PROTEIN IN MALTED PREPARATIONS

By F. Wokes and Chloe Klatzkin

From the Ovaltine Research Laboratories, King's Langley, Herts

Received July 8, 1949

Previous communications[1,2,3,4] from these laboratories have dealt with the general food value of malted preparations, particularly malted barley and malt extract, giving data on the content of B vitamins (aneurine, nicotinic acid and riboflavine), of carbohydrates and of "protein." The present communication deals with the nature of the nitrogenous constituents included in the term " protein."

The nitrogenous constituents of malt extract have received poor recognition from official sources. The British Pharmacopœia, 1932, specified a minimum protein content of 4·5 per cent. based on 6·25 times total N_2, but the British Pharmaceutical Codex, 1934, attributed the nutritive action of malt extract to carbohydrates and B vitamins, ignoring the protein, and the United States Pharmacopœia XII also ignored protein as a constituent. Since the malt extract of U.S.P. XII also contained 10 per cent. of glycerin, giving a more readily fermentable product for which sterilised containers had to be prescribed, it was perhaps fortunate that it was excluded from the U.S.P. XIII. The 4·5 per cent protein minimum of the B.P. 1932, can be considerably exceeded in a good malt extract, as one of us (F.W.) showed in 1943. Nevertheless, the protein minimum in the B.P. 1948 has been lowered to 4·0 per cent.. A moderate diastatic value was introduced and then almost immediately withdrawn. Previous workers[5] have recommended the use of malt extracts with very low diastatic values for malt and oil manufacture, but this would involve the loss of protein and B vitamins. We hope to show that the proteins of malt extract and of other malted preparations are of greater importance than these pharmaceutical divagations indicate.

Methods

Total nitrogen (micro-method). A quantity of material containing about 3 mg. of N_2 was heated with 2 ml. of concentrated sulphuric acid and a trace of mercuric sulphate and 50 mg. potassium sulphate for half an hour after charring had been completed. The cooled solution was placed in a micro-Kjeldahl flask, 10 ml. of 40 per cent. sodium hydroxide solution and 1 ml. of 40 per cent. sodium sulphide solution were added and the contents of the flask were distilled with steam into 10 ml. of N/20 sulphuric acid. The latter was boiled to remove carbon dioxide and titrated against N/20 sodium hydoxide using methyl red as indicator. The difference betwen the reading and that given by a blank with reagents only represented the nitrogen from the sample. The micro method was employed to estimate minute amounts of nitrogenous constituents in germinated seeds of which only small quantities were available. It was controlled against ammonium sulphate and gave good agreement with the B.P. 1948 method.

Salt soluble nitrogen. The method of the Association of Official

Agricultural Chemists[6] was used with 1 per cent. of sodium chloride instead of 5 per cent. sodium sulphate, to minimise bumping during concentration of solutions. This alteration made no difference to the results.

Non-protein nitrogen. The method of the Association of Official Agricultural Chemists[6] was used.

Amino acids were estimated chemically by the methods described by Block and Bolling[7]. Some of these (e.g. for tryptophane) did not give very reliable results and our findings with them must be considered only tentative. However, with arginine, one of the most important amino-acids in our materials, we used Dubnoff's[8] modification of Sakaguchi's method[9], and obtained on a series of oats samples good agreement with results obtained at the Cereals Research Station, St. Albans, by Mr. J. G. Heathcote, who has had considerable experience of this problem, and to whom we are indebted for advice and help.

Aneurine was estimated fluorimetrically by the method[10] previously described which has given good agreement with microbiological methods.

Nicotinic acid was estimated colorimetrically using either *para*-amino-acetophenone[11] or *para*-aminopropiophenone[12] as the aromatic amine. Both of these gave satisfactory agreement with microbiological assays.

Riboflavine was estimated fluorimetrically by a method[13] giving good agreement with microbiological assays.

Diastatic index was estimated as previously described[1].

Trypsin inhibitor of soya and other foods was estimated by a modification of the method of Bowman[14] using skimmed milk instead of casein as substrate, and following the process of digestion by formol titrations.

