CCXXIX. THE BIOLOGICAL VALUES OF PROTEINS.

IV. THE BIOLOGICAL VALUES OF THE PROTEINS OF WHEAT, MAIZE AND MILK.

BY MARGARET AVERIL BOAS FIXSEN
AND HESTER MARY JACKSON.

From the Division of Nutrition, Lister Institute, London.

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THE relative efficiencies of the proteins of wheat, maize and milk in supplying the amino-acid requirements of the body have gained particular interest in connection with current theories of the etiology of pellagra [Wilson, 1921; 1930], a disease now generally accepted to be of dietetic origin. The majority of recorded outbreaks of pellagra have occurred among the poorer classes in districts where maize and maize products form the staple foodstuffs of the population and its occurrence among populations subsisting on wheat and rice, though recorded, is extremely rare. The disease, if not too far advanced, is cured after change to a "good diet" in hospital or elsewhere, and milk, meat and yeast have been shown to possess preventive and curative powers when added to a pellagra-producing diet [Goldberger, Waring and Tanner, 1923; Goldberger and Tanner, 1924; Tscherkes, 1930]. It is therefore reasonable to expect that any hypothesis as to the cause of the disease should account satisfactorily for these facts [see Aykroyd, 1930, 1].

Goldberger's vitamin theory [Goldberger and Tanner, 1925; Goldberger, Wheeler, Lillie and Rogers, 1926; Goldberger, 1927] attributes pellagra to a deficiency of the heat-stable vitamin B_2 component of the yeast vitamin B complex and would explain the curative action of the foodstuffs mentioned above on the ground of their high content of this vitamin. It does not, however, account for the association of pellagra with the consumption of maize, for Aykroyd and Roscoe [1929] have demonstrated that maize contains appreciable quantities of vitamin B_2, being in this respect in no wise inferior to wheat.

Turning to the theory elaborated by Wilson [1921; 1930] that pellagra is due to an amino-acid deficiency, *i.e.* to the ingestion of diets containing proteins of low biological value, the evidence is somewhat conflicting. Our present information as to the biological values of the mixed proteins of foodstuffs is derived from experimental work in which the three following methods have been employed.

(*a*) McCollum and Simmonds [1917], following a method used by Osborne and Mendel [1916] in their work on pure proteins, fed young rats on diets in which the foodstuff under investigation was the sole source of protein and determined the minimum percentage of protein in this form which must be given in the diet in order to prevent loss of weight. Using this method they concluded that the proteins of whole wheat and whole maize were of approximately equal nutritional value.

(*b*) In later work [McCollum, Simmonds and Parsons, 1921] the diets containing the foodstuffs to be investigated were so constituted that in each the proportion of protein was the same. The performances of rats fed on the different diets were then compared, not only as regards rate of growth, but also as regards longevity, reproduction and lactation, during, if possible, at least two generations. On the basis of experiments of this nature they were able to range the proteins investigated in the following descending order of merit as regards their biological value:

$$\text{Ox kidney} > \text{wheat} > \left\{\begin{array}{l}\text{milk}\\ \\ \text{ox liver}\end{array}\right. > \left\{\begin{array}{l}\text{ox muscle}\\ \text{barley}\\ \text{rye}\end{array}\right. > \left\{\begin{array}{l}\text{maize}\\ \\ \text{oats}\end{array}\right. > \left\{\begin{array}{l}\text{soya beans}\\ \text{navy beans}\\ \text{peas}\end{array}\right.$$

In this series the high value assigned to wheat and the low position of maize are noteworthy, and afford support to Wilson's theory of the aetiology of pellagra. Since, however, in these experiments the foodstuff tested was frequently the sole source not only of the protein, but also of the vitamin B complex, variations in the supply of one or other of the constituents of this complex might have influenced the results.

