy in the so-called hepatic insufficiency to warrant the assumption of a clinical symptom-complex corresponding to it.

The hydrogen sulphide test (appearance of an odour of hydrogen sulphide in the expired air after the use of enemata of hydrogen sulphide-containing water) is very uncertain. The intermittent and cyclic excretion of methylene blue which Chauffard regards as characteristic of hepatopathies, is in no way peculiar to diseases of the liver. Still less is this the case with indicanuria, which Beaudeleu claims as a sign of hypohepatia. The significance of the inconstancy of alimentary glycosuria is still very much over-rated in this regard. The proposal of Kolisch to recognise an indication of hepatic insufficiency in the increased ammonia excretion which follows the overloading of the liver with large amounts of nitrogenous material (50 grammes of nutrose) has found no general application. Likewise the idea of E.Schwarz that the excretion of administered lactic acid should be regarded in the sense of a hepatic insufficiency has not proven itself to be practical. Similarly the investigations conducted with a view of obtaining a test of the functional activity of the liver by administration of sodium butyrate and by estimation of the volatile fatty acids excreted in the urine, have not proven very satisfactory, because the increase of fatty acids is not sufficiently constant. The so-called alimentary levulosuria, which was first advocated by Strauss as characteristic of disordered hepatic function, has alone received general recognition through numerous confirmations".

However, it is now recognised that the value of the levulose test has also been over-rated, for it has been found that no alimentary levulosuria occurs in a relatively large percentage of cases of marked hepatic lesion, while its occurrence is quite unequivocal in certain cases in which the liver is perfectly normal.

More recently attention has been directed by Mellanby (1908)[1] to the urinary excretion of creatin and creatinin in liver affections and his results have been confirmed by

Van Hoogenhuyze and Verploegh (1908)[2], while some suggestive work on the total amino-acid excretion in hepatic disease has been recently published by Frey (1911)[3].

Accordingly, the present experimental research was primarily undertaken to try to determine what degree of importance might be ascribed to the several constituents into which the urinary nitrogen is divided, especially with regard to the role of creatin and creatinin and of the total amino-acid content, as furnishing evidence of functional liver insufficiency.

Interesting results were obtained as regards the ammonia-urea relationship during hepatic disturbance. A small but constant increase in the total amino-acid content was found, while creatin and creatinin would appear to depend on other factors, especially on their direct relation to carbohydrate metabolism.

In contra-distinction to this hypothesis of the chemical relationship of creatin and creatinin, the conclusions of some recent work have pointed to their being dependent on an enzymatic basis, as it is asserted that enzymes, which bring about the conversion and destruction of creatin, have been detected in various tissues. It has been suggested that these results depend on bacterial action, and accordingly it was determined to try to eliminate this

factor by extracting the ferments in a dry sterile state by means of a method which has given excellent results in the extraction of bacterial enzymes.

The evidence obtained was altogether negative, so a further development of the hypothesis of a direct chemical relationship between creatin excretion and carbohydrate metabolism was attempted by investigating the relationship between the sugar content of the blood and an artificially produced creatinuria.

Thus this thesis embodies the results of work along three different lines of research.

1. The investigation of the nitrogen partition of the * urine during the hepatic disturbance in dogs produced by the subcutaneous injection of hydrazine sulphate.
2. The question of the presence or absence of creatin-converting and creatin-destroying ferments in the various tissues, as determined by a method of extracting enzymes in a dry sterile state.
3. The relationship of creatinuria to changes in the sugar content of the blood in rabbits, with a discussion of the nature of the rôle played by carbohydrates in connection with creatin metabolism.

* This part of the work has already been published in the Journ. of Path. and Bact. 1913. Vol.xviii. p.p.281-305.

Part I.

HEPATIC INSUFFICIENCY AS ESTIMATED FROM THE NITROGEN PARTITION OF THE URINE.

Introduction.

The aim of Part I has been to try to determine experimentally how far the investigation of the several constituents of the urinary nitrogen, or of their relationships viewed as a whole, might serve as evidence of functional liver disturbance. In the older metabolic work there was the tendency to concentrate attention on the estimation of one or other of the component parts of the nitrogen of the urine, and the relationships of these various elements were altogether neglected. Recent workers have, however, recognised that the isolated estimation of any one of the several substances in which the nitrogen is distributed is useless as regards their value in giving an indication of the extent and type of metabolic disturbance present.

Numerous investigations into the relationship of the urea and ammonia output have been carried out, and very diverse results have been obtained in the estimation of both the relative and absolute amounts of urea and ammonia excreted in hepatic affections. Clinically, Von Noorden

(1907)[4] records cases of 15 to 18 per cent. ammonia content, while Stadelmann and Weintraud (1893)[5] consider there is no such increase except when the factor of acidosis plays a rôle. In support of this hypothesis they have shown that on administering ammonium salts, such as the carbonate or citrate, in cases of hepatic cirrhosis, the power of synthetising ammonia into urea has not been lost. In a recent clinical investigation on hepatic disease Frey (1911)[3] concludes: "The ammonia value stands high in liver diseases, and especially high in cirrhosis." He adds, however, that the diagnostic value of this observation is adversely affected through the occurrence of similar high values after excessive flesh-eating, in fevers, and in various affections which lead to acidosis. Again, Williams' (1906)[6] observations on the ammonia excretion in the vomiting of pregnancy led him to divide such cases into two groups :-

1. Those with a high ammonia percentage of the total nitrogen - over 10 per cent, which he called the "toxæmic" variety.
2. Those with a low ammonia percentage - considered as the "neurotic" type.

Wolf (1906)[7] has shown that most probably this difference in ammonia output depends on the quantity of reserve fat stored in the individual, since an excess of body fat leads, in conditions simulating starvation, to

the excretion of acids with corresponding increase in the ammonia of the urine. On the other hand, Ewing (1908)[8] has pointed out that, since ammonia is essentially connected with the formation of urea and the functions of the liver, an increased excretion of ammonia may be directly dependent on the hepatic disturbance to which the coexistent acidosis is also secondary. Thus the experimental work of Salaskin and Zaleski (1908)[9] shows marked differences between the effects after complete extirpation of the liver and those in Eck fistula animals. In both sets of experiments systhesis of ammonia into urea is defective, but where the liver has been completely removed the ammonia output is still further increased by the production of lactic and other acids in excess.

Pearce and Jackson (1907)[10], from the results of their work on liver necrosis produced by the injection of haemolytic immune sera, also emphasise the danger of overrating the importance of the relationship between hepatic necrosis and high ammonia percentage. Combining their histological and urinary findings, they have attempted to differentiate two types of hepatic disturbance.

1. Hepatic degeneration of a granular and vacuolar type, but no necrosis. The accompanying urinary changes were diminution in percentage urea, increase in percentage ammonia, but no change in total nitrogen.

2. Destruction of the whole cell with autolytic changes — true hepatic necrosis. The urinary changes consisted in a marked increase in total nitrogen with no alteration in percentage urea, but an actual diminution in percentage ammonia, while the undetermined nitrogen (including polypeptids and amino-acids) was higher.

They ~~simply~~ explain the occurrence of those two types on the hypothesis that the simple granular degeneration of the cells is associated with "a disturbance of enzymatic equilibrium" resulting in an interference in the production of urea from ammonium compounds without an increase in total nitrogen output; while in the type of true hepatic necrosis the urine presents all the results of autolytic change, viz, increase in total nitrogen along with the appearance of the products of autolysis.

Although Frerichs and Städeler (1858)(11) demonstrated as early as 1856 that in necrotic affections of the liver the amino-acids, leucine and tyrosine, are present in crystalline form in the urine, yet knowledge as regards the importance of the amino-acid content in liver disease in general has been exceedingly vague and indefinite. Recent work has shown that the presence of those individual amino-acids, leucine and tyrosine, is not of the importance that was previously ascribed to them, for they have been found in small quantities in the urine of various pathological

conditions - hepatic cirrhosis, gout, most infectious diseases, etc. The advent of Sorensen's (1908)[12] formol titration method has given a decided impetus to their more minute investigation, and Frey(1911)[3] using a modification of this method, as described below,concludes that the total excretion of amino-acids is of considerable diagnostic import in hepatic disturbance. Pearce and Jackson (1907)[10], however, suggest that the amino-acid content of the urine has no relation to the type of hepatic lesion present, because, although amino-acids may be found during the autolysis of liver tissue, yet their appearance in the urine is dependent on the condition of the hepatic cells not involved in the lesion. If sufficient normal liver parenchyma is available for the conversion of the amino-acids, then these acids will not appear in the urine.

Somewhat contradictory results have been obtained in feeding patients suffering from liver disease on glycocoll and other amino-acids. Glaessner (1907)[13] found that the ingestion of 20 grms. glycocoll was followed by an increased excretion of amino-acids in hepatic affections (fatty degeneration, phosphorus poisoning, and especially liver cirrhosis) as compared with the output of healthy subjects. On the contrary, Frey's (1911)[3] investigations showed that the feeding with such amino-acids as glycocoll, asparagin, or alanin, of patients with all the clinical evidence suggestive of hepatic cirrhosis led to no increase of the

amino-acids in the urine. Light may be thrown on this conflicting evidence by the recent observations of Damask (1913)[14], who administered the same amount of glycocoll dissolved in a large amount of water (1 litre). He found that patients with diseased livers can then convert this dose of glycocoll quite as easily as the healthy; and he explains the excretion of amino-acids found under such conditions by earlier workers as due to the concentration of the solution.

Considered apart from feeding experiments, the amount of amino-acid nitrogen excreted is considered by Frey to be in itself an important diagnostic feature in hepatic affections, especially in cirrhosis; and he holds that the total output altogether depends on the quantitative relation of the degenerative stages to the healthy cells left. In my experiments, histological examination shows that the degenerative and fatty changes in the liver are diffuse. It is interesting to note that a similar diffuse degenerative lesion was found by Clark (1908)[15] in his observations on the liver changes in chloroform poisoning, in which condition an increase in amino-acid nitrogen has also been found as one of its effects on the protein metabolism of the dog.(Lindsay,1911)[16].

In the present investigation special attention has been directed to the excretion of creatin and creatinin, as their importance as criteria of hepatic disturbance has been suggested by Mellanby (1908)[1] from his researches on

the excretion of these substances in various pathological conditions of the liver. He found that both in hepatic cirrhosis and in carcinoma of the liver the creatinin output is greatly reduced, while in the latter condition large quantities of creatin appear in the urine. These observations were confirmed by Van Hoogenhuyze and Verploegh (1908)[2].

Of the drugs, phosphorus, chloroform, brombenzol, hydrazine, which have a toxic action on the liver, the last named has been used because it has been shown by Gideon Wells (1908)[17] to have the most selective action on the hepatic parenchyma. This limitation of its toxic effects has been confirmed by histological examination in the course of the present experiments.

PREVIOUS WORK ON THE EFFECTS OF HYDRAZINE ADMINISTRATION.

Borissow (1894)[18] first pointed out the markedly toxic effects produced by the subcutaneous injection of 0.1 grm. of hydrazine sulphate per kilo of body weight. The symptoms observed and the post-mortem findings were subsequently confirmed by the work of Poduschka (1900)[19] and of Pohl (1902)[20]. Of its effects on the nitrogen distribution in the urine, Underhill and Kleiner (1909)[21] record the results of one experiment on a dog which lived

for four days after receiving a dose of 0.1 grm. per kilo. As the animal refused all food after the injection, those workers compared their results with those obtained during a fourteen days' fasting experiment conducted on the dog several weeks before. They found that after the administration of the hydrazine sulphate, there was a lower output of ammonia and a higher urea excretion than in the same dog during fasting. The proportion of preformed creatinin nitrogen was somewhat decreased, with a corresponding increase in the creatin nitrogen, while the ratio of the creatinin nitrogen to the total nitrogen was somewhat higher than during starvation. The relation of allantoin nitrogen to total nitrogen remained almost constant during hydrazine poisoning and during starvation, and hence they conclude that a starving condition is the important factor in allantoin elimination, and that hydrazine has no specific action in this respect. The amino-acid nitrogen was not estimated.

AUTHOR'S EXPERIMENTS.

Two sets of experiments on dogs have been carried out: in the one a sublethal, in the other a lethal, dose of hydrazine sulphate in a 2.5 per cent. solution was injected subcutaneously after the preliminary use of cocaine.

1. Injection of a sublethal dose.- Four experiments are recorded in which the dog eventually recovered. In

the first three, the dose was 0.05 grm. per kilo of body weight; while in the fourth it was 0.07 grm. per kilo,- a dose intended for a lethal experiment, - from which the dog made a good recovery.

In all four experiments symptoms of a mild toxæmia were produced. During the first twenty-four hours there was more or less sickness and vomiting, so that the urine was usually contaminated, while the dog was dull and listless, refusing all food and drink. On the second day the animal was considerably better, although showing evidence of general malaise and of lack of stability of equilibrium. By the fourth day the dog was resuming its usual appearance, and was partaking moderately of porridge and milk. Altogether, the symptoms of the animal suggested what is known as a "bilious attack" in the human subject. Microscopical examination of the urinary sediment revealed nothing suggestive of kidney involvement.

2. Injection of a lethal dose.- Owing to the variation in toxic effects produced by the hydrazine injection in different dogs, some difficulty was experienced in arriving at a suitable dose, in order that the urine of several consecutive days might be obtained before the death of the animal. Accordingly, the dosage varied between 0.07 grm. to 0.1 grm. per kilo of body weight.

Four of the dogs lived less than three days, so that urinary investigation was impossible, owing at first to contamination with vomitus and latterly to suppression of urine. In one experiment (No.VII) the urine was obtained directly from the bladder immediately after the death of the animal, about twenty-six hours from the time of the injection of the drug. Another dog lived till the fourth day, while the most successful experiment was one in which the animal survived six days. The dose administered in each experiment recorded is noted in the protocols.