RESULTS

Differentiation of nitrogenous constituents. (a) *Total nitrogenous constituents.* The B.P. 1948 method estimates total nitrogen by the Kjeldahl method and multiplies the result by 6·25 to determine the protein content of malt extract. This involves two faulty assumptions—that the factor 6·25 is accurate for cereal proteins, and that the nitrogenous constituents are all protein. We have overcome both these difficulties by quoting our results as nitrogen instead of as protein content. On this basis Extract of Malt B.P. 1948 should contain at least 0·64 per cent. of total nitrogen. This seems quite a low limit. Our results on 37 samples from 13 manufacturers, summarised in Figure 1, show that only two samples fell significantly below this lower limit, but seven samples fell below the B.P. 1932 limit. Our own samples were all well above this higher limit. Confirmation was provided of our previous finding that a low total nitrogen is usually accompanied by a low diastatic value. Data on malted barleys also given in Figure 1 show a similar tendency for the total nitrogen content to fall with decrease in diastatic value. However, we think that the wide variation in the total nitrogen of malt extracts is probably due partly to differences in brewing conditions.

(b) *Protein nitrogen* (from albumin and globulin plus " less soluble " protein). Our results in Table I show that only 12 to 44 per cent. of the total nitrogen content of malt extract represents true protein, as distinct from protein digestion products.

(c) *Salt-soluble nitrogen.* This figure includes nitrogen from albumins and globulins as well as from the breakdown products of these and other proteins, all of which are important in infant feeding. It therefore provides valuable information on the nutritive value of malted preparations. Our results (see Table I) show that it can vary significantly in different samples.

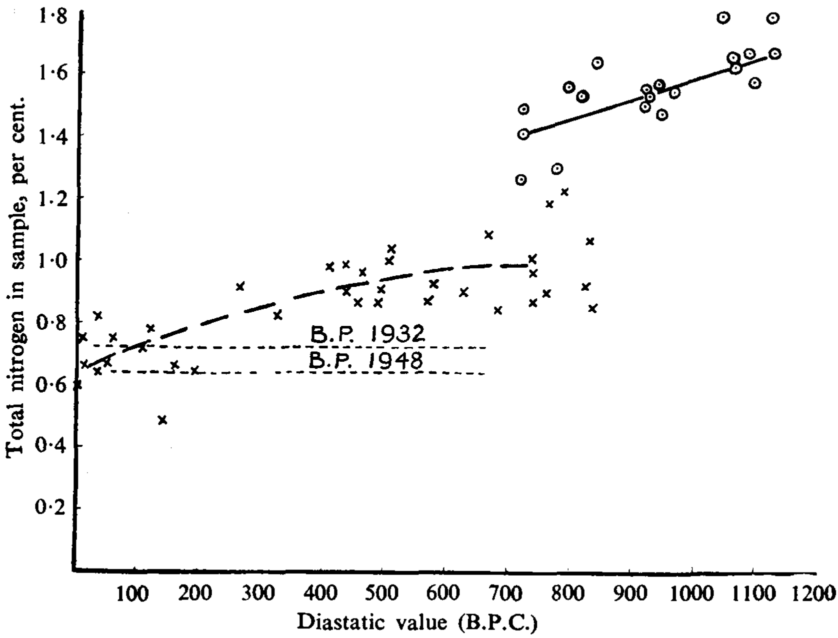

FIG. 1. Relation between diastatic value and total nitrogen content of malted barley (indicated thus ⊙——⊙) and of malt extract (indicated thus X— —X).

(d) *Non-protein nitrogen.* This figure includes the soluble breakdown products of the cereal proteins, with possible slight traces of asparagine and glutamine (which are important in plant metabolism[15] but have yet

TABLE I

DIFFERENTIATION OF NITROGENOUS CONSTITUENTS IN MALT EXTRACT

Sample	Salt-soluble nitrogen	" Non protein " nitrogen	Albumin and globulin nitrogen	" Less soluble " nitrogen	Total nitrogen
3 C	1·60	0·98	0·62	0·08	1·68
3 B	1·47	0·98	0·49	0·13	1·60
3 D	0·94	0·57	0·37	0·07	1·01
9 B	1·10	0·87	0·23	0·03	1·13
9 A	0·93	0·72	0·21	0·06	0·99
9 C	0·93	0·72	0·21	0·05	0·98
5 A	0·815	0·73	0·085	0·015	0·83
7 A	0·74	0·56	0·18	0·08	0·82
8 A	0·72	0·53	0·19	0·08	0·80
3 A	0·51	0·40	0·11	0·03	0·54

