XXX. THE DISTRIBUTION OF NITROGEN AND SULPHUR IN THE URINE DURING CONDITIONS OF INCREASED CATABOLISM.

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As the result of a series of analyses of the urine of normal subjects on standard diets of varying nitrogen and sulphur content but practically free of purine, creatine and creatinine, Folin [1905] advanced the hypothesis that there are two essentially different forms of protein catabolism, the constant or endogenous catabolism yielding chiefly creatinine and neutral sulphur and to a less extent uric acid and ethereal sulphates, and the variable catabolism yielding chiefly urea and inorganic sulphates. Folin stated that "the more the total catabolism is reduced the more prominent become these representatives of the constant catabolism, and the less prominent become the two representatives of the variable catabolism."

Whilst Folin's general hypothesis has been accepted, doubt has been expressed as to whether these various excretory products can be exactly divided into two such groups.

More recent work has demonstrated the difficulty of correlating the creatinine output with the total tissue or endogenous metabolism. Shaffer [1907; 1908, 2], Spriggs [1907, 1, 2], Paton [1910] and Benedict and Osterberg [1923] consider that creatinine is an index of only one phase of this tissue metabolism—that which is peculiarly related to muscle. Spriggs also concluded from his observations that the bulk of the endogenous creatinine output is "a product of the internal structural metabolism of muscle and not of its contraction." Some 30 years ago Sivéń [1901] and Burian and Schur [1901] showed severally that uric acid is to a large extent independent of the total amount of nitrogen eliminated. Folin states that while uric acid does not exhibit the quantitative constancy that is found in the case of creatinine at least a certain part of it represents the breaking down of living protoplasm which must be supposed to be essential to the continuation of life. The reduction in the uric acid output when the total metabolism of protein is reduced is irregular and different for different individuals. Pohl [1917] has more recently suggested that uric acid might be included as a principal endogenous catabolite.

Practically all experiments bearing on this aspect of metabolism have been concerned with studying the effect of different levels of food intake on the partition of the nitrogen- and sulphur-containing catabolites of the urine. Starvation may be taken as an extreme example of this type of experiment. Benedict [1907] found that preformed creatinine and neutral sulphur decreased persistently during fasting. The interesting point arises however as to the effect of increased catabolism on the distribution of the nitrogen and sulphur of the urine where the food intake is kept constant. The main causes generally accepted for such increased protein catabolism are increased muscular work, disuse of muscle, injuries to and toxic conditions of the tissues including febrile states, exophthalmic goitre and various other wasting diseases.

Gregor [1900] concluded that hard physical exercise produces a decided increase in creatinine elimination particularly on the day following the exercise. Campbell and Webster [1921, 1922] and Rakestraw [1921] have also noted an increased output.

While Shaffer [1908, 1] found that muscular work had no influence on the excretion of uric acid, Kocher [1914], Rakestraw [1921] and Levine *et al.* [1924], have since found that it caused a slight increase. Others like Dunlop *et al.* [1897], Campbell and Webster [1921, 1922] and Hartmann [1924] however record a decrease.

While Shaffer [1908, 1] and Campbell and Webster [1921, 1922] have found that work exercises but little influence on the output of ammonia, Dunlop *et al.* [1897] have observed a rise, and Kocher [1914] a definite reduction (nitrogen-free diet) in the output of this catabolite.

In more recent carefully controlled experiments on the human subject, Cathcart and Burnett [1926] have detected a slight but constant rise in the urinary excretion of nitrogen and sulphur after work. This was accompanied by a slight uniform rise in the excretion of ammonia, creatinine and urea, although the latter was not directly analysed. Dunlop *et al.* [1897], Shaffer [1908, 1], Kocher [1914] and Campbell and Webster [1921, 1922] have also found that for the most part the definite rise in the nitrogen after work is in the form of urea.