(*c*) The third method involves experiments of comparatively short duration and consists in determining the relative efficiency of different proteins for replacing the daily nitrogenous expenditure of the body—the "wear and tear" nitrogen—and for supplying the material for growth. The determination is made by the balance sheet method elaborated by Thomas [1909; 1910]. The figures obtained by Thomas in a heroic series of experiments on himself assigned the lowest value to the proteins of maize. The relative quantities of body nitrogen which could be replaced by 100 parts of the protein-nitrogen supplied from the following foodstuffs were as follows: from meat 109; milk 100; white wheat flour 40; maize 30. Thomas's figures were used by Wilson [1921] in his development of the theory that pellagra is the result of an amino-acid deficiency. It is obvious that calculations made on this basis would show diets containing a large proportion of maize to be markedly inferior in the biological value of their total proteins to those consisting largely of wheat, even if other deficiencies were present in the latter diets. Thomas's technique and conclusions have, however, been severely criticised from many points of view by Martin and Robison [1922].

McCollum [1911; 1914] also used the balance sheet method, working with pigs, and obtained results which, calculated by Thomas's formula [see

Chick, 1930] yielded the following estimates of biological value; milk, 74; caseinogen, 67; maize, 51; wheat, 45. A further series of experiments on the pig [Hart and McCollum, 1914] also showed the biological values of the proteins of wheat, maize and oats to be about equal. In these experiments the diets contained high percentages of protein.

The results obtained by Mitchell and his co-workers [Mitchell, 1924, 1, 2; Mitchell and Carman, 1924; 1926; Mitchell and Beadles, 1926] with young growing rats are also of interest. When the diet contained 8 to 10 % of protein, they obtained the following estimates of the biological value of the proteins of certain foodstuffs: whole milk, 85; liver or kidney, 77; ox muscle, 69; wheat, 67; maize, 60; white flour, 52. In these experiments the vitamin B complex was supplied as Harris yeast vitamin.

From the above facts it is apparent that the available data as to the relative nutritional values of the proteins of the different foodstuffs are extremely conflicting, whether the criterion employed in the tests has been the value for maintaining nitrogenous equilibrium in short-period tests or the value for supporting growth and development in long-period trials. The whole subject is reviewed in great detail in a critical article by Mitchell [1924, 3], to which reference should be made.

On the whole there is evidence for the superiority in nutritive value of animal protein over cereal protein, though McCollum and his co-workers found wheat proteins better than those of milk or ox muscle. They also found wheat superior to the other cereals tested including maize and in this advantage of wheat over maize they confirmed the result obtained by Thomas. But in the other researches cited, no significant difference has been demonstrated between the values of the two cereals.

The contradictory nature of these results suggested that a fresh assay of the biological values of some of the more representative foodstuffs, more especially wheat and maize, would be of interest, particularly as it is now possible to supply the constituents of the vitamin B complex in concentrates of relative purity, thus obviating some of the difficulties inherent in many of the estimations carried out previously [see Boas Fixsen, 1930].

EXPERIMENTAL.

Method.

The balance sheet method was used and the technique employed was that fully described in previous papers [Chick and Roscoe, 1930; Boas Fixsen, 1930] with the modifications given in the preceding paper [Boas Fixsen and Jackson, 1932].

Materials used.

Wheat products: whole wheat, white flour and wheat germ prepared from the same sample of soft red English wheat.

Maize products: whole maize and maize endosperm from the same sample of South American yellow corn.

Cow's milk: a preparation of whole winter milk dried by the roller process.

Caseinogen: "light white casein" (British Drug Houses), a sodium caseinogenate, manufactured by the method of Hammarsten.

Heated caseinogen: "light white casein" heated for 3 days at 112°, being stirred at regular intervals.

Lactalbumin: a sample prepared from whey.

"*Whole rat.*" After removal of the skin, six adult rat carcases were placed in boiling water for 10 minutes. The lungs, livers, hearts, kidneys and muscular tissue were minced, dried at 37° for 36 hours and finely ground. 1 g. of the product was the equivalent of 3·2 g. of the fresh material and contained 10·37 % of nitrogen.

Diets.

The nitrogen-free diet used had the following composition.

Corn starch	735 g.
Sugar	90
Clarified beef dripping	100
Cod-liver oil	20
Salt mixture	50
Calcium carbonate	8

The nitrogen content of different batches varied from 0·2 to 0·4 % of the dry weight and the calculated calorie value of the dry diet was 460 kg.-cal. per 100 g.[1]

In the experiments with "whole rat" these materials were administered by the method described in a previous communication in connection with heated caseinogen [Boas Fixsen, 1930]. The other substances tested were incorporated in diets so constituted that the ratio of fat to carbohydrate remained approximately the same. Table I gives the composition of two

Table I. *The constitution of two representative diets—WG and WF—in which the protein was derived from wheat germ and white flour respectively.*

The weights given refer to the air-dry materials.