Notes—Albumin and globulin nitrogen = salt-soluble − non-protein nitrogen.
" Less soluble " nitrogen = total nitrogen − salt-soluble nitrogen.

to be established as significant in animal metabolism). The figure provides a useful measure of the extent of enzyme action during malting and brewing.

(e) *"Less soluble" nitrogen.* This figure represents the structural protein of plants, but would also include protein coagulated by heat during manufacturing processes. Little is yet known about its degree of availability to infants or its importance in infant nutrition. Our results in Table II show that it can vary widely in different samples, and we would suggest that preference be given to malted preparations in which the "less soluble" nitrogen has been reduced to a low level.

TABLE II

PERCENTAGE DISTRIBUTION OF NITROGENOUS CONSTITUENTS IN TOTAL NITROGEN IN MALT EXTRACT

Sample	Percentage of total nitrogen represented by nitrogen from		
	Albumin and globulin	Predigested protein	"Less soluble" protein
3 C	37	58	4·8
3 B	31	61	8·1
3 D	37	56	6·9
9 B	20	77	2·7
9 A	21	73	6·0
9 C	21	74	5·1
5 A	10	88	1·8
7 A	22	68	9·8
8 A	24	66	10·0
3 A	20	75	5·5

Protein value of malted preparations in infant feeding.

The experience of one of us (C.K.) shows that malted preparations are widely employed in London hospitals as dietary supplements for young children, especially those who are undernourished. Whilst some of the nutritive virtues of these malted preparations are doubtless due to their vitamin content, the presence of readily assimilated protein and carbohydrate must be of value. As long ago as 1865 Liebig[16] showed by experiments on his breast-fed grandsons that a deficiency in the supply of human milk could be made good by "Malzsuppe," a malted infant food prepared by heating an aqueous extract of malted barley with wheat flour and milk. The milk provided about one-third of the total solids and two-thirds of the total protein. This led to the introduction of malt extract for infant feeding, and perhaps provided the inspiration for the Italian brewing chemist, Caprino, to devise during wartime emergencies milk substitutes based on malted cereals. In Italy oats are a staple cereal, and hence were used by Caprino, leading to the name "Maltavena." Ward Perkins[17], a British Red Cross worker interested in the Italian experiments passed on the idea to U.N.R.R.A. in London and experiments were begun in England. Here oat flour was replaced by malted barley and wheat flour, and soya flour was added to improve the protein value, but the name "Maltavena" was still retained. A series of experimental batches were made in our laboratories, and tested clinically on babies and physiologically on rats. The clinical tests were not sufficiently comprehensive to show more than that babies could tolerate Maltavena. The physiological tests, carried out at Cambridge by Chick and Slack[18], showed that some of the samples closely resembled milk in their growth-promoting properties.

In view of these physiological results it was decided to institute large scale clinical trials in Germany. We prepared some of the material used in these trials, sending over a ton to Germany during 1947. The results of these large-scale clinical trials have not yet been published. However, a few clinical tests of our materials have also been made in this country. Sufficient data is not yet available to permit publication of precise figures, but the indications are that malt and soya baby foods containing about 10 per cent. of "protein" (based on a total nitrogen content of 1·67 per cent.) are inadequate for satisfactory growth. Better results have been obtained with these foods containing about 12 per cent. of "protein" (based on a total nitrogen content of 2·08 per cent.). At this point, however, a new factor came into view, viz. the occurrence in raw soya of a trypsin inhibitor[14] which might affect the digestibility of the Maltavena protein *in vivo* and thus its availability for growth promotion. On applying *in vitro* tests to the soya used as raw material we found that it contained the trypsin inhibitor, which could be removed by acid extraction and destroyed by autoclaving or enzyme action. Moreover, the Maltavena which we had sent to Germany for the clinical trials also contained the inhibitor. Our method of testing for the inhibitor did not permit its precise evaluation using milk as substrate. Such evaluaton was effected by Dr. Borchers of Nebraska University, a leading American investigator in this field, who very kindly examined some of our Maltavena samples and confirmed that the inhibitor was present (see Table III).

