XVI. THE VALUE OF GELATIN IN RELATION TO THE NITROGEN REQUIREMENTS OF MAN.

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THE story of the earliest attempts to discover the value of gelatin as a food-stuff has been told by Carl Voit [1872] in the introduction to his paper on this subject. It commences in 1682 when Dionys Papin prepared gelatin from bones by means of his digestor, and from the gelatin made soup with which he fed the poor. Such attempts were zealously renewed during the French Revolution by Cadet de Vaux, d'Arcet and others, and were supported by the Government, who issued official instructions extolling the nourishing pro-perties of gelatin soup above those of beef tea. The approbation of the Institute of France and of the Academy of Medicine was also forthcoming, but in spite of d'Arcet's attempts to improve the flavour of the soup with spices, it did not meet with very great approval from the poor, who were expected to consume it.

Gannal, a manufacturer of gelatin, fed himself and his family on gelatin, with and without bread, for some weeks until compelled to desist owing to the unsupportable nausea caused by the diet. The effects on the health of these people led him to conclude that gelatin is not only valueless as a food but actually harmful.

Magendie's Report in 1841 to the Paris Academy on the results of the investigations of the second Gelatin Commission was scarcely more favourable. Gelatin was considered to have no food value by itself and to reduce the value of other foodstuffs when fed in combination with them. A similar opinion was expressed by the Academy of Medicine in 1850 but less extreme views were held by some physiologists among whom were Boussingault [1846] and Frerichs [1845]. The latter ascribed to gelatin the same significance as that of the "Luxus" protein, *i.e.* the excess protein in the diet over the require-ments of the body as represented by the protein decomposition during starva-tion. Though unable to replace the body protein gelatin could, he held, be utilised in the same way as the nitrogen-free foodstuffs ("Respirationsmitteln").

Somewhat similar views were held by Bischoff [1853] while Donders [1853] considered that gelatin might reduce the body's needs for protein since these are not restricted merely to the replacement of tissues.

Voit's own experiments and those carried out in conjunction with Bischoff on dogs form the first systematic study of the nitrogen balances on diets containing varying quantities of meat, gelatin and fat. As the result of a large number of experiments he concluded that gelatin always spares protein and in a greater degree than fat or carbohydrate, but that gelatin plus fat reduces the protein decomposition more than does gelatin alone. On the other hand, however much gelatin and fat are given, body protein will be lost.

The energy requirements of the animal were not sufficiently considered in these investigations and a large part of the so-called sparing action of gelatin can be ascribed to its ability to furnish energy and so to reduce the use of body protein for this purpose. I do not suggest that the whole effect is to be explained in this way, but to what extent gelatin can satisfy any portion of the dog's specific nitrogen requirements cannot be ascertained from Voit's results.

During the next thirty years experiments upon dogs by feeding with gelatin were also carried out by Oerum [1879], Pollitzer [1885], Munk [1894], Kirchmann [1900] and Krummacher [1901]. In Oerum's experiments the dog received a basal diet of starch, butter and meat extract equivalent to over 80 calories per kilo body weight. During successive periods of from four to eight days this diet was supplemented by meat or the equivalent amount of gelatin. Unfortunately only the urea nitrogen was determined (by Liebig's titration method) and the faeces were not analysed, so that a nitrogen balance sheet cannot be made out, but the results appear to indicate that gelatin can save about half the amount of nitrogen excreted by a dog when receiving a carbohydrate diet of sufficient calorie value.

Pollitzer also gave his dog abundant carbohydrate to which, during successive periods, were added equivalent quantities of meat, digestion products of meat (peptone, etc.) and gelatin. Positive nitrogen balances were obtained with all except gelatin.

Kirchmann determined the amount of body protein spared by different amounts of gelatin, no other food except water being given. Taking the nitrogen output during starvation as 100 he found a saving of 25 % when the gelatin given was sufficient to satisfy only 7·5 % of the energy requirements of the animal, while eight times this amount was required to save 35 %. He estimated that a maximum saving of 39 % might be expected if the amount of gelatin could be increased to meet these energy requirements in full.

Krummacher continued these experiments with still greater quantities of gelatin, and obtained a result closely agreeing with Kirchmann's calculated figures. It is clear however that unless we also know the minimum nitrogen output when the energy requirements are fully met by nitrogen-free foodstuffs, the above relationships offer no evidence as to the capacity of gelatin to satisfy any of the specific nitrogen requirements of the animal. This value was not determined by either Kirchmann or Krummacher.

Munk's experiments were on a different plan from those mentioned above.

He gave a dog a diet of rice, fat and meat equal to 58 calories per kilo and containing 0·6 g. nitrogen per kilo body weight, which was more than twice the starvation output. He was then able to replace five-sixths of this protein by the equivalent amount of gelatin and still keep the animal in nitrogen equilibrium.

Kauffmann [1905] carried out a series of experiments on a similar plan but with the precaution of reducing the nitrogen intake of the standard diet to a much smaller amount than that given by Munk. This standard diet consisted of milk, rice, caseinogen (plasmon) and fat and was given in amount equal to 0·32–0·39 g. N and 63–72 calories per kilo body weight. Not more than one-fifth of the nitrogen of this diet could be replaced by gelatin nitrogen without an increase in the nitrogen output occurring. Kauffmann also investigated the possibility of improving the value of gelatin by supplementing it with tyrosine, tryptophan and cystine and concluded from his experiments on dogs and on himself that with these additions gelatin becomes of equal value with caseinogen.

At a much earlier date Escher [1876] had fed dogs and pigs with gelatin supplemented by tyrosine and found that their body weight was maintained, but Lehmann [1885] was unable to obtain this result in experiments on rats.

Rona and Müller [1906] carried out a series of very careful experiments with dogs on the same plan as those of Kauffmann but were unable to confirm the latter's conclusions. Their standard diet gave 0·2 g. N and 91 calories per kilo body weight. With this the animal was in nitrogen equilibrium, but when a portion of the milk was replaced by gelatin plus tyrosine and tryptophan a negative balance was found.

The value of gelatin fed in conjunction with other proteins has also been investigated by Murlin [1907, 1] both by experiments on dogs and on himself. With dogs on a diet containing one-fourth more than the fasting requirement of nitrogen, half of this being in the form of cracker meal and half in the form of caseinogen, it was not possible to replace the caseinogen nitrogen by gelatin nitrogen without increased loss of body protein. With other diets however, in which the protein was in the form of meat, up to 58 % could be replaced without loss of body protein. The fuel value of all diets was greater than the energy requirements of the animal but Murlin attributes the high replacement value obtained in some diets largely to the greater proportion of calories supplied by carbohydrate in place of fat.