		Diet WG g.	Diet WF g.
Wheat germ*, 220 g.	Protein	88	—
	Fat	26	—
	Carbohydrate	54	—
	Undetermined	52	—
White flour*, 600 g.	Protein	—	74
	Fat	—	8
	Carbohydrate	—	507
	Undetermined	—	11
Corn starch		600	226
Sugar		90	90
Clarified beef dripping		104	124
Cod-liver oil		20	20
Salt mixture		50	50
Calcium carbonate		8	8

* The figures for composition are taken from Osborne and Mendel [1919].

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[1] The calorie value of this diet was previously wrongly given as 380 kg.-cal. per 100 g. [Boas Fixsen, 1930].

representative diets in which the protein was provided by wheat germ and wheat endosperm respectively, and Table II shows the estimated nitrogen and water contents and the calculated calorie values of the fat and carbohydrate of each of the diets used.

Table II. *Details of the diets used.*

Figures for water and nitrogen obtained by direct analysis.

Source of protein	Water content %	Nitrogen content of dry diet %	Protein content of dry diet (N × 6·25) %	Non-protein kg.-cal. per g. of dry diet
Whole wheat	10·10	0·890	5·6	4·1
White flour	10·32	1·125	7·0	4·2
Wheat germ	10·38	1·093	6·8	4·0
Whole maize	12·34	1·283	8·0	4·1
,,	8·00	0·781	4·9	4·4
Maize endosperm	9·50	1·072	6·7	4·6
Whole milk	8·66	1·097	6·9	4·4
Caseinogen	9·68	0·919	5·7	4·4
Heated caseinogen	9·02	1·110	6·9	4·5
Lactalbumin	9·54	1·074	6·7	4·5

Influence on biological value of the proportion of protein in the diet. Thomas [1909; 1910] assumed that, if sufficient fat and carbohydrate were supplied to cover the energy requirements of the organism, the capacity of any protein to replace the nitrogenous waste of the body tissue would remain constant at all levels of intake. Martin and Robison [1922] questioned the validity of this assumption on general grounds but found, nevertheless, in their own experiments made with the proteins of whole wheat, that the figure obtained for biological value remained the same when the diet contained the protein in widely different proportions. One of the present authors found the same to be true of heated purified caseinogen [Boas Fixsen, 1930]. Mitchell and other workers [Mitchell, 1924, 1; Morgan, 1931] on the other hand, found the biological values of many proteins to decline as the proportion in the diet was increased. In all the above tests the balance sheet method was used.

In the case of the proteins forming the subject of the present investigations, all those that have as yet been studied in different proportions in the diets have been found to show an increased biological value when given at the lower levels. This point is being made the subject of further study.

When the present investigation was planned it was not anticipated that the estimates of biological value would be influenced by the proportion of the protein in the diet. For this reason this level was not made uniform for all the proteins used. Whole wheat and caseinogen were studied in experiments so arranged that the proteins formed 6 % of the diet, with whole maize the levels of protein intake were 8 and 5 % and, with the other substances tested, 7 %. This has to be taken into account when deducing relative biological values from the results obtained.

RESULTS.

The results of the experimental work are collected in Table III. The average biological values, given in the last column, agree fairly well with those of Mitchell.

Table III. *Details of the experiments in which the biological values of proteins in various foodstuffs were determined.*

The figures represent average daily values.

