ABSTRACTS OF COMMUNICATIONS

The One Hundred and Thirteenth Meeting of The Nutrition Society was held in the Fletcher Memorial Hall, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W. 7, on Saturday, 7 December 1957, *at* 10.15 *a.m., when the following papers were read:*

The net protein utilization (N.P.U.) of some human diets.

By D. S. MILLER and I. S. DEMA, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

The table provides data of value in appraising diets for human protein requirements (Platt & Miller, 1958).

Food	N.P.U.* Alone (%)	N.P.U.* + 0·25% DL-methionine (%)	Protein (%)	Net value† (%)
U.K. survey‡	67	73	11·3	7·5
U.K. meal 1	62	71	11·3	7·0
2	66	85	8·8	5·8
3	67	85	10·8	7·2
4	81	80	10·3	8·3
5	73	76	10·2	7·4
Nigerian survey§ 1	52	59	16·0	8·3
2	44	60	6·8	3·0
3	52	49	10·5	5·5
4	59	60	13·5	8·0
5	53	64	8·0	4·2
Gambian meal 4	35	50	17·7	6·2
13	60	67	19·1	11·0
15	38	48	17·8	6·8
18	55	61	9·0	5·0
25	63	65	8·9	5·6

* By the method of Miller & Bender (1955).
† N.P.U. × protein %.
‡ Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1957).
§ Nicol (1949, 1952, 1956).

We are indebted to the Ministry of Agriculture, Fisheries and Food for use of the vacuum contact drier.

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Biochemical evidences of protein malnutrition. By B. S. PLATT and C. R. C. HEARD, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Renewed interest in comparatively recent years in proteins, amino-acids and their metabolism led to greater interest in the widespread occurrence of protein malnutrition in human populations. The recognition of this syndrome as being due to insufficiency of protein has led in turn to expanded interest in its biochemical aspects (Platt, 1956).

Studies on the distribution of body water and biochemical changes in the blood and other tissues including hair, skin, eyes, muscle, liver, pancreas and digestive tract have all been reported, some in great detail (Trowell, Davies & Dean, 1954). The report of the London School of Hygiene and Tropical Medicine for 1955–6, summarizes, under the Department of Human Nutrition, the work being done by this Unit. Since enzymes are largely proteins it is not surprising that in protein malnutrition many of the biochemical changes observed have been in amounts of enzymes in various tissues. Despite all this work no single biochemical test has been accepted as a reliable indicator of the degree of protein deficiency or of its progress during treatment.

The urine has been neglected as a source of information and it is suggested that the determination of urea nitrogen and ammonia nitrogen as proportions of the total urinary nitrogen offers a simple method of assessing nutritional status with respect to protein.

Folin (1905) first proved that rise or fall in total urinary nitrogen is accompanied by almost identical rise or fall in urea nitrogen. The proportion of total nitrogen present as urea nitrogen ranges between 90% on a high-protein diet and less than 30% in malnourished human subjects. Low values of urea nitrogen as percentage of total nitrogen are typical of protein malnutrition and reflect low total urinary nitrogen output following prolonged low dietary intake of protein. Absolute as well as relative increase in ammonia nitrogen is an additional feature of protein malnutrition. In protein-deficient pigs a relative excess of dietary carbohydrate intensifies this effect.

Increase in dietary protein does not immediately produce a corresponding rise in urinary nitrogen output in chronically protein-malnourished infants or pigs. This may indicate abnormally high nitrogen retention not necessarily accompanied by gain in weight (Platt, 1954), or disturbances in urea metabolism induced by chronic protein malnutrition.

Determining urea and ammonia nitrogen as a percentage of total nitrogen has for clinical purposes the merit that a single morning specimen of urine provides all the required information.

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The amounts and distribution of the nitrogenous components of the breast milk of British, Indian and African mothers, with special reference to the curd : whey protein ratio. By PHYLLIS LUTZ and B. S. PLATT, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Most of the material for this work was obtained from abroad and therefore it was necessary to devise a reliable method of preservation. Five ml. of milk were absorbed on 9 sq. in. of filter paper (Ford's D 4C); the papers dried in a desiccator and sealed in heavy polythene bags. This was found to be effective in preserving the milk for short periods, provided it was dried quickly and completely. Results of analyses of milk samples taken after the 1st month of lactation only have been considered, in view of the high initial level of total nitrogen in human milk after parturition.

Most workers, attempting to correlate dietary protein with milk protein concentration, have determined the latter from the values for total nitrogen $\times$ 6·25 or 6·38 and make no distinction between the various components comprising this fraction.

In view of the difference in behaviour of curd and whey in the infant animal's stomach (Platt, 1954), it was thought that the whey proteins may have some special physiological significance, and it would therefore be of interest to study the relation of curd : whey (C : W) proteins in the milk of well- and mal-nourished mothers.

Milk samples obtained from India and Africa were analysed for total nitrogen, non-protein nitrogen, curd- and whey-protein nitrogen.

Table 1. *Comparison of milks of English, African and Indian mothers*

Country	England	Tanganyika	Ghana	Belgian Congo	India
No of samples*	20	42	7	15	30
Stage of lactation (months)	1–2	1–18	1–7	1–15	2–30
Total protein N (mg/100ml.)	137	121	120	117	116
S.D.	12	23	39	24	23
C : W mean	1·08	1·17	1·21	1·39	1·30
S.D. and range	0·07(0·95–1·18)	0·17(0·94–1·75)	0·21(0·80–1·50)	0·48(0·71–2·44)	0·24(0·93–2·05)

* These were all from different subjects except for the English women where two samples were obtained from each of ten women.

The most noteworthy of the results shown in the table is the difference between the values for the C : W ratios for the milks from the English and Tanganyikan mothers on the one hand and the Indian and Belgian Congo mothers on the other; the ratios in the latter are higher, show a wider scatter and include a number of high values which are never found in the former. The latter included many cases diagnosed as suffering from malnutrition.

After concentration of the whey, three whey-protein fractions, A, B and C, were separated by paper electrophoresis in McIlvaine buffer pH 7·6; $\mu = 0{\cdot}15$. After drying, staining and washing, the strips were scanned for optical density which could, by previous calibration, be related to protein concentration. No statistically

significant differences in these fractions for English, African and Indian milks were found.

Analyses of urine samples, reconstituted from acidified filter-paper, revealed low ratios of urea nitrogen : total nitrogen from malnourished Indian and Belgian Congo women.