It is possible that this factor may have influenced the result, though according to Zeller [1914] the nitrogen requirements are not affected by the proportion of fat to carbohydrate in the diet so long as this does not become greater than about 4 : 1.

There seems however to be insufficient reason for assuming that the amount of meat given in some of these diets was the minimum required for nitrogen equilibrium, and unless this were so the fact that a part could be replaced by gelatin without affecting the balance would prove nothing.

On the other hand, on the cracker meal diets a negative balance was always obtained, which was recognised by Murlin as evidence of the lower availability of this form of protein. On these diets it was not possible to replace any part of the protein by gelatin without increasing the relative loss of body nitrogen.

The criticism that the protein in the diet after part of it had been replaced by gelatin may have still been in excess of the minimum required, applies even more forcibly to the experiment on himself, in which the basal diet contained 14·25 g. N, i.e. about 10 % more than his nitrogen output during starvation. When two-thirds of this had been replaced by gelatin nitrogen he was still receiving 5·33 g. N (0·076 g. per kilo) derived from eggs, cream, butter and cereals. During the two days on which this diet was taken a positive balance was obtained, but this cannot be accepted as convincing evidence of the value of gelatin nitrogen.

In a later paper Murlin [1907, 2] brought forward satisfactory proof that in a dog the reduction (about 30 %) of the fasting nitrogen output produced by small amounts of gelatin, was much greater than could possibly be accounted for by the dextrose which might be synthesised in the body from this gelatin[1].

From the investigations so far considered it may be taken as definitely established that:

1. Gelatin when given as the sole source of nitrogen is unable to maintain the animal body in nitrogen equilibrium.

2. With dogs gelatin is able to reduce the loss of body nitrogen considerably below that occurring during starvation, and this effect is not proportional to the amount of potential energy thus supplied and cannot therefore be simply explained on these grounds.

3. Some of the experiments indicate that when gelatin is mixed with other proteins, they may complement one another so that a proportion of the nitrogen of gelatin is utilisable.

A critical examination of the results of these experiments does not enable us to form any definite conclusions as to the capacity of gelatin alone to satisfy any part of the specific nitrogen needs of the body in man, although some of the results obtained with dogs indicate a limited capacity in this direction if the nitrogen output on an abundant nitrogen-free diet is taken as representing these specific requirements. There is however a difficulty in accepting this since the results obtained with dogs do not fall into line with those obtained with man and some other animals, and suggest that the nitrogen metabolism of the carnivora varies from that of the omnivora and herbivora in some details.

The fasting output of a man is equal to about 0·2 g. N per kilo body

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[1] A brief account of other researches by Ganz, Gerlach (1891), who investigated the value of gelatin peptones, Gregor (1901), who used gelatin for feeding infants, and by Brat (1902) and Mancini (1905), who fed it to convalescents, will be found in Murlin's paper [1907, 1].

weight, that of a large dog is of the same order. On an abundant carbo-hydrate diet the nitrogen output of man can be reduced to one-quarter of this amount, *i.e.* 0·05 g. N per kilo whereas according to most observations under the same circumstances the nitrogen output of a dog is only reduced by 10 % to 20 %.

The difference in detail between the nitrogen metabolism of man and dog also emerges on comparison of the ratios of the total nitrogen to that excreted in the form of creatinine during starvation and on abundant nitrogen-free diets (see Table I). The constancy of the creatinine output and its probable re-lationship to the endogenous metabolism has been noted by Folin [1905], McCollum [1911], Zeller [1914] and others.

Table I

Observer	Animal	Weight Kg.	Diet	Total nitrogen in urine per kilo body weight	Creatinine nitrogen per kilo body weight	Total urine N / Creatinine N
Cathcart [1907]	Man V.B.	62·0	Fasting, 4th day	0·221	0·0056	39
,, ,,	,, ,,	60·0	,, 8th ,,	0·159	0·0053	30
Benedict and Osterberg [1914]	Dog 39	7·6	,, 3rd ,,	0·360	0·0099	36
,, ,,	,, 33	12·7	,, 3rd ,,	0·280	0·0112	25
Towles and Voegtlin [1912]	,, 3	9·0	,, 2nd ,,	0·294	0·0129	23
Murlin [1907, 2]	,, C	13·0	,, 4th ,,	0·257	0·0080	32
Folin [1905]	Man H.B.H.	85·7	Starch, cream, 1 g. N	0·0420	0·0070	6·0
Graham and Poulton [1912]	,, G.G.	62·4	Starch, cream, ·912 g. N	0·0445	0·0093	4·8
,, ,,	,, E.P.P.	72·4	Starch, cream, 1·23 g. N	0·0468	0·0107	4·4
af Klercker [1907]	,, a.K.	88·0	Low N	0·0319	0·0079	4·0
Robison [1922]	,, C.J.M.	60·5	Carbohydrate, fat, ·3 g. N	0·0352	0·0072	4·9
,, ,,	,, R.R.	58·0	,,	0·0355	0·0084	4·2
McCollum [1911]	Pig	10·9	Carbohydrate	0·0495	0·0095	5·2
,, ,,	,,	68·4	,,	0·0387	0·0069	5·6
Mendel and Rose [1911]	Rabbit	1·74	,,	0·126	0·0172	7·3
Murlin [1907, 2]	Dog C	11·3	,,	0·158	0·0104	15

There is a close parallelism between the figures for men and dogs during starvation and between those for men, pigs and rabbits on abundant nitrogen-free diets. The creatinine excretion for Murlin's dog *C* on such a diet is also in good agreement with the corresponding figures for men and pigs but the ratio $\frac{\text{Total urine N}}{\text{Creatinine N}}$ is about three times as high as the same ratio for other animals. It is of course not possible to state on such evidence alone that the real endo-genous metabolism of this dog should be represented by a nitrogen output of one-third the observed amount, but it is clear that the nitrogen metabolism of dogs differs in some way from that of man, and that caution must be used in applying conclusions from experiments with these carnivora to other animals and man.

These criticisms however do not apply to the experiments of McCollum [1911] on pigs, for in these the constancy of the proportion of the endogenous

metabolism represented by creatinine nitrogen was recognised and was used as a criterion for judging when the minimum nitrogen excretion of the animals had been reached.

The pigs were fed on a basal nitrogen-free diet of ample fuel value consisting of starch, a salt mixture and water, until the nitrogen output had reached the minimum, whereupon an amount of the protein under examination equivalent to this minimum (urine nitrogen only) was added to the diet during a further period, after which the basal diet alone was fed until the output had again fallen to the minimum, the nitrogen excreted during this last period being also included in the calculation. In the experiment recorded by McCollum 2·62 g. of gelatin nitrogen was given daily during eight days, i.e. 20·96 g. in all. The total output during these eight and the following four days on which no nitrogen was given, amounted to 41·71 g. in the urine and 12·48 g. in the faeces, i.e. 54·19 g. in all, making a negative balance of 33·23 g.