In addition to the symptoms manifested after the sublethal dose, the animals showed signs of progressive weakness, with marked dyspnœa, and fell gradually into a comatose condition before death ensued, all food having been refused since the administration of the drug.

The animals used were dogs and bitches averaging 10-15 kilograms weight, and the urine was collected in the funnel of special metabolic cages, a few c.c of a chloroform mixture containing 10 per cent thymol being added previous to collection. The dogs were not catheterised, but the urine was always regularly obtained except where noted in the protocols, so that the output recorded per diem is approximately the total urine formed in the twenty-four hours. Before the

injection of the hydrazine sulphate, a daily analysis of the urine was carried out until the animal had been for three successive days (indicated in arabic numerals in protocols) in tolerably stable nitrogenous equilibrium. During the whole experiment the dog was on a creatin-free diet, namely, porridge and milk. Dog biscuit was not given, as on analysis it was found to contain an appreciable amount of creatin.

Methods of Analysis, etc.

Total nitrogen was estimated by Kjeldahl's method. Urea, ammonia, creatin, and creatinin were determined by Folin's methods.

Allantoin was estimated in some experiments. Wiechowski's method was used in one experiment, but did not give very satisfactory results, considering the amount of time the method demands for its performance. In the other experiments the "differential" method described by Miss Lindsay (1909)[22] was used.

Amino-acids were estimated by a modification of Sorensen's formal titration method. In the first experiments phenol-phthalein, as recommended by Jager and Malfatti (1909)[23], was used as indicator in the first stage of the process. In Experiment III., however, during the control days and on the fourth day after the

injection when the dog was again approaching normal condition, negative values were obtained, the figures given for amino-nitrogen being lower than for ammonia nitrogen alone. It was accordingly determined to use the litmus solution recommended by Henriques (1909)[24], very cogent objections having been raised by Malfatti (1910)[25] to the use of ordinary litmus. Positive values for the amino-acid nitrogen were then always obtained. In Experiment V. both indicators were used, and the disparity in the values given are recorded in Table I.

TABLE I.

Day.	Amino-Nitrogen. Phenol-phthalein.	Amino-Nitrogen. Litmus.	Ammonia Nitrogen. Folin's Method.
1.	0.690	0.720	0.710
2.	0.417	0.444	0.474
3.	0.546	0.608	0.624
II.	0.378	0.426	0.330
III.	0.455	0.511	0.350
IV.	0.191	0.213	0.137
V.	0.312	0.390	0.201
VI.	0.302	0.352	0.195

It is doubtless this question of indicator which has led to the disparity in the results of investigations on the presence or absence of amino-acids in normal urine.

The results of both methods, however, show a similar increase in amino-acid nitrogen after hydrazine injection.

Uric acid is included under undetermined nitrogen, the amount being practically negligible since its place is taken by allantoin in the economy of the dog.

TABLES AND CHARTS OF EXPERIMENTS.

In Experiments I. - IV. sublethal doses were administered, in V.-VII. a lethal dose was given.

Experiment I.

Whippet Bitch (Weight 9 kilos).

Eighteen c.c. of a 2.5 per cent. solution hydrazine sulphate injected (50 mgrms. per kilo).

The loss of weight for the first three days following injection amounted to 0.6 kilo, after which there was a gradual recovery.

From the fourth day onwards the dog began to take food again, - approximately half the amount of that before the hydrazine injection.

Except for a trace of albumin on the second day, no other abnormal constituent was detected, - no bile, blood, sugar, acetone or diacetic acid.

Day.	Output.	Total Nitrogen.	Urea Nitrogen.	Ammonia Nitrogen.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.	Percentage of Total Nitrogen.					
									Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.
	c.c.													
1 . . .	660	6·867	5·853	0·517	0·020	0·120	0·015	0·342	85·2	7·5	0·3	1·7	0·2	5·1
2 . . .	540	4·250	3·543	0·232	0·029	0·113	0	0·333	83·4	5·4	0·6	2·6	0	8·0
3 . . .	460	5·432	4·769	0·281	0·017	0·132	0	0·233	87·8	5·3	0·3	2·4	0	4·2
I. . . .	No analysis. Urine contaminated.													
II. . . .	380	7·724	6·319	0·394	0·158	0·128	0·040	0·685	81·9	5·1	2·0	1·5	0·5	9·0
III.† . .	70	2·540	2·152	0·148	0·042	0·030	0·014	0·154	84·7	5·8	1·6	1·1	0·5	6·3
IV. . . .	700	7·379	6·539	0·360	0·079	0·129	0·035	0·237	88·6	4·8	1·0	1·7	0·4	3·5
VI. . . .	640	7·050	6·326	0·367	0·038	0·134	0·011	0·174	89·7	5·2	0·5	1·9	0·1	2·7
VIII. . . .	890	6·096	5·505	0·365	0·013	0·110	0·005	0·098	87·3	5·9	0·2	1·7	0·0	4·9
X. . . .	780	5·449	4·707	0·337	0·021	0·126	0·002	0·258	86·4	6·2	0·4	2·3	0·0	4·7

† Partial urine only, some having been lost.

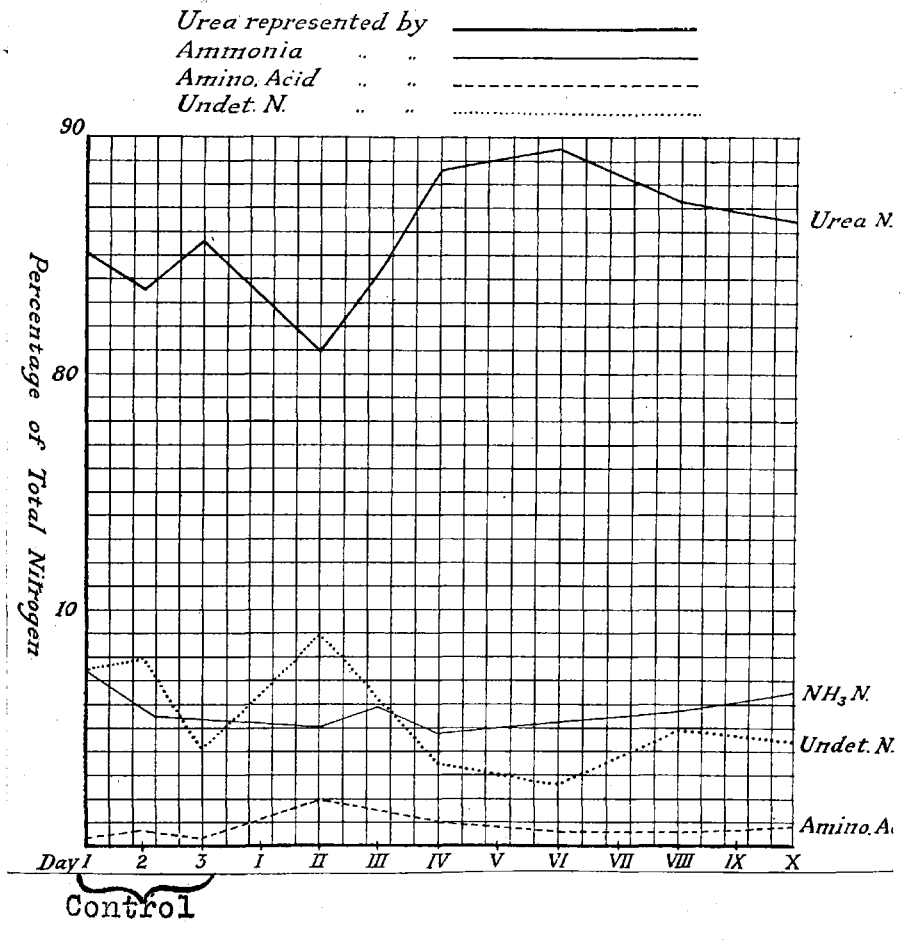
Relation of Creatin and Creatinin to Total Nitrogen.

Day.	Total Nitrogen.	Total Creatinin.	Preformed Creatinin.	Creatin.	Total Creatinin as Percentage of Total Nitrogen.
1 . .	6·867	0·366	0·324	0·042	5·3
2 . .	4·250	0·306	0·306	0	7·2
3 . .	5·432	0·355	0·355	0	6·5
I. . .	Urine contaminated.				
II. . .	7·724	0·453	0·345	0·108	5·8
III.* . .	2·540	0·120	0·081	0·039	4·7
IV. . .	7·379	0·444	0·348	0·096	6·0
VI. . .	7·050	0·394	0·362	0·032	5·5
VIII. . .	6·096	0·327	0·313	0·014	5·3
X. . .	5·449	0·343	0·338	0·005	6·3

* Partial urine.

CHART I.

A graphic representation of the inter-relationships of the urea, ammonia, aminoacid and undetermined nitrogen excretion in Experiment I.



The size of the chart is reduced by leaving out the middle part between 10 per cent. and the lowest percentage to which the urea nitrogen fell.

Experiment II.

Irish Terrier (Weight 11.6 kilos)

24 c.c. hydrazine sulphate (2.5 per cent. solution) injected (=50 mgrms. per kilo.)

After four days 0.7 kilo. was the loss of weight.

By the third day the dog was recovering, some food being taken on the fourth day.

No abnormal constituents were found in the urine during the course of the experiment, except that the urines of days II. and III. became darkly pigmented on standing. None of the reactions to the tests for the ordinary pigments were given.

Day.	Output.	Total Nitrogen.	Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.	Percentage of Total Nitrogen.					
									Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.
		c.c.												
1 . . .	1410	5·073	4·311	0·347	0·035	0·102	0·023	0·255	84·9	6·8	0·6	2·0	0·4	5·3
2 . . .	1400	5·037	4·234	0·313	0·054	0·104	0	0·332	84·0	6·2	1·0	2·0	0	6·8
3 . . .	1240	5·659	4·951	0·326	0·032	0·101	0	0·249	87·4	5·7	0·5	1·7	0	4·7
I. . . .	Urine	contaminated		with	vomitus.	No	analysis	made						
II. . . .	155	4·500	3·774	0·240	0·088	0·066	0·082	0·250	83·9	5·3	1·9	1·3	1·8	5·8
III. . . .	1250	9·957	8·785	0·385	0·118	0·125	0·127	0·417	88·2	3·8	1·1	1·2	1·2	4·5
IV. . . .	1375	6·044	5·290	0·292	0·128	0·098	0·03 3	0·203	87·5	4·8	2·1	1·6	0·5	3·5
V. . . .	1540	6·115	5·259	0·375	0·089	0·144	0·026	0·222	86·0	6·1	1·4	1·8	0·4	4.3
VII. . . .	1560	7·054	6·321	0·318	0·036	0·111	0	0·268	89·6	4·5	0·5	1·5	0	3·9
IX. . . .	1450	6·090	5·262	0·462	0·018	0·118	0	0·230	86·3	7·6	0·3	1·9	0	3·9

Relation of Creatin and Creatinin to Total Nitrogen.

Day.	Total Nitrogen.	Total Creatinin.	Preformed Creatinin.	Creatin.	Total Creatinin as Percentage of Total Nitrogen.
1 . . .	5·073	0·276	0·213	0·063	5·4
2 . . .	5·037	0·280	0·280	0	5·5
3 . . .	5·659	0·272	0·272	0	4·8
I. . . .	Urine contaminated.				
II. . . .	4·500	0·396	0·178	0·218	8·8
III. . . .	9·957	0·778	0·300	0·478	7·8
IV. . . .	6·044	0·358	0·268	0·090	5·9
V. . . .	6·115	0·378	0·296	0·082	6·1
VII. . . .	7·054	0·308	0·300	0·008	4·3
IX. . . .	6·090	0·319	0·319	0	5·2

Experiment III.

Scotch Terrier (Weight 9.35 kilos.)

Nineteen c.c. of a 2.5 per cent. solution hydrazine sulphate injected (50 mgrms. per kilo.)

Weight fell to 8.6 kilos by third day, i.e., a loss of 0.75 kilo., but following on this there was a gradual rise.

This dog began to take food again on the third day.

No albumin, sugar, blood, bile, acetone or diacetic acid was found to be present.

The urine of the second control day was alkaline in reaction.

Day.	Output.	Total Nitrogen.	Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.	Percentage of Total Nitrogen.					
									Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.
	c.c.													
1 . . .	1400	3·332	2·823	0·235	..	0·090	0	0·183	84·7	7·0	..	2·7	0	5·6
2 . . .	1100*	3·850	2·975	0·474	..	0·119	0	0·282	77·2	12·2	..	3·0	0	7·6
3 . . .	1480	3·765	3·083	0·313	..	0·142	0	0·227	81·9	8·3	..	3·7	0	6·1
I. . . .	730†	4·823	3·947	0·406	..	0·084	0	0·386	80·2	8·4	..	1·7	0	9·7
II. . . .	260	6·674	5·677	0·346	0·072	0·085	0·073	0·494	85·0	5·1	1·0	1·2	1·0	6·7
III. . . .	1300	7·389	6·611	0·305	0·040	0·095	0·034	0·304	89·4	4·1	0·5	1·2	0·4	4·4
IV. . . .	1250	4·060	3·500	0·245	0·028	0·083	0	0·204	86·2	6·0	0·7	2·0	0	5·1
V. . . .	1050	4·351	3·828	0·288	..	0·065	0	0·170	87·9	6·6	..	1·4	0	4·1
VI. . . .	1025	4·678	3·776	0·401	0·011	0·080	0	0·410	80·7	8·5	0·2	1·7	0	8·9
VIII. . . .	1325	4·229	3·666	0·415	..	0·082	0	0·056	86·6	9·8	..	1·9	0	1·7

* Alkaline in reaction.
† Urine partial only.