Blood-serum analyses showed, in general, normal total-protein values but low albumin : globulin ratios. It was found in several individuals that a very high γ-globulin concentration in the blood serum was correlated with a high concentration of the whey-protein fraction C.

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Environment and nutrition of the Papuan child. By H. A. P. C. OOMEN, *Nutrition Section, Institute of Tropical Hygiene and Geographical Pathology, Amsterdam, Holland*

In connexion with studies on maternal and infant diets, on behalf of the South Pacific Commission, a comparative investigation was made in different regions in New Guinea, representing typical Papuan environments.

In Kambuaja village (Vogelkop peninsula) the staple food is taro, with some additions. Fish and crayfish are eaten. The average adult intake is 1530 Cal. and 37 g protein, partly animal. Moderately severe malaria is prevalent. Breast feeding is well maintained throughout the 2nd year. Other food is introduced early and growth is good. Children are slender and have livers of normal size; signs of malnutrition are rare. Child nutrition is rated reasonably good and resources satisfactory.

Nubuai village (Waropen coast) in mangrove swamps is built on stilts on mud flats. The staple food, sago starch, provides 93% of the calories, but is scarce. Only one semi-aquatic vegetable (*Ipomoea reptans* Poir.) is eaten in fair quantities. Small fish, shellfish and shrimps contribute a little protein. The average adult intake is 1460 Cal.; most pregnant and lactating women subsist on 10–12 g protein. Breast feeding is protracted but often ceases owing to lack of milk. Sago paste, introduced in the first few weeks, is often the infant's only other food until well into the 2nd year. Growth is poor after the 3rd month and malnutrition (marasmus) is common. Many toddlers and schoolchildren have enlarged livers, not due only to malaria. Mortality is high in the 1st and 2nd years and rickets not unusual. Child nutrition is rated bad and resources unsatisfactory.

In Tambanum (Sepik river) sago and yams are staple foods; banana, taro, cassava and coconut are also important. River fish and shrimps provide some protein, and greens and fruits are eaten in fair quantities. The average adult intake is 1530 Cal. and 19 g protein. There is some malaria. Breast feeding is reasonably maintained in the 2nd year. The first other food is well balanced and introduced timely. Growth rate is satisfactory, livers are of normal size and signs of malnutrition rare. Child nutrition is rated reasonably good and resources satisfactory.

In Jobakogi (Central Highlands, 6000 ft.) 92% of the calorie intake is from sweet potatoes. Considerable quantities of various greens provide important nutrients; intake of animal food is trifling. The average adult intake is 1900 Cal., the protein intake about 25 g; women would get considerably less. People are naked and the climate is cold. Breast feeding is very protracted and the milk supply still significant in the 3rd year. Sweet potato is introduced late. Growth in the 1st year is better than would be expected, but kwashiorkor in older infants and malnutrition in nursing mothers is common. Endemic goitre and cretinism occur and child mortality is high. Child nutrition and resources are rated poor.

Arrested growth lines in the bones of pigs on low-protein diets. By R. J. C. Stewart and B. S. Platt, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, Mill Hill, London, N.W.7*

The radii of two pigs (A and B) on a low (4·5%) vegetable-protein diet (see Knowles, 1957) increased in length by only 10 and 11 mm between the 15th and 56th day of life; in a control animal the increase was 30 mm. Radiographs showed the bones of A and B to be reduced in diameter as well as length and the distal epiphyseal cartilage was thin. Pig B was then given a higher-protein diet by replacing 5% of the starch by casein, the caloric intake being unchanged. During the next 60 days radiographic 'white lines', representing hypercalcified layers, appeared at the cartilaginous border in both pigs. As bone length increased these lines passed deeper into the bones and other lines developed, which in their turn were replaced. Thus a series of transverse lines appeared, those in animal B being less distinct and more widely separated than those in A. Histologically these 'lines' were identified only as bands of thickened or clubbed trabeculae, but cleared slices of bone show a distinct lattice formation.

In animal C, fed for 13 months on the low-protein diet, the epiphyseal cartilages of the radius were very thin and separated from the marrow by a single hypercalcified line. On an unlimited 9·5% vegetable-protein diet the distal cartilage thickened and showed signs of renewed activity, until at 17 months of age a transverse line was seen 14 mm within the metaphysis, this distance corresponding with the increased length of the bony shaft. All growth was at the distal end, for, as would be expected at this age, the proximal epiphysis had closed. Histologically this 'line' appeared as a transverse bar of bone and undoubtedly had, before the diet change, effectively 'sealed' off the cartilage (Frandsen, Nelson, Sulon, Becks & Evans, 1954).

The changes described presumably have a common origin, that in animal C representing a more severe and complete stage of that seen in A and B. The striated appearance was unexpected and it may be that when growth is markedly retarded, through dietary inadequacy, even minor disturbances might be 'recorded' in the bone.

Many of the evidences of protein malnutrition seen in children can be reproduced in the pig (Knowles, 1957). Arrested growth lines have frequently been described in the bones of children in a poor state of health and nutrition; it is suggested in view of the foregoing observations that protein malnutrition may play a major role in their pathogenesis.

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The influence of the dietary fat on the kidneys of rats deficient in vitamin E. By T. MOORE, I. M. SHARMAN and K. R. SYMONDS, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Martin & Moore (1939) first reported degeneration of the renal cortical tubules in rats deficient in vitamin E. Later Emmel (1956, 1957) gave rats diets free from vitamin E and containing the fatty acids of linseed oil, and found that the histological changes caused by vitamin E deficiency occurred during post-mortem autolysis. A combination of highly unsaturated fat with avitaminosis E was considered necessary for the production of the abnormality. In recent work (Moore, Sharman & Symonds, 1957) we confirmed that the typical histological picture, due to vitamin E deficiency and characterized by disintegration of the contents of the tubules, is developed during autolysis. As a routine the effect was conveniently observed by postponing the dissection of the kidneys until 3 h after death. Autolysis could be prevented by combining the lack of vitamin E with the removal of fat from the diet. Conversely, the inclusion of cod-liver oil greatly increased autolysis. In advanced avitaminosis E, induced by our standard diet containing lard, degenerative changes in the nuclei of the renal tubules, without complete disintegration of their contents, could be observed even when autolysis had been prevented by dissection and fixation of the kidneys immediately after death.

