Quality of Protein in Nutrition.¹

By DR. R. H. A. PLIMMER.

THE normal diet of man and animals contains certain nutritional elements every one of which is essential for the maintenance of life and health. These elements are:

- (1) Proteins, complex nitrogenous substances found in meat, milk, eggs, cereals, and plant tissues.
- (2) Carbohydrates, such as starch in cereals, sugars in fruits, milk, etc.
- (3) Fats, such as butter, lard, suet, and vegetable oils.
- (4) Salts, or the mineral constituents in meat, milk, cereals, vegetables, etc.
- (5) Vitamin A, contained in butter, cod-liver oil, eggs, green vegetables, etc.
- (6) Vitamin B, contained in yeast, germ of cereals, meat, eggs, etc.
- (7) Vitamin C, contained in some fruits and some vegetables.
- (8) Water.

If we examine these food elements in fuller detail we find that in whatever form the carbohydrate is taken in the food it is converted during digestion in the body into a simple sugar, such as grape-sugar; so that for nutritional purposes all carbohydrates can be considered the same. They are burnt up like coal to supply the body with heat and energy.

Fat of almost every source consists mainly of three triglycerides, palmitin, olein, and stearin. The consistency of fats depends simply on the relative proportions of these substances. Certain fats are the vehicles of the A vitamin, but, leaving the vitamin out of consideration, fats are of equal value in nutrition, and, like carbohydrates, they supply fuel for heat and energy. Fats can be built up in the body from the carbohydrate in the food. Some very recent feeding experiments by Osborne and Mendel indicate that fat, as such, can be omitted from the diet if the vitamin A is supplied in a specially prepared form. The special value of fat in nutrition thus depends on the A vitamin associated with it, and not on its chemical constitution.

The mineral salts in an ordinary mixed diet do not need to be supplemented, but generally some sodium chloride is added. Animals on cereal diets must be supplied with this common salt.

Whatever is the source of the three vitamins, so far as we know the A vitamin is the same whether it be in butter or cod-liver oil, B vitamin is the same in yeast and cereal germ, and C vitamin the same in orange-juice or cabbage.

Thus, since each of these elements of the diet is reduced to a simple common basis during digestion, we cannot speak of quality of carbohydrate, fat, or vitamins.

The protein constituent differs from all the others by its endless variety. This is obvious to the naked eye. For instance, the protein in white of egg is in solution, and sets to a hard mass on boiling. Meat protein is already in a solid form. Milk contains two kinds of protein, the casein which is used to make cheese and an albumin like egg-albumin in the whey. The presence of protein in cereals is scarcely recognised, as it is obscured by the large amount of starch, yet about one-tenth of flour is protein; in fact, two very special proteins are present, the one soluble in alcohol, the other insoluble but soluble in dilute alkali.

Our usual classification of proteins already indicates their differences, but the variety is really far greater. We need only refer to their chemical analysis. Fischer, Kossel, and their pupils have shown that proteins on hydrolysis break down into some eighteen or twenty amino-acids. These numerous units can be arranged for convenience into eight groups:

- (1) *Simple Mono-amino-Acids*: Glycine, alanine, valine, leucine, and isoleucine
- (2) *Mono-amino-Dibasic Acids*: Aspartic acid and glutamic acid.
- (3) *Hydroxyamino-Acids*: Serine and hydroxy-glutamic acid.
- (4) *Heterocyclic Acids*: Proline and hydroxy-proline.
- (5) *Mono-amino-Acids with Aromatic Nucleus*: Phenylalanine and tyrosine.
- (6) *Mono-amino-Acid with Indole Nucleus*: Tryptophan.
- (7) *Hexone Bases or Diamino-Acids*: Lysine, arginine, and histidine.
- (8) *Thio-amino-Acid*: Cystine.

The chemical analysis of the proteins shows that the various proteins yield different amounts of the amino-acids. Some of the data are shown in Table I. The peculiarities of each protein are indicated by the figures in heavy type.

TABLE I.