TABLE III

TRYPSIN INHIBITOR IN SOYA AND IN MALT AND SOYA FOOD

Materials examined	Inhibitor found by	
	Borchers	Ovaltine Research Laboratories
Soya beans		+++
Soya meal, defatted	100	+++
„ „ full fat...		+++
„ „ heated dry 1 hr. at 100°C.		++
„ „ autoclaved ½ hr. at 115°C.	0	0
„ „ digested with papain	0	0
Malt and soya food 1945*	5	
„ „ 1947	2	trace

Notes—
* Tested physiologically for growth promoting properties by Chick and Slack[18].
Results show relative amounts of inhibitor found using as substrates hæmoglobin (Borchers, private communication) and milk (O.R.L.). Dr. Borchers does not consider that his results indicate the presence of any significant amount of the inhibitor in the two samples.

It was then decided to make further samples in which the inhibitor would be destroyed by autoclaving. Unfortunately this treatment affected the thermoplastic nature of the product, so that vacuum drying became impracticable, and before this difficulty could be overcome the opportunity had passed for taking part in the further German trials.

Vitamin B in Malt and Soya Baby Foods. Before applying growth results to measure the relative protein values of different samples of malt and soya foods it is perhaps advisable to consider their content of B vitamins. In contrast with vitamins A, C and D, these were not supplied as supplements in all the clinical trials. Hence, if they were deficient in any of the samples this might have affected the growth results and thus confused the issue. Jeans and Marriott[19] in their well-known textbook on Infant Nutrition, state that when infants are fed on milk substitutes based on soya the diet will need supplementing with B vitamins. Table IV shows the content of aneurine, nicotinic acid and riboflavine as

TABLE IV

COMPOSITION OF MALT AND SOYA BABY FOOD

No. of samples examined	2 *
Total solids per cent. w/v	95·0
Composition of total solids :—	
Total nitrogen per cent.	1·68
carbohydrate ,,	84·2
fat ,,	2·6
ash ,,	2·6
Ca mg./100 g.	559 †
Fe ,,	6
aneurine μg./g.	4·6
nicotinic acid ,,	100 ‡
riboflavine ,,	2·3
percentage of total calories from total nitrogen	10
,, of total calories from soluble nitrogen	6·0

Notes—

* From over a ton of dried product which had been thoroughly mixed to ensure uniformity. Dr. R. G. Booth's results on the samples were in good agreement with ours.

† Confirmed by microbiological assays by Dr. F. W. Norris. In calculating percentage of total calories from total nitrogen and from soluble nitrogen allowance was made for lower nitrogen factors of vegetable protein.

‡ Most of this comes from soluble calcium salts used in the formula.

μg./g. in the "10 per cent." sample. These data have now to be compared with the actual requirements of babies, and the amounts in human milk, which are given in Table V on the same basis of μg./g. total solids of diet and of human milk respectively. The figures for aneurine and nicotinic acid in human milk are considerably lower than the suggested requirements, and it seems safer to assume that the latter are more reliable. The nicotinic acid figure especially has been queried by later workers[20]. On this requirement basis our samples probably contained

TABLE V

VITAMIN B VALUE OF MALT AND SOYA FOOD AND ITS RAW MATERIALS

Material	content as μg./g. total solids		
	aneurine	nicotinic acid	riboflavine
Malt and soya food "10 per cent. protein"*	4·6	100	3
Human milk, solids of	2·4‡	16	2–7·5§
Requirements in diet of baby†	5·5	55	8
Malt extract solids*	2·5 to 4·5	100 to 150	2 to 4·5
*Per cent. from raw materials**—			
Malt extract	75	95	88
Soya	19	2	7
Wheat	6	3	3—4

* Results obtained in Ovaltine Research Laboratories. † Calculated from data from U.S.A. National Research Council [22, 23]. ‡ Calculated from data by Knott, Kleiger and Schlutz [20]. § Calculated from data of Marriott and Jeans [21].

enough aneurine, and certainly supplied enough nicotinic acid. They may, however, have been deficient in riboflavine. Later samples have been fortified with this vitamin.