Further experiments have now been made on the effect of the dietary fat. The promotion of kidney autolysis by the inclusion of 5 or 10% of cod-liver oil has been confirmed. Moreover, the kidneys of rats which had received 10% of cod-liver oil for 8 months, and which had been fixed immediately after death, showed nuclear degeneration in the tubules as advanced as in the kidneys of rats which had been kept for over a year on our standard vitamin E-deficient diet containing 10% of lard. Coconut oil, as might be expected from its low degree of unsaturation (iodine value 11), did not promote autolysis. Linseed oil (iodine value 181), as 10% of the diet, induced autolysis in the kidneys of only one out of six rats, and was thus less active in causing autolysis than lard (iodine value 50). Analyses of the various fats for tocopherols failed to explain why linseed oil did not cause autolysis. The possibility must be considered that acids of the arachidonic type are more active in promoting kidney abnormalities than are linoleic and linolenic acids.

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An improved diet for the assay of vitamin B_{12} with rats. By KATHLEEN M. HENRY and J. W. G. PORTER, *National Institute for Research in Dairying, Shinfield, near Reading*

A basal diet with the following percentage composition was used: soya protein 35, lactose 17, arachis oil (semi-hardened) 12, glucose 30, salts (de Loureiro, 1931) 4, vitaminized arachis oil (Cuthbertson & Thornton, 1952) 2. The diet was supplemented with the vitamin mixture of Cuthbertson & Thornton (1952) together with 0·3 g choline chloride/kg.

The table shows the mean weight gains, in 4 weeks, of groups of twelve littermate male rats weaned from normal mothers. Results are given for four of the experiments done with two soya-protein preparations, Drackett (The Drackett Co., Cincinatti) or α-protein (The Glidden Co., Chicago).

Exp. no.	Dietary protein	Weight gain (g) with vitamin B_{12} supplement (μg/day) of: 0	0·05	0·10	0·20	0·40
1	Drackett	46·1	—	82·8	87·4	95·5
	Dracket + 1·4% DL-methionine	52·1	—	82·6	93·0	98·4
2	Drackett	58·2	—	84·1	—	—
	Drackett + 0·7% DL-methionine	61·9	—	100·1	—	—
	Drackett + 1·4% DL-methionine	63·9	—	92·3	—	—
	Drackett + 2·1% DL-methionine	58·9	—	89·4	—	—
3	Drackett + 1·4% DL-methionine	44·4	—	74·3	—	—
	α-Protein + 1·4% DL-methionine	48·4	—	85·4	—	—
4	α-Protein + 0·7% DL-methionine	54·4	85·3	101·2	102·5	—

In Exps. 1 and 2 the effect of additions of methionine to Drackett protein was studied, and in Exp. 3 Drackett and α-protein were compared. Exps. 2 and 3 were done at the same time and from them it was concluded that 0·7% methionine was the optimum supplement and that α-protein was probably superior to Drackett protein. This combination was used in Exp. 4 and maximum growth, nearly as good as that of stock-colony males, occurred with a daily supplement of 0·1 μg compared with about 0·2 μg vitamin B_{12} with the 'Drackett' diets.

The error of the method tends to be large, probably because of variable reserves and the possibility of intestinal synthesis of the vitamin in individual rats. It may be possible to reduce the reserves by giving the mother rats the deficient diet during lactation (cf. Hartman, Dryden & Riedel, 1956); intestinal synthesis might be reduced by changes in the nature or proportion of the dietary carbohydrates.

https://doi.org/10.1079/PNS19580021 Published online by Cambridge University Press

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The incidence of xerophthalmia in relation to age and sex in Java. By H. A. P. C. OOMEN, *Nutrition Section, Institute of Tropical Hygiene and Geographical Pathology, Amsterdam, Holland*

Several authors have stressed the high incidence of xerophthalmia in the Indonesian archipelago. It has been said that the severer manifestations, keratomalacia and xerosis of the cornea, occur most often in the youngest age groups, whereas xerosis of the conjunctiva and Bitôt's spots are more frequently found in the child of school age, and that, though the incidence of keratomalacia is the same in male and female infants, xerophthalmia occurs twice as often in male as in female children over 2 years old.

To investigate the distribution of the affection the clinical notes on 11,000 patients treated in an eye hospital in Jogjakarta and on about 1000 treated in an eye polyclinic at Semarang were studied. These are large cities in Central Java, all diagnoses were made by competent ophthalmologists, and the notes on about 8000 patients, believed to represent random samples of the population, could be used for comparative studies. The distribution as to age and sex showed similar trends in both places.

The term xerophthalmia is used to cover all visible signs connected with xerosis of the eye; keratomalacia is reserved for conditions in which a colliquative necrosis affects the entire cornea.

About 4% of the patients were affected during the 1st year of life and about 80% in the 2nd–6th year, the peak being often towards the end of the 3rd year. The disease is thus typical of the toddler period.

Up to the 6th year 58% of the affected children were male, after the 10th year 85% were male, which suggests two levels of predominantly male susceptibility depending on age.

It was impossible to distinguish clearly between keratomalacia and other manifestations of xerophthalmia, but keratomalacia was probably most prevalent in the youngest age groups. Of infants with xerophthalmia, under 6 months of age 66% were male and from 6 months to 1 year 52% were male. The reservation is made that age and sex distribution may be different in keratomalacia and in xerosis of the conjunctiva and cornea and that rates during the 1st year may be different from those in older children. Aetiologically and epidemiologically, xerosis of the conjunctiva and cornea may be a different disease from keratomalacia.

It is believed that early dietary protein deficiency, poor carotene utilization and infectious disease are responsible for the high incidence in the toddler period. The difference in incidence between the sexes in older children is attributed to a sex difference in requirement or availability of vitamin A.

The present-day endemic nature of xerophthalmia in Central Java closely resembles the picture in Japan described by Mori in 1904.

Postoperative nutritional failure presenting the kwashiorkor syndrome.

By Z. A. LEITNER, 52 *Welbeck Street, London, W.1*

A man, aged 61, suffering from obstructive jaundice, was submitted to pancreas-head resection with partial gastrectomy. He was well for nearly 2 years, but 22 and again 28 months after operation he had to be admitted to hospital with steatorrhoea, hypoproteinaemia, and mild normocytic anaemia. Recovery occurred both times quickly with a diet high in protein and low in fat, together with pancreatin, vitamins and iron therapy. Soon afterwards a third severe relapse followed. In spite of very energetic treatment, not only his clinical condition deteriorated, but also the hypo-proteinaemia and anaemia were intensified, and obvious signs of severe avitaminosis with biochemical evidence of lack of vitamins A, C and E and thiamine occurred whilst in hospital. Furthermore, during the last few months of life he developed a pigmented, pellagra-like dermatosis covering nearly the whole body with the exception of places exposed to light. The skin was dry, wrinkled, xerotic, in some regions it was hyperpigmented with crazy-pavement dermatosis, exfoliation and deep cracks. In between purpura-like, punctate spots and striae were scattered.