Had the pig received no nitrogen at all its total output during these twelve days would have amounted to 31·44 g. in the urine and 12·48 g. in the faeces, making 43·92 g. in all, so that a saving of 10·69 g. nitrogen has been effected by 20·96 g. of gelatin nitrogen. This implies a utilisation of 50 % of the nitrogen given in this form, which was confirmed by five other similar experiments the details of which are not given. If the result is stated in terms of body protein saved, this amounts to 1·34 g. per day (if reckoned on eight days), i.e. 37 % of the minimum output in urine and faeces or 51 % of that in the urine only, which is taken by McCollum as representing the essential tissue metabolism of the animal.

Boruttau [1919] has recently attempted to determine the biological value of gelatin by two experiments on dogs, using the method and formulae adopted by Karl Thomas [1909] and has obtained the figures 49·1 % calculated by formula I and 67·3 % calculated by formula II. These values would agree much more closely had Boruttau not made an error in his use of formula I by taking the total food nitrogen as denominator in place of this amount less the nitrogen of the faeces, as intended by Thomas. In any case however such figures have no real significance in the case of gelatin since they will necessarily vary with the amount of the intake, and moreover the experiments were of too short duration to possess much value.

Apart then from the experiments of McCollum no very satisfactory evidence has been produced regarding the value of gelatin alone to satisfy any of the nitrogen requirements of the animal body. Most of the investigations have in fact been concerned with its value when fed in conjunction with other proteins and this introduces the possibility of complementary effect, about which very little is definitely known. That such effect is possible is shown by the experiments of Osborne and Mendel [1912] on rats. With gelatin as the sole protein the animals rapidly declined in weight but recovered when half of the gelatin was replaced by gliadin, a protein incapable of inducing more

than a very slight growth when fed as the sole protein constituent of the diet. Further, almost all the previous work, including that of McCollum, has been carried out on animals, and the results might not necessarily apply to man. The whole question is of very great theoretical and practical interest because of its bearing on protein metabolism in general and the nature of the body's requirements for particular compounds of nitrogen.

EXPERIMENTAL.

The investigation about to be described was an attempt to obtain more light on the problem by direct experiments on man.

The subject of the experiment was myself, age 37 years, medium build, weight 59 kilo, height 173·5 cm. My minimum nitrogen output had been determined by previous experiments which will be discussed in another paper.

In the second of these experiments, in which a diet containing about 0·3 g. N and equivalent to 2600–3000 calories (45–52 cals. per kilo) was taken for a period of seven days, the nitrogen output in the urine fell to a fairly constant level of 2·06 g., while the average amount of nitrogen excreted in the faeces was 1·13 g. per day.

In the present investigation the basal diet supplemented by different quantities of gelatin was taken for periods of ten days, the nitrogen intake being kept absolutely constant during each period.

Profiting by the experience of the previous experiments the basal diet was somewhat altered, the original attempt to introduce some variety and palatability being given up in favour of greater simplicity and uniformity of the food intake. The proportion of calories supplied by fat and the total nitrogen in the diet were both reduced. In the later experiments the process of simplification was carried to its furthest extent, the diet consisting of corn starch, lactose, sucrose and a salt mixture. Minimal quantities of lemon juice and cod liver oil were added to supply the antiscorbutic and fat soluble A accessory factors and agar-agar was taken to increase the bulk of the faeces and prevent constipation. The corn starch, lactose, salt mixture and agar for each day's ration were weighed out and mixed together before the experiment began. The mixture was taken in the form of a cream made with cold or warm (but not boiling) water and washed down with more water. The uncooked starch grains were very well absorbed, extremely few being found in the faeces. Usually a third of the day's ration was taken at 8 a.m., 1 p.m. and 7 p.m., but sometimes it was found necessary to increase the number of meals in order to consume the prescribed amount. The gelatin was dissolved in warm water and taken either by itself or mixed with some of the starch and lactose. The lemon juice, sweetened with cane sugar, was taken as a drink and a little weak tea with lemon was also permitted. The very small amount of nitrogen in the tea was assumed to be due to caffeine and to be excreted unchanged in the urine. It was therefore always subtracted from the total nitrogen intake and from the output.

The salt mixture had the following composition:

Calcium diacid phosphate $CaH_4(PO_4)_2, H_2O$ 20   Na   2·5 %
Calcium lactate $(C_3H_5O_3)_2Ca, 5H_2O$ 30   K   13·5
Potassium hydrogen phosphate K_2HPO_4 30   Ca   7·1
Sodium dihydrogen phosphate NaH_2PO_4, H_2O 15   P   13·6
Magnesium carbonate $MgCO_3$ 3   Mg   ·85
" Iron carbonate "   2   Fe   ·7

It was intended that 10 g. of this mixture with the addition of 5 g. sodium chloride should be taken daily. The amounts of calcium and phosphorus would then correspond with those recommended by Sherman as 50 % above the minimum requirements of the body for these elements [Sherman, Wheeler, and Yates, 1918; Sherman, 1920]. It was found necessary however to reduce these quantities to 6 g. and 4 g. respectively on account of the diarrhoea caused by the diet. The ash of the above salt mixture is markedly alkaline, a point of importance in view of the observations by McCollum and Hoagland [1913] on the increased nitrogen output caused by diets having an acid ash.

The urine was collected from 8 a.m. to 8 a.m. and stored under toluene. The faeces were collected over the whole period and mixed with dilute sulphuric acid, those passed during the morning being considered as belonging to the previous day. Owing to the fluid consistency of the faeces the use of markers was found to be impracticable, but in view of the regular evacuation of the intestines and the length of the experiment, no serious errors can have been introduced in this way. Estimations of nitrogen in urine, faeces and in all components of the diet were carried out by the Kjeldahl method in duplicate. Creatinine was estimated by the method of Folin.

The percentages of nitrogen found in the constituents of the diet and their fuel values are given in Table II.

Table II.

	Nitrogen per 100 g.	Calories per 100 g.
Gelatin (Coignet's "Extra." Gold Label)	14·16	324
Corn starch (a)	0·039	360
,, ,, (b)	0·027	360
Dextrin	0·065	360
Agar-agar	0·242	—
Lactose	0·013	370
Sucrose	—	395
Butter	0·080	775
Cod liver oil	Not determined	930
Lemon juice (per 100 cc.)	0·067	40
Vermouth ,,	0·005	140
Tea infusion* ,,	0·008	—

* The strength of the tea infusion was kept as nearly constant as possible but the total nitrogen intake from this source was checked by removing an aliquot portion of all tea drunk during an experiment and estimating the nitrogen in the whole quantity.