	Ox muscle.	Casein.	Lact-albumin.	Gelatin.	Wheat gliadin.	Wheat glutenin.	Maize zein.	Maize glutenin.	Edestin.	Sturin.
Glycine ...	2.1	0	0	19.3	0	0.9	0	0.3	3.8	
Alanine ...	3.7	1.5	2.5	3.0	2.0	4.7	9.8		3.6	
Valine ...	0.8	7.2	0.9		3.4	0.2	1.9		+	
Leucine ...	11.7	9.4	19.4	6.8	6.6	6.0	19.6	6.2	20.9	
Phenylalanine...	3.2	3.2	2.4	1.0	2.4	2.0	6.6		3.1	
Tyrosine ...	2.2	4.5	0.9	0	1.2	4.3	3.6	3.8	2.1	
Serine ...		0.5		0.4	0.2	0.7	1.0		0.3	
Cystine ...				0	0.5	0.02			0.3	
Proline ...	5.8	6.7	4.0	10.4	13.2	4.2	9.0	5.0	4.1	
Hydroxyproline		0.3		6.4					2.0	
Aspartic acid ...	4.5	1.4	1.0	1.2	0.6	0.9	1.7	0.7	4.5	
Glutamic acid...	15.5	15.6	10.1	1.8	43.7	23.4	26.2	12.7	18.7	
Tryptophan ...	+	1.5		0	1.0	+	0	+	+	
Arginine ...	7.5	3.8	3.2	9.3	3.2	4.7	1.6	7.1	14.4	58.2
Lysine ...	7.6	6.0	9.2	5.0	0.2	1.9	0	3.0	1.7	12.0
Histidine...	1.8	2.5	2.1	0.4	0.6	1.8	0.8	3.0	2.4	12.9
Ammonia ...	1.1	1.6	1.3	0.4	5.2	4.0	3.6	2.1		
Total ...	67.5	66.5	57.0	65.4	83.0	59.72	85.4	45.7	81.9	83.1

In general, the albumin group of proteins contains all the amino-acids, except glycine, in various proportions. The globulin group is similar, but contains glycine, and has, in addition, a higher amount of glutamic acid, especially those globulins of vegetable origin. The phospho-proteins resemble the albumins, with no striking preponderance of any single amino-acid. The gliadin group of cereal proteins is peculiar in its high content of glutamic acid and proline. The members of the sclero-protein group (horn, hair, and gelatin) are heterogeneous, and here we may note that silk-fibroin is composed mainly of three mono-amino-acids, and is the very antithesis of sturin (the protein of fish sperm), which is made up of the three hexone bases with no, or very little,

¹ From a discourse delivered at the Royal Institution on Friday, April 8.

mono-amino-acids. Gelatin lacks cystine, tyrosine, and tryptophan. Hair is richest in cystine. These are simply some of the most obvious differences. Proteins thus differ markedly in quality.

Our analytical data are far from complete; in no case do the totals of the amino-acids add up to 100. The incompleteness is chiefly due to the great difficulty of separating and estimating the individual amino-acids. There may be still some unknown amino-acids in small quantities; e.g. hydroxyglutamic acid has been discovered recently by Dakin by a new extraction method. This method may yet lead to new results; once again it has proved that every new process in connection with the chemistry of the proteins has given a valuable result.

Rather too great stress has been laid upon the analytical figures. The methods scarcely give exactness as far as the decimal figure, and it would have been better if the data had been returned to the nearest whole number. Many workers still give their data to two places of decimals, so that an entirely wrong impression is given of the accuracy of the method. Fischer pointed out that his method was not quantitative, but others have neglected this important statement.

The figures for the hexone bases are more accurate, but it is still not sufficient to express results to two decimal places. Kossel considers that the hexone bases form a special nucleus on account of their presence in all proteins. We might value a protein by its content of hexone bases, but it is not sufficient, because their total only tells us about a third or less of the whole molecule.

Tryptophan, discovered by Hopkins and Cole, is perhaps the most important unit in the protein molecule. It is not estimated except by direct isolation—a method which is laborious and requires considerable skill. Its amount is not known except in casein and a few other proteins. By its distinctive colour reaction with glyoxylic and sulphuric acids it can readily be proved to be a constituent of most proteins.

The amount of cystine in proteins is known only in a few cases, but its amount can be gauged by the sulphur content of the protein. It is the one unit known which contains sulphur, but there are indications that there is another sulphur-containing unit.