Relation of Creatin and Creatinin to Total Nitrogen.

Day.	Total Nitrogen.	Total Creatinin.	Preformed Creatinin.	Creatin.	Total Creatinin as Percentage of Total Nitrogen.
1 . . .	3·332	0·248	0·248	0	7·4
2 . . .	3·850	0·321	0·321	0	8·3
3 . . .	3·765	0·384	0·384	0	10·2
I.* . .	4·823	0·228	0·228	0	4·7
II. . . .	6·674	0·426	0·231	0·195	6·3
III. . . .	7·316	0·351	0·258	0·093	4·7
IV. . . .	4·060	0·225	0·225	0	5·5
V. . . .	4·351	0·175 +	0·175	Trace	4·0
VI. . . .	4·678	0·215	0·215	0	4·6
VIII. . . .	4·229	0·223	0·223	0	5·2

* Partial urine.

Experiment IV.

Retriever Dog (Weight 16 kilos)

Forty-five c.c. of a 2.5 per cent. solution hydrazine sulphate injected (=70 mgrms. per kilo of body weight.

This was intended for a lethal dose, but the dog, after the manifestation of a considerable degree of toxæmia, showed signs of recovery on the fifth day, when it partook of food and appeared comparatively normal on the last day of the experiment, when it took porridge and milk with relish.

On the fifth day there was a loss in weight = 1.8 kilo, after which there was a gradual increase.

On the second and third days albumin was present, while there was also a trace of bile. No other abnormal urinary constituent was detected.

Day.	Output.	Total Nitrogen.	Urea Nitrogen.	Ammonia Nitrogen.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.	Percentage of Total Nitrogen.					
									Urea Nitrogen.	Ammonia Nitrogen.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.
	c.c.													
1. . .	850	6·350	5·315	0·355	0·079	0·220	0	0·381	83·7	5·5	1·2	3·6	0	6·0
2. . .	760	5·937	4·916	0·425	0·107	0·209	0·021	0·259	82·8	7·1	1·8	3·5	0·3	4·3
I. . . .	Urine contaminated													
II. . . .	310*	3·281	2·566	0·211	0·139	0·071	0·054	0·240	78·2	6·4	4·2	2·1	1·6	7·3
III. . . .	510	11·395	9·725	0·271	0·264	0·155	0·153	0·827	85·3	2·3	2·3	1·3	1·3	7·2
IV. . . .	480	7·392	6·415	0·184	0·158	0·165	0·110	0·360	86·7	2·5	2·1	2·2	1·5	4·8
V. . . .	650	14·651	12·762	0·349	0·278	0·187	0·139	0·936	87·1	2·3	1·8	1·2	0·9	6·3
VI. . . .	650	9·373	8·030	0·251	0·181	0·174	0·045	0·692	85·6	1·8	1·9	1·8	0·4	7·3
VIII. . . .	720	4·810	3·904	0·151	0·047	0·163	0·007	0·538	82·1	5·1	1·3	3·3	0·1	8·1

* A portion of the urine was lost on this day through contamination.

Relation of Creatin and Creatinin to Total Nitrogen.

Day.	Total Nitrogen.	Total Creatinin.	Preformed Creatinin.	Creatin.	Total Creatinin as Percentage of Total Nitrogen.
1. . .	6·350	0·591	0·591	0	9·3
2. . .	5·937	0·618	0·562	0·056	10·4
I. . .	Urine contaminated.				
II.* . .	3·281	0·337	0·191	0·146	10·2
III. . .	11·395	0·825	0·415	0·410	7·2
IV. . .	7·392	0·738	0·443	0·295	9·9
V. . .	14·651	0·876	0·503	0·373	5·9
VI. . .	9·373	0·588	0·468	0·120	6·2
VIII. . .	4·810	0·457	0·438	0·019	9·5

* Urine partial.

Experiment V.

Whippet Bitch (Weight 8 kilos.)

Thirty-two c.c. of a 2.5 per cent. solution hydrazine sulphate injected (100 mgrms. per kilo.)

The loss of weight up to day before death amounted to 0.95 kilo. During the fourth day the animal appeared to recover somewhat, but fell into a comatose condition on the following day, and died on the sixth.

Bile and Protein were present in small amounts in the urine on the fifth and sixth days - just before death.

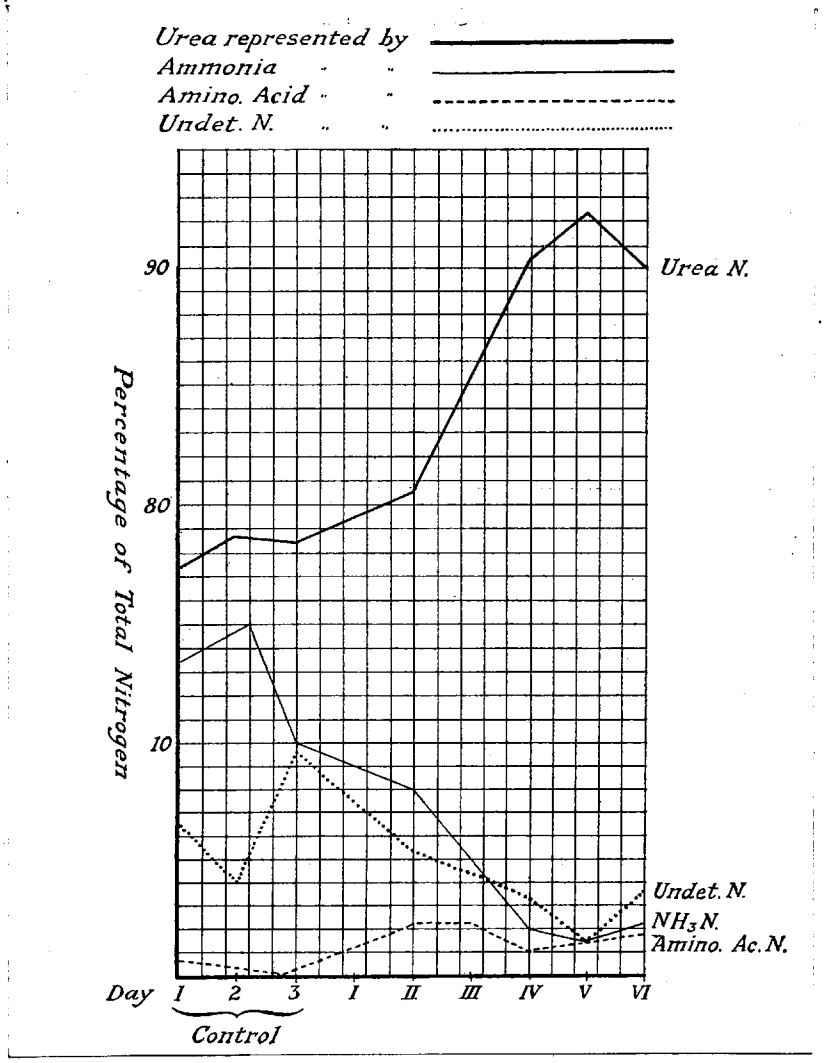
Day.	Output.	Total Nitrogen.	Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.	Percentage of Total Nitrogen.					
									Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.
	c.c.													
1 . . .	1410	5·191	4·027	0·710	0·010	0·128	0	0·316	77·5	13·6	0·19	2·4	0	6·3
2 . . .	2230	4·121	3·247	0·624	..	0·091	Trace	0·159	78·7	15·1	..	2·2	0	4·0
3 . . .	1925	4·689	3·676	0·474	..	0·080	0	0·459	78·4	10·1	..	1·7	0	9·8
I. . . .	Urine	contaminated.												
II. . . .	160	3·897	3·177	0·330	0·096	0·047	0·030	0·217	81·5	8·4	2·4	1·2	0·7	5·8
III. . . .	250+	7·168	6·118	0·350	0·161	0·059	0·149	0·331	85·3	4·8	2·2	0·8	2·0	4·9
IV. . . .	350	6·507	5·939	0·137	0·076	0·042	0·130	0·183	91·2	2·1	1·1	0·6	1·9	3·1
V. . . .	310	11·561	10·649	0·201	0·189	0·086	0·234	0·202	92·1	1·7	1·6	0·7	2·2	1·7
VI. . . .	225	8·190	7·377	0·195	0·157	0·050	0·140	0·271	90·0	2·3	1·9	0·6	1·7	3·5

Relation of Creatin and Creatinin to Total Nitrogen.

Day.	Total Nitrogen.	Total Creatinin.	Preformed Creatinin.	Creatin.	Total Creatinin as Percentage of Total Nitrogen.
1 . . .	5·191	0·345	0·345	0	6·6
2 . . .	4·121	0·245+	0·245	Trace	5·9
3 . . .	4·689	0·216	0·216	0	4·6
I. . . .	Urine contaminated.				
II. . . .	3·897	0·208	0·127	0·081	5·3
III. . . .	7·168	0·562	0·160	0·402	7·8
IV. . . .	6·507	0·462	0·113	0·349	7·1
V. . . .	11·561	0·864	0·232	0·632	7·4
VI. . . .	8·190	0·513	0·137	0·376	6·2

CHART II.

A graphic representation of the inter-relationships of the urea, ammonia, amino-acid and undetermined nitrogen excretion in Experiment V.



The size of the chart is reduced by leaving out the middle part between 10 per cent and the lowest percentage to which the urea nitrogen fell.

Experiment VI.

Black Retriever Bitch (Weight 20.1 kilos.)

5.5 c.c. of a 2.5 per cent. solution hydrazine s sulphate injected (= 70 mgrms. per kilo.)

Similar symptoms manifested to those in Experiment V.

Dog found dead on morning of fifth day after injection.

Urine of second, third, and fourth days showed trace of albumin and presence of bile, but no sugar acetone, or diacetic acid.

As in Experiment V., the dog refused all food from the time of the hydrazine injection until its death.

Day.	Output.	Total Nitrogen.	Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.	Percentage of Total Nitrogen.					
									Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.
	c.c.													
1 . . .	1450	4·222	3·476	0·259	0·046	0·116	0	0·325	82·3	6·1	1·1	2·7	0	7·8
2 . . .	1400	5·252	4·414	0·211	0·083	0·109	0	0·435	84·0	4·0	1·5	2·0	0	8·5
3 . . .	1250	4·873	3·888	0·263	0·073	0·136	0	0·513	79·8	5·4	1·5	2·8	0	10·5
I. . . .	Urine	lost.												
II. . . .	450+	4·228	3·340	0·289	0·119	0·106	0·056	0·238	79·0	6·8	4·7	2·5	1·3	5·7
III. . . .	300	6·375	5·112	0·330	0·363	0·145	0·168	0·257	80·2	5·1	5·6	2·2	2·6	4·3
IV. . . .	110	7·926	6·709	0·295	0·229	0·131	0·198	0·364	84·7	3·7	2·9	1·6	2·5	4·6

Relation of Creatin and Creatinin to Total Nitrogen.

Day.	Total Nitrogen.	Total Creatinin.	Preformed Creatinin.	Creatin.	Total Creatinin as Percentage of Total Nitrogen.
1 . . .	4·222	0·312	0·312	0	7·3
2 . . .	5·252	0·292	0·292	0	5·5
3 . . .	4·873	0·365	0·365	0	7·5
I. . . .	Urine	lost.			
II. . . .	4·228	0·435	0·285	0·150	10·2
III. . . .	6·375	0·841	0·390	0·451	13·1
IV. . . .	7·926	0·881	0·351	0·530	11·1

Experiment VII.

Retriever Dog (Weight 16.5 kilos.)

Sixty c.c. of a 2.5 per cent. solution hydrazine sulphate injected (= 90 mgrms. per kilo.)

During the rest of the day the dog was very restless and vomited repeatedly. Next morning it was found in a comatose condition, and showed symptoms of nervous iritation, there being a steady rhythmic action of the limbs, simulating the act of running. The dog was killed, and 65 cc. of urine, obtained directly from the bladder, were diluted up to 200 c.c. Specific gravity 1024. No protein, sugar or bile, and no acetone or diacetic acid detected.

Day.	Output.	Total Nitrogen.	Urea Nitrogen.	Ammonia Nitrogen.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.	Percentage of Total Nitrogen.					
									Urea Nitrogen.	NH_3 N.	Amino-Acid Nitrogen.	Creatinin Nitrogen.	Creatin Nitrogen.	Undetermined Nitrogen.
	c.c.													
1	1150	4·937	4·117	0·385	0·025	0·105	0·008	0·297	83·4	7·8	0·5	2·1	0·1	6·1
2	980	4·141	3·402	0·268	..	0·114	0	..	82·0	6·4	..	2·7	0	..
3	1020	4·226	3·570	0·287	0·014	0·120	0	0·235	84·5	6·7	0·3	2·8	0	5·7
	Urine obtained directly from bladder of dog after death.													
I.	65	2·066	1·677	0·151	0·101	0·032	0·014	0·091	81·2	7·3	4·9	1·5	0·7	4·4

Relation of Creatin and Creatinin to Total Nitrogen.

Day.	Total Nitrogen.	Total Creatinin.	Preformed Creatinin.	Creatin.	Total Creatinin as Percentage of Total Nitrogen.
1	4·937	0·303	0·283	0·020	6·1
2	4·141	0·306	0·306	0	7·3
3	4·226	0·321	0·321	0	7·6
I.	2·066	0·124	0·086	0·038	6·0

SUMMARY AND DISCUSSION OF RESULTS.

A. Experiments with Sublethal doses.

Total Nitrogen.- All the experiments show a marked increase in the total nitrogen after hydrazine injection, although no food was taken before the fourth day. This increase is most marked on the third and fourth days, after which there is a gradual fall, but the figure usually remains somewhat higher than it was during the control period.

