# LXXVIII. THE INFLUENCE OF FAT AND CARBOHYDRATE ON THE NITROGEN DISTRIBUTION IN THE URINE.

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In previous papers it has been shown that the complete withdrawal of carbohydrate from the diet produces well marked changes in the metabolism. Landergren [1903] in his original paper showed clearly that (1) on an exclusively carbohydrate diet the output of total nitrogen steadily fell and (2) when the diet was changed to one composed exclusively of fat the output of total nitrogen rose. This finding I confirmed and extended [1909]. The series of experiments detailed in the present paper were intended to extend further our knowledge of the subject. They were all carried out previous to July 1914 on Mr R. Lang and it was intended to complete the series with the effects of such limited diets on the capacity of the individual to do muscular work, but unfortunately Mr Lang on his demobilisation was no longer available and so far no other subject, either willing or suitable to undergo the necessary privation, has been found.

## Methods.

Previous to the ingestion of the various experimental diets the subject on each occasion attempted for two or three days to consume roughly the same diet so that the base line would be approximately the same. Unfortunately owing to circumstances over which the subject had no control this was not always possible. The life led by the subject was uniform throughout.

The oil used was the finest olive oil procurable. It was always emulsified before taking by shaking, after the addition of about 1 g. of potassium carbonate dissolved in water. In this form the oil was fairly readily consumed and during the three days of the duration of the experiment it gave rise to no untoward digestive disturbance or diarrhoea. As a matter of fact in one only of the three day experiments did a single movement of the bowels take place. Mr Lang found it impossible to carry on for more than three days on account of the nausea induced by the mere sight and smell of the emulsified oil. The addition of even 150 g. of glucose did not materially lessen this disgust although the subject always declared that a feeling of "tiredness" which was prominently associated with the ingestion of pure oil was distinctly reduced when sugar was added even in comparatively small amounts. The sugar used was chemically pure anhydrous dextrose. The calorie values of the various diets were kept approximately constant; they varied between 3102 C. with pure oil and 2978 C. with oil plus 150 g. sugar. The analytical methods employed were: total nitrogen, Kjeldahl; ammonia, Folin; urea, Folin and urease; uric acid, Hopkins-Folin; creatinine and creatine, Folin.

## RESULTS.

### *Output of Nitrogen.*

| | | In g. | | | | | | In percent. of T.N. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet | Day of experiment | Total | Urea | Ammonia | Total creatinine | Uric acid | Undetermined | Urea | Ammonia | Total creatinine | Uric acid | Undetermined |
| 323 g. olive oil | 1 | 10·00 | 7·31 | 0·32 | 0·50 | 0·094 | 1·76 | 73·1 | 3·3 | 5·0 | 0·94 | 17·6 |
| | 2 | 14·24 | 11·08 | 0·66 | 0·62 | 0·031 | 1·84 | 77·8 | 4·6 | 4·4 | 0·22 | 12·9 |
| | 3 | [10·75] | 7·75 | 1·07 | 0·63 | 0·033 | 1·27 | 72·1 | 9·9 | 5·8 | 0·31 | 11·8 |
| 323 g. olive oil | 1 | 10·95 | 7·88 | 0·29 | 0·49 | 0·123 | 2·16 | 72·0 | 2·7 | 4·5 | 1·12 | 19·7 |
| | 2 | 14·35 | 11·05 | 0·56 | 0·52 | 0·059 | 2·17 | 77·0 | 3·9 | 3·6 | 0·41 | 15·1 |
| | 3 | 14·18 | 10·44 | 1·13 | 0·59 | 0·029 | 1·99 | 73·6 | 8·0 | 4·2 | 0·20 | 14·0 |
| 310 g. olive oil | 1 | 9·41 | 6·94 | 0·21 | 0·58 | 0·136 | 1·53 | 73·7 | 2·2 | 6·2 | 1·44 | 16·2 |
| 30 g. dextrose | 2 | 11·72 | 7·48 | 0·44 | 0·57 | 0·087 | 3·15 | 63·8 | 3·7 | 4·9 | 0·74 | 26·7 |
| | 3 | 10·23 | 6·60 | 0·58 | 0·60 | 0·066 | 2·38 | 64·5 | 5·7 | 5·9 | 0·64 | 23·2 |
| 297 g. olive oil | 1 | 9·78 | 7·36 | 0·26 | 0·52 | 0·142 | 1·50 | 75·3 | 2·6 | 5·3 | 1·45 | 15·3 |
| 60 g. dextrose | 2 | 9·31 | 7·07 | 0·31 | 0·49 | 0·119 | 1·32 | 75·9 | 3·4 | 5·3 | 1·28 | 14·2 |
| | 3 | 8·60 | 5·86 | 0·38 | 0·50 | 0·108 | 1·76 | 68·1 | 4·4 | 5·8 | 1·25 | 20·4 |
| 279 g. olive oil | 1 | 7·97 | 5·38 | 0·26 | 0·48 | 0·133 | 1·73 | 67·5 | 3·2 | 5·9 | 1·67 | 21·7 |
| 100 g. dextrose | 2 | 7·75 | 5·24 | 0·25 | 0·54 | 0·118 | 1·60 | 67·6 | 3·3 | 7·0 | 1·52 | 20·6 |
| | 3 | 7·12 | 4·79 | 0·18 | 0·54 | 0·145 | 1·47 | 67·3 | 2·5 | 7·6 | 2·03 | 20·6 |
| 257 g. olive oil | 1 | 10·18 | 7·66 | 0·37 | 0·56 | 0·121 | 1·47 | 75·2 | 3·6 | 5·5 | 1·18 | 14·4 |
| 150 g. dextrose | 2 | 9·95 | 7·28 | 0·24 | 0·56 | 0·152 | 1·68 | 73·2 | 2·4 | 5·6 | 1·52 | 16·8 |
| | 3 | 7·37 | 5·05 | 0·16 | 0·56 | 0·143 | 1·46 | 68·5 | 2·2 | 7·6 | 1·94 | 19·8 |

