# Further Observations on the Nutritive Value of the Proteins contained in Wheat Flours of Different Extraction Rates

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Chick (1942) described experiments in which comparison was made of the growthsupporting value of the proteins contained in flours of 100, 85 and 70% extraction of the wheat grain, the method employed being that of Osborne, Mendel & Ferry (1919). In these tests three groups of weanling litter-mate rats were fed *ad lib*. amounts of three different diets containing equal suboptimal amounts of protein provided by the respective flours, the protein being the factor limiting growth in an otherwise adequate regime. Determinations of the protein efficiency ratio (g. weight increase/g. protein consumed, henceforth referred to as the P.E.R.), for the proteins of the different flours indicated an advantage over the proteins of white flour of 17-26% for those of wholemeal, and of 13-16% for those of flours of 85% extraction.

The value of results yielded by this type of experiment had previously been criticized by Mitchell (1924) and others, and carefully appraised by Fixsen (1935) and by Barnes, Maack, Knights & Burr (1945). The chief grounds for criticism were: that the method does not differentiate between the value of the protein for maintenance and for growth; that under conditions of ad lib. feeding the diet containing the protein judged to be of higher nutritive value will probably be more palatable to the experimental animal and, for this reason rather than because of its higher nutritive value, will be consumed in greater amount, with a consequently greater weight increase; that the weight gain of larger, more rapidly growing, animals will contain relatively more fat and, therefore, will not be a measure of the increase in body protein. The last objection is effectively answered in the accompanying paper by Slack (1948), at least in so far as the present experiments are concerned, and, in a recent review of the methods available for comparing the nutritive values of different proteins, Chick (1947) has replied to some of the other arguments. Barnes et al. (1945) concluded that fairly trustworthy results are given for the nutritive value of most proteins by P.E.R. values with ad lib. feeding if the protein content of the ration is 10-12%. Graves (1945, 1946), however, in repeating the above criticisms, found a basis for discrediting the conclusion arrived at by Chick (1942) that proteins of whole wheat had a higher nutritive value than those of white flour.

The present paper presents additional evidence of the value of the method of Osborne *et al.* (1919) for comparing the nutritive efficiency of proteins in wheat by determining their P.E.R. In the first experiment (Exp. 1), of which a preliminary account has already been published (Chick, Copping & Slack, 1946), in addition to measurements of weight increase, food and nitrogen intake, faecal nitrogen and weight of faeces,

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determinations were also made of the proportion of the ingested nitrogen which was retained in the bodies of groups of comparable young rats fed on diets in which the protein was derived from flours of 70, 80, 85 and 100% extraction, respectively. In order to do this, analyses were made of the composition of the bodies of the rats in the different groups at the end of the test and of comparable litter-mates killed at the beginning of the experiment. This work is fully reported in the accompanying paper by Slack (1948) and only the final results will be alluded to here. They show, however, a close correlation between the proportion of ingested nitrogen retained in the animal's body, the growth rate, and the P.E.R. values of the proteins of the different flours.

A second experiment (Exp. 2) of a different type is also reported in this paper. The results confirmed the superiority of the nutritive value of the proteins contained in the whole wheat over those of white flour. Equal rates of growth were obtained when the protein content of the diet containing the wholemeal flour was 25% lower than that of the diet containing the white flour.

#### EXPERIMENTAL

*Exp.* 1. The flours (K series) were supplied by the Cereals Research Station, St Albans, and were all milled from the same grist. With the exception of the wholemeal flour they were prepared on a semi-commercial scale in 1 ton lots. Analysis revealed a nitrogen content, on the dry weight, of 2.55, 2.52, 2.42 and 2.34% for the 100, 85, 80 and 70% extraction flours, respectively. The flours were incorporated as the sole source of nitrogen in a series of four diets, adequate in other essential nutrients but suboptimal in protein content, which was about 12%. The nitrogen content of the diets was equalized by the addition of pure maize starch to the flours of higher percentage extraction (see Table 1). The four diets were given to four groups of six rats