Urea Nitrogen. - There is always an absolute increase in the total amount excreted during the experiment, while the percentage of urea nitrogen to total nitrogen is slightly higher than, and never falls below, the figures for the control period.

Ammonia Nitrogen. - In Experiments I. and II. the total amounts are slightly increased, but in III and IV. the figures are somewhat lower than those of the control. All over, however, the percentage of ammonia nitrogen to total nitrogen shows a tendency to fall during the height of the toxic symptoms, gradually rising again by the last day recorded. In Experiment IV. the diminution in the percentage figure is quite

unequivocal, and is comparable to that obtained in the six-day lethal experiment (Experiment V.)

Amino-Acid Nitrogen. - As mentioned above, the excretion on the control days shows some disparity. In Experiments I., II., and IV. an average of 0.06 grm. is found, while in III. no excretion is recorded. All the experiments, however, show similar results after hydrazine injection. There is always a definite increase during the toxic period both in the total amount and in the percentage of total nitrogen.

Preformed Creatinin Nitrogen. - In I. and II the total amounts remain fairly constant, while in III. and IV. there is a distinct fall. There is always a decrease in the percentage of creatinin nitrogen to the total nitrogen.

Creatin Nitrogen. - Creatin is found only occasionally and in the merest traces during the control days. From the second day after the injection of hydrazine there is always a considerable output, equal to 1 to 2 per cent. of the total nitrogen. In III it only appears on two days, at the height of the toxæmia, but in this dog the metabolic disturbances are less marked than in the others.

Undetermined Nitrogen. - All the experiments tend to show an increase in the values recorded during

the first few days after injection. No definite variation in the percentage of undetermined nitrogen to total nitrogen is to be noted. There is, however, a quite definite rise in the output of undetermined nitrogen, as also in that of the amino-acid nitrogen, coincident with the fall in the percentage of urea nitrogen to the total nitrogen, which occurs immediately after the hydrazine injection.

B. Experiments with Lethal Doses.

The experiments give results showing similar changes, but to a more marked degree than with the non-lethal doses. As regards the urea-ammonia relationship in VI. and VII., the results are in accordance with those of the non-fatal doses, the urea percentages being very little affected, while the ammonia figures show a slight fall. In V., however, there is a very marked rise in the urea nitrogen, from 78 per cent to 90 per cent. of the total nitrogen; while the ammonia falls considerably, reaching 2.1, 1.7, and 2.3 per cent. on the fourth, fifth, and sixth days respectively.

The amino-acid increase is more marked than in the sub-lethal experiments. The creatin nitrogen shows a similar increase, while the values for creatinin are usually lower than during the control period.

DISCUSSION OF RESULTS.

Total Nitrogen. - In all the experiments there was a definite increase in the excretion of total nitrogen except on the second day, when the low output often recorded is doubtless associated with the difficulty of completely collecting the urine on that day. In the experiments with the sublethal doses the increase was confined to the days of most marked symptoms, and was followed by a gradual return to the normal. In Experiment IV., where the largest sublethal dose was given, the increased output of total nitrogen lasted until the sixth day, the average excretion from the second till the sixth being as high as 10.7 grms. as compared with 6.1 grms. during the control period. It is to be noted that no food was taken until the fifth day, and then it only amounted to half of that ingested before the injection of the hydrazine. Where a lethal dose was administered, the increase in total nitrogen became progressively larger, although no food at all was consumed. Thus in V., while the average during the control days was 4.6 grms.,after the injection of hydrazine it was 7.4 grms., the highest excretion recorded being 11.5 grms. on the day before death. Underhill and Kleiner (1909)(21) record a similar increase in total nitrogen; while in Mendel and Rose's (1911)(26) experiments on phosphorus poisoning, where lesions in the liver

tissue, in addition to disturbances of the glycogenic function, are very marked, large excretions of nitrogen, as compared with the output during simple fasting, occur.

The protein of liver tissue may be a source of this nitrogen, while there is also evidence of considerable breakdown of muscle protein. (p.p.42-44.) The cause of this rapid katabolism is still uncertain. From a consideration of the small increase in the total nitrogen output during muscular activity, provided that the supply of oxygen and carbohydrates were adequate, Noël Paton (1905)[27] suggested that in the katabolism of muscle the nitrogen containing part of the protein molecule could be resynthetised if sufficient carbohydrate were available, and hence that under such conditions a comparatively small supply of protein was necessary for ordinary muscle metabolism. Cogent evidence in support of this hypothesis has been furnished by Cathcart (1909)[28] as the result of his investigations on the output of total nitrogen under the influenceof different diets. He concludes that "carbohydrates are absolutely essential for intracellular synthetic processes in connection with protein metabolism", and that, if the glycogenic power of the liver is impaired, an insufficient supply of sugar will be available for protein synthesis, and hence an increased excretion of nitrogen will result. That such

an impairment of glycogenic function and consequent upsetting of the carbohydrate metabolism occurs after the injection of hydrazine is shown by the histological findings of extensive degenerative and necrotic changes in the liver parenchyma, as well as by the condition of hypoglycæmia, which Underhill (1911)[29] found invariably to accompany hydrazine poisoning.

Urea Nitrogen and Ammonia Nitrogen.- Various hypotheses, as described in the Introduction p.p.5-8, have been put forward to account for the great diversity in the results which have been obtained in the estimation of both the relative and absolute amounts of urea and ammonia excreted in hepatic affections. The importance of the high ammonia value obtained in liver disease is discounted by the constant presence of the factor of acidosis. This criticism has been confirmed by the researches of Williams (1906)[6] and Wolf (1906)[7], the latter of whom suggests that this difference in ammonia output depends on the amount of reserve fat stored in the individual, since it is known that an excess of body fat leads to the excretion of acids, with a corresponding rise in the urinary ammonia content.

On the other hand Ewing (1908)[8] holds that the explanation of the increased ammonia excretion is to be

found in the diminished synthesis of ammonia into urea - a metabolic disturbance due to disordered hepatic function to which the coexistent acidosis is also secondary. Finally we have described how Pearce and Jackson (1907)[10] from their investigations on liver necrosis produced by injections of hæmolytic sera explain the occurrence of the two distinct urinary chemical pictures as dependent on the diverse types of hepatic lesion produced, and histologically demonstrated.

In the light of these findings the results recorded in this paper are interesting. The urea percentage in none of the experiments fell: on the contrary, there was in some a tendency to rise, while in the lethal Experiment V. there was a marked increase from 78 per cent. during the control period to 90 per cent., while in the sublethal Experiment IV. it rose to 87 per cent. as compared with 83 per cent. during the days preceding injection. The ammonia output generally remained fairly constant, but in Experiment V. it showed a marked fall, from 13.5 per cent. during the control days to 4.2 per cent. after hydrazine, while on the day before death it reached 1.7 per cent. It may be noted that the urea was rather low and the ammonia high for the normal dog during the control period. This, however, rather tends to accentuate the remarkable

nature of the results, for it offers no explanation for this marked fall in ammonia after the administration of a toxic drug. Likewise, in Experiment IV., where a large dose (0.07 grm. per kilo. body weight), which did not prove fatal, was administered, the ammonia percentage of the total nitrogen for the first six days after hydrazine injection averaged 3 per cent. In none of the experiments were acetone or diacetic acid ever obtained in the urine, so that probably no ammonia was required for the neutralisation of acids. It is, however, to be remembered that those substances - acetone and diacetic acid - are seldom, if ever, found in dog's urine, even under conditions comparable to that of acidosis.

As the dogs refused all food in the fatal experiments, and never partook of either porridge or milk before the fourth day after hydrazine injection in the sublethal experiments, the starvation factor has to be considered. Underhill and Kleiner (1909)[21] record the results of the nitrogen partition of the urine during the fast of a dog for twelve days. Their figures show a slightly increased ammonia nitrogen per-centage, and correspondingly decreased urea percentage compared with the total nitrogen, while in the one experiment which they record on the changes in

the nitrogen distribution during hydrazine poisoning, a lower output of ammonia and higher output of urea was obtained than in the same dog during starvation. Likewise, in accordance with the results recorded in this paper, there is a marked fall in the ammonia percentage of total nitrogen, while the urea percentage also falls to a slight extent.

Allantoin. - In the protocols, allantoin is included in the urea content. On the occasions on which it was separately estimated by the method described above, the excretion after hydrazine injection did not show any proportionate increase, when due allowance was made for the amount found to be excreted in fasting alone. This corroborates the findings of Underhill and Kleiner, who first pointed out the increased excretion of the substance in starving dogs, and who conclude that "hydrazine has no specific action in causing an elimination of allantoin."

Amino-Acid Nitrogen. - From our review of previous work on the subject (see Introduction p.8-10) it has been seen that the total amino-acid content of the urine, as distinguished from the estimation of individual amino-acids, appears to be of considerable diagnostic import in liver affections. This observation of Frey has been confirmed by the results of the present experiments; for, of the

changes produced by hydrazine, the increase in amino-acid nitrogen is the most marked and most constant feature. In the sublethal experiments the absolute increase is sometimes small, but when compared with the values for the control period, or considered as percentage of the total nitrogen, the rise is quite appreciable. In Experiment VI., on the second and third days it is equal to 5 per cent. of the total nitrogen, while on the latter day the output is greater than that of the ammonia. This increase is always found very soon after the hydrazine injection; indeed, it is invariably the earliest abnormal relationship to make its appearance. The percentage increase is most marked on the second and third days and definitely precedes the rise in total nitrogen, for with the increased output of total nitrogen the amino-acid percentage is always found to decrease, although the total amount excreted is, if anything, increased. In VII., in the urine of the first day, the amino-acid content is equal to 4.9 per cent. of the total nitrogen as compared with 0.4 per cent. during the control days, while in both V. and VI. the increase is marked.

If one accepts the deaminisation hypothesis, this increased amino-acid excretion is due to the amino-acids which result from the katabolic products of the protein

of the tissues escaping undeaminised, presumably from the liver. The fact that the amino-acid excretion is greatest so soon after the onset of symptoms, and before the liver is called upon to deal with the nitrogen resulting from the greatly increased protein katabolism, points to the compensatory functional hypertrophy of the remaining functioning healthy liver parenchyma. In spite of this apparently marked increase in amino-acid output, it is to be noted that it bears a very small proportion to the total urea and ammonia excretion, which at the same time tends to increase rather than fall; while the tendency of recent work goes to show that the degree of deaminisation which takes place is probably much less marked than was formerly thought. Hence, there is the probability that the amino-acid excretion will be found not to be of great importance from the point of view of metabolism, although, as a possible test of liver inefficiency as borne out by the results of the present experiments, it may be of considerable value.

The relationship of the amino-acid nitrogen to the undetermined nitrogen is interesting, in consideration of the fact that the amino-acids were formerly considered to form the bulk of the undetermined nitrogen residue. The latter, in all the experiments, except

in II and VI., forms an increased percentage of the total nitrogen and is usually highest on the second or third day after hydrazine injection, when often the highest percentage of amino-acids is also recorded. It is of interest to note that this increased percentage value for both amino-acid nitrogen and undetermined nitrogen coincides with the slightly diminished urea percentage of the total nitrogen which immediately follows hydrazine injection. A high undetermined nitrogen value can, however, be accounted for, apart from the presence of amino-acids, especially in cases of necrosis. The residue remaining after extraction with absolute alcohol and ammoniacal alcohol is usually very small in amount, but in the case of necrotic tissues this amount is markedly increased. Salkowski (1905)[30] found that this nitrogen in the urine of a case of acute yellow atrophy formed 20 per cent. instead of a normal 3 to 4 per cent., while no crystals of leucine or tyrosine could be found. This "colloidal nitrogen" which consists chiefly of proteoses, is what one would expect as the result of autolytic change. The removal of such products by way of the blood stream leads to an increase in the urine of the undetermined nitrogen residue - an increase which used to be ascribed to amino-acids. No

precipitate of any importance, however, was found after the addition of ammoniacal alcohol in the course of the present experiments.

Creatin and creatinin. - Some recent workers have urged the importance of hepatic activity in the metabolism of these substances. As mentioned above, according to the investigations of Mellanby[1] and Van Hoogenhuyze and Verploegh,[2][3] the creatinin output both in hepatic cirrhosis and in carcinoma of the liver is greatly reduced, while in the latter condition large quantities of creatin appear in the urine. Further Mellanby concludes "In the formation of creatinin muscle plays a small part", and "the liver is intimately connected with the production of creatin and excretion of creatinin".

But cases of cirrhosis of the liver are far from being the only cases of low creatinin excretion. Indeed, the excretion of an abnormally small amount is peculiar to no one special disease, but may be present in very diverse conditions, e.g., various anæmias and muscular atrophies. Shaffer (1908)[32] holds that creatinin is not an index of total endogenous protein katabolism, but of that part of endogenous metabolism which is an expression of muscle "tonus" or efficiency, so that the amount of creatinin nitrogen per kilo body weight shows a direct parallelism to the muscular development

or strength. Thus, in exophthalmic goitre, where the general metabolic processes are excessively active, there is a low creatinin excretion, which Shaffer correlates with the limp and toneless condition of the muscles. In connection with this view of creatinin metabolism, it is interesting to note that in the case of myotonia congenita reported by Findlay (1912)[33], where the muscles were in a condition of marked tonus as shown by both physical and electrical modes of examination, I found that the urine of the man, who was on a creatin-free diet, had per diem a large creatinin content, the creatinin coefficient (creatinin nitrogen per kilo of body weight), which normally averages 5 to 9, being found to lie between 12 and 14. Further, Pekelharing (1911)[34] found a rise in the output of creatinin when muscle tonus was raised, while there was no such increase after the increased muscular contraction occasioned by a walk of 20 kilometres. He concluded that there was quite a distinct difference between those two metabolic processes occurring in muscle. Spriggs (1907)[35] records cases of progressive muscular atrophy, amyotonia congenita and myasthenia gravis - conditions associated with muscular wasting and lack of "tonus", in all of which the excretion of creatinin was below normal, while in a case of locomotor ataxia it was unaffected, and in two

cases of tetanus and one of spastic paraplegia there appeared to be a slight increase. His conclusions agree with those of Shaffer, and he considers that "the creatinin is connected with the nutritional metabolism of the muscle fibre, and is not a substance formed in the act of contraction."