Source of protein used	% of protein in diet	Date of exp.	Rat no.	Body weight g.	Change in body weight g.	Intake of non-protein Calories per kg. body weight kg.-cal.	Intake of nitrogen (true) mg.	Output of nitrogen			Biological value	Average
								Urine mg.	Endogenous faecal mg.	Total mg.		
Whole wheat	6	29. iii.–2. iv. 31	21	379	+1	174	122·4	117·1	16·8	133·9	77	
		13–17. iv. 31	20	460	−3	124	98·6	138·9	20·8	159·7	67	
		"	21	399	−2	138	98·6	121·7	16·8	138·5	67	
		27. vi.–1. vii. 31	23	503	−2	144	133·7	141·7	18·4	160·1	59	68
Wheat germ	7	16–20. xi. 31	26	420	−2	129	127·0	126·8	18·0	144·8	67	
		"	31	406	−1	142	134·2	133·8	19·7	153·5	71	
		"	32	416	−1	138	129·9	140·2	17·1	157·3	51	
		"	27	408	−1	142	130·5	126·4	17·8	144·2	75	
		"	34	401	−1	155	136·7	130·7	19·4	150·1	83	69
Wheat endosperm	7	11–15. v. 31	26	362	−3	136	123·6	123·7	16·2	139·9	60	
		27. iv.–1. v. 31	20	460	−2	117	129·9	159·3	19·4	178·7	56	
		4–8. v. 31	23	454	+1	139	151·0	145·7	17·5	163·2	57	
		20–24. iv. 31	27	321	+2	192	155·9	133·1	21·8	154·9	69	
		"	28	328	−1	154	123·1	120·5	17·6	138·1	62	61
Whole maize	8	17–24. iii. 31	12, 15	393	+1	185	195·6	147·0	22·9	169·9	63	
		"	11, 16	383	+2	188	177·5	138·6	15·7	154·3	71	
		17–21. iii. 31	13, 14	375	+1	142	144·9	108·9	32·9	141·8	68	67
	5	26. iv.–1. v. 31	11, 15	418	−2	139	79·6	97·0	19·5	116·5	85	
		"	12, 16	416	−2	160	99·5	96·2	20·3	116·5	78	
		"	13, 14	392	−1	151	97·3	92·2	22·9	115·1	81	
		29. xii. 30–2. i. 31	20	414	−3	119	76·5	114·1	18·1	132·2	92	84
Maize endosperm	7	9–13. ii. 31	21	371	−2	187	135·0	134·9	16·8	151·7	66	
		"	22	389	−4	173	135·4	136·0	19·5	155·5	71	
		16–20. iii. 31	18	546	−2	133	150·8	142·6	23·2	165·8	77	
		"	20	441	−3	123	122·4	149·3	20·8	170·1	65	70
Whole milk	7	20–24. iii. 32	37	342	+1	136	103·4	111·3	15·6	126·9	93	
		"	41	305	−1	172	119·1	84·3	26·1	110·4	88	
		"	35, 36	345	−1	129	89·3	104·6	13·3	117·9	77	
		4–8. iv. 32	31	408	−1	136	118·3	105·7	19·7	125·4	87	86
Lactalbumin	7	1–5. ii. 32	42	308	−4	126	83·1	108·6	11·3	119·9	70	
		"	26, 31	406	−3	131	121·8	137·3	19·2	156·5	66	
		"	32	416	−2	139	128·7	135·7	17·0	152·7	63	
		8–12. ii. 32	36	345	−4	147	106·6	130·6	14·4	145·0	60	65
Caseinogen	6	23–27. xi. 31	35	262	+2	169	94·0	78·0	13·2	91·2	94	
		"	37	403	−1	144	118·0	124·2	18·3	142·5	75	
		"	39	388	−3	116	87·9	95·6	19·9	115·5	79	
		10–14. i. 32	38	337	−1	133	91·9	100·1	7·5	107·6	81	
		25–29. i. 32	39	316	−6	157	106·9	125·4	18·2	143·6	57	
		"	37	344	−1	156	114·1	127·5	17·6	145·1	75	
		"	35	300	−1	159	94·4	101·6	13·0	114·6	74	76
Heated caseinogen	7	22–26. ii. 32	41	299	+1	167	115·6	119·3	26·1	145·4	57	
		"	43	361	−4	146	121·2	169·0	18·4	187·4	49	
		28. ii.–3. iii. 32	42	297	−2	180	117·9	135·0	11·3	146·3	57	54
"Whole rat"	7	27. vi.–1. vii. 32	31	431	−1	>122	139·7	157·3	17·6	174·9	44	
		"	35	377	0	>142	143·0	132·9	16·8	149·7	64	
		"	41	355	−1	>145	134·9	135·4	22·8	158·2	53	
		"	43	424	−1	>130	140·0	149·0	19·5	168·5	57	
		25–29. vii. 32	41	361	−1	>141	145·1	137·2	20·7	157·9	57	55

Seeing that the figures obtained from different experiments with the same protein showed variations of from 10 to 20 %, only large differences between the average figures obtained for the different proteins are considered to be of significance. When this is allowed for, the following conclusions can be drawn.