The present writer [Cuthbertson, 1929], found that subjects in nitrogenous equilibrium showed within a day or two from the commencement of a period of rest a rise in the excretion of sulphur, nitrogen, phosphorus and calcium. The S:N ratio suggested a sulphur-rich source of the excreted material. It was found that the rise in the excretion of sulphur was due to a slightly greater proportionate increase in the inorganic sulphate fraction. Ethereal sulphates tended to decrease; neutral sulphur remained more constant. The rise in the excretion of total nitrogen was mainly due to a proportionate increase in the amount of urea. Ammonia tended also to rise proportionately while the absolute amounts of creatinine and uric acid remained practically unaltered. A very slight rise in the excretion of creatinine was sometimes noted, particularly as the period of enforced rest lengthened.

In various febrile conditions, it has been found that on the average 82 % of the total nitrogen in the urine is in the form of urea [von Noorden, 1907]. Ammonia excretion is also increased considerably, but the percentage increase is generally not greater than the percentage increase in the total nitrogen. The endogenous output of uric acid is also increased, the increase coinciding and terminating with the fever [Cathcart, Kennaway and Leathes, 1908]. Although the output of creatinine is almost always increased in febrile conditions the rise is not proportional to the increased elimination of total nitrogen [Leathes, 1906; Shaffer, 1908, 2]. Leathes [1906] for example found that while there was a 50 % increase in the total nitrogen, there was only a 20 % increase in the creatinine nitrogen in one particular case. In the case of uric acid the proportion excreted was considerably increased.

Sandiford *et al.* [1926] found that the injection of thyroxine on the 30th day of a protein-free diet in the human subject produced a subsequent rise in the excretion of nitrogen mainly in the form of urea, creatinine and uric acid remaining constant. Shaffer [1907 and 1908, 2] had many years ago noted that the amount of creatinine excreted in exophthalmic goitre was very low, despite the increased tissue catabolism.

The purpose of the short foregoing review has been to demonstrate that in conditions where the nitrogen and sulphur reserves of the body are being catabolised to any great extent and where there is little or no change in the food intake the main increase in the urinary excretion is accounted for as urea. The proportion of this catabolite eliminated may even be slightly greater. Creatinine on the other hand exhibits practically no change in the absolute amount excreted. Occasionally when the increased catabolism is at a high level this substance may be excreted in slightly greater amount, but it is never increased proportionately. The excretion of uric acid, while not so constant as that of creatinine, is relatively stable, such fluctuations as occur are usually in the form of a rise. It may be that there is a special metabolism of nucleoprotein. Ammonia generally tends to rise with the increased catabolic level.

Concerning sulphur not so much is known. It may be generally stated that a rise in total sulphur is due to a practically proportionate increase in the inorganic sulphates. Neutral sulphur remains practically constant. The ethereal sulphates may diminish.

Such findings as have been described appear in agreement with the laws governing the composition of the urine as enunciated by Folin [1905].

More recently the present author [Cuthbertson, 1930] has investigated, in part, the effect of tissue injury on metabolism. It was found that the urinary excretion of sulphur, nitrogen and phosphorus rose rapidly to a maximum generally within 3 to 6 days from the time of injury. The S:N ratio suggested that some sulphur-rich tissue, such as muscle, might be the main source of the material catabolised. The partitions of the nitrogen and sulphur were not determined at that time. They are amplified in the present investigation.

Table I. *The partition of nitrogen and sulphur in the urine of patients at rest in bed during their recovery from fractures of bone or minor operations.*