			Ехр. 1.	Series 1	K flours		Exp	p. 2. Sei	ries G flo	urs
	Ingredient	Nitrogen content (%)	Diet 1	Diet 2	Dict 3	Diet 4	Nitrogen content (%)	Diet 5	Diet 6	Diet 7
Elawa	( 70	2.34	92				2.27	92	—	
Piour	80	2.42	_	88	•	- '-				
% extrac	<sup>-</sup> ] 85	2.52		_	85					
tion	100	2.25	_	_	<u> </u>	85	2.44		78.3	62
Maize sta	arch	0.03	-	3.2	6.8	6.8	0.03		14	30
Arachis o	il, hardened	—	4.6	4.6	4.6	4.6		4	4	4
Salt mixt	ure*		2,	2	2	2	<u>.</u>	2	2	2
Cod-liver	· oil	—	I	I	I	I	-	I	I	I
Average r	nitrog <mark>en c</mark> on-	—	2.10	<b>2</b> · I I	2.08	2.08		1.99	1.91	1.23

Table 1. Percentage composition in terms of dry ingredients of diets used in Exps. 1 and 2

Each rat received daily 1 ml. of protein-free yeast extract ( $\equiv 0.5$  g. dry yeast) to provide extra B vitamins.

• McCollum, Simmonds & Pitz (1917).

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each, selected from four newly weaned litters to give groups comparable in heredity, sex and initial body-weight. The rats were caged and fed singly but in other ways each group was treated as a unit, estimations being made of total dry food and nitrogen consumed and of the total faeces and faecal nitrogen passed by the group. Further details of the methods used were published by Chick (1942).



Fig. 1. Exp. 1. Average weight curves for groups of six rats maintained on diets in which the nitrogen, c. 2·1% of the dry weight, was derived from flour of 70 ●----●, 80 ◎ ----●, 85 +----+ and 100% ●----●, extraction.
---- 'Normal' growth curve of similar rats fed on a complete synthetic diet containing 25% casein.

The results set out in Table 2 show that the flours formed a descending series in respect of the nutritive value of their proteins which corresponded to the progressive fall in the extraction rate. In Fig. 1 is shown the average increase of weight of the rats in the different groups. At the end of the 7 weeks' test the rats receiving the wholemeal diet had attained an average body-weight of 126 g.; the corresponding weights for the groups receiving the 85, 80 and 70% extraction flours were 110, 99 and 91 g., respectively. In addition it was noticeable that the rats receiving the whole-meal and

85% flours were in a better and healthier condition than the others, as judged by the condition of their coats, eyes and muscular development. As was to be expected, the more rapidly growing animals had better appetites than their outstripped litter-mates and consumed more food, but comparison of intake with growth showed that they used it more economically.

The values for the P.E.R. of the proteins in the wholemeal, 85, 80 and 70% extraction flours were respectively 1.63, 1.63, 1.47 and 1.35. If the P.E.R. values were calculated on the basis of the proteins absorbed from the different flours, the advantage shown by the 100 and 85% extraction flours would appear even greater owing to the lesser availability of their proteins. In the last three columns of Table 2 are given figures taken from the paper by Slack (1948) which show for the different flours the average proportion of the nitrogen eaten which was incorporated in the bodies of the rats during the period of the test. The figures were 26.7% for the wholemeal nitrogen and 22.9% for that of the white flour of 70% extraction; the corresponding figures for the proportion of the absorbed nitrogen which was retained were 33 and 25.9%. In capacity to support growth, in P.E.R. value and in the proportion of ingested nitrogen retained, the proteins of the 70 and 80% extraction flours fall into a group of about the same order; those of the wholemeal flour were in all respects more efficient and those of the 85% extraction flour were intermediate.

The apparent digestibility of the nitrogen in the four flours was found to fall progressively as the percentage extraction was increased; the coefficients determined for the flours of 70, 80, 85 and 100% extraction were 88.8, 86.7, 85.5 and 81.0 respectively.