Creatin appears in the urine in quite appreciable quantities in necrosis of the liver, both clinically and in that experimentally produced. Thus the amounts recorded, expressed as percentage of total nitrogen, are in acute yellow atrophy, 0.7 per cent. (1908)[2]; chloroform poisoning, 0.5 to 0.8 per cent (1911)[16], and in the present experiments on hydrazine poisoning, 1 to 2.5 per cent. As mentioned above, it is also obtained in carcinoma of the liver. But it does not follow that its presence has any direct relation to defective liver metabolism. There is, no doubt, marked disturbance of hepatic function, but there are also profound disturbances, in all these various conditions, of other metabolic processes. Thus muscle metabolism is considerably upset, as shown by the rapid breakdown of muscle protein with the simultaneous excretion of creatin. One may say that it is now generally agreed that the urinary creatin has its source in the creatin of muscle, but it does not seem necessary to regard liver inefficiency as the essential metabolic disturbance to

explain its excretion in hepatic disease rather than in acute fever, the acute stages of exophthalmic goitre, or during the puerperium, when as much as 1.5 grm. has been found to be excreted per diem. Recent experimental work tends to support this view. Towles and Voegtlin (1912)[36], from their feeding experiments in Eck fistula dogs, conclude that the "exclusion of the portal circulation from the liver has no effect upon the creatinin metabolism"; while Noël Paton (1912)[37] has recently demonstrated that the exclusion of the liver in the bird, by ligature of both portal vein and hepatic artery, has no effect upon the proportion of creatin, although, as Minkowski had shown, there are marked changes in the distribution of the urinary nitrogen.

According to Cathcart (1909)[28], the output of creatin induced by fasting at once disappears on the ingestion of carbohydrates, just as there is a fall in the output of urinary nitrogen. Hence creatinuria will occur if no proper supply of sugar be available through defective glycogenic function. Thus this large excretion of creatin after hydrazine injection can be explained through an upsetting of the balance of carbohydrate metabolism. That such a disturbance of glycogenic function occurs is shown by the marked hypoglycæmia already mentioned as occurring as a result

of hydrazine poisoning. No doubt hepatic activity is the chief factor in one stage at least of the glycogenic function, but it is only one of several factors concerned in the process. Muscle metabolism also plays a part in the utilisation of carbohydrates, and creatin may be excreted in conditions where the pathological lesion is chiefly a muscle involvement, as in the muscular atrophies (1910)[38]. Recently Mellanby (1913)[39] has suggested that the creatin excretion of parturient women has probably some relation to the activity of the mammary glands. He notes the fact that creatin is also excreted during pregnancy, and admits that there is nothing specific about this suggested relationship. The creatinuria of both pregnancy and puerperium, however, may be related to a disordered carbohydrate metabolism, for Bar (1910)[40] has shown that in the former condition there is a distinct lowering of the tolerance for sugars, while the puerperal period is associated with an alteration in carbohydrate equilibrium occasioned by the establishment of the function of lactation.

In the present experiments rapid muscle breakdown appeared to be an important feature when the toxic symptoms were most marked, and an estimation of its amount was attempted by the method suggested by Noël Paton (1910)[41] when the preformed creatin in is regarded as a

derivative of the creatin of muscle, and the total creatinin as a measure of muscle katabolism. In Table II. (page 44) the "total flesh" katabolised is calculated from the total nitrogen, and "muscle flesh" from total creatinin, while the ratio $\frac{\text{muscle flesh}}{\text{total flesh}}$ is also recorded. The interpretation of these figures is by no means unequivocal, especially as there is the complication involved by the introduction of the food factor, since porridge and milk was always taken by the dogs after the fourth day in the sublethal experiments. Further, it may be noted that although the ratio which "muscle flesh" bears to "total flesh" during the control days of any individual experiment shows considerable uniformity, yet this ratio varies considerably not only for the different experiments, but also at different times in the same dog. Thus in Experiment IV. the ratio on both the control days averages 1.07, thus pointing to some retention of nitrogen, while the same dog, in Experiment VII. five weeks afterwards, no longer shows this condition. From the figures representing "muscle flesh" katabolised, it is, however, clearly evident that in all the experiments there is considerable muscle disintegration, especially during the second, third, and fourth days after the administration

of the hydrazine. This muscle breakdown is most marked in the lethal Experiments V. and VI., distinct evidence being furnished not only by the total amounts of "muscle flesh", but also by the ratio of "Muscle flesh" to "total flesh". Thus in the former, the ratio for the first four days after hydrazine injection averages 0.76 in contrast to 0.62 during the control period, while in VI. it is 1.26 as compared with 0.74. In these two experiments it is also found that while the total creatinin shows a considerable increase as compared with the excretion before injection, there is a marked fall in the preformed creatinin coincident with the substantial rise in the excretion of creatin. Experiment IV. also shows this relationship, but to a much less extent.

TABLE II.

Experiment 1.

Day.	Total Flesh.	Muscle Flesh.	Muscle Flesh / Total Flesh.	Remarks.
	grms.	grms.		
1.	190.	111.	0.57.	Food taken.
2.	118	93	0.78	"
3.	150	108	0.72	"
II.	214	137	0.64	Fast.
III.	70	36	0.51	Fast. Partial urine only.
IV.	204	134	0.65	Some food taken.
VI.	195	119	0.61	"
VIII.	169	99	0.59	"
X.	151	104	0.68	"

Experiment II.

Day.	Total Flesh.	Muscle Flesh.	Muscle Flesh / Total Flesh.	Remarks.
	grms.	grms.		
1.	140	84	0.60	Food taken.
2.	139	85	0.61	"
3.	156	82	0.52	"
II.	125	120	0.96	Fast.
III.	276	236	0.85	"
IV.	167	108	0.64	Food taken.
V.	169	114	0.67	"
VII.	195	93	0.48	"
IX.	169	96	0.57	"

Experiment III.

1.	92	75	0.81	Food taken.
2.	102	97	0.95	"
3.	104	116	1.11	"
I.	133	69	0.59	Fast.
II.	185	129	0.69	"
III.	203	106	0.52	"
IV.	112	68	0.60	Food taken.
V.	120.	53	0.44	"
VI.	129	65	0.50	"
VIII.	117	67	0.57	"

Experiment IV.

1.	176	179	1.01	Food taken.
2.	164	187	1.14	"
II.	91	102	1.12	Fast. Partial urine only.
III.	315	250	0.79	Fast.
IV.	205	223	1.08	"
V.	406	265	0.65	Food taken.
VI.	259	178	0.66	"
VIII.	133	138	1.03	"

Experiment V.

1.	143	104	0.72	Food taken.
2.	114	74	0.65	"
3.	129	65	0.50	"
II.	108	63	0.58	Fast.
III.	198	170	0.86	"
IV.	180	140	0.77	"
V.	320	262	0.82	"
VI.	226	155	0.60	Dog died.

Experiment VI.

Day.	Total Flesh.	Muscle Flesh.	Muscle Flesh / Total Flesh.	Remarks.
	grms.	grms.		
1.	117	94	0.80	Food taken.
2.	145	88	0.60	"
3.	135	110	0.81	"
II.	117	132	1.12	Fast.
III.	176	255	1.44	"
IV.	219	267	1.22	Dog died.

Experiment VII.

1.	137	92	0.67	Food taken.
2.	115	93	0.80	"
3.	117	97	0.82	"
I.	57	37	0.65	(Partial urine) only. Dog died) at end of (twenty-four hours

In the results of none of the experiments can any parallelism be traced between the excretion of total nitrogen and total creatinin, in contrast to the findings of Mendel and Rose[26] in both their diet and phosphorus experiments. From their investigations they conclude that total nitrogen and total creatinin appear to have a common source. It must be admitted, however, that such a conclusion cannot be pushed very far, because we are still without evidence as to the factors governing the selection for katabolism of muscle and non-muscle protein, apart altogether from the question of the possible resynthesis of a portion of the nitrogen. They themselves, however, admit that all ~~the~~ methods of

calculating tissue loss on this basis must be inaccurate.

The consideration of the relation of total creatinin to loss of body weight was found to be useless owing to vomiting being a more or less constant symptom during the first days of the toxæmia, and this leads to a marked loss of weight, not only from the depletion of the animal of fluid, but also from the loss of the hydrochloric acid of the gastric juice leading to a reduction in the salt content of the tissues.

CONCLUSIONS.

I. The estimation of any one element of the nitrogen partition of the urine is useless as a guide to hepatic efficiency; their inter-relationships have to be considered.

II. After the injection of hydrazine sulphate there is an increased excretion of total nitrogen, as found also in phosphorus poisoning. It is probably due to the disturbance of the glycogenic function with the subsequent direct or indirect disintegration of the muscle protein.

III. The increase in amino-acid nitrogen, both absolutely and relatively to the total nitrogen, is the most constant feature of the urinary nitrogen partition during the hepatic disturbance produced by hydrazine. Further, this increase is invariably the

earliest abnormality to be detected.

IV. After hydrazine injection the urea percentage of the total nitrogen increases, and the ammonia diminishes, slightly in sublethal, markedly in lethal, experiments. Neither acetone nor diacetic acid was detected in the urine during the course of any of the experiments.

V. The excretion of creatinin is found to be practically constant, while there is a large excretion of creatin, which appears to be dependent on impaired glycogenic function exerting its influence on the muscle metabolism, rather than on liver inefficiency leading to a decrease in the conversion of creatin to creatinin. This seems to be indicated by the increased katabolism of "muscle flesh" as calculated from the output of total creatinin.

PART II.

THE PRESENCE OR ABSENCE OF CREATIN-DESTROYING OR CREATIN-CONVERTING FERMENTS IN THE VARIOUS TISSUES.

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Introduction.

From Part I. it was concluded that in disturbances of hepatic function, the excretion of creatin appears to be dependent on impaired glycogenic function exerting its influence on muscle metabolism rather than on liver inefficiency leading to a decrease in the conversion of creatin to creatinin. If such conversion of creatin occurred as the result of hepatic action per se, one would expect to find evidence of the action of the enzymes which initiated and carried through these changes. But the presence or absence of such enzymes, as would lead to the production of creatin, the conversion of creatin into creatinin or other substance (creatinase), and the destruction of creatinin (creatininase) in liver tissue and indeed in any tissue of the organism is still undecided.

The results of the early work of Seemann (1907)(42) seemed to prove the production of creatin in autolysis, as he found that the creatin content of muscle was thereby increased.

His technique, however, was decidedly faulty and his creatinin estimations were determined by the gravimetric method. A much more important research is that of Gottlieb and Stangassinger (1907)[43] who appeared to find numerous ferments - or different aspects of the same ferment - which produce various products from their action on creatin and creatinin. Their results are thus summarised -

" I. In the autolysis of muscle and other organs creatin is first produced.
II. The creatin becomes converted in part into creatinin during autolysis by a ferment.
III. As autolysis progresses, creatin and creatinin are destroyed by ferments - creatinase and creatininase.
IV. From the interaction of those processes there is obtained a complex curve representing the creatin and creatinin value in autolysing organ extracts, and dependent on the ferments resulting in creatin formation, its conversion into creatinin, and the destruction of both bodies. "

These conclusions are open to considerable criticism. It has now been definitely shown that creatin alone, but never creatinin except in extremely minute amounts, exists in fresh muscle, and that, as muscle undergoes autolysis through bacterial action, all the creatin in muscle disappears. Mellanby (1908)[44] found that when the autolysing tissues e.g. guinea-pig liver, cat liver, hedge-hog liver or rabbit muscle, were kept strictly aseptic, no creatinin could be detected, while the creatin

content remained unchanged. Nor was creatinin found when creatin solution was left in contact with sterile mixtures of muscle and liver or muscle and kidney; while this same investigator working in conjunction with Twort (1912)[45] demonstrated the normal existence of creatin-destroying bacteria in the intestine.

Mellanby also points out a grave defect in the technique of Gottlieb and Stangassinger in their mode of estimation of preformed creatinin. They evaporated their organ extracts to dryness on a water-bath, whereas it is known that at 100° C. the conversion of creatin into creatinin is very easily brought about.

The results of Gottlieb and Stangassinger that creatin is partly converted into creatinin, and partly destroyed, have been subsequently confirmed by Rothmann (1908)[46], another worker in their laboratory. He carried out part of his experimental work by the use of Mellanby's method, by means of which overheating was avoided, and admits its superiority over that of Gottlieb which is only useful for estimations of total creatinin. Further he concluded that bacterial action plays no part in this conversion and destruction of creatin - a finding quite at variance with that of Mellanby.

Accordingly, it was determined to tackle the question of the presence or absence of those enzymes by utilising a method which has given excellent results

in the extraction of bacterial enzymes in the hands of Hahn and Cathcart (1902)[47]. By this method a dry organ residue is obtained with the action of certain known ferments undiminished, or at least unequivocably active; while asepsis is more easily attained, and the use of antiseptics such as toluol and chloroform, which at least delay enzyme action, if not sometimes altogether inhibiting it, is avoided.

METHOD EMPLOYED IN PRESENT EXPERIMENTS.