Table V shows the proportion of these B vitamins supplied by the different raw materials. It will be seen that by far the greater part of the vitamins comes from the malt extract. Since commercial malt extracts vary widely in their content of B vitamins, stress is laid on the importance of ensuring that this is as high as possible in any malt extract used in the preparation of malt and soya baby foods. This applies particularly to riboflavine, of which soya is rather a poor source. By taking sufficient care in the malting and brewing processes it is possible to obtain malt extracts which are considerably better sources of this vitamin than soya is.

The nicotinic acid content of malt and soya foods made from malted barley should not present much difficulty, judging by the fairly narrow range in commercial malt extracts. If, however, malted barley were replaced by malted oats, as Caprino originally employed, the nicotinic acid content might be more critical. Unmalted oats contain only about one-tenth as much of this vitamin as unmalted barley[24] and in contrast with the latter, the nicotinic acid content can at least double during germination, but will still be only one-fifth of that in malted barley. A similar objection applies to the use of maize in baby foods.

Another point to be considered is the possible loss of B vitamins during removal or destruction of the trypsin inhibitor in soya. Our experiments indicated that the destruction of the inhibitor by autoclaving would involve losing 10 to 20 per cent. of the aneurine in the soya. Table V shows that this would be only 2 to 4 per cent. of the total aneurine in the " 10 per cent." sample. This loss is insignificant. Much greater losses of aneurine can occur by heat treatment during the preparation of soya flour from soya beans. Removal of the inhibitor by extraction with very dilute acid as used by American workers[25] removed much more aneurine and also a good deal of nicotinic acid and riboflavine.

The trypsin inhibitor of soya may also be destroyed by dry heating at a sufficiently high temperature. Recent work[26] shows that such dry heating may, if carried on long enough (say five hours), cause the protein to be less rapidly digested by the enzymes of the gastro-intestinal tract. The amino-acid lysine seems to be particularly affected.

American workers[26] suggest that with optimal processing conditions 50 to 65 per cent. of the protein in soya should be extractable with water. American samples of soya vary widely in their content of water-soluble protein, some containing practically none. In the nitrogenous constituents of the soya we used in manufacturing our samples about 20 per cent. was water-soluble, which is higher than is indicated by the protein figures for some American samples but well below the optimum. Table II showed that in commercial malt extracts at least 90 per cent. of the nitrogenous constituents are soluble. Since this is a much higher proportion than the corresponding figure for soya, increase in the proportion of the latter in Maltavena would be expected to lower the proportion of soluble in total nitrogenous constituents.

Table II showed that in the total nitrogenous constituents of malt extract 56 to 88 per cent. represented protein digestion products. The corresponding figures in barley before malting are 7 to 8 per cent. and after malting 22 to 33 per cent. according to the efficiency of malting. Our " 10 per cent." sample of malt and soya food gave a figure of 36 per cent., our results showing that the nitrogenous constituents of soya almost entirely come from undigested protein. When the soya was subjected to enzyme treatment to destroy the trypsin inhibitor, a certain amount of the soya protein was also predigested, as shown by the increase to 43 per cent. for the 12 per cent. sample.

Amino-acid composition of proteins used in infant feeding. (a) *Essential amino acids from malted barley and other staple cereals.* The nutritive value of the protein in malted preparations depends on its content of essential amino acids. The available data are too scanty and unreliable to permit precise evaluation, but, it may in general terms be said that the proteins from malted barley and other staple cereals contain more of the sulphur containing amino acids than the protein from soya, and compare fairly well with that from milk[27]. This advantage of cereal over soya protein is particularly well marked with cystine. The protein of malted barley contains about twice as much cystine as that of soya, and that of oats about 3 times. Those of rice, wheat and maize are intermediate. Turning to other essential amino acids, soya has an advantage over cereals as a source of arginine and lysine, in which it compares favourably with milk. Whilst the protein of oats contains as much arginine as that of soya or of milk, it contains only half as much lysine. The protein of malted barley contains rather more lysine than that of oats, but much less arginine. The proteins of maize and wheat are also deficient in these two essential amino acids. Thus it seems clear that in the development of a milk substitute based on malted cereals and soya, the malted cereals can make a valuable contribution to the protein value of the product only if they are carefully selected and blended with suitable proportions of soya.