Case	Total daily excretion of nitrogen g.	Daily excretion and (partition) of nitrogen					Total daily intake of N g.	Daily excretion and (partition) of sulphur (SO ₃)			Remarks		
		Urea	Ammonia	Amino-acid	Total creatinine	Pre-formed creatinine		Uric acid	Total	Inorganic		Ethereal	Neutral
1	32.06*	26.09 (81.4)	1.56 (4.9)	1.14 (3.5)	1.29 (4.0)	—	0.195 (0.6)	11.70	4.96 (83.1)	0.24 (4.8)	0.60 (12.1)	Fourth day following a fracture of both bones of one leg of a boy aged 16 years. Average daily output of faecal nitrogen 1.51 g.	
2	9.29	7.69 (82.8)	0.20 (2.2)	0.06 (0.7)	0.51 (5.5)	0.45 (4.8)	0.18 (1.9)	9.32	1.73 (72.5)	0.155 (9.0)	0.32 (18.5)	Prior to operative removal of torn cartilage from knee joint of man. Third day after operation	
	21.31*	17.23 (80.9)	1.12 (5.2)	0.315 (1.5)	0.52 (2.4)	0.33 (1.5)	0.15 (0.70)	9.32	3.66 (83.1)	0.26 (7.1)	0.36 (9.8)		
3	7.66	6.41 (83.7)	0.35 (4.6)	0.04 (0.5)	0.40 (5.2)	0.39 (5.1)	0.09 (1.2)	7.06	1.79 (74.9)	0.09 (5.0)	0.36 (20.1)	Fourth day after fracture of both bones of one leg of a man. Seventh day after	
	12.70*	11.44 (81.0)	0.28 (2.2)	0.03 (0.2)	0.42 (3.3)	0.41 (3.2)	0.18 (1.4)	7.06	2.30 (77.8)	0.14 (6.1)	0.37 (16.1)		
4	6.83	5.63 (82.5)	0.42 (6.1)	0.06 (0.9)	0.50 (7.3)	0.50 (7.3)	0.13 (1.9)	7.78	1.66 (55.2)	0.175 (10.5)	0.57 (34.2)	Prior to operative removal of torn cartilage from knee joint of man. Third day after operation	
	16.06*	11.96 (74.5)	0.72 (4.5)	0.09 (0.6)	0.50 (3.1)	0.47 (2.9)	0.22 (1.3)	7.78	2.51 (73.7)	0.16 (6.4)	0.50 (19.9)		
5	16.90*	13.49 (79.8)	0.495 (2.9)	—	0.47 (2.8)	0.475 (2.5)	0.14 (0.83)	8.36	2.57 (84.05)	0.17 (6.6)	0.24 (9.35)	Eighth day after fracture of the femur of a man	
6	10.08	8.51 (84.4)	0.515 (5.1)	0.03 (0.3)	0.58 (5.7)	0.55 (5.4)	0.10 (1.0)	12.67	2.14 (77.1)	—	—	Third day after a dislocation of the ankle of a man. Sixth day after	
	19.74*	17.24 (87.3)	0.45 (2.3)	0.22 (1.1)	0.70 (3.5)	0.70 (3.5)	0.23 (1.2)	12.67	3.29 (86.6)	—	—		
7	13.33	9.85 (73.8)	0.90 (6.8)	0.16 (1.2)	0.57 (4.3)	0.57 (4.3)	0.17 (1.3)	10.77	2.79 (80.0)	0.15 (5.3)	0.41 (14.7)	Third day after fracture of tibia of a man. Sixth day after	
	20.58*	18.45 (89.6)	0.66 (3.2)	0.31 (1.5)	0.68 (3.3)	0.625 (3.0)	0.26 (1.2)	10.77	3.81 (80.6)	0.19 (5.0)	0.55 (14.4)		
8	11.86	8.73 (73.6)	0.51 (4.3)	—	0.41 (3.5)	0.40 (3.4)	0.23 (1.9)	12.10	2.06 (74.7)	0.15 (7.3)	0.37 (18.0)	Prior to bone-grafting operation for ununited fracture of humerus of 6 years standing in a man. Fourth day after operation	
	17.53*	15.05 (85.8)	0.91 (5.2)	—	0.63 (3.7)	0.36 (2.0)	0.25 (1.5)	12.10	2.97 (85.9)	0.09 (3.0)	0.33 (11.1)		

* Maximum daily excretion of nitrogen.

The excretion of urinary nitrogen was determined daily throughout the periods of observation, but these values are omitted except on the days on which the distribution was detailed.

Methods. Total N, Kjeldahl; urea, urease; ammonia, aeration; creatine and creatinine, Folin; amino-acids, Sorenson; uric acid, Hopkins; total sulphur, Denis; inorganic and ethereal sulphates, Folin.

The figures for excretion are g.; those for partition %.

EXPERIMENTAL.

