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ABSTRACTS OF COMMUNICATIONS

*The One Hundred and Twenty-first Meeting of The Nutrition Society was held in the London School of Hygiene and Tropical Medicine, London, W.C.*1, *on Saturday,* 13 *December* 1958, *at* 10.45 *a.m., when the following papers were read:*

Species differences in the occurrence of xanthine oxidase in milk. By V. V. Modi, E. C. Owen and R. Proudfoot, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Using the triphenyltetrazolium method of Zittle, Dellamonica, Custer & Rudd (1956), Modi & Owen (1956) confirmed earlier reports that xanthine oxidase occurs in cow's milk but not in human milk. We have also examined the milk of goats, sheep, pigs and a mare. The milks of all the ruminants, cow, sheep and goat, contained xanthine oxidase. Milk from the sow and the mare resembled human milk in being devoid of the enzyme. To test whether these differences had a dietary basis the rabbit, which is herbivorous but non-ruminant, was examined and each of two does gave milk which contained xanthine oxidase. Oxytocin had to be used to obtain milk from the sow and rabbit. Analyses by an adaptation (Owen, 1957) of the dithiol method of Clark & Axley (1955) showed the presence of 30–60 μg molybdenum/l. in cow's milk. In human milk and pig's milk ashed with H_2SO_4 + $HClO_4$ the molybdenum dithiol colour at 680 mμ in the *iso*-amyl acetate extract was no greater than the blank for the reagents used. The failure to secrete xanthine oxidase may be a consequence either of a small intake of molybdenum or of an inability of the mammary epithelium of the non-secreting species to metabolize molybdenum. Comparative xanthine oxidase titres expressed as in Crossland, Owen & Proudfoot (1958) were: cow, 0·08–0·56; goat (fed on bean, oats, dried grass and hay), 0·10–0·35; goat (artificial diet of Crossland *et al.* 1958), 0·16–0·25; goat (dosed with Na_2WO_4, see Crossland *et al.* 1959), 0·003; sheep, 0·1; rabbit, 0·18–0·24; human, pig, horse, nil.

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The effect of tungstate on xanthine oxidase in goat's milk. By A. Crossland, E. C. Owen & R. Proudfoot, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Crossland, Owen & Proudfoot (1958) found that xanthine oxidase which is a compound of protein, flavinadenine dinucleotide, iron and molybdenum (see Underwood,

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1956) is present in the milk of goats maintained on a diet deficient in riboflavin and free from greenstuff. Dick (1953) showed that both the absorption of molybdate and its excretion in urine are much enhanced when the diet of the sheep contains sulphate. We have found, however, that xanthine oxidase continues to be present in the milk of the goat when molybdate is omitted from the salt mixture of Crossland *et al.* (1958) at the same time as nearly all the sulphate in it is replaced by chloride. While receiving such a diet goats were each dosed with 1 g sodium tungstate ($Na_2WO_4.2H_2O$) which, presumably by competition with molybdate, inhibits the formation of xanthine oxidase in both the rat and the chick (Higgins, Richert & Westerfeld, 1956). Milk from the goats then showed much reduced titres of xanthine oxidase. In these experiments there was evidence that doses of riboflavin (100 mg), of sulphate (6 g K_2SO_4 + 6 g Na_2SO_4) or of sodium molybdate (0·4 g) caused increases of xanthine oxidase in the milk. Since no harmful effects were observed from the Na_2WO_4, goats were later given much larger doses when they were eating beans, oats, dried grass and straw. One of these goats received 6 g $Na_2WO_4.2H_2O$ on 6 January, 5 g on 4 March and another 5 g 10 days later. After each dose the xanthine oxidase in the milk was depressed. In three other goats on the same diet similar depressions of xanthine oxidase in the milk were produced by doses of 5 or 6 g $Na_2WO_4.2H_2O$. The riboflavin content of the milk was also measured but showed no change as a result of treatment with tungstate.