5-10 grms.of the organ - liver, muscle, kidney, - are taken and minced very finely. After being rubbed up in a mortar to which 30 c.c of acetone are added, the mixture is thrown on a filter paper in a large Buchner funnel with full negative pressure applied. As soon as the minced tissue appears tolerably dry, the rubbing up in the mortar with acetone and drying in the Buchner funnel is repeated two or three times according to the consistency of the mixture. From 100 to 130 c.c. acetone should altogether be used. Then the same process is repeated using 100 c.c. pure ether instead of acetone. The filtering in the "Buchner" under full pressure is continued until the residue is bone dry, when it is transferred to a vacuum-drying oven with toluol and kept at its boiling point 111° C. for 20-30 minutes. The

organ residue is then transferred to a sterile bottle with glass stopper, when it was found to retain its enzymatic properties for several months at least. Each stage must be carried out as rapidly as possible.

Previous to carrying out experiments for the attempted detection of creatin and creatinin destroying enzymes, tests for other ferments were applied. All the liver residues revealed the presence of an active diastase and lipase. The test for the former was carried out in three test-tubes containing the following mixtures which were incubated for six hours. -

A. .4 gm. Glycogen + 5c.c. distilled water

B. .4 gm. Glycogen + 5c.c. distilled water + .02gm. liver residue.

C. 5c. c distilled water + .02 gm. liver residue.

To each tube 5c.c. of Fehling's solution was then added, and the mixture boiled when there occurred -

A. Minute trace of reduction.

B. Quite marked reduction.

C. No trace.

A very active lipase was obtained from all the liver residues. Thus the following was the product obtained with dog's liver, utilising the production of acid from monobutyrin as the test of lipolytic activity after half-an-hours incubation, phenol phthalein being

the indicator. A quantitative estimation of the acidity was made in terms of $\frac{N}{10}$ Na.O.H.

(1) Monobutyrin 3 drops + .02 gm. Liver + 10 c.c. distilled water required 2.1 c.c. $\frac{N}{10}$ Na.O.H.

(2) Monobutyrin 3 drops + 10 c.c. distilled water required .05 c.c. $\frac{N}{10}$ Na.O.H.

(3) .02 gm. Liver + 10 c.c. distilled water required 0 $\frac{N}{10}$ Na.O.H.

Similarly in the muscle preparations an active diastatic ferment was found present by quantitative estimation, after the reduction due to the glucose in the muscle had been allowed for. In the case of the kidney residues, no enzymatic test was found suitable, and accordingly the value of the mode of preparation had to be deduced from the results obtained in the case of the liver and muscle preparations.

Freshly prepared creatin solution was always used, a strength of .2% being found most convenient for the colorimetric readings in 500 c.c. dilution. The following mixtures were put up -

A. .02 gm. organ residue + 15 c.c. sterile water

B. 5 c.c. sterile water + 10 c.c. .2% creatin.

C. .02 gm. organ residue + 5 c.c. sterile water + 10 c.c. .2% creatin.

D. 5 c.c. .15% Na_2CO_3 + 10 c.c. .2% creatin.

E. .02 gm. organ residue + 5 c.c. .15% Na_2CO_3 + 10 c.c. .2% creatin.

Two sets of test-tubes were incubated at 37° C., and estimations of (a) Preformed creatinin, (b) Total creatinin, were made after three hours in the one set, and after twenty-four in the other.

In the first few experiments a 6th tube F, containing 15 c.c. .15% Na_2CO_3 + .02 gm. organ residue, was put up, but it was subsequently omitted as being unnecessary, since it was found that the slightly alkaline medium gave the same negative result as the sterile water medium in A.

SUMMARY OF RESULTS.

I The question of the conversion of creatin into creatinin.

To this an answer is given by a consideration of the values obtained for preformed creatinin estimated after incubation for (a) 3 hours, (b) 24 hours. By the application of Jaffe's test a very slight coloration was obtained, but an attempted estimation of the amount by the colorimetric method gave no reading, even when the solution was only diluted to 62.5 c.c. No quantitative difference of tint was found present by the use of Nessler's glasses, nor was any creatinin obtained on evaporation of the fluid at 43° C. in Haskin's apparatus. A deeper tint was constantly found in the alkaline solutions, yet the difference was so slight that one can conclude that

it was not the result of enzyme action which would doubtless produce much greater changes.

II <u>The question of the decomposition of creatin into a body or bodies unknown</u>. i.e. is there any change produced in the amount of total creatinin present ?

Estimations were made after incubation at 37° C. for 3 hours and 24 hours respectively. In the accompanying table the average of 10 colorimetric readings at 500 c.c. dilution is recorded as the result of the action of the various organ residues on the .2% solution of creatin.

Table III (Next page)

TABLE III.

Total Creatin in estimated after { I. 3 hours' incubation. / II. 24 hours' incubation.

Average of 10 Colorimetric Readings at 500 c.c. dilution.

Organ-residue.	.02gm.Organ-residue + 15c.c. sterile H_2O.	10c.c.Creatin solution + 5c.c. sterile H_2O.	10c.c.Creatin solution + 5c.c. sterile H_2O. + .02gm.Organ-residue.	10c.c.Creatin solution + 5c.c. .15% $Na_2C.O_3$	10c.c.Creatin solution + 5c.c. .15% $Na_2C.O_3$ + .02 gm. Organ-residue.
I. After 3 hours' incubation.					
Liver-Rabbit.	-	7.2m.m.	7.1m.m.	7.5m.m.	7.7m.m.
Liver-Dog	-	8.3	8.3	9.2	9.0
Liver-Hen	-	9.5	9.1	10.2	10.4
Muscle-Rabbit	-	8.5	8.4	8 .5	8.5
Muscle-Duck	-	9.9	9.6	9.8	10.2
Kidney-Duck	-	8.1	8.3	8.9	8.7
Kidney-Dog	-	7.9	7.8	8.3	8.3
II. After 24 hours' incubation.					
Liver-Rabbit	-	7.5	7.4	8.0	7.7
Liver-Dog	-	8.3	8.5	9.3	9.3
Liver-Hen	-	9.3	8.3	10.1	10.4
Muscle-Rabbit	-	8.4	8.4	9 .2	9.3
Muscle-Duck	-	9.9	9.7	10.1	9.9
Kidney-Duck.	-	8.3	8.2	9.1	9.0
Kidney-Dog	-	7.9	8.0	8.3	8.5

DISCUSSION OF RESULTS.

The answers given to these two questions by the mode of investigation described above seem quite unequivocal.

No conversion of creatin into creatinim was obtained in the presence of any of the organ residues, - liver, kidney, or muscle. The somewhat deeper coloration obtained with von Jaffe's test in the tubes containing the slightly alkaline medium was found to be due to the alkaline medium itself.

Secondly, that no creatin destroying ferment was present, is shown by Table III. where the average colorimetric readings obtained for the creatin solution in contact with the organ residues as compared with those for the control tubes in each experiment after both three and twenty-four hours' incubation, is sufficiently close to be within the limits of experimental error. Slightly higher colorimetric readings were again uniformly obtained in the alkaline media.

The most obvious criticism which may be offered adverse to these results is that although diastatic and lipolytic enzymes were obtained in quite an active condition, yet the conditions present or the mode of preparation employed were incompatible with the continued vitality of the ferments which bring about the decomposition or conversion of creatin. It must be admitted that this criticism cannot be satisfactorily

answered, except that the more important defects in the technique of the work where positive results were obtained have been eliminated while the conclusions of Mellanby have been in the essential confirmed.

The contention of Rothmann that bacterial action plays no part in the conversion and destruction of creatin appears quite erroneous. In some experiments in the course of the present work in which bacterial contamination occurred, considerable destruction of creatin was found; while, as mentioned above, the existence of creatin-destroying bacteria in the healthy intestine has been demonstrated by Mellanby and Twort.

Pekelharing and van Hoogenhuyze (1910)[48] have suggested that enzyme action is diminished during inanition, and they thus explain the excretion of creatin in starvation, ignoring altogether the rôle which carbohydrates play. No definite evidence has been brought forward in support of this theory, while the present experiments, taken in conjunction with those of Part III., appear to negative such an explanation as well as any hypothesis associating the creatinuria of hepatic insufficiency with defective enzymatic activity.

CONCLUSION.

By the methods used in the present experiments, no ferment bringing about the conversion of creatin into creatinin, and no creatin-destroying ferment was detected in any of the organ residues - liver, kidney, or muscle, although active diastatic and lipolytic ferments were obtained.

PART III.

THE RELATIONSHIP OF CREATINURIA TO CHANGES IN THE SUGAR CONTENT OF THE BLOOD.

Previous Work and Object of Present Research.

An increase in the normally constant sugar-content of the blood has long been known to be an associated phenomenon of certain pathological conditions, especially pancreatic diabetes or the glycosuria artificially produced by the injection of certain drugs, e.g., phloridzin, uranium. Of recent years it has been shown that a diminution in the normal sugar content, or a hypoglycæmia, may follow the injection of certain other drugs, which cause considerable hepatic disturbance accompanied by the excretion of creatin. This creatinuria is what would be expected if the conclusions of Part I. that carbohydrates are essential for the conversion of creatin into creatinin or into some as yet unrecognised substance or substances be true. Thus Frank and Isaac (1911)[49], by the administration of phosphorus to rabbits, produced a distinct hypoglycæmia along with marked liver necrosis.

The effect of hydrazine sulphate on the percentage of sugar in the blood has been investigated by Underhill (1911)[50] both in dogs and rabbits. As the result of numerous experiments in dogs, he found that a dose of 50 mgrms. per

kilo. invariably produces an appreciable hypoglycæmia, although considerable variations in the degree of diminution of the sugar content occur.

His experiments with rabbits were not so conclusive. On the administration of hydrazine in doses of 50 mgrms. per kilo. a constant decrease in the sugar content was not obtained; for, although a hypoglycæmia of varying amount was found to occur in the majority of the experiments, yet in a certain proportion of the animals, the blood sugar remained unaltered. On the administration of 100 mgrms. per kilo. the animals died within 24 hours, and Underhill concludes "From this diversity of results it is obvious that the rabbit cannot be relied on to invariably exhibit hypoglycæmia after hydrazine injection".

No attempt, however, was made by the administration of intermediate doses to allow for the idiosyncrasy of individual rabbits, nor has there been any investigation of the dependent or associated factors of this hypoglycæmia. Accordingly, the object of the following experiments has been to try to determine experimentally whether this irregular occurrence of hypoglycæmia in rabbits depends upon the dose of hydrazine injected, and if it is in any way associated with the simultaneous occurrence of creatin in the urine.

Author's Experiments and Methods of Analysis.

The rabbits were kept in small cages somewhat similar to the metabolic cages mentioned in Part I. in connection with the experiments on dogs. From the cage a funnel-shaped frame descended, by means of which the urine was collected in a vessel containing 5c.c. of 5% H_2SO_4 to prevent alkaline changes in the urine. At first a difficulty was experienced in obtaining the urine creatin-free during the control days. This was in most instances surmounted, or at least so diminished that the amount of creatin still present was considered negligible; while in a few cases persistent creatinuria was found to be dependent on pregnancy, and the rabbits were accordingly discarded. No attempt was made to obtain the absolute quantitative amounts of creatin and creatinin excreted, partly owing to the difficulty of being able to completely empty the bladder of the animal at the end of each 24 hours, while the absolute figures have no direct bearing on the object of the present research. Thus, the figures in Table IV. represent the amounts of creatin and creatinin which would be present in a 100c.c. sample of each day's urine. Although wide divergencies in the daily figures are thereby obtained, the relationship of the excretion of creatin to total creatinin is unaltered.

Control estimations of the sugar content were made from 7 - 10 days previous to the injection of a 2.5%

solution of hydrazine sulphate. This was done without doing any injury to the vascular system of the animal, for after some practice no difficulty was experienced in obtaining from 20-30c.c. of blood from the auricular vein. The blood was dropped directly into a 50c.c. flask containing 5c.c. of a 2% solution of Pot.Oxalate, and by the addition of the requisite amount of distilled water from a burette to bringthe total amount of fluid up to 50c.c., the quantity of blood obtained was accurately determined. In two of the experiments recorded where the rabbits were comatose, it was found impossible to obtain blood from the auricular vein, and hence it was drawn directly from the jugular vein and heart. The sugar content was then estimated by the method of Allihn in terms of CuO.

Here I may mention that I had the opportunity of testing the accuracy of the technique in the case of the dog used in Experiment VII. Part I. which became comatose within 24 hours.after the injection of 90 mgrms. per kilo. of hydrazine. The dog was killed and from its jugular vein two quantities of blood 32.2c.c. and 60c.c., were obtained, the sugar content of which was found to be .064% and .068% respectively. The sugar content of the normal dog is constant within narrow limits and averages .1%, so that it is interesting to note that the above estimations corroborate the work of Underhill (50) who found

a sugar content of .05% in the blood of a dog one day after hydrazine injection of 100 mgrms. per kilo.

In the initial experiments, doses of 50 mgrms. per kilo. body weight were administered, and 48 hours after, blood was withdrawn as above mentioned for sugar estimation, while daily extimations of the urinary excretion of preformed creatinin and creatin were carried out.

If no symptoms were exhibited, and no creatinuria resulted, the same rabbit was injected again but with a larger dose in order to try to just exceed the tolerance of the animal's tissues to the poison. The varying doses used in each experiment are recorded in the protocols.

Since the animals, which showed any metabolic disturbance, refused all food after the injection, two experiments (X. and XI.) with the rabbits fasting for two days were carried out in order to eliminate the starvation factor from the results.

Table IV. (next page)

(The days of the control period in each experiment, during which an attempt was being made to obtain the urine creatin-free, are indicated by arabic numerals, while the Roman figures refer to the days following the injection of the hydrazine.)