The subjects were patients at rest in bed during recovery from fractures of bone or minor operations and generally within 1 or 2 days following the injury were put on a constant intake of food, the quality and quantity of which they chose from the following list: milk, brown and white bread, digestive biscuits, butter, eggs (with the exception of case 5), tea (2 tea-spoonsfull infused daily: no tea in cases 5, 7 and 8), steak (except in cases 3, 4, 5, 7 and 8), potatoes, lettuce, apple and orange. As they had been on a normal diet prior to admission, it was thought that the nitrogenous intake would be closer to this normal if a meat-containing diet was consumed. Subsequently meat-free diets were selected in order to assess the endogenous metabolism. The analytical results are summarised in Table I.

Nitrogen.

Total nitrogen. The maximum total daily output of urinary nitrogen of this series occurs between the 3rd and 8th days following the injury. There does not appear to be any definite relationship between the time interval for the urinary excretion of nitrogen to reach a maximum and the total amount passed on that particular day. The maximum daily loss may even exceed 20 g. (Case 1).

Urea. There is an absolute increase in the amount of urea, which increase is practically proportional to the raised urinary output of total nitrogen (81–82 %).

Ammonia. The absolute amount of ammonia tends to vary but there is generally a relative diminution in the proportion of the total nitrogen excreted in this form.

Amino-acids. There is generally a rise in the absolute amount passed (as measured by the nitrogen content) and this rise is practically proportional to the rise in total nitrogen.

Creatinine (preformed). Except for slight increases in cases 6 and 7 there is little or no alteration in the absolute amount passed.

Uric acid. There is generally an absolute rise in the excretion of uric acid, but this rise may or may not be proportional to the increased output of total nitrogen.

Creatine. A creatinuria with a normal (or even supernormal) output of creatinine has been noted following amputations of limbs [Bürger, 1919; Cameron and Gibson, 1922], and also sometimes after fractures, particularly of the thigh or leg bones. Hirst and Imrie [1928] found that in such fractures the amount varied from traces to over 500 mg. daily. It disappeared gradually during the healing. Administration of thyroid increased the output threefold.

In cases 2, 4 and 8 only was a pre-injury period available. Case 2 was on a meat diet and cases 4 and 8 on a non-meat diet. It is seen that whereas in cases 2 and 4 the total creatinine was constant during the pre-operative

and post-operative periods there was in case 2 in the post-operative period of maximum catabolism a slight increase in the creatine fraction and in case 4 there was only a trace of creatine on the 3rd day following the operation. Prior to operation case 8 had no creatinuria, but on the day after operation there was a very definite amount present, the creatinine fraction being slightly diminished. Case 3, who was also on a meat-free diet, showed no change in the total creatinine excretion nor was any creatine present. No creatinuria was found at the height of catabolism in case 6 although on the 3rd day after admission there was a trace present. This case had a dislocated ankle but there was no fracture or surface wound present. In case 7 no creatine was present shortly after admission but on the day of the appearance of the maximum excretion of nitrogen a trace was found. It appears then that a temporary creatinuria may follow injury. It may be that this is due to an interference with the normal transformation of creatine into creatinine, such as appears to occur in conditions of heightened catabolism.

Proteinuria. On the 3rd day following injury in case 1 there was a faint trace of coagulable protein; on the 4th day there was a definite trace (day of appearance of maximum nitrogen excretion); on the 5th day a faint trace, on the 6th day a very faint trace, thereafter it was absent. In case 4 a trace was noted on the 2nd day following operation only. No evidence of a proteinuria was noted in the other cases described here, although unsystematised testing had often revealed faint traces in cases of recent fracture admitted to hospital.

Sulphur.

In a previous investigation on the disturbance of metabolism produced by injury, it was found that the maximum daily output of sulphur practically always ran parallel with that of nitrogen. As the result of this finding day-to-day determinations of the total nitrogen only were performed and when the maximum excretion was noted the partitions both of nitrogen and of sulphur were determined on that day and also on an earlier date, *i.e.* prior to the appearance of the obvious increase in catabolism. A marked increase in the excretion of sulphur was again noted to follow injury.

Inorganic sulphate. Not only is there an absolute increase in the amount of inorganic sulphate excreted but the percentage of sulphur excreted in this form is greater.