The differences in proteins are not confined to such quantitative data; they are still more involved. Fischer's synthetical work with the amino-acids has proved that the amino-acids are combined together in a polypeptide form, i.e. the amino-group of one amino-acid is combined with the carboxyl group of another, the amino-group of this acid being united with the carboxyl group of still another. We therefore consider a protein molecule to be a chain of amino-acids, thus:

This method of combination allows theoretically of endless variation. If we take three amino-acids we can arrange them in six different ways: Glycylalanyltyrosine, glycyltyrosylalanine, alanyltyrosylglycine, alanyltyrosylglycine, tyrosylglycylalanine, and tyrosylalanyltyrosine. With eighteen or twenty amino-acids the number of arrangements is almost infinite.

Differences in arrangement may be the cause of differences in proteins. Two proteins may perhaps have exactly similar amounts of amino-acids and yet be different; a difference could be expressed by the interchange of one amino-acid. We may imagine the proteins of the blood or milk of different species to differ thus: one may have the arrangement *a, b, c, d, e, f*, the other *d, a, b, f, e, c*.

Another important difference may exist in the so-

called tautomerism of the amino-acids and polypeptides. With the same arrangement of the amino-acids we may have several formulæ representing the polypeptide structure. Certain of the properties of the polypeptides can be explained on this basis.

Fischer and Kossel have revolutionised our conception of protein nutrition. We no longer think, like Liebig and others, that the protein of the food becomes directly the protein of the body, for it has been demonstrated by the physiologists that the protein of the food undergoes hydrolysis during digestion to amino-acids, that the amino-acids circulate in the blood, and that the tissues receive amino-acids from which they build up their protein. Proteins must be regarded as a mixture of amino-acids.

We can look upon a protein as we look upon the contents of a box of assorted biscuits, arranged in rows and layers of various kinds. Each biscuit should be connected to its neighbour so that we have a continuous chain. The general appearance of the contents of two boxes is different; in one case we may find sugary biscuits on the top, in another plain ones. In the process of digestion the protein is acted upon by acid in the stomach with the formation of metaprotein. No great chemical change occurs, but we can imagine that the change consists in a tautomeric re-arrangement in preparation for the action of pepsin. Pepsin hydrolyses the protein at certain junctions, forming proteoses and peptones. Their formation can be compared with the separation of the layers of the biscuits. Pancreatic and the further digestion which follow in the intestine separate the individual amino-acids or biscuits entirely. The separate parts circulate to the tissues; the tissues select the ones they require, and form another arrangement of the units or simply replace those which have been used in their metabolism. Digestion and metabolism are a sort of re-shuffling of the units. In the absence of any particular unit the tissue can no longer rebuild its substance, and consequently suffers. The old example of the inadequacy of gelatin is now explained; the tissues require tryptophan, tyrosine, and cystine, and gelatin cannot provide them.

In nutrition there are essentially two problems to study: the formation of new tissue, as in the growth of young animals, and the maintenance of tissue, which undergoes so-called wear-and-tear, in adult animals. In the latter case we have ultimately to ascertain if every unit of the molecule breaks down or certain selected units only. If these are in the middle of a chain it would follow that the whole molecule would undergo metabolism, and not units at the ends alone. The problem resolves itself into ascertaining the function of each amino-acid.

Since the practical difficulties of feeding animals with a mixture of pure amino-acids are far too great, advantage may be taken of feeding incomplete proteins and adding to them the missing unit or units.

Wilcock and Hopkins made the first experiment of this kind in 1906. They selected zein as protein and fed it to mice, in one set alone and in another set with the addition of 2 per cent. of its amount of tryptophan. Young mice on zein alone immediately began to lose weight and generally died in sixteen days; decline in weight also occurred in the other set, but with the added tryptophan death did not occur until the thirtieth day. Adult mice lived twenty-seven days without tryptophan, and forty-nine days with tryptophan. Tryptophan had thus an appreciable effect on the survival period of the animals. Zein is incomplete in respect of other units, and death was probably on this account.

The experiment was repeated in 1916 by Ackroyd and Hopkins under different, but better, conditions.

The animals were first given a mixture of amino-acids from casein (*i.e.* without tryptophan, which is destroyed in hydrolysis by acid) to which tryptophan was added; on the twelfth day the tryptophan was omitted, and included once more on the thirty-fifth day. There was growth during the first period, decline in weight during the second period, followed by growth on inclusion once more of the tryptophan. This is shown by the continuous lines in Fig. 1. The upper dotted line shows continuous growth on com-

FIG. 1.—After Ackroyd and Hopkins.

plete mixture. The lower dotted line shows loss of weight in absence of tryptophan.