On post-mortem a large, fatty liver (fat content 32·4%) with slight fibrosis of the portal tract was found. The pancreas showed complete atrophy of the parenchyma with extensive fibrous replacement; shrinkage of acinar tissue with reduction of zymogen granules and hypertrophy of the islet tissue.

It is submitted that the postoperative malabsorption syndrome which led to the fully developed picture of a secondary kwashiorkor in this case—the first so far reported in the literature—had the following sequel of events. After the operation a mild postgastrectomy syndrome developed with chronic pancreatitis, which for more than $2\frac{1}{2}$ years easily responded to routine treatment. During this whole time of 'gastrointestinal hurry', however, absorption suffered in various ways: on one hand the decreased pancreatic secretion was not adequately mixed with the quickly moving chyme, leading to faulty absorption; on the other hand, owing to the same increased motility the pancreas-stimulating hormones (secretin, pancrozymin) could not be fully absorbed; both of these interferences led not only to a qualitative, but also to a quantitative change in the protein absorption, which further decreased the pan-creatic enzyme secretion. The diminished enzyme secretion on its own account intensified the development of pancreatic fibrosis, and the destruction of acinar tissue, leading ultimately to cessation of all enzymic activity.

Thus, a vicious circle was completed within 3 years of the operation, which during the last year of life gradually produced severe progressive hypoproteinaemia with reversion of the albumin : globulin ratio, extreme pancreatic fibrosis, gross fatty infiltration of the liver, and ultimately the fully established protein-malnutrition syndrome (secondary kwashiorkor).

'Available lysine' in wheat, flour and bread. By K. MARY CLEGG and NANSI DAVIES, *Research Association of British Flour-Millers, Cereals Research Station, St. Albans, Herts*

The 'available lysine' in samples of wheat, flour, bread and gluten has been determined by the modified procedure of Bruno & Carpenter (1957). This chemical method gives a measure of free ϵ-amino-groups of lysine, and Carpenter, Ellinger, Munro & Rolfe (1957) have shown a correlation between the 'available lysine' and the feeding value of a range of protein materials. In the present work values for 'available lysine' in wheat products determined chemically have been compared with the total lysine content of hydrolysates of these materials using the technique of Moore & Stein.

The apparent 'available lysine' in a range of wholemeal and white flours was about 80% of the total lysine. The difference between the two methods of lysine estimation may however be attributed almost entirely to the interference of carbohydrates during hydrolysis in the 'available lysine' determination (cf. Bruno & Carpenter, 1957). This has been established by experiments with gluten. The 'available' and total lysine in freeze-dried gluten were found to be similar. But addition of increasing amounts of starch to the gluten, up to a starch : gluten ratio of 1 : 1, reduced the value obtained for 'available lysine'. With this proportion of starch the apparent 'available lysine' figure was about 90% of the value for total lysine. But no further reduction in the 'available lysine' value occurred when the starch : gluten ratio was increased beyond the value 1 : 1. That is, the proportion of 'available lysine' to total lysine became constant with starch contents similar to those in wheaten flour.

When white flours were baked into bread analyses of the crumbs showed that the 'available' : total lysine ratio was not significantly different from that for flour. For bread crust McDermott & Pace (1957) found that the total lysine was 15% less than in the corresponding flour and in the present work a similar reduction in the 'available lysine' value of crust has been found. This may be contrasted with heat-damaged fish fillets, where the total lysine fell by 11% and the 'available lysine' by 40% (Carpenter *et al.* 1957).

The results so far obtained from the chemical method for the determination of 'available lysine' indicate that in the conversion of wheat into flour, and the baking of flour into bread, there is no significant reduction in the number of free ϵ-amino-groups of lysine.

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Some observations on the nutritive value of a dehydrated meat bar. By JEAN I. SCOTT and E. J. ROLFE, *Ministry of Agriculture, Fisheries and Food, Research Establishment, The Experimental Factory, Greyhope Road, Aberdeen*

The meat bar has been developed as a new ration item. It contains equal parts of

vacuum-dried minced cooked beef and pork compressed into blocks and vacuum-packed into foil laminated pouches (Gooding & Rolfe, 1957).

Microbiological methods (Analytical Methods Committee, Society of Public Analysts and other Analytical Chemists, 1956; Barton-Wright, 1954; Clegg, Kodicek & Mistry, 1951; Kodicek & Pepper, 1948; Shrimpton, 1956) have been used to study the fate of certain vitamins of the B complex, namely riboflavin, nicotinic acid, pantothenic acid and vitamin B_{12} during preparation of the meat bar and subsequent storage in Singapore (average temperature 82°F.). Of these vitamins the only loss observed in the dehydrated product was approximately 10% of the pantothenic acid, and this occurred during the precooking process. There was an apparent increase in riboflavin content but this may be due to other substances stimulating the growth of the assay organism. A similar effect has been reported by Rice & Robinson (1944).

Thiamine was assayed chemically and it was found that almost 90% was lost during the first 6 months' tropical storage. After 12 months only a negligible quantity remained. In contrast to this, riboflavin, nicotinic acid, pantothenic acid and vitamin B_{12} were found to be stable over a 21 months' storage period.

Vitamin content (mg/g non-fatty solids) of freshly prepared meat bar

Thiamine	4·8	Pantothenic acid	23·5
Riboflavin	9·5	Vitamin B_{12}	0·051
Nicotinic acid	193·6		

During tropical storage, vacuum-packed meat bars slowly deteriorate due to non-enzymic browning—a reaction involving amino-nitrogen of the dehydrated meat. The development of a dry texture is evidence that the proteins are affected and it was feared that a marked loss in their biological value would also occur. However after 15 months' storage the biological value (expressed as net protein utilization) had dropped by only a small amount from the initial figure of 76 to 69 when assayed by the shortened method of Miller & Bender (1955).

It is evident therefore that the meat bar when packed in this way is a stable product and during protracted storage in the tropics loses little of its nutritive value except for destruction of thiamine.

The assistance of Dr A. E. Bender by carrying out the N.P.U. assays is gratefully acknowledged.