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Growth and reproduction of mice fed on wheat grown under different systems of soil management. By J. P. GREAVES and PATRICIA P. SCOTT, *Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.*1

To compare the nutritive value of tenth generation wheat (Atle variety; 1957 crop) from the three sections of the Soil Association's experimental farm at Haughley (described by Milton, 1956) samples have been fed without supplements to T.O. mice. Mineral and vitamin analyses have been made by Dr R. F. Milton. The three sections are farmed using different systems of management, and have received continuously, for over 10 years, the following fertilizer treatments: artificials only on the Stockless section; muck and artificials on the Mixed section; compost only on the Organic section.

Three experiments have been carried out, each using seventy-five male and seventy-five female mice, 21 days old. The mice were divided into equal groups and each fed one of the wheats, the source of which was not known till the conclusion of the experiment. In the first experiment two groups were fed diet 41.B as controls.

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Although growth on the wheat alone was poor, weight increments for individual mice were calculated for consecutive fortnights and averaged for each group: tests of significance were applied between the groups from which it was possible during the 1st or 2nd fortnight to rank the wheats in the following order of merit: 'Stockless' > 'Organic' > 'Mixed'.

Mice of the middle-weight range were mated at 10 and 7 weeks of age on the monogamous pair system and breeding was continued without supplements for 14 weeks, as shown below.

	Breeding performance of mice during 14 weeks on: Wheat from section			
	Stockless	Organic	Mixed	Diet 41 B
Total no. breeding pairs	17	17	17	6
Total no. live born	37	39	28	81
Total no. actually weaned (at 21 days)	28	22	10	71
Maximum no. weaned*	31	31	13	71

* Includes mice under 21 days old at conclusion of experiment.

At post-mortem it was observed that although many females on the wheat were pregnant, many conceptuses were being resorbed.

The results of this pilot experiment suggest that the 'Mixed' wheat was nutritionally the least satisfactory, but the differences obtained are not taken to imply respective merits of the different farming systems involved; they do however show, in contrast to Miller & Dema (1958), that it is possible to distinguish similar wheat varieties grown under different conditions, and indicate that the work might usefully be extended to a full-scale experiment.

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Leaf protein in rations of growing pigs. By R. S. Barber, R. Braude and K. G. Mitchell, *National Institute for Research in Dairying, Shinfield, Reading*

In a preliminary test leaf protein, produced by Dr N. W. Pirie of Rothamsted, was compared with white fish meal as a protein supplement in rations for growing pigs. The methods of production of the leaf protein used, which was of mixed origin (barley, grass, oats, wheat, rye, kale), have been described by Pirie (1957*a*,*b*). The material was kept at $-20°$ as a pressed cake containing 50–60% water and was thawed out at room temperature just before each batch of diet was made up (at 3–4 week intervals). It contained 10–11% nitrogen. The remainder was mainly lipids, including chlorophyll and starch; ash and fibre totalled only about 5%. The comparison was made at both what is currently considered an adequate level of protein supplementation and at a subnormal level. The control ration consisted of cereals and a vitamins A and D supplement plus either 7 or $3\frac{1}{2}$% white

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fish meal. In the experimental rations the fish meal was replaced by leaf protein, on a protein-content basis, and limestone and salt were added. The pigs were fed individually according to live weight and a scale which was adjusted for the pigs fed the leaf protein to allow for the additional moisture content of the ration.

Eight groups of four, 10–11 week old litter-mate pigs were allocated at random to the four experimental treatments. There was only sufficient leaf protein available to carry four pigs on each treatment to bacon weight, the remaining four being on test for 9 weeks only. Results in the table are for eight replicates all of which were on test for 9 weeks. No significant differences were recorded between the fish-meal and leaf-protein rations at the two levels of supplementation tested. Differences between the two protein levels were significant.