Similar experiments have been made by Osborne and Mendel in America. They used the gliadin of wheat as protein. This protein is a complete one, but it contains very little of certain amino-acids, especially lysine. Adult rats were maintained for quite long periods, so long as 500 days, but young rats capable of growth, though maintained for long periods, failed to grow.

We may here notice that though the growth of the animal may be suppressed and it reaches maturity in age, the capacity to grow is not lost. Osborne and Mendel illustrated this by a photograph of a rat which had failed to grow for 273 days, but resumed growth on being given a suitable diet.

The small amount of lysine in gliadin led the authors to regard this unit as essential for growth. In a later experiment they added lysine at intervals; growth took place with the lysine, but not without it. Fig. 2 shows the upward curve of growth with lysine, but no growth without it, in four sets of rats.

The effect of lysine on growth was again demonstrated by Buckner, Nollau, and Kastle in the case of chickens living under the natural conditions of a poultry farm. The birds were fed upon grain mixtures of high and low lysine content; growth was more rapid on the mixture of high lysine content.

The element sulphur is present in proteins in the form of cystine, though it is possible that another sulphur-containing unit is present. Little or no cystine in a protein has also an effect upon the growth of rats. This has been most clearly demonstrated in

the case of the protein, phaseolin, of the navy bean. There was slow growth with this protein alone, but normal growth if the protein were supplemented with 2 per cent. of its amount of cystine.

Casein is deficient in cystine. Less casein is required in a diet for producing normal growth; if extra cystine be included 15 per cent. of casein was required by itself, but only 9 per cent. if cystine were added.

The amino-acids containing aromatic nuclei are probably essential units of the protein, but it is difficult to carry out a decisive experiment, since all proteins contain phenylalanine, though they may lack tyrosine. There is plenty of evidence that phenylalanine can be transformed in the body by oxidation; both tyrosine and phenylalanine yield homogentisic acid in cases of alkaptonuria. Totani has shown that the almost complete removal of tyrosine from the mixture of units yielded by casein made no difference to the growth of rats. There was evidently enough phenylalanine for all purposes.

The two hexone bases, arginine and histidine, as shown by Ackroyd and Hopkins, are interrelated in nutrition. Absence of both causes loss of weight; absence of either alone lessens the rate of growth. These two workers further showed that these amino-acids are connected with the production of the purine ring in the animal body, *i.e.* with the production of uric acid.

The function of the whole group of mono-amino-acids has yet to be determined. Are they all necessary? Of glycine we can say that it is not essential, for it is the only amino-acid which the animal can synthesise.

These results remind us of the well-known experiments on the need by plants of all the inorganic elements. Sir Daniel Hall in his "Fertilisers and Manures" gave a striking picture of barley-grains grown on a full food and on foods lacking one constituent. We may thus correlate the amino-acid con-

FIG. 2.—After Osborne and Mendel.

tent of proteins for the growth of animals with the set of inorganic elements needed for the growth of plants.

The relative value of various proteins in nutrition has been studied by Osborne and Mendel. In their experience lactalbumin is superior to casein, and casein to edestin. They found that 50 per cent. more casein and 90 per cent. more edestin were required to produce the same gain in weight; in other terms, a food containing 8 per cent. of lactalbumin was equal to one with 12 per cent. of casein and 15 per

cent. of edestin. Fig. 3 shows that the same amount of growth resulted in the same time with these quantities of proteins.

Suitable mixtures of proteins have also been tested, and attempts are being made to find out the most convenient addenda for making the proteins of cereals more adequate for the growth of animals, i.e. adding what we may call "good" protein to "bad" protein to make the latter efficient as food. Leaf and seed proteins are good as a mixture. Fig. 4 shows that if zein $\frac{1}{4}$ be supplemented with lactalbumin $\frac{3}{4}$, normal growth results.

FIG. 3.—After Osborne and Mendel.