TABLE VI

COMPARISON OF PROTEIN FROM HUMAN AND FROM COWS' MILK FOR THEIR CONTENT OF ESSENTIAL AMINO ACIDS

Amino acid	Percentage of the amino acid in protein from	
	human milk	cows' milk
Arginine	5·0	4·3
Lysine	7·2	7·5
Tyrosine	5·1	5·3
Tryptophane	1·9	1·6
Phenylalanine	5·9	5·7
Cystine + Methionine	5·4	4·4
Threonine	4·6	4·6
Leucine	10·2	11·3
iso-Leucine	7·6	6·2
Valine	9·9	6·6

Note.—Figures published by Block and Mitchell[28] for the amino-acid content of different samples of human milk show wide variations for arginine, tryptophane, cystine and valine, and to a less extent for isoleucine, which may smooth out some of the apparent deficiencies of cows' milk in these essential amino acids. The baby's requirement of arginine may be greater in the earlier stages of lactation, since colostrum contains more than average human milk. The value of malt and soya food as a source of arginine is considered in Table VII.

(b) *Comparison of human and cows' milk proteins as standards.* The high biological value of the proteins in cows' milk justifies its use as the basis of most baby foods. Comparison of the essential amino acid content of cows' milk protein with human milk protein (see Table VI) shows on the whole remarkably good agreement. There are, however, deficiencies in tryptophane, cystine + methionine, *iso*leucine and valine in cows' milk protein, which have led us to prefer human milk protein as a standard for comparison with malted preparations. (Jeans and Marriott[19] suggest that cows' milk protein may be as much as 20 per cent. inferior to human milk protein.)

(c) *Comparison of " malt and soya" protein with human milk protein.* Table VII compares the content of essential amino acids in the protein

TABLE VII

ESSENTIAL AMINO ACIDS IN PROTEIN OF MALT AND SOYA FOOD AS COMPARED WITH THAT OF HUMAN MILK

Each figure shows the concentration of the given amino acid as a percentage of its concentration in the n the protein of human milk.

Animo-acid	10 per cent. sample*	12 per cent. sample*
Arginine	85	92
Lysine	64	68
Tyrosine	54	60
Tryptophane	74	76
Phenylalanine	83	86
Histidine	77	78
Cystine + Methionine	43	41
Threonine	81	82
Leucine	61	62
iso-Leucine	60	60
Valine	45	44

Note.—* Protein contents of 10 and 12 per cent. expressed as percentage of total calories provided by the protein, allowance being made for the lower factor of cereal proteins when calculating calorie value from total nitrogen.

Results by American workers[7,27] indicate that replacement of malted barley by malted oats might be expected to improve considerably the content of arginine, lysine and cystine plus methionine, largely eliminating the deficiency of the samples in these essential amino-acids. Our results on arginine contents of unmalted oats are in agreement with the American results, and we find no appreciable change in arginine content after malting.

In the " 10 per cent." sample about 47 per cent. of the " protein " came from the soya and about 42 per cent. from the malt extract. In the " 12 per cent." sample the corresponding figures were 57 and 34 respectively.

of two of our batches of malt and soya baby foods used in clinical trials with the content of the same amino acids in human milk protein. In both batches the protein is seen to be markedly deficient in a number of these amino acids. The batch of higher protein content was made from a higher proportion of soya and therefore contains more arginine, lysine and other essential amino acids, and slightly less cystine and methionine, but owing to difficulties in estimating these latter amino acids the figures given for them are very approximate and almost certainly, too low.