*Exp.* 2. The comparison of the nutritive value of the proteins of wholemeal and 70% extraction white flour made in the preceding experiment by comparing the values for their respective P.E.R. showed a superiority of the former of about 20%. It therefore seemed possible that diets of equal growth-supporting power might be obtained with proteins supplied respectively from wholemeal and white flour if the proportion of the protein in the first were about 20% lower than that in the second. Exp. 2 was designed to test this possibility and the result showed it to be realized.

The performance of young weanling rats on diet 5 (Table 1), containing white-flour protein at a level yielding 2% of nitrogen on dry weight, was compared with that of litter-mates on two diets, 6 and 7, in which the protein was supplied from wholemeal flour and the nitrogen content on dry weight was respectively 1.9 and 1.5%. Unfortunately the supply of the K series of flours used in Exp. 1 was insufficient for this test and use had to be made of another (series G), the wholemeal flour of which had a nitrogen content of 2.44% and the white flour one of 2.27%, on dry weight. The composition of diets 5, 6 and 7, which were made according to the ordinary procedure, is given in Table 1.

Three litters of newly weaned rats were distributed evenly in three groups of seven rats each, receiving respectively diets 5, 6 and 7. The experiment lasted 6 weeks. The intake of food was measured weekly. The faeces were collected and analysed for nitrogen content in fortnightly periods and thus three checks were provided of the digestibility of the nitrogen in the different diets. Average growth curves of the rats

			aralim in	m percent	uge extration (Nitrogen	content of	un vautes I dry diets 2.6	01 111 Jul	Sroups )	ni vie lo	3			
	Percentage	Dry food	Nitrogen	Protein *	Dry weight of faeces	Nitrogen in	Coefficient of apparent	Body-w	Total	Protein efficiency	Nitrogen contained ] in the	Percentage	retention of	
Diet no.	extraction of flour	eaten (g.)	eaten (g.)	eaten (g.)	passed (g.)	faeces (g.)	digestibility of nitrogen1	r (g.)	increase (g.)	ratio (p.e.r.)	weight gain‡ (g.)	Nitrogen eaten	Nitrogen absorbed	
н	20 20	285	66.5	34'1	8.8	- 6 <u>-</u>	8.88	44-8	46.2	1.35	1-36	6.22	6.52	
N 0	80.20	232.6	0.30 6-04	35'9 30'6	10.6 16	0.04 1.01	84.5	45.0	53°0 64.5	1.62 1.62	6£.1	9.72	28.8 28.8	
ο 4	100	408	8.46	48'2	42.1	09.1	0.18	47:2	78.6	1.63	2.26	26.7	0.88	
		• N × 5.	7.											
		4 8. N e	aten – g. N	in facces ×	100.									
		t Gain ir	g. N eater n weight rec	ı. ckoned fron	n initial (esti	imated) an	d final (mea	sured) ' emp	ty' weigh	ts (see Sla	ick, 1948).			
	Table	}. Exp. 2	Econom	ty of utili.	zation for	growth a	during 6 w	eeks of th	te protei	ns conta	ined in w	heat flour.	6	
			of 70 and	1 100% 6	xtraction.	Mean v	alues per 1	at jor gro	s fo sam	even rais	r Body-weigh	ţ		
						Ċ	,				Inci	rease	ſ	
	Percentage	Nitrogen content of	Drv food	Nitrogen	Protein *	Ury weight of facces	Nitrogen of in di	oemcient f apparent igestibility		l	g./c.	g./g. Protein•	g./100 g.	
Diet no.	extraction of flour	dry diet (%)	eaten (g.)	eaten (g.)	caten (g.)	passed (g.)	faeces of (g.)	f nitrogen† (g.)	Initial (g.)	T`otal (g.)	Nitrogen caten	eaten (P.E.R.)	Food	
ŝ	20	66.1	311.2	61.9	35.3	8.7	99.o	89.3	49.7	48	7-7	1.36	15.4	
9	100	16.1	341.2	6+9	37.0	30.5	21.1	82'2 ?	20.2	61	9.4	1.65	6.41	
1	81	1.53	326.9	4.93	1.82	24.8	o.94	80.7	20.1	50	1.01	1.78	15.3	
					• N×5	ʻ7.								
					2 30 +	eaten – g.	N in faeces	100						
					-	g. N ea	iten							