Economically, it may be better to use an expensive protein as food for animals and produce rapid growth than to feed for longer periods on poor proteins and get slower growth. A simple calculation brings out the problem to be solved. We may wish to build up the casein of milk with 16 per cent. of glutamic acid, and we are provided with wheat gliadin with more than 40 per cent. of this unit. There is waste of glutamic acid. Gliadin further contains 0.2 per cent. of lysine, whilst casein contains 6 per cent. To produce this

FIG. 4.—After Osborne and Mendel.

amount we require thirty times as much gliadin, and, consequently, the waste of glutamic acid is further increased.

Cannibalism is the most economical method of protein nutrition, as the amino-acids of the food are in the exact proportion required by the tissues. The nearest parallel to this is the nursing of the young animal by its mother; the child actually gets the proteins of the mammary glands.

Recent work shows that quality of protein is most probably the primary cause of the disease pellagra, although there are some indications that general in-

sufficiency of protein together with improper salt supply are contributory factors.

Pellagra is a peculiar disease characterised by severe disturbance of the whole digestive tract, by skin lesions, usually bilaterally symmetrical, and often mistaken at first for sunburn or chapping of the hands, face, neck, and other exposed areas. The nervous system is also affected. There is no definite record of pellagra in Europe before maize was introduced into Spain by Columbus. From Spain the disease spread to France, Lombardy, and eastwards, wherever maize was extensively used for food in the poorer agricultural districts. The relation of maize to the disease puzzled the medical profession for nearly two hundred years, for the disease also occurred where maize was not used, while in some districts maize was used but there was no pellagra. Roussel in 1866 showed that it could be cured by good food, and Lorentz (1914) and Willets (1915) successfully treated advanced cases with a generous diet. Goldberger also cured and prevented the seasonal appearance of pellagra in lunatic asylums and orphanages by increasing the quantity of meat and milk; previously the diet had been deficient in these respects. Goldberger, by the offer of a free pardon from the Governor of Mississippi, was enabled to obtain eleven convicts as volunteers for a feeding experiment to determine if pellagra could be produced by an unbalanced diet in healthy white men. The "pellagra squad," as they were called, were fed on white wheat flour, various maize preparations, polished rice, sugar, sweet potatoes, pork fat, cabbage, and turnip-tops. The food had an energy value of 2950 Calories, and was amply sufficient in this respect, but after the second month on this diet the men complained of weakness, headache, abdominal pain, and other minor discomforts. After five months six of them developed a rash which was pronounced by experts to be identical with that seen in pellagra, and during the last four weeks all the prisoners had shown marked loss of weight and were much out of health. Pellagra would probably have developed in the remainder, but the experiment had to be abandoned owing to the refusal of the men to continue. A control was carried out at the same time; their diet contained some meat, eggs, and buttermilk; there was not a single case of pellagra and no progressive loss of body-weight.

These and other facts clearly point to the diet as the controlling factor in the cause and prevention of the disease. The determining factor seems to be the quality of the protein. Good evidence on this point has been furnished by Wilson, of Cairo. In 1916 pellagra broke out in a camp for Armenian refugees at Port Said. Wilson showed that the diet at first supplied was inadequate both in energy supply (2200 Calories) and in protein supply; indeed, 92 per cent. of the protein was of vegetable origin—three-quarters from wheat and one-quarter from maize.

By determining the least daily amount of a protein required to keep a man from loss of body protein, Thomas was able to assign a series of values to proteins representing their biological efficiency. The comparative values according to the quantity required to maintain a man without loss of nitrogen and body-weight were:

Ox-meat	...	104	Rice	...	88
Cows' milk	...	100	Potato	...	79
Fish	...	95	Peas	...	56
Casein	...	70	Wheat-flour	...	40
			Maize-meal	...	30

The biological value of meat is therefore three times that of maize. Wilson calculated that the diet as given to the refugees was equal to 22 gm. of

casein. On improvement to a casein equivalent of 41 gm. no more cases of pellagra occurred.

Chick and Hume (1920) succeeded in producing in three monkeys symptoms very like those of human pellagra. The diet was very carefully selected, and was deficient only in respect that it contained no animal protein. One monkey refused the food after a short time; he lost weight and showed signs of incipient pellagra. The second monkey also lost weight, but the loss was lessened by adding tryptophan, though the addition of other amino-acids lacking in maize had no appreciable effect. This monkey

had signs of pellagra, and was cured by giving a normal diet. The third monkey had its loss of weight arrested by including tryptophan and hexone bases. This monkey showed some of the characteristic symptoms of pellagra, such as the symmetrical bilateral rash.