Concerning the purity of the gelatin used in the experiments.

The source from which commercial gelatin is obtained and the methods employed in its manufacture are not likely to produce a pure product. One would expect to find it contaminated with traces of other animal proteins or their decomposition products. Such included impurities being colloids could not be removed by washing, and might conceivably possess a high value for the replacement of body nitrogen.

Kirchmann drew attention to the fact that the best French gelatin gave a slight precipitate with Millon's reagent and with potassium ferrocyanide and acetic acid, and claimed to have succeeded in removing the impurities to which these reactions were due. The unoxidised sulphur was also reduced from 0·387 % to 0·263 %. He considered that the difference between his own results and those of previous workers was to be attributed largely to the presence of this protein in their gelatin. One of his methods consisted in soaking the gelatin first in water, then in 10 % sodium chloride solution, again in water and finally in alcohol. Murlin [1907, 1], using the same methods, was unable to detect any improvement in the purity of the product.

The gelatin used in the present investigation also gave a slight positive reaction with Millon's reagent and with potassium ferrocyanide and acetic acid, and an attempt was therefore made to purify it by soaking it for 24 hours in $N/20$ HCl followed by $N/20$ NaOH, then for some days in running water. No appreciable reduction in the intensity of the colour produced with Millon's reagent was observed after such treatment.

Folin and Denis [1912] have recorded finding a trace of tyrosine in gelatin, using Folin's colorimetric method, and Dakin [1920] has recently obtained a similar result using a gravimetric method. He estimates the amount of tyrosine at about 0·01 % and considers that it cannot be an integral part of the gelatin molecule.

I attempted to estimate the amount of tyrosine present by means of Folin's method, using relatively large quantities of gelatin. The tyrosine in a sample of dried ox muscle was also estimated by the same method. The results are shown in Table III. Millon's reaction is not well adapted for colorimetric measurement but under suitable conditions it was found possible to make approximate determinations by comparing the colour with that developed by different amounts of pure tyrosine, and the results agree reasonably well with those obtained by Folin's method.

Table III.

	Tyrosine estimated by Folin's method	Tyrosine estimated with Millon's reagent
Gelatin (Coignet's extra)	0·57 %	0·6 % to 0·7 %
„ after purification	0·45	—
„ Swiss	0·55	—
Glue	1·47	—
Ox muscle	5·8	—

The accuracy of Folin's method has been called in question by Abderhalden [1913, 1, 2] who has suggested that other amino-acids, tryptophan, hydroxy-tryptophan and hydroxyproline give the same colour reaction. Of these the first two are not present in gelatin but Dakin estimated the amount of hydroxy-proline as 14·1 %. Through the kindness of Prof. Leathes, F.R.S., who supplied me with a specimen of this amino-acid, I was able to test its behaviour with Folin's reagent and found that a slight colour developed under the conditions laid down by Folin and Denis for the estimation of tyrosine, but that the intensity was about $\frac{1}{375}$ of that produced by the latter compound.

A slight colour, similar to that given by hydroxyproline, was also obtained from a specimen of phenylalanine.

In the face of the results given by gravimetric methods it would be rash to assert that the gelatin actually contained 0·57 % of tyrosine, but the colour produced with Folin's reagent does not appear to be due to any of the other amino-acids known to be present. It is also probable that the same compound, tyrosine or other amino-acid, is the cause of the colour produced with Millon's reagent.

If the percentage of tyrosine is correct and if it is present as a constituent of another protein similar to ox muscle, the proportion of the latter in the gelatin would be about 10 %. This calculation however is based on too many assumptions to be of more than speculative interest.

Up to the present neither cystine nor any other compound containing sulphur has been isolated from gelatin though the presence of such unidentified compounds has been noted by Dakin [1920].

The gelatin used in these experiments after purification in the manner described above, contained 0·24 % of total sulphur, calculated on the dry substance. Krummacher [1903] after purifying gelatin by Kirchmann's method found 0·28 % S (of which 0·02 % was in the form of SO_3 and SO_4). The original commercial product used by Krummacher contained 0·62 % total S, of which 0·4 % was present as SO_3 and SO_4. Such an amount (0·24 %) of unoxidised sulphur would correspond with 0·9 % of cystine (or other com-pound containing a like proportion of S) and this can hardly be ascribed to impurities in the gelatin.

RESULTS OF THE EXPERIMENTS ON GELATIN DIETS.

It was proposed to carry out three diet experiments in which low, medium and high amounts of gelatin nitrogen should be given in addition to the basal diet, in order to determine

(1) whether the minimum nitrogen loss on abundant nitrogen-free diet can be still further reduced by gelatin, and if so,

(2) what relation the amount of this reduction bears to the amount of gelatin ingested.

Two experiments were completed during the early part of 1921, but the

third had to be broken off through illness shortly after it was begun. It was repeated in September 1921 the conditions being somewhat modified on account of certain results that had in the meantime been obtained from other experiments carried out with Prof. C. J. Martin. These appeared to indicate that the amount of certain proteins required for nitrogen equilibrium could be greatly reduced if a carbohydrate diet very much in excess of the energy requirements was taken. In this last experiment, therefore, I increased the fuel value of the diet to the maximum that could be tolerated, so that the body weight was maintained and even increased during the first half of the period (see Fig. 1, Curve C). Synthesis of fat from the carbohydrate of the food was also indicated by the high respiratory quotient.

The diets for the three experiments are given together in Table IV. The diet taken during the experiment in which my minimum requirements were determined is also included for purposes of comparison.

Table IV.

Food	Nitrogen minimum 29/11/20—5/12/20 Total calories=2605 Calories per kilo=45[1] Calories supplied as Fat=31%		Gelatin I 28/1/21—6/2/21 Total calories=2525 Calories per kilo=44 Calories supplied as Fat=6·4%		Gelatin II 18/4/21—27/4/21 Total calories=2757 Calories per kilo=47 Calories supplied as Fat=6·5%		Gelatin III 8/9/21—17/9/21 Total calories=3256 Calories per kilo=54 Calories supplied as Fat=0·86%	
	Wt g.	N g.	Wt g.	N g.	Wt g.	N g.	Wt g.	N g.
Gelatin	—	—	66·67[2]	12·000	27·14[2]	4·885	41·89[2]	7·540
Corn starch	280	0·118	350	0·137	340	0·143	500	0·135
Dextrin	50	0·033	16	0·010	—	—	—	—
Butter and margarine	105	0·074	20	0·016	20	0·002	Cod oil 3	—
Honey	55	0·013	—	—	—	—	—	—
Sucrose	25	—	—	—	70	—	30	—
Lactose	65	0·008	180	0·023	250	0·032	300	0·039
Lemon juice	30 cc.	0·020	25	0·017	25	0·017	20	0·013
Vermouth	25 cc.	0·001	50	0·002	—	—	—	—
Tea	1200 cc.	0·072	350	0·028	250	0·020	600	0·043
Agar-agar	15	0·036	13	0·031	10	0·024	10	0·024
Salt mixture	5	—	10[3]	—	10[3]	—	10[3]	—
	—	0·375	—	12·264	—	5·123	—	7·794
Total fluid	—	—	2200—2500	—	2000	—	2200	—

[1] During the first five days of the experiment the fuel value of the diet was equal to about 52 cals. per kilo.