Table 2. Exp. 1. Economy of utilization for growth during 7 weeks of the proteins contained in wheat flours

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in the three groups are shown in Fig. 2, and figures for intake of food and nitrogen and excretion of nitrogen in the faeces are given in Table 3.

The results showed an almost identical performance by the rats on the white-flour diet 5 containing 1.99% nitrogen and by those receiving the wholemeal diet 7



Fig. 2. Exp. 2. Average weight curves per rat for groups of six rats maintained on diets in which the nitrogen was derived from flour of: 70% extraction providing 1.99% nitrogen on dry weight of diet ●----●, 100% extraction providing 1.53% nitrogen on dry weight of diet ④----●, and 100% extraction providing 1.91% nitrogen on dry weight of diet ●----●.

- 'Normal' growth curve of similar rats fed on a complete synthetic diet containing 25% casein.

containing 1.53% nitrogen, about 25% less protein of a lower degree of digestibility. The average food intake per rat on these two diets was 311 and 327 g., the total weight increases 48 and 50 g., and the coefficients of digestibility of the nitrogen 89.3 and 80.7 respectively. These figures for digestibility are in close agreement with those (88.8 and 81.0 respectively) obtained in Exp. 1 for that of the nitrogen in 70 and 100% extraction flours.

From these results one may conclude that the proteins of the wholemeal flour had

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a nutritive efficiency 25% greater than that of the white-flour proteins, a result which confirms that of Exp. 1. Comparison of the P.E.R. would seem to indicate an even greater advantage for the wholemeal proteins, but this conclusion would be unjustified, as one of the conditions for determining P.E.R. is that the proteins under test should be given in diets of equal protein content. It is, however, permissible to compare the value of the P.E.R., 1.36, obtained with diet 5 of nitrogen content 1.99%, with that of 1.65 obtained with diet 6 of nitrogen content 1.91%. These figures show an advantage of 21% for the wholemeal proteins, a result which is in agreement with that derived from the tests with diets 5 and 7 by a different type of experiment. In those tests comparison is made between the different proportions in the diets of white-flour proteins and of wholemeal proteins, respectively, which correspond to an equal growth performance; from the results obtained with diets 5 and 6 comparison is made between the values of P.E.R. obtained with diets containing the same proportion of protein derived, respectively, from the two flours.

### DISCUSSION

The nutritive advantage demonstrated for the proteins in flours of higher, over those of lower, percentage extraction of the wheat grain is due to the fact that the proteins contained in the tissues of the outer layers of the grain, which include the aleurone, are of better quality than those situated in the endosperm. They are richer in the essential amino-acids lysine, arginine and tryptophan (Jones & Gersdorff, 1925; Barton-Wright & Moran, 1946). The results of tests of the growth-promoting value for young rats of the proteins of white flour alone and of admixtures with those of wheat bran showed an advantage for the latter which was in direct proportion to the amount of bran proteins in the mixture, in other words to the amount of extra lysine (or tryptophan) introduced with the bran (Chick, Cutting, Martin & Slack, 1947). The bran is included in the fractions of the wheat discarded in the milling of white flour; these fractions comprise, besides the fibrous pericarp, the aleurone layer, the germ and the outer portions of the endosperm. The aleurone layer, although only one cell thick, occupies from 7.5 to 9 %of the volume of the grain and has a protein content of about 22%, amounting to about 16% of the total protein in the grain (Girard, 1884; Osborne & Mendel, 1919; Booth, Carter, Jones & Moran, 1941; Hinton, 1947; Chick et al. 1947). Also the outer layers of the endosperm are richer in protein than the inner portion and the protein has a higher nutritive value (Cobb, 1905; Morris, Alexander & Pascoe, 1945; Moran, 1945; Moran & Drummond, 1945; Hinton, 1947; Chick et al. 1947). The germ is even richer in protein of high quality, but as its weight is only about 1.5-2.5% of that of the grain, its contribution of protein is relatively small. As the percentage extraction of the flour is progressively raised, more and more of these outer tissues richer in protein of higher nutritive value are included.

