XXXVI. THE NUTRITIONAL VALUE OF TUBERIN, THE GLOBULIN OF POTATO.

By STANISŁAW KAZIMIERZ KON.

From the Biochemical Laboratory, Cambridge.

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THERE is much controversy concerning the value of potato as a source of nitrogen, and especially divergent opinions are encountered when the results of human maintenance experiments are compared with experiments carried out on growing rats. The high value of potato nitrogen for human beings seems to be established beyond doubt, not only by the experiments of Hindhede [1913, 1, 2; 1917], Rose and Cooper [1917], and Kon and Klein [1928], but also by the large scale natural experiment of potato-eating nations. On the other hand, McCollum, Simmonds and Parsons [1918] and Hartwell [1927] state that the potato is not a good source of nitrogen for the rat, young rats either growing very slowly or failing to grow on a diet in which the nitrogen was solely derived from whole potato. These rats also had bad fur. Adult rats, however, could be maintained in fair condition for a period of 9 months on a diet consisting of potato supplemented with NaCl, CaCO₃ and butter fat. In the opinion of the former investigators "maintenance on 8 % of potato protein ($N \times 6.27$) compares favourably with that obtained with a diet properly adjusted but containing only 7 % of wheat or oat protein."

All these experiments relate to the total nitrogen of potato. According to König [1903] only 43–65 % of the potato nitrogen is of protein nature. In view of these facts it seemed worth while to investigate the nutritional value of the isolated potato protein.

The chief, and probably only, protein of potato is a globulin called tuberin by Osborne and Campbell [1896].

EXPERIMENTAL.

Preparation of the protein.

The method described by Sjöllema and Rinkes [1912] was first used. The pressed and filtered potato-juice was saturated with common salt, the precipitate purified by solution in 10 % NaCl and dialysis, and then dried in the usual way. This method was associated with heavy losses, due chiefly to incomplete precipitation of the protein by the salt, incomplete solubility of the precipitate in 10 % NaCl, and extremely slow and incomplete precipitation during dialysis. Some 200 g. of protein were obtained in this way.

This batch was very dark in colour. Feeding experiments with this resulted in complete failure. When the protein was incorporated in an otherwise complete diet and fed to young rats at a 10 % level there was no growth at all, a marked loss of appetite was soon manifested and the rats died in the course of a few days, rarely surviving one week. They were always in a pitiful condition, thin and emaciated and continuously losing weight. When caseinogen replaced the potato protein in the diet the rats grew normally. On post-mortem examination of the potato-fed rats retention of urine was frequently found, but nothing else of interest. The symptoms clearly pointed to the presence of some toxic substance in the protein and it was thought that this might be solanin. There is much controversy regarding the toxicity of solanin and its rôle in cases of so-called potato poisoning. In any case, when taken in concentrations found in good sound potatoes it is obviously absolutely harmless to man and animals. It is therefore probable that if solanin is the cause of the symptoms of poisoning it must be concentrated along with the tuberin in the course of preparation of the latter. By extracting a 70 g. portion of the tuberin three times with boiling 85 % alcohol and treating the extract as described later in this paper, 0.1 g. of solanin, identified by the various colour reactions, was obtained. The potatoes used were old, and were sprouting when extracted (May, 1927). In view of these facts it was decided to prepare the protein by heat coagulation, and the following method was finally adopted: the potatoes (new "Eclipse" potatoes) after careful washing were finely minced in a meat mincer in 12.5 kg. batches and pressed in a hand press through cheese cloth, the juice was collected under toluene, the residue reminced and pressed in a hydraulic press. The combined pressings were then filtered clear through a thick mat of filter pulp on a Büchner funnel, the mat being changed 2-3 times. The yield was between 9-10 l. from 12.5 kg. of potatoes. The clear filtrate was then placed in an enamelled iron pan and steam introduced until the temperature reached 90°, when the fluid was allowed to settle. The bulk of the clear fluid was then syphoned off and the residue transferred to a Büchner funnel and sucked dry. It was then shaken with distilled water, filtered, suspended in 85 % alcohol and boiled under a reflux condenser for 20 minutes. This was repeated twice. The residue was then dried in the usual way by suspending in increasing concentrations of alcohol and finally in absolute alcohol and ether. It was found most convenient to make the suspension by shaking the protein with the fluids on a mechanical shaker. After washing with absolute ether the protein was kept in a vacuum desiccator until sufficiently dry, then powdered and dried in a steam-oven for 3 hours at 95°. 192 kg. of potatoes yielded 553 g. of a very light grey powder containing 14.8 % N, 1.1 % S, and 1.8 % ash. The 85 % alcoholic extracts were evaporated *in vacuo* to a small bulk, acidified with sulphuric acid, heated on a water-bath and filtered. The filtrate was then made alkaline with ammonia and a gelatinous precipitate was formed after a few minutes' heating. This was filtered off, extracted with