It appears thus that pellagra is caused by a continuous shortage in the supply of certain amino-acids in the food. A diet containing animal protein in small quantities will supply the needful amino-acids, but a large supply of vegetable protein may not be equally efficient.

The Cawthron Institute, Nelson, N.Z.

THE building and grounds in which the staff of the Cawthron Institute of Scientific Research has commenced its work were formally opened on Saturday evening, April 2, by his Excellency Lord Jellicoe, Governor-General of the Dominion of New Zealand. The building is a fourteen-roomed house, formerly the residence of the late Mr. John Sharp, and has been fitted up with chemical and biological laboratories, a library, a museum, and offices. The grounds provide room for a considerable amount of investigational work, but an experimental orchard and a site for an arboretum have been secured elsewhere. After being shown over the building by the trustees and staff, Lord and Lady Jellicoe adjourned to the School of Music, where a very enthusiastic gathering of citizens awaited them.

In opening the proceedings the chairman of the Trust, the Lord Bishop of Nelson, gave a short résumé of the events which led to the founding of the institute under the will of the late Mr. Thomas Cawthron, and explained the nature of the difficulties which had been met in attempting to carry into effect the provisions of the will. He also stated that the trustees had been fortunate in securing the unique entomological library of Dr. David Sharp, the editor of the *Zoological Record*.

Lord Jellicoe, in declaring the institute open, emphasised the importance of the co-operation of the workers in pure science with those engaged in industry. He had seen sufficient of the Cawthron Institute and its staff to convince him that the work carried out in the institute would be of very great value indeed.

An account of the work of the staff was then given by the director, Prof. T. H. Easterfield, who stated

that the staff had been working steadily for about eight months. In the chemical laboratory Mr. Rigg, the soil chemist, had obtained sufficient data for the preparation of a preliminary soil-map of the Waimea district, and this was already being eagerly examined by the farmers and fruit-growers of the district. A careful comparison of the chemical constituents of New Zealand mineral oils from various sources had been made by Mr. McClelland. Dr. R. J. Tillyard, the chief biologist, had paid much attention to the question of the control of plant diseases both by inoculation and by the use of natural enemies of insect pests. He had been successful in establishing *Aphelinus mali*, one of the enemies of the woolly aphis. Several entomological papers by Dr. Tillyard and Mr. Alfred Philpott, the assistant entomologist, were already in the press. The relation of hawthorn hedges to the spread of fire-blight and other plant diseases had also been the subject of close inquiry. Dr. Kathleen Curtis, mycologist to the institute, was working out the life-history of several fungoid diseases under New Zealand conditions, and the work was being followed with great interest by the fruit- and tomato-growers. The rapidity with which the building had been converted into a convenient research institute was very largely due to the energy and effectiveness of the curator, Mr. W. C. Davies, whose arrangement of the museum was admirable.

The director announced that during the week following the official opening the institute would be thrown open for four afternoons and one evening, and that the staff would explain the various activities to the public. More than a thousand visitors took advantage of the opportunity to visit the institute.

Institute of Historical Research in London.

THE opening of the new Institute of Historical Research of the University of London in Malet Street, close to the British Museum, on July 8 is a notable event on which warm congratulations may be tendered to the University and to Prof. Pollard. London University has always led the van in the recognition of research, and the new institute is to be devoted to the extension of knowledge. The inauguration of the building has been happily made the occasion of an Anglo-American Conference of Professors of History. London University, a pioneer in so many directions, created in 1920 the first post in England for the history of medicine. We have already referred to the systematic courses in the history of science that are being developed at University College, and it was in harmony with this London tradition that a sectional meeting of the congress was

held on Wednesday, July 13, to discuss "Anglo-American Co-operation in the Publication of Documents and Results of Research on Medieval Science and Thought." The meeting was well attended, and the chair was taken by Mr. A. G. Little, who spoke of the immense amount of important medieval material by English writers still waiting to be edited. He emphasised the need of scholars keeping in touch with one another's work.

Dr. Singer spoke of the educational value of the history of science and of the advantages accruing both to professor and student when to specialised research in a purely scientific field is added a general training as a qualification for a degree. Mr. Charles Johnson, of the Public Record Office, suggested that the editing of a text formed an excellent training for historical research, and suggested that such work, carried