At 80% extraction the flour resembled that of 70% extraction in consisting almost exclusively of endosperm, but it contained more of the outer layers (see Cobb, 1905), a fact which was reflected in its higher growth-promoting power and higher P.E.R. value; the proportion of the ingested nitrogen retained was not, however, any greater

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(see Table 2). At 85% extraction the flour contains, besides the outer endosperm, some of the germ, a fair proportion of the aleurone and relatively little of the pericarp. The nutritive advantage shown over the 80% flour is probably due to the amount of the aleurone it contains. The separation of the aleurone in amounts sufficient for a biological test has not been accomplished, but fractions rich in this tissue have been shown to favour retention of nitrogen when compared with white flour in tests on rats and on human subjects (Guillemet & Jacquot, 1944; Jacquot, Trémolières, Guillemet & Erfman, 1944).

Flour of 85% extraction produces an acceptable loaf which is off-white in colour. In 1942 it was selected by the authorities in this country as National wartime flour for ordinary bread making.

#### SUMMARY

1. Four groups of rats from the same litters were maintained for 7 weeks on diets containing equal amounts of protein derived from wheat flours of 70, 80, 85 and 100% extraction of the grain respectively. The diets were adequate in other essential nutrients, but the proportion of protein was suboptimal, about 12% (N × 5.7) on dry weight.

2. The average rates of weekly weight increase in the four groups were respectively  $6\cdot6$ ,  $7\cdot6$ ,  $9\cdot2$  and  $11\cdot3$  g.; the corresponding values of the P.E.R. (g. weight increase/g. protein consumed) were  $1\cdot35$ ,  $1\cdot47$ ,  $1\cdot63$  and  $1\cdot63$ . The percentage of the nitrogen ingested which was incorporated in the bodies of the rats during the period of the test were respectively  $22\cdot9$ ,  $22\cdot1$ ,  $24\cdot6$  and  $26\cdot7\%$ .

3. Equal rates of weight increase were observed when rats consumed approximately equal amounts of diets, otherwise similarly composed but containing respectively 1.53% nitrogen derived from whole wheat (100% extraction) flour, and 1.99% nitrogen derived from white flour of 70% extraction. This result showed a 25% inferiority in efficiency for supporting growth, of the proteins in the white flour. The P.E.R. calculated from this experiment for the proteins of the white flour was 1.36: for those of whole-wheat proteins in a similar diet containing 1.91% nitrogen it was 1.65%.

4. From the results summarized in 2 and 3 above, it may be concluded that the nutritive efficiency for support of growth in young rats, as measured by the value of the P.E.R., is about 20% greater for the proteins of whole-wheat flour than for those of white flour of 70% extraction. The value of the proteins in the specimens of 85% extraction flour investigated, as judged by the same criterion, was equal to that of the whole-wheat proteins; the value of those of the 80% extraction flour was much lower and nearer to that of those in the flour of 70% extraction.

5. The figures found for the coefficients of apparent digestibility of the nitrogen in the flours of 70, 80, 85 and 100% extraction were respectively  $88\cdot8-89\cdot3$ ,  $86\cdot7$ ,  $85\cdot5$  and  $80\cdot7-81\cdot0\%$ . The lower digestibility of the nitrogen in the flours of higher percentage extraction was more than compensated by their greater nutritive value.

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