hot 85 % alcohol, the alcohol evaporated on the water-bath, the residue re-dissolved in acidified water and precipitated by adding an excess of ammonia. The white gelatinous flakes were filtered off and dried. They had a very bitter taste and gave all the colour reactions of solanin. The yield from 533 g. of crude tuberin was 0.3 g.

FEEDING EXPERIMENTS.

Of the various methods employed for estimating or comparing the nutritive values of proteins, two seem to be more generally adopted. One of them, originated by Thomas [1909] and based on the estimation of nitrogen balance, is known in its present greatly improved form as the method of Mitchell [Mitchell, 1924, 1; Mitchell and Carman, 1926]. The other, relating the nitrogen intake to growth, has been evolved by Osborne, Mendel and Ferry [1919]. Both use rats as experimental animals. It was thought advisable to make use of both of them in the investigation of the nutritional value of tuberin, not only in order to obtain more reliable results, but also to gain a direct comparison of both biological methods.

Mitchell's method.

Six young pure-bred piebald rats were used. Their initial weights were 50–70 g. The methods of Mitchell were followed with a few small modifications. The cages were higher than those described by Mitchell and the screen supporting the rat was raised 5 cm. above the bottom, thus preventing any possibility of ingestion of filter-paper. The food was placed in special feeders and scattering almost entirely avoided. The faeces were collected daily, urine every second day. For cleaning the rats were transferred to fresh cages and any loss of urine or faeces thus avoided. The urine was washed out with 400 cc. of very hot weak sulphuric acid. The faeces were collected under acidified alcohol. The estimations were carried out on the weekly samples. The composition of the diets is given in Table I.

Table I.

	Egg diet	Tuberin diet	Caseinogen diet
	g.	g.	g.
Tuberin	—	8.5	—
Caseinogen	—	—	8.6
Dried egg	5.35	—	—
Butter fat	17	17	17
Rice starch	45.65	42.5	42.4
Potato starch	10	10	10
Cane sugar	17	17	17
Salts	5	5	5

Each rat received, separately from the diet, 2 drops of cod-liver oil and an aqueous extract of yeast equivalent to 0.3 g. daily. The daily intake of 3.7 mg. N from this source was not taken into consideration when calculating the nitrogen balance. The rations were prepared in quantities of 1 kg. and kept in tightly stoppered glass jars in a cool place. The potato starch added to the diet took the place of roughage owing to its low digestibility.

Table II. *Nitrogen metabolism data.*

First group of rats.													
Rat No.	Initial weight g.	Final weight g.	Daily food intake g.	Daily N intake mg.	Faecal N mg.	Body-N in faeces mg.	Food-N in faeces mg.	Ab-sorbed N mg.	N in urine mg.	Body-N in urine mg.	Food-N in urine mg.	Food-N re-tained mg.	Bio-logical value
Low egg-nitrogen ration (0.71 % N)													
1	70	76	8.00	—	22	2.8*	—	—	18	25+	—	—	—
2	63	69	8.05	—	22	2.7*	—	—	14	22+	—	—	—
3	62.5	63.5	6.53	—	18	2.8*	—	—	14	22+	—	—	—
Tuberin ration (1.29 % N)													
1	82	90	8.11	104	23	22	1	103	45	20	25	78	76
2	77	81	6.82	88	24	18	6	82	30	17	13	69	84
3	71	80	7.21	93	22	19	3	90	34	16	18	72	80
Caseinogen ration (1.29 % N)													
1	94	100	8.08	104	26	21	5	99	59	22	37	62	63
2	84	89	7.04	91	20	19	1	90	54	17	37	53	59
3	86	92	8.15	105	22	20	2	103	60	17	43	60	58
Low egg-nitrogen ration (0.71 % N)													
1	103	111	8.71	—	21	2.5*	—	—	21	20+	—	—	—
2	92	97	7.57	—	20	2.7*	—	—	18	19+	—	—	—
3	97	104	8.79	—	22	2.5*	—	—	18	18+	—	—	—
Second group of rats.													
Low egg-nitrogen ration (0.71 % N)													
4	47	53	6.48	—	19	2.9*	—	—	13	26+	—	—	—
5	55.5	58.5	6.15	—	19	3.1*	—	—	14	25+	—	—	—
6	61.5	65.5	7.00	—	20	2.9*	—	—	14	23+	—	—	—
Caseinogen ration (1.29 % N)													
4	62	69.5	6.70	86	23	20	3	83	29	15	14	69	83
5	64	70	6.57	85	22	20	2	83	37	16	21	62	75
6	72	76	6.97	90	22	19	3	87	42	16	26	61	70
Tuberin ration (1.29 % N)													
4	75	78	6.31	81	20	18	2	79	50	16	34	45	57
5	75	78	6.55	84	29	19	10	74	46	17	29	45	61
6	84	90	7.54	97	26	20	6	91	48	18	30	61	67
Low egg-nitrogen ration (0.71 % N)													
4	81	84	7.47	—	22	2.9*	—	—	15	18+	—	—	—
5	82	88	7.87	—	22	2.8*	—	—	17	20+	—	—	—
6	91.5	97	8.43	—	22	2.6*	—	—	18	19+	—	—	—