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Copper and zinc supplements for fattening pigs. By M. M. ALLEN*, R. S. BARBER, R. BRAUDE and K. G. MITCHELL, *National Institute for Research in Dairying, Shinfield, near Reading*

Observations of Wallace (1956) indicate that the addition of zinc to the diet of fattening pigs enhances the growth-promoting effect of copper supplementation. In continuation of our studies on high-copper supplements for pigs (last communication: Barber, Braude, Mitchell, Rook & Rowell, 1957), we have compared the effect of copper added to the basal diet at 0·1% as $CuSO_4 \cdot 5H_2O$ (supplying approximately 250 p.p.m. Cu) with that of zinc (added at 0·045% as $ZnCO_3 \cdot 2ZnO \cdot 3H_2O$, supplying approximately 250 p.p.m. Zn) and of the two supplements added together. The basal diet consisted of 52% barley meal, 38% fine wheat offal, 10% white-fish meal, plus a supplement of vitamins A and D_3, and the pigs were fed as much as they could clear up in 20 min at two daily feeds, up to a maximum of 6½ lb./pig/day.

The results are summarized in the table, and show that the growth-promoting effect of the copper supplement was again confirmed. Zinc, in the amount fed, had no effect, and the combination of copper and zinc did not produce a better response than copper alone.

Mean daily live-weight gain, efficiency of food conversion and liver storage of copper in pigs receiving supplements of copper or zinc or both during fattening from a mean weight of 48·1 lb. to 207·3 lb.

Treatment no.	1	2	3	4
Supplement	None	Zn (250 p.p.m.)	Cu (250 p.p.m.)	Zn + Cu (250 p.p.m.) (250 p.p.m.)
No. of pigs	12	12	12	12*
Daily gain (lb.)	1·40	1·42	1·56	1·55
Food consumed (lb.): total	535·2	533·5	505·3	5·162
/lb. gain	3·4	3·3	3·2	3·3
Copper in liver (mg/kg dry tissue): value	57·5	52·8	887·5	593·3
range	40·4–93·1	38·7–64·5	316·8–2559·7	118·8–1060·0

* One pig died and missing values were calculated by the missing-plot technique (Yates, 1933).

The amount of copper stored in the liver of pigs that received the copper supplement was significantly higher than that of the control pigs. When zinc was fed in addition to the copper supplement, the amount of copper stored was reduced in comparison with that found when copper alone formed the supplement.

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* In receipt of an Agricultural Research Council Scholarship.

The use of chromium sesquioxide to determine 'instantaneous' digestibilities. By E. C. OWEN and R. PROUDFOOT, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Because of the variations from day to day in the weight of faeces from ruminants maintained at constant food intake, digestibilities of nutrients cannot normally be determined in a single day. By mixing Cr_2O_3 intimately with that part of the food which contains the nutrient whose digestibility is sought this difficulty was overcome by Chanda, Clapham, McNaught & Owen (1950, 1951) who were studying the fate of carotene in the ruminant. A similar method has since been used by us to compare the digestibility of carotene, fed separately from the food, with that of carotene fed as greenstuff. Whenever an inert marker such as Cr_2O_3 is used a problem arises as to how well it has been mixed with the nutrient being studied and whether it has become unmixed with the nutrient in the gut. By using both carotene and Cr_2O_3 inside one capsule this efficacy of mixing can be tested.

Gelatin capsules (Parke, Davis & Co. Ltd) were filled with 2 g of a mixture of vitamin-free margarine, Cr_2O_3, β-carotene and α-tocopheryl acetate (e.g. 32:40:2·5:1·5 by weight). Mixing was continued till duplicate estimations of the Cr_2O_3 and carotene in it agreed to within 1% on 20 mg samples from different parts of the mixture. One such capsule was fed to a goat which had been deprived of greenstuff till its faeces showed a just detectable trace of carotene.

Every subsequent defaecation was separately analysed for carotene and Cr_2O_3. The results showed that the carotene and Cr_2O_3 remained mixed, for they gave superimposable excretion curves which were nevertheless not dependent on the amount of dry matter simultaneously excreted. If the ratio of carotene to Cr_2O_3 in the capsule is K, 'instantaneous digestibilities' (d) of carotene are calculable from any faecal sample by using the formula:

$$d = 100 - \frac{(\text{concentration of carotene in faeces}) \times 100}{(\text{concentration of } Cr_2O_3 \text{ in faeces}) \times K},$$

in which the denominator is the concentration of carotene which would have been expected had none been digested. Table 1 shows a selection of results from such an experiment in which K = 0·0206 and in which the capsule, containing 23·3 mg carotene, was given, by means of a dosing gun, 24 h before the first sample of faeces was taken for analysis. The dose was 0·364 mg carotene/kg body-weight.

Table 1. *Digestion of carotene by a goat*

Time	Faeces (g)	Carotene (mg/100g)	Cr_2O_3 (mg/g)	Digestibility of carotene	
9–10 a.m.	77	0·75	7·5	81	
11 a.m.–noon	91	1·28	9·0	72	
2–3 p.m.	161	1·34	16·1	84	Mean
3–4 p.m.	39	1·57	17·8	83	78
4–5 p.m.	62	1·22	10·6	77	
9–10 p.m.	80	1·36	8·4	69	

Results will be published in detail elsewhere.

https://doi.org/10.1079/PNS19580021 Published online by Cambridge University Press

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Further observations on the utilization of glucose and maltose in the young pig. By R. BRAUDE, A. M. DOLLAR, K. G. MITCHELL and J. W. G. PORTER, *National Institute for Research in Dairying, Shinfield, near Reading*

Results of further studies of the concentration of reducing sugars in the blood of young pigs receiving aqueous solutions of maltose agreed with our earlier findings (Dollar, Mitchell & Porter, 1957) that the 2–4 days old pig is unable to utilize this sugar, but suggested that utilization begins when the pig is about 5 days old. That this inability is due to a deficiency of maltase was shown by the marked rise in the level of reducing sugars in the blood of 2- or 3-day-old pigs given together solutions of maltose and of maltase (a crude maltase (amyloglucosidase) of fungal origin).

That the 2–5 days old pig is unable to use maltose was fully confirmed by the results of feeding experiments in which groups of pigs received either the basal diet or the basal diet with glucose or maltose. The composition of the diets was:

	Basal*		Basal with carbohydrate†	
	g/100 g	Calories (percentage of total)	g/100 g	Calories (percentage of total)
Casein	61	57	25·4	24·8
Arachis oil	19·6	42	8·3	17·9
Carbohydrate	—	—	58·2	57·0
Vitamin and mineral mix	19·4	1	8·1	0·3

* Offered *ad lib.* as 7·3% aq. solution supplying 34 Cal./100 g.
† Offered *ad lib.* as 16% aq. solution supplying 78 Cal./100 g.