[2] Weight calculated as dry gelatin.

[3] Includes 4 g. sodium chloride.

No purification was attempted for experiment I. For II and III the gelatin, after purification by the method described in the text, was dissolved in hot water. Weighed amounts of this solution were transferred to bottles and sterilised in the autoclave. The figures for the nitrogen intake are based on a number of analyses of samples from different bottles.

Gelatin I (28th Jan. to 6th Feb. 1921).
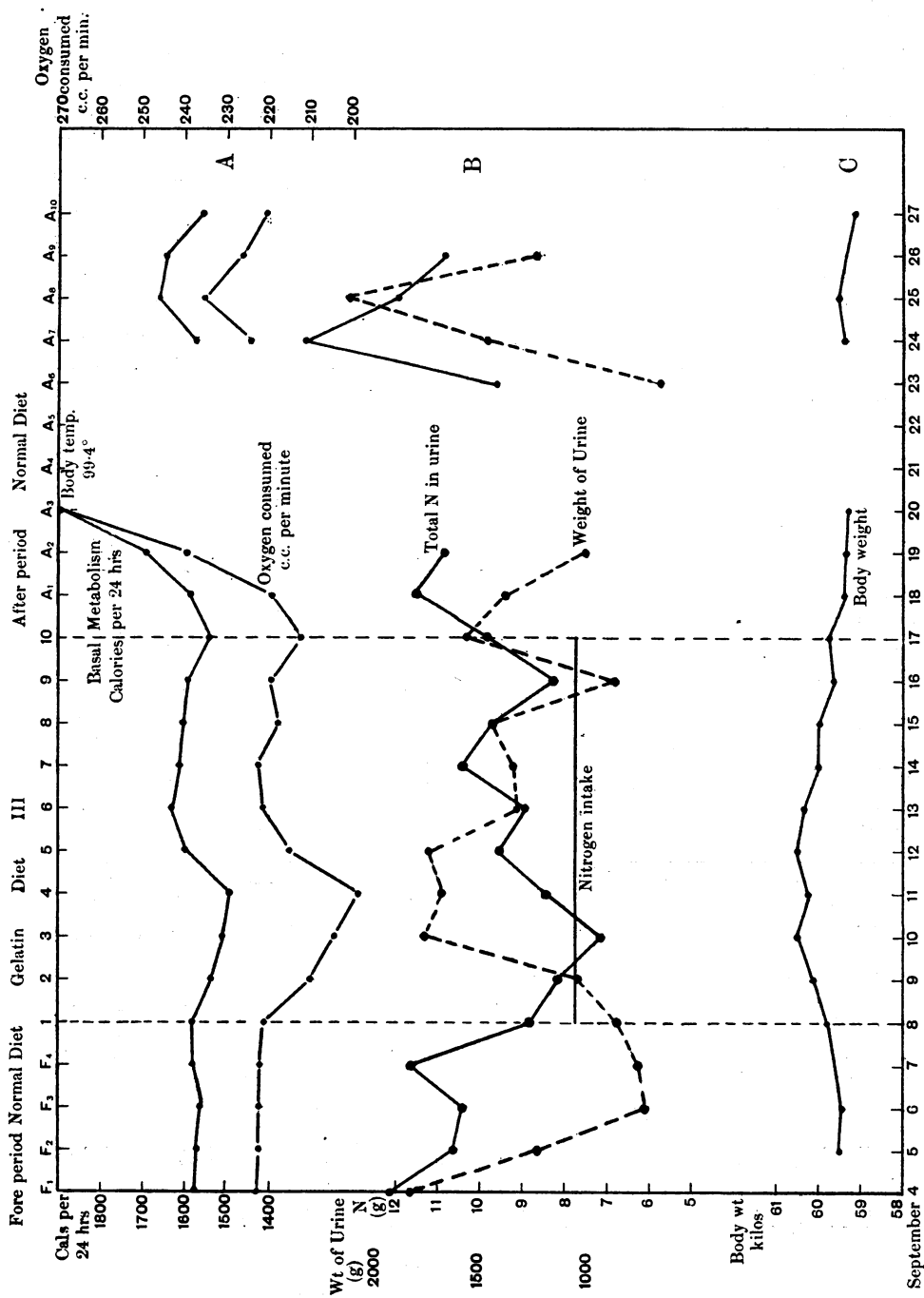
During the three days previous to the experiment a mixed diet containing about 12 g. N was taken.

The experimental diet is given in Table IV. It included:

N in the form of gelatin ...	12·00 g.
N in accessories[1] (excluding tea)	0·23
Total N	12·23

[1] The nitrogen in the constituents of the basal diet.

Fig. 1.

A few nitrogen-free biscuits made from starch, dextrin and agar were chewed at each meal to increase the flow of saliva. The rest of the starch etc. was taken in the raw state as already described.

The results of the experiments are shown in Table V. It will be seen that the nitrogen output in the urine remained almost stationary for three days, then rose and a series of oscillations set in.

Table V.

Date	Day of exp.	Body weight k.	Weight of urine g.	Sp. gr. of urine g.	Creatine N g.	Total N in urine[1] g.	N in faeces g.	Total N (U + F) g.	Balance
Jan. 28	1	58·75	1447	1·0175	0·44	12·37	(1·38)[2]	(13·75)	(−1·50)
29	2	—	1790	1·014	0·53	12·42	1·38	13·80	−1·57
30	3	—	714	1·023	0·46	12·38	1·38	13·76	−1·53
31	4	—	1236	1·015	0·48	14·05	1·38	15·43	−3·20
Feb. 1	5	—	1550	1·013	0·52	12·84	1·38	14·22	−1·99
2	6	—	1496	1·015	0·53	14·42	1·38	15·80	−3·57
3	7	57·80	1200	1·016	0·51	13·13	1·38	14·51	−2·28
4	8	—	1300	1·015	0·53	14·20	1·38	15·58	−3·35
5	9	—	1601	1·014	0·50	12·27	1·38	13·65	−1·42
6	10	57·55	1914	1·014	0·54	14·44	1·38	15·82	−3·59
Average for whole period					0·50	13·25	1·38	14·63	−2·40
Average for the last six days					0·52	13·55	1·38	14·93	−2·70

[1] The "caffeine N" = 0·03 g. (from tea) has been subtracted from the total urinary nitrogen.