* These figures represent the metabolic faecal nitrogen per g. of food consumed and are used in estimating the body-N in the faeces in the intermediate periods from the amounts of food consumed.

† These figures represent the endogenous urinary N per 100 g. of body weight and are used in estimating the body-N in the urine in the intermediate periods from the average body weight.

Table III. *Summary of biological values.*

Rat No. ...	1	2	3	4	5	6	Average
Tuberin diet	76	84	80	57	61	67	71
Caseinogen diet	63	59	58	83	75	70	68

Method of Osborne, Mendel and Ferry.

The protein was fed at three levels of intake, namely 8.5, 10.5 and 12.7 %. Rice starch was added to 51 and the composition of the rest of the diet was as stated in Table I. According to Osborne and Campbell [1896] tuberin contains 16.24 % N so that the actual amounts present in the above diets were 7.8, 9.7, 11.8 g. respectively ($N \times 6.16$).

Young pure-bred piebald rats were used. They were kept in individual screened cages. The food was mixed with a little water and placed in tall, narrow porcelain jars to avoid scattering. It was offered slightly in excess of the intake. Each rat received separately from the diet 2 drops of cod-liver oil and aqueous extract of yeast equivalent to 0.4 g. daily.

At the end of the experiment all the rats were in very good condition and their fur was smooth and soft. It is possible that the maximal gain per g. of protein ingested would be found still below the 7.8 % level and it is to be regretted that the protein was not fed at a lower level.

Table IV.

Diet	Rat	Sex	Days	Total intake		Gain in weight g.	Gain per g. protein g.
				Food g.	Protein g.		
8 % tuberin	7	F.	30	260	20.4	40.0	2.0
	8	M.	30	218	17.1	33.5	2.0
10 % tuberin	9	F.	30	233	22.6	43.5	1.9
	10	F.	30	235	22.8	32.5	1.4
	11	M.	28	203	19.6	32.0	1.6
	12	M.	30	213	20.6	34.5	1.7
	13	M.	28	201	19.5	33.0	1.7
12 % tuberin	14	F.	16*	121	14.3	25.0	1.7
	15	F.	28	212	24.9	42.5	1.7

* This rat was taken ill on the 17th day.

DISCUSSION.

It would appear from the above data that tuberin is a good and complete protein. The fact that it is sufficient not only for maintenance, but also for continued though subnormal growth when fed at the low level of 7.8 %, places it among the best of the isolated vegetable proteins. The gain per g. of protein is at the 7.8 % level distinctly higher than that found by Osborne, Mendel and Ferry [1919] for caseinogen at a 9.3 % level. Moreover, the nutritional value of isolated tuberin compares very favourably with values found by Osborne and Mendel [1920] for the entire proteins of barley, oat, rye and wheat kernels at an 8–10 % level. The biological value of the entire nitrogen of the potato has been estimated by Mitchell [1924, 2] at two levels of intake, called approximately 5 and 10 % protein ($N \times 6.25$). In reality these levels were found after calculation to be 5.5 and 7.9 % respectively. It is therefore possible to compare Mitchell's higher level with the results found in the present work for the isolated protein. The difference between the biological values (67 for the entire potato nitrogen and 71 for tuberin) is well within the limits of experimental error. It would therefore appear, as judged by Mitchell's methods, that the non-protein nitrogenous constituents of the potato are well utilised by the rat for repair of the body tissues. The value of potato-nitrogen for growth of young rats has been estimated by McCollum, Simmonds and Parsons [1918] and Hartwell [1927]. The former workers found that when whole cooked dried potato was supplemented with