TABLE IV.

Day.	Total Creatinin. mgrms in 100c.c. urine.	Preformed Creatinin.	Creatin as Creatinin.	Creatin / Total Creatinin.	Sugar content of blood.	Remarks.
	I. Rabbit A.-Weight 3.20 kilos.					
1.	127.4	127.4	-	-	.114%	
2.	101.5	98.3	3.2	3.1%		
	Injection of 50 mgrms. Hydrazine sulph. per kilo.					
II.	87.0	87.0	-	-	.108%	Animal showed no metabolic disturbance.
	II. Rabbit B.-Weight 2.73 kilos.					
1.	71.0	65.5	5.5	7.4%	.095%	
2.	107.7	90.0	17.7	16.4%		
3.	-	-	-	-		
4.	81.5	81.5	-	-		
	Injection of 50 mgrms. Hydrazine sulphate per kilo.					
I.	72.5	74.1	-	-		
II.	124.5	121.5	3.0	2.4%	.102%	Nothing abnormal was detected in the rabbit's condition, food being taken as usual.
III.	110.	115.0	-	-		
	III. Rabbit B. Weight 2.47 kilos.					
1.	126.5	126.5	-	-	.087%	
2.	107.5	98.5	9.0	8.3%		
3.	94.3	94.3	-	-		
	Injection of 75 mgrms Hydrazine sulphate per kilo					
I.	139.5	146.0	-	-		No metabolic disturbance noted
II.	124.5	126.5	-	-	.107%	
III.	85.7	82.6	3.1	3.6%		

Day.	Total Creatinin.	Preformed Creatinin.	Creatin as Creatinin.	Creatin / Total Creatinin.	Sugar content of Blood.	Remarks.
	mgrms in 100c.c.urine.					
IV. Rabbit C. Weight 1.77 kilos.						
I.	206.5	204.3	2.2	1.0%	.101%	
2.	87.3	87.3	-	-		
3.	132.1	134.8	-	-		
Injection of 75 mgrms. Hydrazine sulphate per kilo.						
I.	*142.1	135.0	7.1	5.0%	-	Rabbit died within 12 hours of injection. No sugar estimation.
V. Rabbit D. Weight 2.32 kilos.						
1.	165.0	139.0	26.0	15.7%	.098%	
2.	-	-	-	-	-	
3.	158.5	158.5	-	-		
4.	72.0	73.5	-	-		
5.	107.5	107.5	-	-		
Injection of 75 mgrms. Hydrazine sulphate per kilo.						
II.	158.5	62.8	95.7	60.3%	.047%	Rabbit showed considerable malaise, refusing all food etc. Blood obtained from jugular vein.

* Urine drawn from bladder - post-mortem.

Day.	Total Creatinin. mgrms. in 100c.c urine.	Preformed Creatinin.	Creatin as Creatinin.	% Creatin / Total Creatinin	Sugar content of blood.	Remarks.
VI. Rabbit A. Weight 2.82 kilos.						
1.	155.7	155.7	-	-	.098%	
2.	128.4	125.2	3.2	2.4%		
3.	99.8	101.2	-	-		
Injection of 75 mgrms. Hydrazine sulphate per kilo.						
II.	112.5	112.5	-	-	.110%	No ill effects produced.
III.	154.0	156.5	-	-		
VII. Rabbit A. Weight 2.96 kilos.						
1.	171.0	162.0	9.0	5.2%		
2.	-	-	-	-		
3.	62.5	64.5	-	-		
4.	149.0	144.0	5.0	3.3%		
Injection of 100 mgrms. Hydrazine sulphate per kilo.						
I.	217.0	135.0	82.0	37.8%	.150%	The rabbit from the 1st day after injection showed considerable malais Blood was obtained with difficulty from the auricular vein, only 19.7c.c. being obtained.
III.	279.0	208.0	71.0	25.4%		
VIII. Rabbit B. Weight 2.87 kilos.						
1.	159.6	148.7	10.9	6.8%		
2.	-	-	-	-		
3.	78. 3	78.3	-	-		
4.	122.1	122.1	-	-		
Injection of 90 mgrms. Hydrazine sulphate per kilo.						
II.	172.9	114.2	58.7	33.9%	.060%	The usual symptoms of metabolic disturbance. Rabbit recovered but not used for further experimts.
III.	96.3	68.4	27.9	20.9%		

Day.	Total Creatinin.	Preformed Creatinin.	Creatin as Creatinin.	% Creatin / Total Creatinin	Sugar content of blood.	Remarks.
	mgrms. in 100cc. urine					
IX. Rabbit F. Weight 1.70 kilos.						
1.	137.3	137.3	-	-	.090%	
2.	106.0	103.8	2.2	2.0%		
3.	143.4	145.2	-	-		
	Injection of 75 mgrms. Hydrazine sulphate per kilo.					
I.	43.6	39.1	4,5	10.3%	.094%	Rabbit found comatose 24 hours after injection. 45cc. urine were removed from bladder - acid in reaction. Blood obtained directly from the heart.
X. Rabbit G. Weight 2.14 kilos.						
1.	66.5	63.5	3.0	4.5%	.104%	
2.	130.0	130.0	-	-		
3.	-	-	-	-		
4.	106.5	102.6	3.9	3.6%		
	Fasting Experiment.					
II.	187.4	143.5	43.9	23.4%	.140%	After two days' starvation.
III.	124.2	101.6	22.6	18.2%		
XI. Rabbit K. Weight 1.91 kilos.						
1.	126.0	128.2	-	-	.092%	
2.	137.0	137.0	-	-		
	Fasting Experiment.					
II.	132.5	95.7	36.8	27.7%	.116%	After two days' starvation.
III.	87.2	59.6	27.6	31.6		

Day.	Total Creatinin. mgrms. in 100c.c. urine.	Preformed Creatinin.	Creatin as Creatinin.	Creatin / Total Creatinin %	Sugar content of blood.	Remarks.
	XII. Rabbit G. Weight 2.0 kilos.					
1.	124.5	115.5	9.0	7.2%	.095%	
2.	97.4	99.2	-	-		
3.	131.4	128.0	3.4	2.5%		
	Injection of 75 mgrms. Hydrazine sulphate per kilo.					
II.	92.8	58.8	34.0	36.6%		Rabbit showed usual symptoms of considerable malaise.
III.	128.5	95.0	33.5	26.1%	.071%	
	XIII. Rabbit M. Weight 2.54 kilos.					
4.	102.5	74.3	28.2	27.5%	.105%	Could not be obtained creatin free.
5.	144.5	135.0	9.5	6.5%		
6.	58.6	54.3	4.3	7.3%		
	Injection of 50 mgrms. Hydrazine Sulphate per kilo.					
II.	108.0	84.5	23.5	21.7%	.114%	Animal aborted after withdrawal of blood.
	XIV. Rabbit K. Weight 1.7 kilos.					
1.	-	-	-	-	.101%	
2.	134.0	136.0	-	-		
3.	98.4	98.4	-	-		
	Injection of 90 mgrms. Hydrazine sulphate per kilo.					
II.	89.4	56.4	33.0	37.0%	.042%	Rabbit found comatose morning of 2nd day, and 18.3c.c. blood removed from jugular vein. Urine obtained directly from bladder - acid in reaction.

SUMMARY OF RESULTS.

In the four experiments (I.II.III.VI.) where the hydrazine administered was insufficient to produce any obvious metabolic disturbance no creatin was excreted, while the sugar content of the blood, as compared with the control estimation previously made, was within normal limits, when due allowance has been made for experimental error.

Of the six experiments, where creatinuria followed hydrazine injection, four (V.VIII.XII.XIV.) showed a condition of hypoglycæmia; one (VII) a state of hyperglycæmia, while in the remaining one (IX) the sugar content remained unchanged. In this last experiment it may be noted that the sugar estimation was carried out on blood obtained directly from the heart 24 hours after the administration of the hydrazine; while the percentage $\frac{\text{creatin}}{\text{total creatinin}}$ was small when compared with the figures obtained in the "hypoglycæmia" experiments. Indeed it was not much higher than the percentage occasionally recorded during the control days.

In Experiment XIII. where the rabbit aborted after the withdrawal of the second quantity of blood, and where pregnancy had not been previously noticed, there is little difference between the two estimations although there is a distinct increase in the creatin percentage

of the total creatinin.

In the two fasting experiments, the rabbit of Experiment X showed quite a marked hyperglycæmia, while in XI. the sugar content of the blood showed a slight increase when compared with the control estimation. In both a marked creatinuria resulted.

In the remaining experiment (IV.), Rabbit C died within 12 hours. No blood was obtained, while the urine drawn from the bladder post-mortem showed a trace of creatin. This was the only rabbit which exhibited such toxic symptoms after a dose of 75 mgrms. per kilo.

DISCUSSION OF RESULTS.

The four experiments in which no alteration in the sugar content of the blood, and no creatinuria, followed the injection of hydrazine, are interesting in relation to the previous work done on the subject. Underhill[50], from his results, considers that rabbits are unsatisfactory for experimental work planned to furnish data regarding carbohydrate metabolism. From the wide differences in the effects which were produced in rabbits after the administration of the drug, he concludes that their unreliability is especially marked when the alterations

in the sugar content of the blood are under review. Thus he says "When hydrazine is administered in the doses indicated (50 mgrms. per kilo) it is clear that in the majority of cases the drug is capable of decreasing the sugar content of the blood, in some instances to a remarkable degree, in others only slightly, while a practically normal blood sugar content is maintained by a third group of individuals. From this diversity of results it is obvious that the rabbit cannot be relied upon to invariably exhibit hypoglycæmia after hydrazine introduction. Hence results of experiments planned to supply data of carbohydrate metabolism from the standpoint of hypoglycæmia may be of questionable value"

In the present series of experiments the control estimations, carried out previous to the administration of hydrazine, show a reasonably constant sugar content of the blood, the average being approximately 0.10 per cent, thus corroborating previous investigations; while the divergence in the sugar estimations after inoculation appears dependent on the differences in susceptibility of the rabbits to the amount of the drug injected. Thus Rabbit A. was used in Experiments I. and VI., and Rabbit B in II. and III., when 50 and 75 mgrms. per kilo. body weight were respectively administered with no resulting abnormal

symptomatic or urinary manifestations, while a dose of 75 mgrms. per kilo. had such a remarkably toxic effect on Rabbit C. that it died within 12 hours of injection. After an interval of approximately one month, a larger dose was administered, (100 mgrms. per kilo. to Rabbit A (Experiment VII.) and 90 mgrms. per kilo. to Rabbit B (Exhibit VIII) with the result that both showed a distinct alteration in the percentage of their blood-sugar, associated with the production of creatinuria. In all Underhill's experiments in which the blood-sugar content was estimated, there were administered only 50 mgrms. per kilo, a dose which, in the case of the rabbits used in the present research, was never found to produce any alteration in sugar percentage. In the two other experiments he records, in which a dose of 100 mgrms. per kilo. was used, the rabbits died within five and twenty-four hours respectively, no sugar estimation being carried out. Hence it would appear that the rabbit can be relied upon to exhibit a constant hypoglycæmia after the administration of the amount of hydrazine requisite for each individual animal.

In the six experiments in which distinct creatinuria followed hydrazine injection, and in which no obvious extraneous factors can be detected, the sugar estimations present unequivocal evidence in the direction of a well-

marked associated hypoglycæmia. If the relationship of creatin to the total creatinin is considered, it is seen that after hydrazine injection, the lowest percentage of creatin is 20.9, the highest being 60.3%; whereas during the three control days previous to inoculation, the highest figure recorded, even in the case of those rabbits which were not obtained creatin-free, is 7.2%. The only exception was Experiment II, in which on the third day previous to injection a percentage of 16.4 was recorded. However, before administration of the drug, no creatin was obtained, while after a dose of 50 mgrms. per kilo. the relationship of creatin to total creatinin remained within what was found to be normal limits. The $\frac{\text{creatin}}{\text{total creatinin}}$ ratio in the two fasting experiments ranged from 18.2% to 31.6% - figures quite in accordance with previous results.

As regards the sugar content of the blood in those four experiments where the occurrence of hypoglycæmia and creatinuria were associated, the control figures ranged from .087% - .101%, while the figures which pointed to a condition of hypoglycæmia lay between .042% and .071%, there being thus always a distinct decrease in sugar percentage beyond the limits of experimental error.

Of the two experiments in which creatinuria was not associated with the simultaneous occurrence of hypoglycæmia, no alteration in the sugar percentage figure was found in

Experiment IX.; while in Experiment VII. Rabbit A., in which two months previously no change in the sugar content had been produced by doses of half and three fourths of the amount administered in the present experiment, a marked hyperglycæmia resulted. To account for this contradictory result, no very satisfactory explanation can be offered. The rabbit certainly showed considerable malaise and symptomatic disturbance very shortly after receiving the injection. Considerable diffuculty was experienced in obtaining the requisite amount of blood from the auricular vein, and this may have been partly due to the less fluid character of the blood. This possibility would in itself tend to raise the percentage figures of the sugar content; and in this connection it may also be mentioned that owing to a similar difficulty in obtaining sufficient blood from the veins in Experiment IX., it was drawn directly from the heart.

Thus we may safely conclude that after the administration of the optimum dose of hydrazine for the production of an alteration of the sugar content, a hypoglycæmia results which is invariably associated with creatinuria; while if no change in the sugar content is produced, creatin is not found in the urine. It has also to be noted that only the rabbits which showed this combined hypoglycæmia and creatinuria exhibited any

marked symptoms of metabolic disturbance, the animals being quite dull and listless and refusing all food and drink.