[2] The faeces were only collected for the last nine days.

Gelatin II (15th–27th April 1921).

During the two days previous to this experiment the diet consisted of eggs, milk, bread, potatoes, butter and apples, and contained 11·2 g. nitrogen. The experimental period was divided into two parts.

During the first three days the basal diet was supplemented by 250 g. of egg-white while during the last ten days this was replaced by gelatin. The two diets contained:

	Three days 15th–17th April	Ten days 18th–27th April
N in form of egg-white ...	5·0	—
„ gelatin ...	—	4·88
N in accessories (excluding tea)	·21	·22
Total N	5·21	5·10
Fuel value	2580 cals = 44 cals per kilo	2757 cals = 47 cals per kilo (6·5 % supplied by fat)

The results are shown in Table VI.

A pronounced negative balance occurred on the egg-white diet and the nitrogen output during the first few days of the gelatin period is slightly above the average of the last six days.

Gelatin III (8th–17th Sept. 1921).

During the preceding four days a mixed diet containing about 12 g. N and equal to about 2400 calories was taken. On the 7th Sept. 100 g. lactose and 100 g. starch were consumed in addition to the above, making the total calories 3130.

Table VI.

Date	Day of exp.	Body weight k.	Weight of urine g.	Sp. gr. of urine g.	Creatin-ine N g.	Total N in urine[1] g.	N in faeces g.	Total N (U + F) g.	Balance
First period:									
April 15	E.1	59·3	1351	1·017	—	7·30	—	—	—
16	E 2	—	1155	1·019	—	5·91	—	—	—
17	E 3	—	962	1·019	—	6·09	—	—	—
Second period:									
April 18	1	58·85	1517	1·013	0·52	7·42	1·28	8·70	− 3·60
19	2	—	1779	1·012	0·56	6·32	1·28	7·60	− 2·50
20	3	—	904	1·018	0·55	6·85	1·28	8·13	− 3·03
21	4	—	1125	1·015	0·53	6·73	1·28	8·01	− 2·91
22	5	—	833	1·019	0·55	5·68	1·28	6·96	− 1·86
23	6	—	1148	1·017	0·54	7·12	1·28	8·40	− 3·30
24	7	—	1523	1·013	0·54	6·18	1·28	7·46	− 2·36
25	8	58·35	817	1·021	0·53	6·12	1·28	7·40	− 2·30
26	9	—	1209	1·015	0·52	6·77	1·28	8·05	− 2·95
27	10	—	1020	1·017	0·53	6·56	1·28	7·84	− 2·74
28	—	58·15	—	—	—	—	—	—	—
Average of whole period	...	...	...		0·54	6·57	1·28	7·85	− 2·75
Average of last six days	...	...	...		0·53	6·41	1·28	7·69	− 2·59

[1] The "caffeine N" = ·02 g. (from tea) has been subtracted from the total urinary nitrogen.

The experimental diet is given in Table IV. It included:

N in the form of gelatin	7·54 g.
N in accessories (excluding tea)	·21
Total N	7·75

From Sept. 14th the fuel value was increased by 100 calories taken in the form of starch biscuits and honey. The increased nitrogen is negligible.

The daily exercise consisted of a walk of from five to seven miles.

The results are shown in Table VII.

Here as in Gelatin I the nitrogen output during the first four days is below the average for the whole period.

Table VII.

Date	Day of exp.	Body weight k.	Weight of urine g.	Sp. gr. of urine g.	Creatin-ine N g.	Total N in urine[1] g.	N in faeces g.	Total N (U + F) g.	Balance
Sept. 8	1	59·8	850	1·0225	0·59	8·77	1·54	10·31	− 2·56
9	2	60·1	1038	1·0175	0·59	8·12	1·54	9·66	− 1·91
10	3	60·5	1764	1·011	0·60	7·11	1·54	8·65	− 0·90
11	4	60·25	1680	1·013	0·58	8·43	1·54	9·97	− 2·22
12	5	60·5	1735	1·013	0·60	9·49	1·54	11·03	− 3·28
13	6	60·35	1320	1·0165	0·59	8·87	1·54	10·41	− 2·66
14	7	60·0	1347	1·0155	0·57	10·37	1·54	11·91	− 4·16
15	8	59·93	1439	1·015	0·59	9·68	1·54	11·22	− 3·47
16	9	59·65	859	1·0215	0·59	8·20	1·54	9·74	− 1·99
17	10	59·78	1555	1·0145	0·60	9·84	1·54	11·38	− 3·63
Average for the whole period	...	...			0·59	8·89	1·54	10·43	− 2·68
Average for the last six days	...	...			0·59	9·41	1·54	10·95	− 3·20

[1] The "caffeine nitrogen" = ·04 g. (from tea) has been subtracted from the total urinary nitrogen.

BASAL METABOLISM.

My basal metabolism was determined each day while on the experimental diet and during the periods immediately before and after.

The method adopted was that of the Douglas bag, the expired air being analysed in Haldane's gas analysis apparatus. From the 4th to the 7th of September the estimation was made at 9 a.m., fasting, after a walk of one mile followed by a resting period of at least 30 minutes. All the remaining determinations were made between 7.30 a.m. and 8 a.m. on waking. The results are shown in Table VIII.

A slight decrease in the basal metabolism occurred at the commencement of the experimental diet but the normal level was regained by about the fourth day. This decrease coincides with the lower nitrogen output, as may be seen in Fig. 1 A and B, but it is not possible to say whether the two are in any way connected. No corresponding decrease occurred in the creatinine output which was constant throughout the period.

In calculating the basal metabolism in calories per 24 hours the protein oxidation has been ignored but the error thus introduced is less than $+ 1 \%$. For those days in which the R.Q. is greater than 1 a correction has been made for the nett heat production due to synthesis of fat from carbohydrate by adding 0·3 of the calories equivalent to the excess of the CO_2 output over the oxygen consumed. I am aware that this is an arbitrary estimate, but the possible error involved is only slight.

Table VIII.