(d) *Protein value of " malt and soya" compared with that of human milk.* Before attempting to relate the approximate data in Table VII with the clinical results given by the two samples, we thought it desirable to make some allowance for the total protein content. The protein content of human milk decreases rapidly in the earliest stage of lactation. Data from a large number of subjects summarised in Figure 2, show that by the end of the first month the average protein content has fallen to about 8 per cent., after which it levels out between 7 and 8 per cent. on a calorie basis. Since milk substitutes are hardly ever given until after the first few weeks of lactation it seems fair to compare them with human milk containing about 8 per cent. of protein (on a calorie basis). This

TABLE VIII

COMPARISON OF MALT AND SOYA FOOD WITH HUMAN MILK AS A SOURCE OF ESSENTIAL AMINO ACIDS

Amino-acid	" 10 per cent." sample	" 12 per cent." sample
Arginine	106	138
Lysine	80	102
Tyrosine	68	90
Tryptophane	93	114
Phenylalanine	104	129
Histidine	96	117
Cystine + Methionine	54	62
Threonine	101	123
Leucine	76	93
iso-Leucine	75	90
Valine	56	66

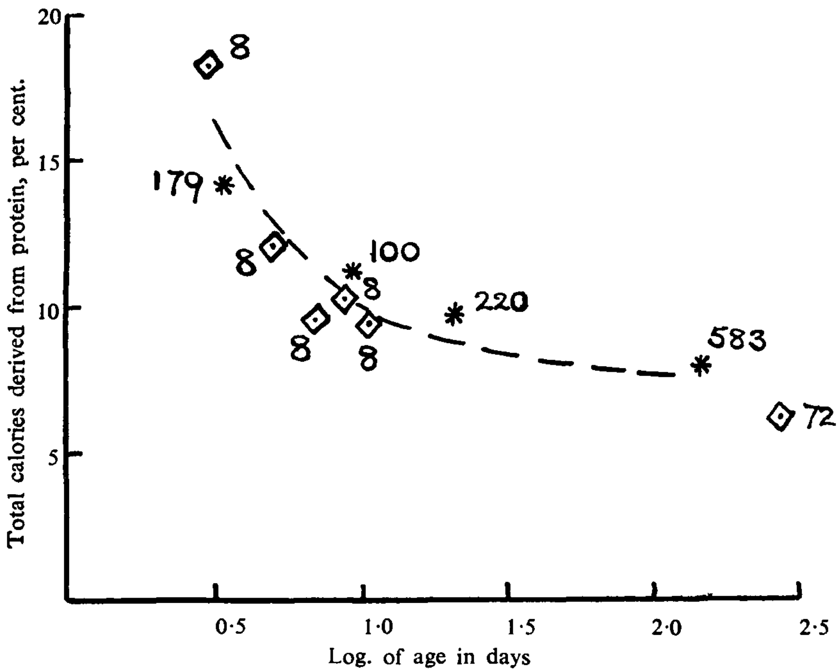

FIG. 2. Relation between protein content of human milk (on calorie basis) and stage of lactation plotted from data published by Gardner and Fox[29] (indicated thus *) and Hammett[30] (indicated thus ◇). Figures against each point refer to number of samples analysed, the majority of these representing all but the first month of lactation gave an average of 8·0.

suggestion is in agreement with Jean and Marriott's[19] findings. An average value of 8 per cent. would imply that in Table VII the figures for the "10 per cent." sample could be multiplied by 1·25 and those for the 12 per cent. sample by 1·5 to obtain their amino acid values as compared with human milk. Table VIII shows the effect of doing this. In the 12 per cent. sample all the amino acid values have been brought close to those of human milk, except those for valine, whose importance is perhaps not so great, and for cystine+methionine, for which figures quoted are almost certainly too low. On this basis the protein value of human milk should be definitely superior to that of the 10 per cent. sample, but perhaps not greatly superior to that of the 12 per cent. sample. Moreover, it should be possible to prepare from cows' milk by careful adjustment of the proportion of curd and whey proteins a baby food containing about 8 per cent. of protein (all from milk) which would have a protein value similar to that of human milk. The clinical data so far obtained are in line with these assumptions.

Summary

1. The total nitrogen content of malt extract may range from 0·2 to 1·2 per cent. but is usually well above 0·64 per cent. which corresponds to the B.P. 1948 minimum content of 4 per cent. (total nitrogen x 6·25).