Wheat and maize proteins. There is little significant difference between the values found for whole maize (67 and 84 at 8 and 5 % levels, respectively) and whole wheat (68 at a 6 % level). Wheat germ gave a value of 69 (7 % level) and wheat endosperm 61 (7 % level) which is in accord with the observation of Klein *et al.* [1926] that in wheat the proteins of the germ are superior to those of the endosperm.

Milk protein. The proteins of whole milk were found to possess the highest value of the series tested, with an average figure of 86 when given in a diet containing 7 % protein. Caseinogen and lactalbumin were both inferior, giving figures of 75 and 65 for protein intakes of 6 % and 7 % respectively, suggesting that these two proteins exert a supplementary action upon one another in their mixture in milk. The slight superiority of caseinogen over lactalbumin is contrary to what would be expected from the available data concerning the amino-acid make-up of these two proteins, seeing that analysis of the latter shows a larger proportion of the important amino-acids tryptophan, cystine and lysine. Osborne and Mendel [1916] found lactalbumin to be superior to caseinogen for both growth and maintenance of young rats.

Purified caseinogen (extracted with acidified water and with dilute alcohol and then heated for 3 days at 115°) when evaluated previously [Boas Fixsen, 1930], was found to have an average biological value of 45 (50 when calculated by the modified method now used) at all levels. The discrepancy between this figure and the higher values obtained by other workers [McCollum, 1914; Mitchell, 1924, 2] was at that time tentatively ascribed either to differences in technique, or to the removal during purification of traces of a contaminating protein with a biological value higher than that of caseinogen. Morgan's work [1931] has shown, however, that it should be attributed to the marked deterioration in biological value suffered by a protein as the result of prolonged heating at a relatively high temperature. This is confirmed in the present work, in which unpurified caseinogen was evaluated at 76 (6 % level), while caseinogen which had been heated but not extracted had sunk in value to 54 (7 % level).

The low value of 55 given by the "whole rat" preparation is of interest. Theoretically one would expect the proteins of the rat to have for that animal a value approaching 100, by analogy with Michaud's [1909] experiments in which dog's flesh was found to have an efficiency of 100 % in replacing the dog's daily nitrogen excretion.

APPLICABILITY OF THE EXPERIMENTAL RESULTS TO THE CURRENT THEORIES OF THE AETIOLOGY OF PELLAGRA.

It is difficult to reconcile the above observations on wheat and maize with the theory that pellagra is caused by an inferiority in the quality of the protein consumed on the maize diets, *i.e.* to an amino-acid deficiency. Since 58 % of the protein of maize [Osborne and Mendel, 1913] is the notoriously incomplete zein, one must conclude that maize glutelin, which constitutes 36 % of the total protein, forms an effective supplement to zein in maintaining nitrogenous equilibrium in the adult animal. Osborne and Mendel [1914], indeed, found maize glutelin to possess a high nutritive value.

On the other hand it is not easy to decide how far results of the kind described in this paper obtained in short term experiments on rats are applicable to the case of human beings subsisting for long periods on a diet consisting largely of maize or to what extent they are of importance in the aetiology of pellagra, a human disease developing after many months' subsistence on a faulty dietary regime.

It is worth noting, nevertheless, that six days (the duration of each metabolism experiment including the preliminary period) in a rat's life is roughly equivalent to 4 to 5 months of a man's life. In Goldberger's Rankin farm experiment [Goldberger and Wheeler, 1920] the first symptoms of pellagra appeared after 5 months' subsistence on the experimental diet.

The balance sheet method as used by us is concerned only with the relative efficiency of proteins in replacing the daily nitrogenous expenditure of the body, *i.e.* with the function of maintenance. In McCollum's long-term experiments, on the other hand, growth, reproduction and lactation were also involved. It is probable that for these functions a disparity exists between proteins shown to be of equal value as regards maintenance[1]. The inferiority of maize to wheat, when given as sole source of protein in diets employed for rearing young rats to maturity, has received some measure of confirmation in recent experiments in this laboratory. Pellagra, however, as was pointed out by Mitchell [1924, 3] in this connection, is a disease mainly of adult life and not specifically associated with periods of growth or adolescence.