### *Total nitrogen.*

The table clearly shows that with increasing amounts of carbohydrate there is a general tendency for the output of total nitrogen to decrease. On the pure oil, although in each instance there is a rise in the output of total nitrogen on the second day of the experiment, there is not the further rise noted on the third day of the experiment which both Landergren and I found in previous experiments. This may be due to the fact that in this series, in contradistinction to the earlier ones, olive oil emulsified by alkali was used in place of butter fat or cream. There is no doubt that the olive oil and alkali is much more readily tolerated by the intestine than either cream or butter. Instead of experiencing the acute diarrhoea which usually results from the ingestion of these products when taken alone, as above noted Mr Lang was constipated throughout the experiments.

Zeller [1914] has also carried out a series of observations, both on dog and man, on the effect of varying the fat and carbohydrate values of practically protein-free diets. It is to be regretted that his experimental periods on the fat-rich diets were so short, two days with the 75 % fat diet and only a single day with 100 % fat. Experiments of such very short duration do not permit of the metabolism adjusting itself to the new conditions. The effect of the fat-rich diet had however the usual result, viz. a marked rise in the output of total nitrogen both in dog and man.

### *Urea.*

The urea output resembles very closely that of total nitrogen. It is interesting to note that when the percentage outputs of urea and ammonia nitrogen are compared there is a very marked fall in the output of urea after the sugar, even with the smallest dose, although at the same time there is also a fall in the output of ammonia, whereas it might have been expected that with the decrease in the ammonia output there would have been a rise in the urea output. In the case of the experiments with oil alone there does seem to be a balance between the two outputs, a rise in the ammonia output being associated with a fall in the urea.

When the outputs of both ammonia and urea are taken together it may be definitely stated that when oil is given alone there is a definite and steady rise in the excretion of these nitrogenous materials, whereas in the experiments in which sugar is added there seems to be just as general a tendency for the united output to fall during the three days of the experiment.

Zeller in his series also found that the urea output followed very closely the curve for the output of total nitrogen. His percentage output of urea reached however a far lower level than any that I obtained. Thus when a pure carbohydrate diet was given immediately after a pure fat diet he found in one instance that urea only formed on the average 49·7 % of the total nitrogen and in the other it reached the very low level of 39·7 %.