The growth of four pigs (initial weight about 1·6 kg) on each diet was:

	Basal with glucose	Basal with maltose	Basal only for 3 days then with maltose	Basal only for 18 days
Gain (kg):				
2–5 days	0·45	−0·04	0	0
5–18 days	2·04	2·25*	2·26†	0·36
Dry matter eaten (kg/kg gain):				
2–18 days	1·36	1·36	1·27	2·86

* One died at 7 days. † One died at 9 days.

It is evident from the results that during the period 2–5 days the pigs receiving the diet with maltose did not grow and that during the subsequent 5–18 days period their performance was similar to that of the group receiving glucose. The group which received the basal diet grew only slightly during the 2–18 days period, but when then given the diet with glucose immediately resumed normal growth. The pigs showed no impairment in their ability to utilize glucose, though abnormal blood-sugar tolerance curves were obtained for the first few days after the changeover.

We are grateful to Glaxo Laboratories Ltd for the maltase and for the basal diet.

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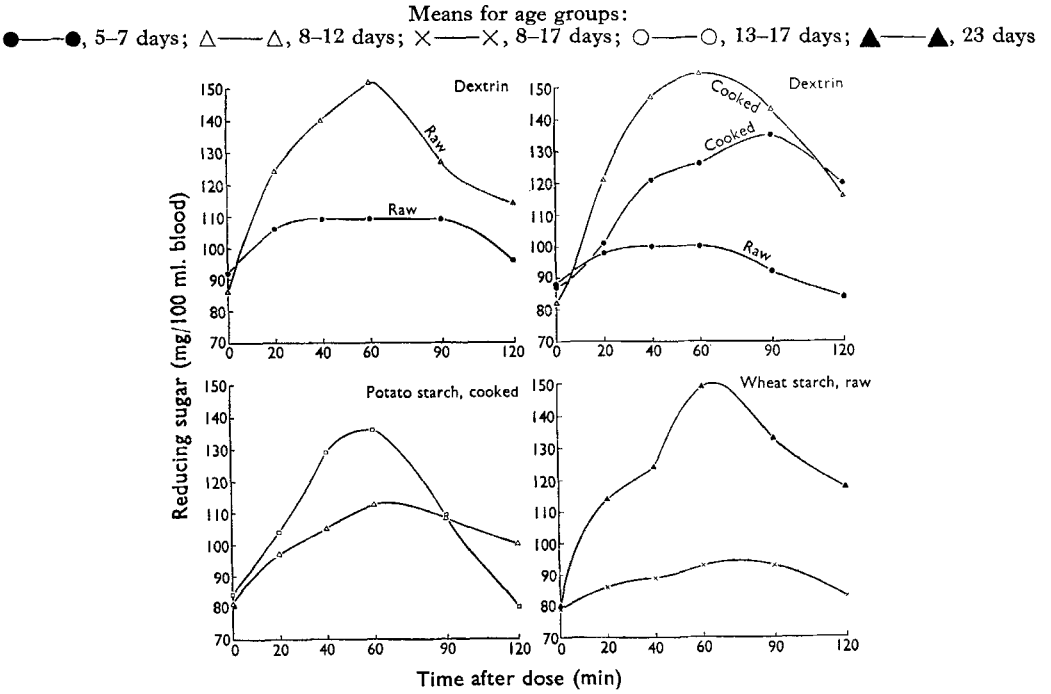
The utilization of raw and cooked starch by the young pig. By R. BRAUDE, A. M. DOLLAR, K. G. MITCHELL and J. W. G. PORTER, *National Institute for Research in Dairying, Shinfield, near Reading*

Dollar, Mitchell & Porter (1957) showed that 2–4 days old pigs were unable to utilize solutions of dextrin, as judged by the rises in reducing sugar in blood, though the ability had developed in 10-day-old pigs.

We now find that already at 5–7 days of age the pig can utilize solutions of dextrin (dose 3·3 g/kg body-weight). It is clear, therefore, that amylase is secreted in effective amount by pigs of this age.



The figure also shows that raw dextrin (dose 1·8 g/kg) and cooked potato starch (dose 3·3 g/kg) caused less marked rises in the blood sugar of 8–12 days old pigs than solutions of dextrin (dose 3·3 g/kg), but with 13–17 days old pigs well-marked rises were obtained. Raw wheat starch (dose 3·3 g/kg) caused only slight rises in the blood sugar of 8–17 days old pigs but gave a good response in 23-day-old animals.

It is evident from these results that rapid digestion of the ingested polysaccharides is necessary to promote a marked rise in blood sugar, and that this rapid digestion depends both on the age of the pig and on the solubility of the polysaccharide. It

is possible, therefore, that the action of amylase is complemented by a solubilizing process that is inadequate in the young pig but that develops with age.

We are studing further the validity of the blood-sugar technique in the assessment of the utilization of polysaccharides by carrying out growth tests with young pigs.

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The separation and the estimation of cerebrosides and gangliosides in brain: some results on the rat brain during growth from 15 to 120 days of age. By SHEILA PAYNE and B. S. PLATT, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Weiss (1956) separated sphingolipids by adsorption chromatography using silicic-acid columns and estimated the hexose component by a modification of the anthrone reaction (Radin, Lavin & Brown, 1955). He found two ganglioside fractions which he combined and analysed, assuming the hexose to be all galactose. We have found three ganglioside fractions; all contain both galactose and glucose but in differing proportions; the galactosamine content of one of the fractions remains constant throughout life, but the proportion of galactosamine in the other two fractions decreases with age (Svennerholm, 1957).

40–60 mg of crude sphingolipid were analysed by the method of Weiss (1956) modified as follows. (a) To avoid a 10% loss of cerebroside fraction 2 during acetone drying, the fresh tissue is homogenized with 1 vol. acetone and dried in vacuo over P_2O_5. (b) To increase the resolving power of the column, silicic acid is ball-milled to 300 mesh; activated by heating to 180°–200° for 2 h (Kay & Trueblood, 1954), and prewashed according to Trueblood & Malmberg (1949). (c) The glucose and galactose in the solid matter in appropriately pooled fractions are determined by hydrolysis in 2N-HCl (Montreuil, Boulanger & Houke, 1953); extracted with a 5% (v/v) solution of *N*-methyldioctylamine in chloroform; portions of the supernatant developed on paper for 72 h in *n*-propyl alcohol–ethyl acetate–water (7:1:2) (Albon & Gross, 1952); sprayed with aniline phthalate (Partridge, 1949); heated to 100° for 4 min in a water-saturated atmosphere. The glucose and galactose spots are cut out with an appropriate blank spot of paper and the colour eluted in 3·5 ml. of 0·7 N-HCl in 80% ethyl alcohol (Leloir, 1951) and read at 400 mμ.