2. Lower diastatic values are usually associated with lower total nitrogen contents, the tendency being observed with malted barley as well as with malt extract.

3. Of the total nitrogen of malt extract only 12 to 44 per cent. is true protein nitrogen. The greater part represents breakdown products of protein, largely formed during malting and brewing.

4. The "less soluble" nitrogen of malt extract may range from 1·8 to 10 per cent. of the total nitrogen, and should be reduced to as low a level as possible in malted preparations.

5. In baby foods prepared from malt extract, wheat flour and soya flour sufficient aneurine and nicotinic acid may be derived from the raw materials if care is taken to use malt extract of high vitamin B content. Fortification with riboflavine may be necessary. If the diet is adequately supplemented by vitamins A, C and D the growth-promoting effect on babies may provide a measure of the protein value of the diet.

We are indebted to Dr. F. W. Norris for microbiological assays, to Dr. R. G. Booth and to Mr. J. G. Heathcote for checking at the Cereals Research Station some of our protein analyses, to Miss Hazel Williams for fluorimetric estimations of aneurine, to Miss Janet Horsford for technical assistance, and to Dr. H. Chick and her colleagues for advice and generous comments on our work.

References

1. Organ, Duncan, Westbrook and Wokes, *Quart. J. Pharm. Pharmacol.*, 1943, **16**, 275.
2. Organ and Wokes, *J. Soc. Chem. Ind.*, 1944, **63**, 165.
3. Organ, James and Wokes, *Quart. J. Pharm. Pharmacol.*, 1944, **17**, 183.
4. Klatzkin, Norris and Wokes, *Quart. J. Pharm. Pharmacol.*, 1946, **19**, 376.
5. Garratt and Woodhead, *Analyst*, 1942, **67**, 194.

6. Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis,* 1945, 6th ed., Washington, D.C.
7. Block and Bolling, *The Amino-Acid Composition of Proteins and Foods,* 1945. C. T. Thomas, Illinois.
8. Dubnoff, *J. biol. Chem.*, 1941, **141,** 711.
9. Sakaguchi, *J. Biochem,* 1., Tokyo, 1925, **5,** 133.
10. Williams and Wokes, *Quart. J. Pharm. Pharmacol.*, 1947, **20,** 240.
11. James, Norris and Wokes, *Analyst*, 1947, **72,** 327.
12. Klatzkin, Norris and Wokes, *Analyst*, 1949, in the press.
13. Klatzkin, Norris and Wokes, *J. Pharm. Pharmacol.*, 1949, **1,** 914.
14. Bowman, *Proc. Soc. Exp. Biol. Med.*, 1944, **57,** 139.
15. Chibnall, *Protein Metabolism in the Plant,* 1939, Yale and Oxford Univ. Press.
16. Liebig, *Liebig's Ann.*, 1865, **133.**
17. Ward Perkins, *Mother and Child, Oct.*, 1947.
18. Chick and Slack, *Lancet*, 1946, **251,** 601.
19. Jeans and Marriott, *Infant Nutrition,* 1947, 4th ed., Henry Kimpton, London.
20. Knott, Kleiger and Schultz, *J. Pediat.* 1943, **22,** 43.
21. Marriott and Jeans, *Infant Nutrition,* 1942, 3rd ed., Henry Kimpton, London.
22. Dann and Davison, *Amer. J. Dis. Child*, 1942, **63,** 366.
23. Butler, *Amer. J. Dis. Child*, 1942, **64,** 898.
24. Klatzkin, Norris and Wokes, *Biochem. J.*, 1948, **42,** 414.
25. Borchers, Ackerson and Sandstedt, *Arch. Biochem.*, 1947, **12,** 267.
26. Melnick and Oser, *Food Technology*, 1949, **3,** 57.
27. Baumgarten, Mather and Stone, *Cereal Chem.*, 1946, **23,** 135.
28. Block and Mitchell, *Nutr. Abstr. Rev.*, 1946, **16,** 249.
29. Gardner and Fox, *Practitioner*, 1925, **114,** 153.
30. Hammett, *J. biol. Chem.*, 1917, **29,** 381.