Ethereal sulphate. While there is generally a rise in the excretion of this fraction during periods of increased catabolism, this rise is not proportional to the increase in catabolism as measured by the increase in total sulphur.

Neutral sulphur. There is not quite the same constancy about this fraction as there is about creatinine, but in only one case (7) was there really a pronounced alteration in the amount eliminated as the total sulphur excretion increased. It is of interest that it was in this particular case that a marked increase in the creatinine excretion was noted. As a consequence of this

relative constancy there is a decrease, during periods of heightened catabolism, of that fraction of the total sulphur represented by neutral sulphur.

DISCUSSION.

While these experiments are totally different in kind from those devised by Folin, yet they have an intimate bearing on the two conceptions of metabolism which he describes. It is obvious in the first place that the source of these nitrogen and sulphur losses cannot be the food intake. They must come from the increased catabolic changes in the tissue cells and body fluids. It must also be remembered that it is really only one phase of the total catabolism which has been examined, namely that particularly related to protein.

As the result of the foregoing analyses, there appear to be two distinct forms of metabolism, one constant the other variable. The constant element may exhibit small fluctuations, for example, it may increase with any general increase in catabolism. It is obvious that whilst creatinine and neutral sulphur do chiefly represent the constant catabolism they are not the main representatives of the endogenous catabolism. Urea and inorganic sulphate are the chief products of the increased endogenous catabolism. It seems necessary therefore to dissociate Folin's terms "constant" and "endogenous", "variable" and "exogenous" when describing these and other allied conditions of increased catabolism, for fluctuations in both endogenous and exogenous metabolism are mainly characterised by fluctuations in the variable group of catabolites.

The work of Morpurgo [1897], Greene [1919], Mandel [1928] and others suggests that muscle has a very considerable capacity either for storage or depletion of protein without seriously damaging the life of the individual cell.

It is suggested here that the disintegration and integration of muscle cells through wear and tear and indeed of the body generally, may be peculiarly represented by creatinine and perhaps by neutral sulphur. This does not mean that they are the sole nitrogen- and sulphur-containing catabolites of this form of metabolism. Fluctuations in the urea and inorganic sulphate fractions, although they represent mainly variations in the improvement or impoverishment quotas, may also be an index of changes in the processes of wear and tear. Ammonia is rather an index of the acid-base balance and as such is subject to pronounced variations. Alterations in the excretion of amino-acids and ethereal sulphates are difficult to interpret. Uric acid, although it appears to fluctuate to some extent with the general increase in the total nitrogen excretion, may also be taken to be an index of wear and tear. Possibly it is a catabolic product of the nuclear part of the cell.

The question arises as to the cause or causes of these various catabolic phenomena. Wasting of tissue, particularly of muscle, may be due to an inadequate supply of normal stimuli, or to an excessive number of abnormal stimuli. It may be due to direct poisoning of the tissue cells such as might be supposed to take place in febrile conditions and in tissue injury. The wasting

on the other hand may represent the response to the demand for a non-nitrogenous residue for energy purposes, or it may be part of the mechanism for the rapid production of repair material to heal an injured part. In the present state of our knowledge it is impossible to define the relative significance of these processes.

SUMMARY.

1. The present series of experiments provides additional evidence of the early catabolic loss of nitrogen and sulphur which results from tissue injury. The rate of loss reached a maximum about the 3rd to 8th day following the injury. The maximum daily loss of nitrogen might even exceed 20 g.

2. The partition of the nitrogen- and sulphur-containing catabolites demonstrated that the increase in nitrogen was due to a practically proportionate increase in the urea excreted, and that the increase in sulphur was due to a slightly greater proportionate increase in the excretion of inorganic sulphates.

While the excretion of ammonia fluctuated, that of amino-acids and uric acid generally rose. Ethereal sulphate tended to diminish slightly. Creatinine and neutral sulphur remained practically constant, any increase being in no way proportional to the total increase of nitrogen or sulphur respectively.

3. Definite traces of creatine were observed particularly during phases of increased catabolism.

4. Traces of heat-coagulable protein were occasionally found, particularly during the time of the maximum excretion of the nitrogenous catabolites.

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