butter fat and Na, Ca and Cl, and fed at a protein ($N \times 6.25$) level of approximately 8 %, some growth was obtained in a few rats over a period of 16 weeks, but the rats were emaciated and in poor condition. Other rats failed to grow on this diet. The authors ascribe their results to the deficiency and poor quality of the protein of the diet. It might be argued that the diet was not optimally balanced with regard to the other constituents and especially it has not been demonstrated that NaCl and $CaCO_3$ are alone sufficient to make good the inorganic deficiencies of potato. Hartwell by feeding cooked potato (containing probably approximately 75 % water) supplemented by the addition of 5 % butter and 1 % salt mixture, obtained better growth than McCollum and his co-workers. It was nevertheless very subnormal and the animals had at a certain period very bad fur. Hartwell thinks that the protein deficiency of potato when fed to rats is rather of a quantitative than a qualitative nature. The amount of fat present (some 20 % of the dry substance) might tend to lower the food intake by increasing the caloric value. That this is really the case seemed to be proved by the present writer by feeding to young rats at first a diet similar to that employed by Hartwell (100 g. of steamed potatoes, 5 g. of butter, 1 g. of salt mixture) with the addition of 2 drops of cod-liver oil and 0.5 cc. of an alcoholic extract of marmite. The rats grew very slowly, and reached a plateau after having gained about 30 g. The butter was then reduced to 1 %; there was then a rapid response, growth was resumed and the fur became better. After a month the general condition of the rats was very markedly improved. It would seem that when the other constituents of the ration are properly adjusted and the caloric value is low, the nitrogen of potato enables young rats to grow at a subnormal rate over long periods of time. It is to be regretted that the food intake of rats receiving whole potatoes has not been determined, as it was therefore impossible to calculate the nutritional value of whole potato-nitrogen according to the method of Osborne, Mendel and Ferry. Judging by the rate of growth of the rats, their behaviour and general appearance, there is little doubt however that the isolated protein is at the same level of intake distinctly superior to the entire nitrogen of potato. It is interesting to note that the difference disappears when the method of Mitchell is used and it seems evident that both methods often give quite discordant results. Thus, for example, the biological values at an 8 to 10 % level of veal, beef, pork, oat, wheat, and potato proteins were found by Mitchell [Mitchell and Carman, 1924, 1926; Mitchell, 1924, 2] to be equal to 62, 69, 74, 65, 67 and 67 respectively, with no significant differences between the various proteins, whereas using the method of Osborne, Mendel and Ferry the following gains per g. of protein ingested have been found: veal 2.9 g. [Hoagland and Snider, 1926, 1]; beef 3.1, pork 2.8 g. [Hoagland and Snider, 1926, 2]; oats 1.3 g., wheat 1.3 g. [Osborne and Mendel, 1920]; tuberin 1.8 g. (present work). Here the difference is very marked and decidedly in favour of the animal proteins. A specific error seems to be inherent in the method based

on the estimation of nitrogen balance, namely, the tendency to give higher biological values in the periods immediately following the standardising nitrogen-free or low nitrogen periods. This is specially marked in the present work, but may also be found in the papers of Mitchell and his co-workers [especially Mitchell and Beadles, 1927] and of Klein, Harrow, Pine and Funk [1926]. The method of Osborne, Mendel and Ferry also has the advantage of greater simplicity. On the other hand, it requires roughly three times as much of the protein for the same number of rats as the method of Mitchell, and is more sensitive to variations of the food intake. Obviously the two methods are not strictly comparable, as the one measures the efficiency of a given protein to make good, over short periods of time, the wear and tear of body tissues, while the other measures the ability of a protein to build new tissues. The question which of these methods is the more adequate for judging the relative values of various proteins in human nutrition by means of animal feeding experiments remains to be answered.

SUMMARY.

The biological value of tuberin, the globulin of potato, was found to be equal to 71 as measured at an 8 % level of intake by the method of Mitchell. The protein was also fed to rats at levels of approximately 8, 10 and 12 %, and a maximal gain of 2 g. body weight per g. of protein ingested was found at a 7.8 % level of intake by the method of Osborne, Mendel and Ferry. The protein was not fed at lower levels. Tuberin appears to be a good, complete protein.

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