### *Ammonia.*

In each case with the pure oil diet a very definite steady rise both of the absolute and the percentage output of ammonia nitrogen occurs on the three days of the experiment. A slight rise is also noted on the third day when 30 and 60 g. of sugar are given although, even with these small amounts of sugar, there is a definite reduction in the total ammonia output. When the larger amounts of sugar are given there is a steady fall in the output of ammonia during the three days of the test.

Zeller too in his experiments found, like other observers, that the giving of fat-rich diets led to a marked rise in the output of ammonia. In all of his experiments, just as he found a much lower percentage output in the case of urea, he obtained much higher outputs of ammonia, both with the carbohydrate-rich and carbohydrate-poor diets, than those given in the present paper. His maximum percentage output of ammonia was 15·4 % with the 100 % fat diet.

### *Uric acid.*

The variation in the excretion of uric acid as the result of the alterations in the composition of the diets is very striking and most interesting. As the table shows it may generally be stated that when carbohydrate is completely removed from the diet there is a well marked reduction in the output and that this output gradually and steadily rises as the amount of glucose added is increased. It was shown in a previous paper [1909] that the output of uric acid varied with the carbohydrate intake; that the output was high when the subject was on a carbohydrate-rich diet and low when on a fat-rich, carbohydrate-poor diet. Graham and Poulton [1913] who studied the influence of carbohydrate and fat on the output of endogenous purine also found that a diet in which fat predominated was associated with a low output and a diet in which carbohydrate predominated with a high output of uric acid. Later Umeda [1915] working in my laboratory confirmed, both in the case of man and the dog, the variation in uric acid output as the result of alteration of the diet. He found in man that, although there was a definite fall in the output of total purines, the output of purine bases on a fat diet was higher than on a carbohydrate one. Graham and Poulton had also noted that when the carbohydrate content of the diet was reduced there was a tendency for the purine base output to rise. In the case of the dog, Umeda found that the allantoin output behaved like uric acid in its relation to the nature of the diet. Zeller also investigated the influence of carbohydrate and fat diets both on the output of uric acid and the purine bases. He too found that there was a reduction in the output of uric acid on a 100 % fat diet but there was little or no influence on the output of the purine bases. As already noted Zeller's experiments were of exceptionally short duration.

In an interesting and suggestive paper Ackroyd and Hopkins [1916] showed very definitely that (1) when both arginine and histidine were removed from the diet the amount of allantoin excreted in the urine of the rat was much decreased; (2) it was somewhat diminished when only one of these amino acids was present and (3) when both were restored to the diet the allantoin excretion returned to normal. Their conclusion that arginine and histidine probably constitute the most readily available raw material for the synthesis of the purine ring in the animal body would seem to be justified. Still, although these two amino acids may be considered to supply the raw material for the synthesis, this does not dismiss the probability that the presence of carbohydrate is necessary as in the experiments of Ackroyd and Hopkins the carbohydrate supply was abundant. Further, the well known experiment of Knoop and Windaus [1905] in which they demonstrated the formation of the iminazole ring when a solution of dextrose was acted upon by ammonia in the presence of zinc and sunlight, shows, at least, that a synthesis from very simple compounds is possible. If the rest of the diet played but a minor part in the synthesis of the purine it would have been expected that the output of purines would have risen when a general increase in the breakdown of the protein molecule took place, as is the case when oil alone is the food material supplied, *i.e.* when there would be in all probability an increased amount of amino acids including arginine and histidine free in the tissues. Further the diminution of the output of uric acid on the fat diet is not due to delayed oxidation of other purine bodies because reference to the output of undetermined nitrogen, which would include purine bases, shows that the excretion on the fat diet is actually lower than when sugar is given. It is probable then that, although arginine and histidine may be regarded as the actual nitrogenous source of the purine, before the synthesis can take place carbohydrate in some form or other must be present.

### *Creatinine and Creatine.*

Inasmuch as many of the estimations of these substances were made without the precautions suggested by Graham and Poulton the total creatinine output only is given. It may be stated that in two other experiments when every precaution was taken the presence of creatine in the urine was definitely shown. The work of Underhill and Baumann [1916] showed very conclusively that acidosis alone cannot account for the presence of creatine in the urine.