Measurements of the amounts of the six successive fractions (1, 2, 3, cerebroside, and a, b, c, ganglioside) have been obtained for preparations from brains of rats at various ages from 15 to 120 days of age on stock diet 41.

The concentration of fraction (a) remains approximately constant throughout growth, and fraction (b) does not increase as rapidly as does the cerebroside fraction 1. The ganglioside–galactose, expressed as a proportion of the total galactose,

decreases; that of fraction (a) especially rapidly with age, indicating that the gangliosides might be precursors of the cerebrosides. After the 40th day of age, the synthesis of ganglioside fraction (a) diminishes whilst that of the cerebrosides increases.

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Observations on the blood of rats fed different levels of protein. By SHIRLEY A. BISSON and G. R. WADSWORTH (introduced by B. S. Platt), *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.*1

Anaemia is commonly observed in clinical protein malnutrition in infants; the precise role and the importance of protein deficiency in the pathogenesis of anaemia have, however, not been fully established (Platt & Wadsworth, 1956). In the study of this problem it is necessary to measure blood volume and carcass protein because total haemoglobin is correlated with 'lean body mass' (Muldowney, 1957) and both may be affected by insufficient dietary protein.

Weanling male rats were fed on a standard diet for 3 weeks and were then divided into two groups. One was given a diet containing 10% casein; the other an isocaloric diet containing 3% casein. After 13 weeks blood volumes were measured, the animals killed and body water determined by drying to constant weight in an oven at 100°.

The mean haemoglobin concentration of twenty-nine rats on 10% casein was 14·1 g/100 ml.; that of twenty-seven rats on 3% casein was 12·5 g/100 ml. The difference could be accounted for by a relatively large plasma volume in the low-protein animals. The mean body water of each group was about 63·5% of the body-weight. Using the formula of Miller & Bender (1955) it was calculated that the mean carcass protein in each group was 19·2% of the body-weight. The ratio, total haemoglobin : carcass protein, was 0·05 in the high-protein group and 0·049 in the low-protein group. Thus dietary protein shortage resulted in a proportionate reduction in haemoglobin and carcass-protein production during growth. In growing rats Belcher & Harriss (1957) found that red-cell volume remained more or less constant in relation to body-weight, but plasma volume progressively diminished. The present results show that plasma volume is also relatively large in rats whose growth has been retarded because of an inadequate diet. This is of importance in assessing haemoglobin levels in relation to nutrition, and emphasizes the importance of blood-volume determinations in such studies.

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The distribution of tritium in the body of a young piglet after administration of ^{3}H–DL–lysine. By J. DONE and P. R. PAYNE (introduced by B. S. Platt), *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.*7

A 9-day-old piglet (3·0 kg) was maintained on an artificial sow milk. 1·05 mg (57·6 μC) of ^{3}H-DL-lysine was injected in two doses, at 11.00 a.m. and 6.00 p.m. The marked lysine was prepared by reduction of 1:5-diamino-1-carboxybut-3-yne (Raphael, 1953). At 11.00 a.m. the next day the animal was anaesthetized by intraperitoneal injection of nembutal and killed by bleeding from neck veins. Tritium was assayed by the procedures described (Done & Payne, 1956) with one modification, i.e. combustion water was absorbed by anhydrous Na_2SO_4.

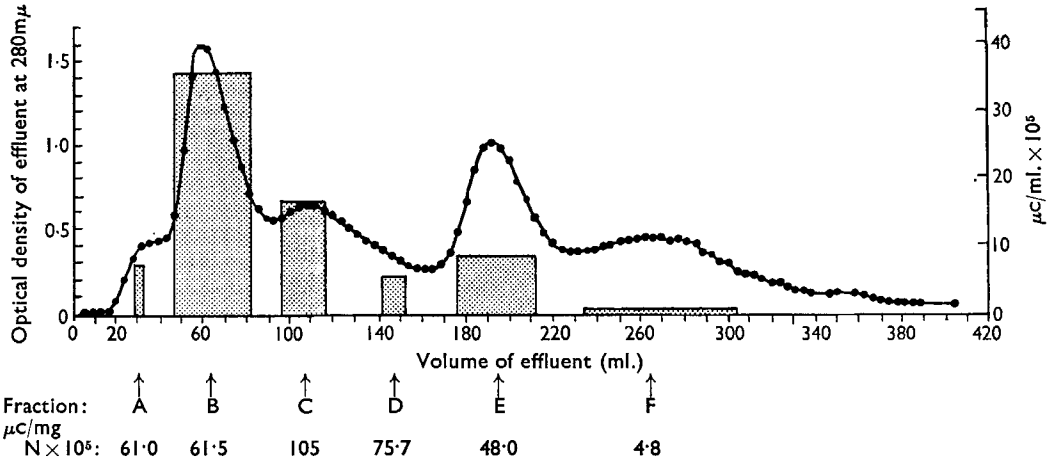

Fig. 1. Electrophoresis of 6 ml. of serum on a cotton-powder column, 4 × 45 cm, in borate–phosphate buffer pH 8·6 (Flodin & Kupke, 1956; Campbell & Stone, 1957). Shaded areas show radioactivity of pooled fractions A to G.

Fig. 1 shows the distribution of activity between serum-protein fractions. Results for other samples prepared from various tissues by washing with hot trichloroacetic acid and solvents (Zamecnik, Lotfield, Stephenson & Steele, 1951) were as follows, the figures given being μC/mg × 10^5:

Intestinal mucosa, 25; spleen, 21; fibrinogen, 21; liver, 17; pancreas, 13; kidney, 10; serum, 7·4; lung, 7·2; brain, 6·5; cornea, 5·8; skeletal muscle, 4·1; R.B.C., 2·0; skin collagen (prepared according to Neuberger & Slack, 1953), 1·8; lens, 0·52.

https://doi.org/10.1079/PNS19580021 Published online by Cambridge University Press

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A simplified method for assay of tritium–labelled water in body fluids. By P. R. PAYNE and J. DONE, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

A sample containing about 0·4 ml. of water is placed in a 1 oz. screw-capped bottle, a lump of anhydrous sodium sulphate, 4 g approx., is introduced and supported above the sample by a spiral of copper wire. The bottle is covered by a rubber disc, and connected to a vacuum line by means of a hypodermic needle. When frothing of the sample occurs, the bottle is disconnected.