In considering the theory linking pellagra with a simple amino-acid deficiency, a fact to be noted is the low calorie value of all the pellagrous diets quoted by Wilson, except that used in Goldberger's Rankin farm experiment. In consequence, much of the already low protein intake must have been used for fuel, so that whatever the biological value of the protein when used solely to supply nitrogen, a deficiency of protein would inevitably result. In every case cited where pellagra was cured by the addition to the diet of animal protein, the calorie value was at the same time raised, thus automatically improving the value of what was previously the sole source of protein. Many of the non-pellagrous diets were also of low calorie value, so that in these also the biological value of the protein must have been much lower than that calculated by Wilson, even if Thomas's figures from which the calculations were made are considered trustworthy.

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[1] It is interesting to note in this connection that in South Carolina mill villages in 1917 the incidence of pellagra per 1000 persons from 20–29 years of age was 61 females to 3 males, while the ratio of married pellagrins to those found among single women was 52 to 12 [Smith, 1931].

It is evident, therefore, that the experimental support for the theory connecting pellagra with a simple amino-acid deficiency in the diet, is not as strong as has been supposed. The superiority in biological value shown by proteins of animal origin is in accord with the proved curative value of animal foods, but the fact that maize has not been found consistently inferior in this respect to wheat or certain other cereals fails to account for the known association of the disease with the consumption of maize. In this respect, therefore, the theory relating pellagra directly to a deficiency of protein of high biological value has not been more fortunate in its experimental support than the theory of vitamin deficiency put forward by Goldberger and his associates (see above).

It must be admitted therefore that as yet there is no satisfactory explanation of the fact, well established in human experience, that maize possesses a nutritive value inferior to that of wheat and that there is a greater liability to nutritional disease on diets consisting largely of the former cereal. Maize is eaten of necessity and never of choice if wheat is available, which fact probably provides an additional proof of its inferiority. The theory has frequently been advanced that a toxic substance is present in certain portions of the maize grain or may be developed under certain conditions. There is, however, very little direct evidence in its support. The oft-quoted experimental work of Horbaczewski [1910; 1912] was interpreted to show that the skin lesions of pellagra were due to an increased sensitiveness to strong sunlight caused by absorption of this toxin. Although his experiments lack confirmation [Aykroyd, 1930, 2] the widespread suspicion that some definitely unwholesome substance may be present in maize deserves further investigation.

SUMMARY.

1. The following figures have been obtained for the biological values of the proteins of certain foodstuffs, using the balance sheet method with adult rats as the subjects, biological value being defined as the number of parts of body nitrogen replaced by 100 parts of the protein investigated,

$$\text{or} = 100 \times \frac{\text{Body N saved}}{\text{Food N absorbed}}.$$

Whole wheat (6 % protein in diet) 68; wheat embryo (7 %) 69; wheat endosperm (7 %) 61.

Whole yellow maize (8 %) 67; (5 %) 84; yellow maize endosperm (7 %) 70.

Whole milk (7 %) 86; lactalbumin (7 %) 65; caseinogen (6 %) 76; heated caseinogen (7 %) 54·5.

"Whole rat" preparation (7 %) 55.

2. From these figures certain conclusions have been drawn.

(*a*) No significant difference is shown between the biological values, as defined above, of the proteins of wheat and maize.

(*b*) The proteins of the embryo of wheat are of slightly superior value to those of the endosperm.

(*c*) Caseinogen suffers a marked decline in biological value as the result of prolonged heating at 112°.

(*d*) Caseinogen is a slightly more valuable protein for maintenance than is lactalbumin.

(*e*) Caseinogen and lactalbumin appear to have a supplementary action towards one another, the proteins of whole milk being superior in value to either protein ingested separately.

3. The experimental study of the biological value of maize and wheat proteins lends little support to the theory that pellagra among maize eaters is the result of a simple amino-acid deficiency. So far no satisfactory explanation is forthcoming of the general experience of mankind that diets consisting largely of maize have been found of inferior nutritive value to these containing wheat as the principal foodstuff.

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