Date	Day of exp.	Diet during previous 24 hours (calories per kilo)	Oxygen consumed per minute (cc)	R.Q.	Calories per 24 hours
Sept. 4	F 1	Normal (40)	223·0	0·831	1575
5	F 2	,, ,,	222·5	0·830	1571
6	F 3	,, · ,,	222·4	0·803	1561
7	F 4	,, ,,	222·3	0·866	1580
8	G 1	Normal + 200 g. starch (54) lactose mixture	221·2	0·904	1582
9	G 2	Carbohydrate + gelatin (54)	210·1	1·012	1537
10	G 3	,, ,,	204·8	1·043	1510
11	G 4	,, ,,	199·0	1·093	1492
12	G 5	,, ,,	215·3	1·065	1600
13	G 6	,, ,,	221·6	1·051	1633
14	G 7	,, ,,	222·8	0·967	1614
15	G 8	,, ,,	218·0	1·040	1604
16	G 9	,, ,,	219·8	0·967	1592
17	G 10	,, ,,	212·9	0·968	1542
18	A 1	,, ,,	218·7	0·979	1587
19	A 2	Normal diet	239·8	0·823	1691
20	A 3	,,	269·3[1]	0·805	1893
24	A 7	,,	224·3	0·801	1574
25	A 8	,,	235·5	0·809	1660
26	A 9	,,	226·3	0·823	1596
27	A 10	,,	220·9	0·830	1559

[1] The body temperature was 99·4° when this determination was made. Several attacks of vomiting had occurred during the previous night. The condition became worse and necessitated some days' rest.

The normal *basal metabolism* calculated from Harris and Benedict's formula for a man of age 37, weight 59·8 kilo, height 173·5 cm. would be $66·4730 + 13·7516 \times 59·8 + 5·0033 \times 173·5 - 6·755 \times 37 = 1506$ calories.

GENERAL CONSIDERATIONS.

My usual mode of life was followed throughout these experiments, eight or nine hours of each day being occupied with laboratory work. The only form of exercise was a walk of from two to seven miles. No great difficulty was found in consuming the diet although a feeling of nausea was frequently experienced. This was greatly intensified by the excessive quantity of food in the third experiment; the tongue became furred and more or less headache was common. Slight diarrhoea occurred in all three periods, faeces of fluid consistency being passed two or three times a day. The diet was however very well assimilated scarcely any starch being found in the faeces. Traces of reducing sugar were regularly present in the urine during the third experiment but only occasionally during the first two.

It was intended that a period on nitrogen-free diet should follow immediately on the third gelatin diet in order to determine my minimum requirements once more. This was not possible, and indeed much difficulty was experienced in completing the ten days proposed for the above experiment.

In the two experiments (G I, G III) which followed a normal mixed diet the average nitrogen output during the first four days was lower than that for the remainder of the period while the reverse of this was observed when the previous diet had been insufficient to satisfy the protein requirements (G II) and a considerable negative balance had occurred. These differences are probably due to the influence of the protein of the preceding diet, a diminishing store of which, perhaps in the form of amino-acids, remains in the body for some days. The first four days have therefore been excluded in considering the results. Another disturbing factor is the variation in the urine nitrogen from day to day. This frequently amounted to more than 20 % of the average output and showed no relationship whatever to the volume of the urine. Consequently it cannot be explained by diuresis.

SUMMARY AND DISCUSSION OF RESULTS.

The results obtained in the three experiments are summarised in Table IX, the average figures being given for the last six days of each ten day period. The average amounts of the nitrogen intake and output on the last three days of the earlier experiment on "nitrogen-free" diet are also included.

Table IX

Date	Body weight (kilos)	Fuel value of diet, cals per kilo	Nitrogen intake		Nitrogen output				
			Gelatin g.	Accessories g.	Urine g.	Faeces g.	Total g.	Creatinine g.	Balance
3. xii. 20–5. xii. 20	58·0	52–44	—	·30	2·06	1·13	3·19	0·49	− 2·89
1. ii. 21–6. ii. 21	57·8	44	12·00	·23	13·55	1·38	14·93	0·52	− 2·70
21. iv. 21–27. iv. 21	58·4	47	4·88	·22	6·41	1·28	7·69	0·53	− 2·59
11. ix. 21–17. ix. 21	60·2	54	7·54	·21	9·41	1·54	10·95	0·59	− 3·20

In attempting to calculate the amount of body protein spared by the gelatin from these results we are met by two difficulties, namely what is to be done with that part of the intake due to the nitrogen of the accessories, and with that part of the nitrogen output due to the faeces.

Nitrogen of the accessories. The nitrogen from the tea does not appear in the table, having been subtracted both from intake and output. The assumption that this is all "caffeine N" is not strictly correct but as the total amount is very small, usually under 0·05 g. any error involved in this mode of treatment must be negligible. The greater part of the accessory nitrogen comes from the corn starch. In McCollum's account of his experiments on pigs, no mention is made of any nitrogen arising from this source although large quantities (1700 g.?) of corn starch were given. If this starch contained as much nitrogen as the samples used by me the nitrogen intake from this source may easily have been 0·5 g. or more. We do not know in what form this nitrogen is present nor its value in the human body and cannot therefore estimate its effect on the nitrogen output. If the amount and nature of such accessories are the same during the determination of the nitrogen minimum and experiments with gelatin, the following argument might be applied. If the value of this nitrogen in the accessories is zero then the real nitrogen requirements will be less than the observed output by the full amount of such nitrogen intake since the latter must be excreted in addition to the nitrogen resulting from the protein metabolism of the body. If the value of this nitrogen for the replacement of body nitrogen is 100 % then it will spare an equal amount of the latter and the observed output on the so-called "nitrogen-free" diet will represent the actual minimum requirements. But in this case an equal amount of gelatin will also be spared when gelatin is taken, and the apparent sparing effect of the gelatin will thus be increased by the same amount.

In either case the real saving of body nitrogen due to the gelatin will be less than the apparent saving, *i.e.* the difference between the negative balance on the gelatin and the minimum nitrogen output on the "nitrogen-free" diet, by an amount equal to the nitrogen of the accessories. This will also hold for all values of the latter between 0 and 100 $^\circ/_\circ$. Unfortunately the proviso that the accessories should be the same on both diets does not strictly hold in the above experiments but if the butter nitrogen be subtracted from the total intake on the "nitrogen-free" diet the remainder is practically the same as the accessory nitrogen on the gelatin diets. The butter nitrogen would probably have a high value and I have therefore assumed that it does not appreciably increase the nitrogen output. The rest of the accessory nitrogen (·22 g. average) has been deducted in calculating the amount of body nitrogen saved by the gelatin. This is not strictly accurate but is probably the best that can be done with the figures. The above argument however ignores the possibility of complementary action of the accessories and the gelatin. It has been already pointed out that such action occurs when gelatin is fed with certain cereal proteins. McCollum, Simmonds and Pitz [1917] have shown that a mixture of oat protein and gelatin has a higher value than either alone or than oat protein plus caseinogen. It is impossible to say whether the results in my experiments were affected by such complementary action but if this did occur the real value of the gelatin alone is still less than the calculations appear to show.