Of the factors which may lead to this alteration from normal metabolism, that of starvation can be eliminated on considering the results of the two fasting experiments (X.and XI). Neither of the rabbits showed any reduction in the percentage of blood sugar. On the contrary, Experiment XI. showed a slight increase, while in X. a distinct hyperglycæmia resulted. In both cases, as already mentioned, there was a marked excretion of creatin.

Thus, although the results of the experiments here recorded, in combination with the conclusions of Part I., all point to the important rôle played by carbohydrates in the metabolism of creatin, yet the exact nature of this rôle, or even its degree of importance is still uncertain. It has been shown that the creatinuria following hydrazine injection is accompanied by a condition of hypoglycæmia, while it is already known that the creatinuria of diabetes and phloridzin poisoning is associated with hyperglycæmia. * On the other hand, no

* Graham and Poulton in a recent communication (51) suggest that an important fallacy underlies the supposed creatinuria occurring in diabetes and in conditions associated with acidosis, owing to the presence of acetone or diacetic acid which tends to lower the value of the preformed creatinin estimations. However, in some unpublished estimations in which this error has been eliminated, a distinct excretion of creatin is still found to occur. It may be noted that this criticism does not apply to the creatinuria obtained in dogs in the experiments of Part I., since, as already mentioned, no acetone or diacetic acid were ever detected in the urine.

such constant change in the sugar content of the blood is found to accompany the creatinuria induced by fasting. Furthermore, some experiments by Underhill and Rand (1910)[52] show that "The perverted creatin metabolism as well as the metabolism of the other nitrogenous constituents of the urine tends rapidly to resume the normal on the rectal administration of dextrose, without necessarily exerting any influence on the pathological condition of the patient".

Nor do the various attempted explanations of the influence of carbohydrates on the excretion of creatin carry us very far. There is no definite evidence in favour of the hypothesis that they are essential for the conversion of creatin into creatinin or other as yet unknown substances, or for its oxidation and excretion as urea. No deductions in this connection can be drawn from the results of Part I, because the creatinuria of those experiments was associated with an increased excretion of urea. Besides, as has been already mentioned, so many factors enter into urea excretion, that it is impossible to eliminate them satisfactorily; and analogies drawn from "in vitro" experiments to the changes occurring "in vivo" are, in regard to the present problem, essentially untrustworthy.

That an adequacy of carbohydrates inhibits muscle

disintegration and consequently prevents the excretion of creatin, is merely a special phase of the more general hypothesis suggested by Mendel and Rose[53] that "the tissue cells may not functionate properly when the normal amount of carbohydrate food is wanting, and in this case the elimination of creatin would be analogous to the production of the acetone bodies which is also inhibited by the administration of carbohydrates". Again, Krause (1913)[54] supports the idea that creatin may be an end product of metabolism, arguing that creatinuria may sometimes be not so much a deviation from normal metabolism but rather an indication that our conceptions of normal metabolism, based as they have been on ~~the~~ somewhat limited observations, may not be comprehensive enough. Recent work on the creatinuria of childhood[55,56], and on its irregular occurrence during the menstrual cycle of normally healthy women[57,58] supports this plea.

A consideration of those various assumptions in conjunction with an attempt to properly appreciate the reason for the divergences in the sugar content of the blood in those various conditions which are associated with the excretion of creatin suggests that an effort must be made to trace the stage or stages farther back in the metabolism of carbohydrates and creatin, before we shall be able to form any chemical picture of their

relationships. In this connection it is interesting to note that Underhill and Fine (1911)[59] found that in dogs no glycosuria results after the removal of the pancreas if the animals have previously received injections of hydrazine. Accordingly they have put forward the tentative hypothesis that hydrazine acts by its inhibition or suppression of the function of the supra-renals. No definite evidence in favour of this theory is produced, while they admit that the hypoglycæmia induced by hydrazine might equally well result from an increase in the output, or of the efficiency of the pancreatic secretion. However, although this explanation is purely theoretical, it signifies a realisation of the fact that the importance of the carbohydrate rôle in creatin metabolism is undoubted, but that it is itself dependent on some obscure process or processes, the mystery of which has yet to be unravelled. In short, all we can at present say is that carbohydrate metabolism is a very delicately poised mechanism, one of the earliest manifestations of any disturbance of which being the excretion of creatin. Indeed, so sensitive is this metabolism of creatin to the least disturbance of carbohydrate equilibrium that its urinary excretion occurs before a slight exaggeration of an otherwise normal physiological process has become a definite pathological phenomenon.

CONCLUSIONS.

1. After hydrazine injection in rabbits, if no alteration in the sugar content of the blood occurs, no creatinuria results; while the excretion of creatin is associated with a condition of hypoglycæmia.

2. This creatinuria is not dependent on the starvation factor per se, for in the two fasting experiments, the excretion of creatin was associated with an increased sugar content of the blood.

3. Creatinuria appears to be produced on the least disturbance of the equilibrium of carbohydrate metabolism of which it is one of the earliest and most sensitive manifestations.

4. The exact nature of the rôle played by carbohydrates in relation to creatin metabolism is still obscure, a short summary of the various hypotheses put forward being given. All the evidence suggests the necessity of tracing farther back the stage or stages in the metabolism of creatin and carbohydrates before any chemical picture of their relationships can be formed.

GENERAL CONCLUSIONS OF THESIS.

From the experiments of Part I. we have seen that -

(1) The estimation of any one element of the nitrogen partition of the urine is useless as a guide to hepatic efficiency; their inter-relationships have to be considered.

(2) The most constant feature of the urinary nitrogen distribution during the hepatic disturbance produced by hydrazine is the increase in amino-acid nitrogen both absolutely and relatively to the total nitrogen.

It has also been noted that this increase is invariably the earliest abnormality to be detected, so that, although the tendency of recent work points to the amino-acid fraction not being of great importance from the point of view of general metabolism, yet, as a possible test of liver inefficiency, as borne out by the results of the present experiments, it may be of considerable value.

(3) The increased excretion of total nitrogen following hydrazine injection, and as found also in phosphorus poisoning, is most probably due to the disturbance of glycogenic function with the subsequent direct or indirect disintegration of muscle protein.

(4) The excretion of creatinin is found to be practically constant; while there is a large excretion of creatin, which appears to be dependent on impaired glycogenic function exerting its influence on the muscle metabolism, rather than on liver inefficiency leading to a decrease in the conversion of creatin into creatinin. This seems to be indicated by the increased katabolism of "muscle flesh" (Table II.) as calculated from the output of total creatinin.

This conclusion, when considered along with our discussion of the results of other workers, suggests that the claim appears unjustified that the clinical estimation of the urinary excretion of creatin may be of significance in disturbances of hepatic function.

In Part II. we have seen that by the use of a method which had given excellent results in the extraction of bacterial enzymes and by means of which active diastatic and lipolytic ferments were obtained in the course of the present experiments, no ferment bringing about the conversion of creatin into creatinin or the destruction of creatin into known or unknown substances was detected, so long as bacterial action was excluded.

This conclusion, while corroborating the earlier findings of Mellanby, is in accordance with the results of Part III which suggest a purely chemical relationship of creatin and creatinin to carbohydrate metabolism rather than an enzymatic

mode of formation.

The main feature of the experiments of Part III. has been the demonstration of the close relationship between the creatinuria following the injection of hydrazine in rabbits and the coexistent hypoglycæmia. We have seen that if no alteration in the sugar content of the blood occurs, no creatinuria results, while the excretion of creatin is associated with a condition of hypoglycæmia. From these results we have eliminated the starvation factor.

We have also seen from a consideration of the previous work done on the subject as well as from a discussion of the various explanatory hypotheses put forward, that although the appearance of creatin in the urine is one of the earliest manifestations of the least disturbance of the equilibrium of carbohydrate metabolism, yet the exact nature of their relationship is still obscure.

All the evidence suggests that, while the importance of the carbohydrate rôle in creatin metabolism is undoubted, it is itself dependent on some obscure process or processes, the nature of which will probably be elucidated when we are able to trace the still earlier stage or stages in the metabolism of creatin and carbohydrates.

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BIBLIOGRAPHY.

1. Mellanby. — Journ. Physiol., London, 1908, vol.XXXVI, p.447.

2. Van Hoogenhuyze und Verploegh. — Ztschr. f. phys. Chem., Strassburg, 1908, Bd. LVII. S.161.

3. Frey. — Ztschr. f. klin. Med., Berlin,1911, Bd. LXXII, S. 383.

4. Von Noorden. — "Pathology of Metabolism", 1907, vol.II.,p.278.

5. Weintraud. — Exper. Archiv., 1893, Bd.XXXI. S.30.

6. Williams. — Johns Hopkins Hosp. Bull., Baltimore, 1906, vol. XVII. p.71.

7. Wolf. — New York Med.Journ., 1906, vol.LXXXIII. p.813.

8. Ewing. — Arch. Int. Med., 1908, vol.II., p.330.

9. Salaskin und Zaleski. — Ztschr. f. phys. Chem. Strassburg, 1908, Bd.XXXV., S.246.

10. Pearce and Jackson. — Journ. Exper. Med., Baltimore, 1907, vol.IX., p.534.

11. Frerichs und Städeler. — Deutsche Klin., Berlin, 1858, No.31.

12. Sorensen. — Biochem. Ztschr., 1908, Bd.VII., S.45.

13. Glaessner. — Ztschr. f. Path. u. Therap.,1907, Bd.IV., S.336.

14. Damask. — Ztschr. f. klin. Med., Berlin,1913, Bd. LXXVII. S.333.

15. Clark. — Proc. Roy. Soc. Edin., 1908-1909, vol.XXIX. p.418.

16. Lindsay. — Biochem. Journ., 1911, vol.V., p.407.

17. H.Gideon Wells. — Journ. Exper. Med., Baltimore, 1908, vol.X., p.457.

18. Borissow.	Ztschr. f. phys. Chem., Strassburg, 1894, Bd.XIX. S.499.
19. Poduschka.	Arch. f. exper. Path.u.Pharmakol., Leipzig, 1900, Bd.XLIV. S.59.
20. Pohl.	Ibid., 1902, Bd.XLVIII. S.367.
21. Under_hill and Kleiner.	Journ. Biol. Chem., 1909, vol.IV. p.169.
22. Lindsay.	Biochem. Journ., 1909, vol.IV. p.448.
23. Malfatti.	Ztschr. f. phys. Chem., Strassburg, 1909, Bd.LXI. S.499.
24. Henriques.	Ibid., 1909, Bd.LX. S.I.
25. Malfatti.	Ibid., 1910, Bd.LXVI. S.152.
26. Mendel and Rose.	Journ. Biol. Chem., 1911, vol.X. p.246.
27. Noël Paton.	Journ. Phys., London, 1905, vol.XXXIII, p.I.
28. Cathcart.	Ibid., 1909, vol.XXXIX. p.311.
29. Underhill.	Journ. Biol. Chem., 1911, vol.X. p.159.
30. Salkowski.	Berl. klin. Wchnschr., 1905, Bd.XLII, S.1581.
31. Van Hoogenhuyze und Verploegh.	Ztschr. f. phys. Chem., Strassburg, 1905, Bd.XLVI. S.415.
32. Shaffer.	Amer. Journ. Phys., 1908.vol.XXIII. p.I.
33. Findlay.	Quart. Journ. Med., 1912, vol.V. p.495.
34. Pekelharing.	Ztschr. f. phys. Chem. Strassburg, 1911, Bd.LXXV. S.207.
35. Spriggs.	Quart. Journ. Med., 1907, vol.I. p.63.
36. Towles and Voegtlin.	Journ. Biol. Chem., 1912, vol.X. p.479.
37. Noël Paton and Mackie.	Journ. Physiol., London, 1912, vol.XLV. p.115.

38. Krause and Cramer. Ibid., Proc.Phys.Soc.,1910, vol.XL. p.LXI.

39. Mellanby. Proc.Roy.Soc.,London, 1913, vol.LXXXVI. p.88.

40. Bar. L'Obstétrique, 1910, vol.III. p.506.

41. Noël Paton. Journ. Physiol., London, 1910, vol.XXXIX. p.498.

42. Seemann. Ztschr. f. Biol., 1907, Bd.XLIX. S.333.

43. Gottlieb und Stangassinger. Ztschr. f. phys.Chem., 1907, Bd.LII. S.I.

44. Mellanby. Journ. Physiol., London, 1908, vol.XXXVI. p.462.

45. Mellanby and Twort. Journ. Physiol., 1912, vol.XLIV. p.43.

46. Rothmann. Ztschr.f.phys.Chem., 1908 /Bd.LVII. S.131.

47. Cathcart und Hahn. Archiv.f.Hygiene,1902, Bd.XLIV. S.295.

48. Pekelharing und van Hoogenhuyze. Ztschr.f.phys.Chem., 1910, Bd.LXIX. S.395.

49. Frank und Isaac. Archiv.f.exper.Path.u.Pharmakol,1911, Bd.LXIV. S.274.

50. Underhill. Journ.Biol.Chem.1911, Vol.X. p.159.

51. Graham and Poulton. Journ. Phys. 1913, vol.XLVI, p.XLIV.

52. Underhill and Rand. Archiv. Inter. Med. 1910, vol.V.p.61.

53. Mendel and Rose. Journ. Biol. Chem., 1911, Vol.X. p.213.

54. Krause. Quart.Journ.Exper.Physiol., 1913, vol.VII. p.87.

55. Rose. Journ.Biol.Chem., 1911, Vol.X.. p.265.

56. Folin and Denis. Journ. Biol.Chem., 1913. Vol.XI. p.253.

57. Krause and Cramer. Journ. Physiol. 1911, vol.XLII.. Proc. Phys. Soc., p.XXXIV.

58. MacAdam. Practitioner, 1914, vol.XCII. p.540.

59. Underhill and Fine. Journ. Biol. Chem. 1911, vol.X. p.270.