### *Undetermined nitrogen.*

Although the absolute amount of undetermined nitrogen daily excreted does not vary very greatly in any of the experiments, yet, when calculated on a percentage basis, it is found that there is a very definite rise when carbohydrate is added to the diet. Zeller in one of his pure fat experiments found the highest percentage output whereas in the other the output was low, if not the lowest of the series. Zeller in addition to the substances referred to in this paper also determined amino and peptide nitrogen. The output of these substances rose when the pure fat diet was given.

## Discussion.

The consideration of the question of isodynamic replacement and the inferences to be drawn from the consideration of experiments on complete replacement such as those described, irrespective of the question as to whether such experiments fall within the normal capacities of the tissues, open up very wide issues. The mere statement of the isodynamic law virtually upholds the thesis that the basis of nutrition is the exchange of energy and not the exchange of material or, at the very least, that *Kraftwechsel* predominates over *Stoffwechsel*. Rubner [1883] who enunciated the hypothesis that fat and carbohydrate are mutually replaceable in a diet in isodynamic amounts, although in a much more recent paper he definitely stated that it was impossible to replace completely any of the proximate principles, definitely selected the term isodynamic after the consideration of such less specific terms as *gleichwertig*.

The work of Rubner is generally stated to be substantiated by the work of Atwater and Benedict [1903]. The experiments of these workers do undoubtedly support it but the type of experiment they adopted could not be expected to determine the ultimate degree of replacement, as only variations in a mixed diet on the human subject under approximately normal conditions were employed in contradistinction to Rubner's method of a rigorous administration of a single food stuff. It is undoubtedly true that within limits fat and carbohydrate may replace one another in the diet and it is obvious that in the average diet such an arrangement is automatically adopted. The statement of the case in the form of a general isodynamic law is simply untenable. At present the evidence is clear and convincing in support of the statement that it is impossible to replace carbohydrate completely by fat but so far the evidence available in support of the view that fat as such is a necessary constituent of a diet is scanty and unsatisfactory.

No one will of course seriously maintain that nutrition can ultimately be reduced merely to the satisfying of the energy demands: the calorie factor may be regarded as strictly secondary to the supply of material. We do not live on calories, yet all our general estimates of food requirements are quite properly for the most part made in terms of calories. Calorie value is simply a very convenient physical standard for the assessment of diets, but merely because such a standard has proved of great utilitarian value there is no real justification for placing this standard as the foundation stone of hypotheses framed to offer an explanation of cellular activity. Many writers are obsessed with the idea of the calorie, forgetting that the organism is certainly not a heat engine. It is perfectly true that calories are a measure of heat, but it must not be forgotten that we do not consume actual heat units but only potential heat-giving substances which can eventually be degraded to the form of heat and be measured as such. The thermal aspect of nutrition is unduly stressed, for, while heat may be a necessary product of tissue activity, it is, after all, a by-product.

The use of the term isodynamic in connection with problems of nutrition should be strictly limited. One can undoubtedly speak of isodynamic quantities of various substances but it does not follow that they are of equal, or indeed of any, value to the organism. When dealing with foodstuffs we ought to keep constantly in view that the material side is fully as important as the energy side. Therefore one ought not to stress so much the equality in energy as the equality in sparing or preventing tissue breakdown, the isoeconomic or, as I prefer to call it, the *isotamieutic* (Gk. tamieuo = to husband or to spare) value. Such a value is more physiological than isodynamic as it covers all phases of cellular activity. At present the data available do not suffice to permit of any adequate explanation of metabolic phenomena. Considerations such as those on which Carl Voit based his theory of metabolism, so actively rebutted by Pflüger, are not dismissed by the more modern hypothesis of Folin. Folin simply dealt with entirely superficial results and within these limitations the hypothesis is admirable. He did not attempt to elucidate the causal factors which lie beneath the phenomena which he correlated in his papers. The old question discussed so energetically by Voit and Pflüger as to whether the newly ingested material becomes an integral part of the living molecule before utilisation is still unanswered.

## Conclusions.

1. The output of total nitrogen, urea, and ammonia rises on a fat diet and falls on the addition of carbohydrate.

2. The output of uric acid is low on the fat diet and increases on the addition of carbohydrate.

3. The output of total creatinine is but little affected by the change of diet. Small amounts of creatine are excreted on a carbohydrate-free diet.

4. The output of undetermined nitrogen is greater on diets containing carbohydrate than on those from which carbohydrate is absent.

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