Faecal nitrogen. The problem of how to treat the nitrogen excreted in the faeces is even more difficult. In McCollum's experiments the urinary nitrogen is alone considered, that in the faeces being estimated merely as a check on the complete absorption of the food protein. He considers that the nitrogen of the urine represents the essential tissue metabolism, while that of the faeces represents losses which may be termed accidental in character.

It has been shown by Rubner [1919] that the increased amount of nitrogen in the faeces of men, when fed on various diets, above that found on a carbohydrate diet cannot be taken as entirely due to undigested food protein, but that a considerable proportion of the increase comes from the body. In my experiments somewhat large variations were observed in the nitrogen of the faeces and these may have been due to the slight diarrhoea which occurred. Probably most of the nitrogen came from the body but the possibility that a small amount of gelatin escaped absorption must not be overlooked. We know very little about the relationship between loss of body nitrogen through the intestines and that excreted in the urine, but there seems to be no evidence that an increase in the former is accompanied by a decrease in the latter. The reverse of this is perhaps more probable.

This question remains at present the limiting factor for the accuracy of such experiments. I have attempted to define the limits between which the true conclusion from my results is to be found, by calculating the amount of body nitrogen saved by the gelatin in two ways. In the first (A) I have assumed that my minimum nitrogen requirements are represented by the sum of the nitrogen in the urine and that in the faeces on the nitrogen-free diet, and that the difference between the latter amount and the corresponding excretion on the gelatin diets represents unabsorbed gelatin nitrogen.

Table X.

| Experiment | N Intake | | N Balance g. | N minimum g. | A Body Nitrogen saved | | B N minimum g. | Body Nitrogen saved | |
	Gelatin g.	Accessories g.			g.	% of minimum		g.	% of minimum
R.R. II	4·88	0·22	− 2·59	3·19	0·38	11·9	3·34	0·53	15·9
R.R. III	7·54	0·21	− 3·20	3·19	0	0	3·60	0·19	5·3
R.R. I	12·00	0·23	− 2·70	3·19	0·26	8·1	3·44	0·51	14·7
McCollum's pig average of last six day	2·62	?	− 2·35	3·68	1·33	36·1	3·66	1·31	36·3

In the second (B) I have assumed that the gelatin is completely absorbed and that the minimum requirements for such periods are represented by the output in the urine on "nitrogen-free" diet plus the nitrogen in the faeces on the gelatin diet under consideration. The truth probably lies somewhere between these two extremes. The results of these calculations are given in Table X. McCollum's figures are also included for comparison. The accessory nitrogen has been in each case deducted from the apparent amount of body nitrogen saved.

The maximum saving in terms of the nitrogen minimum is thus 11·9 % if the first method of calculation is employed and 15·9 % if the second is used.

The fact that this was obtained with the lowest amount of gelatin would appear to prove that the effect is not due to any impurity, in which case there should be a proportionality between the amount saved and the gelatin intake. In this connection however the possibility that the increased protein in the diet entails an increased loss of body nitrogen must not be overlooked, although the creatinine excretion does not lend any support to such an hypothesis.

The results of the first and second experiments agree well between themselves, but in the third a much lower value was apparently indicated. It will be noticed however that the creatinine excretion in the experiment was higher than the normal and the body weight was also higher. This nitrogen minimum may therefore have been higher than the amount shown (perhaps owing to the excessive amount of food taken) in which case the calculated result would be too low.

All these values are much lower than those found by McCollum and this discrepancy cannot be explained except by assuming a difference in the metabolism of man and pig. If the values are calculated in terms of the urinary output alone they are all proportionately increased—the value for my experiment G II then becoming 25·7 % of the minimum, but the difference between the man and the pig still persists.
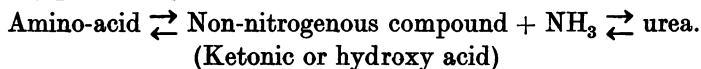
The creatinine output has been shown to bear some close relationship with the minimum nitrogen output. I therefore attempted to compare my results with McCollum's in terms of the amounts of creatinine excreted in the several experiments. I ignored the faeces and calculated the negative balance by subtracting the nitrogen intake from the output in the urine. The ratio of this balance to the average amount of creatinine nitrogen excreted during the same period is shown in the last column of Table XI. The last six days of each period have been alone considered.

Table XI.

Experiment	Nitrogen intake g.	Nitrogen in urine g.	Nitrogen balance g.	Creatinine N g.	$\dfrac{\text{Nitrogen balance}}{\text{Creatinine nitrogen}}$
R.R. II	5·10	6·41	−1·31	0·53	2·47
R.R. III	7·75	9·41	−1·66	0·59	2·81
R.R. I	12·23	13·55	−1·32	0·52	2·54
McCollum's pig	2·62	3·89	−1·27	0·48	2·65

There is obviously no discrepancy between our results when they are considered in this way, though what this agreement implies is not easy to state. It has been shown in many papers by Grafe (1912–1914), Abderhalden [1915], Underhill and Goldschmidt [1913] and others, that many nitrogen compounds other than amino-acids, namely organic ammonium salts, urea etc., have the capacity to spare a certain proportion of the loss of body nitrogen occurring on a carbohydrate diet. The results obtained by these workers do not agree in all points but the amount of nitrogen thus spared appears to be of the same order as that spared with gelatin in my experiments. It may well be that the action of the gelatin is of the same nature as that of these simpler

compounds and consists essentially in the reduction of the waste of amino-acids derived from body protein through deaminisation and subsequent oxidation in the body. The amino-acids of the gelatin and the ammonia and urea produced from them can play a part in the reversible reactions that are constantly proceeding in the body and thus influence the resulting equilibrium.

$$\text{Amino-acid} \rightleftarrows \text{Non-nitrogenous compound} + NH_3 \rightleftarrows \text{urea.}$$
(Ketonic or hydroxy acid)

If this is true the loss of body nitrogen when both carbohydrate and gelatin are fed may represent a "N-minimum" that corresponds more closely with the specific nitrogen requirements of the body than does the output on carbohydrate diet alone. This may perhaps be the explanation of the close agreement between the ratios of such loss to the creatinine nitrogen shown by my experiments and those of McCollum.

In conclusion I would express my very sincere thanks to Prof. C. J. Martin for his constant encouragement and advice throughout this investigation.

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