After several hours, most of the water in the sample is absorbed by the sodium sulphate, which is then removed and placed in a simple reaction vessel constructed from readily available components—a length of 1 in. copper tubing, and a vacuum union and valve. A cup of pleated filter-paper containing 3·0 g of aluminium carbide is inserted in the vessel, which is then closed and evacuated through the valve to a pressure-gauge reading of 3–4 mm Hg. The vessel is then heated in a pressure cooker for 1 h at 120°, and the gas generated transferred to the proportional counter tube.

The counter and associated apparatus will also be demonstrated.

Some biochemical, histological and clinical effects of malnutrition on the eyes of experimental animals. By D. S. MCLAREN and K. BAGCHI, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

The eye is one of the organs best protected against the ill effects of malnutrition. In the severely protein-malnourished rat the eye continues to grow and its water content does not differ from normal. The cornea, and Harderian and lacrimal glands show histological changes. The sulphydryl (–SH) content of the lens falls in both its soluble and insoluble fractions and carbohydrate metabolism is disturbed. Cataract results when the antimetabolite, methionine sulphoximine, is given, and also in the offspring when there is a marked maternal dietary deficiency of protein. Pigs on low-protein diets for long periods also develop cataracts.

Deficiencies of protein and vitamin A frequently co-exist in man. Experiments in the rat suggest that protein deficiency neither hastens the onset nor enhances the severity of xerophthalmia. The healing of corneal wounds is not materially affected in rats deficient in both protein and vitamin A.

Dietary intake and refractive errors: an experimental correlation. By P. A. GARDINER and I. MACDONALD, *Department of Physiology, Guy's Hospital Medical School, London, S.E.*1

A possible relationship between shortsightedness in children and dietary intake has been recorded (Gardiner, 1954). When young rabbits were fed for a period with a diet low in the proportion of green food they became relatively myopic compared with the controls (Gardiner & Macdonald, 1957).

In order to determine whether there was any relationship between the extent of the dietary deficiency when young and the adult state of refraction in rabbits, it was decided to give young rabbits (8 weeks old), which were litter-mates, a diet consisting of equal parts of their normal green food and sucrose, whereas the controls were given solely the green food. Weekly refraction of the horizontal and vertical meridians of each eye was carried out and the findings for each animal expressed as a percentage of the refraction at the commencement of the experiment. The animals were in groups containing four rabbits and the percentage change in refraction and the daily dietary intakes were expressed as the mean for each group.

As the young rabbits were on the diet for varying periods various degrees of shortage of normal green food were produced. This deficiency as compared with the controls can be expressed as protein shortage during the time on the diet. The mean amount of the shortage for each group can be plotted against the mean adult refraction of the group. The original findings (Gardiner & Macdonald, 1957) when the young rabbits were put on one part of green food to three parts sucrose diet can also be plotted on the same graph.

When this is done there is seen to be a straight-line relationship between the percentage alteration in refraction and the extent of the dietary deficiency expressed as protein shortage ($P = 0{\cdot}05$–$0{\cdot}025$).

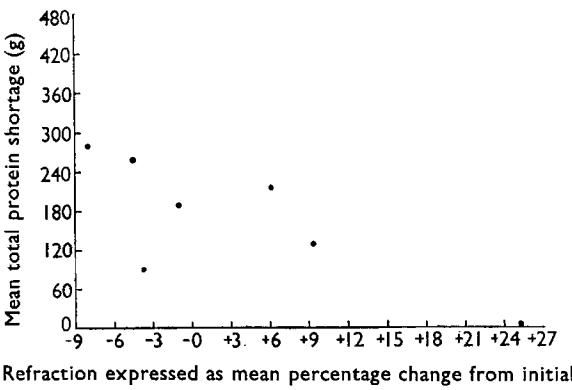

Fig. 1. Mean total protein shortage while on the diet plotted against the adult refraction of the eyes (expressed as mean percentage change from initial value).

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https://doi.org/10.1079/PNS19580021 Published online by Cambridge University Press

Method for the drying of foodstuffs by heating in edible oils in vacuo. By B. S. PLATT, C. R. C. HEARD and P. L. PELLETT, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

This method was demonstrated to the Biochemical Society in 1945 (Platt, 1945) and a full description of the process is given in a British Patent Specification (Platt & Heard, 1944). It originated in an observation by one of us (B.S.P.) made during an investigation of the effect on the nutritive value of foods of a Chinese method of cooking in which ingredients are fried for a short time then stewed in hot water. Green leaves were rapidly dehydrated by immersion in hot oil and retained 80% of their ascorbic acid; this oil-heating (frying) stage is the basis of the present method of food dehydration. The vacuum–oil procedure has been used for the dehydration of leafy green vegetables, sliced roots and tubers and minced or cubed meat and fish. Studies are being made on the suitability of various oils for the process and on the changes in nutritive value that may occur consequent on dehydration.

Amongst the merits of the process are the following: (a) very rapid drying is achieved with uncomplicated apparatus; (b) enzymes are rapidly destroyed by the initial immersion in the hot oil thus avoiding preliminary steam or water blanching with consequent loss or destruction of water-soluble constituents; (c) relatively low temperatures are used throughout the process, thus minimizing the risks of heat damage to the material; (d) the material reconstitutes readily to produce a tender, palatable product.

Trials were made in 1948 in Trinidad where large quantities of local vegetables were dried and used as the basis of school meals; also for tests by a taste panel of twelve student teachers; the flavour and texture of the vegetables were concluded to be highly acceptable.

The vacuum–oil process might be useful for dehydration of foods in some tropical areas where oil would normally be abundant. Gluts of food sometimes occur in these areas and a method of food preservation could well extend the supplies of food beyond the usual seasons. A further use would be in the preparation of mixed dishes; this could be done by individual treatment of the constituent foods, giving each appropriate precooking in hot oil. The mixed, dried foodstuffs could then be reconstituted and their cooking completed in one simple stage by unskilled staff.

Considerable interest in this method of drying foods at the present time is shown by its successful application in New Zealand (Vere-Jones, 1957) to meat. A very similar procedure was patented by Zimmermann (1951) in the United States. During the war Cutting, Reay & Shewan (1956) showed that the incorporation of fat into a foodstuff prior to dehydration improved its texture.

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