Mean growth rate and efficiency of feed utilization of pigs receiving a protein supplement of either leaf protein or white fish meal
(Experimental period, 9 weeks)

	Normal level of protein		Suboptimal level of protein		
	White fish meal	Leaf protein	White fish meal	Leaf protein	S.E.
No. of pigs	8	8	7*	8	
Initial live weight (lb.)	49·7	47·9	47·3	48·1	
Final live weight (lb.)	119·9	122·5	104·6	109·4	
Daily gain (lb.)	1·11	1·19	0·92	0·97	0·0353
Efficiency of feed utilization (lb./lb. gain)	2·91	2·72	3·38	3·26	0·0977

* One pig had to be withdrawn for reasons unconnected with the test. Adjustments were applied using the missing-plot technique.

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The effect of a low-protein diet on the urinary excretion of urea. By G. R. WADSWORTH, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.*1, and T. S. LEE (introduced by B. S. PLATT), *Department of Physiology, University of Malaya, Singapore*

Platt & Heard (1958) drew attention to the possible value of measuring the proportion of total nitrogen excreted as urea as an index of protein malnutrition in children. The present study was undertaken to find out the effect of a sudden diminution of protein intake in two normal adult males.

A diet consisting mainly of cassava, and estimated to give an intake of less than 10 g/protein/day was consumed for 4 whole days. The urea and total nitrogen were estimated on early morning urine samples, and also on 24 h samples. It was found that there was a steady fall in the proportion of nitrogen excreted as urea with a return to normal on the 2nd day of resuming the usual diet.

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The effect of diuresis both in association with a normal and low-protein diet was also investigated. Results suggested that sometimes the ratio could fall to very low values, and on other occasions could increase.

The investigation showed that proportionate urea excretion may be decreased in normal subjects under certain conditions, and this must be taken into consideration in the interpretation of this test.

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The uptake of [^{35}S] cystine by the nail of the rat. By K. O. Godwin, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, London, N.W.7*

Bern, Harkness & Blair (1955), in a study of 'hard' and 'soft' keratin formation, found that isotope, administered as [^{35}S] cystine, was concentrated in the keratogenous zone of the hair follicle; uptake was negligible from either, a protein hydrolysate labelled with [^{14}C], or [^{35}P] phosphate. During the course of work on nail growth, it was noticed, independently, when [^{35}S] cystine was administered to rats and mice the isotope became rapidly and markedly localized in the keratinized part of the nail.

Forty-four animals (twenty-six mice and eighteen young rats) were given intraperitoneal injections of carrier-free [^{35}S] cystine (approximately 100 μc/100 g bodyweight). Presumably it is then reduced to cysteine. Animals were sacrificed at $\frac{1}{2}$, 1, 2, 4, 8 and 24 h after injection. Claws were taken from each animal, fixed in Heidenhain Susa for a few days (to allow decalcification of small particles of bone) and double-embedded in celloidin and paraffin wax. Autoradiographs were made using the stripping-film technique.

The sections show rapid uptake of the isotope by the mature nail. The following features were observed: (a) uptake after 30 min is as great as in any of the samples taken later; (b) activity is present, distal to the nail matrix, above the nail bed, and as far forward as the tip of the nail; (c) after 24 h there is a diminution of activity.

The mechanism of keratinization is still not understood. Two views are usually offered; that it is either (a) 'specific synthesis of fibrous protein', or (b) 'degradation of normal protoplasmic proteins' (Bern *et al.* 1955). Giroud & Leblond (1951) have postulated, without supporting evidence, the incorporation of newly entering sulphur compounds into hard keratin; they explain the increase of sulphur content of tissues as concentration by removal of such constituents as lipid and glycogen.

These observations on cysteine uptake by the nail may be explained by (a) a concentration of the isotope by the nail; (b) exchange, indicating the existence of a dynamic equilibrium, or (c) new synthesis of keratin. If either (b) or (c) obtain, the current concept of keratin as a 'dead' material would have to be revised.

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The equilibration of water and of serum albumin labelled with tritium, in the body fluids of pigs maintained on normal, low-protein and low-protein plus carbohydrate diets. By P. R. PAYNE and J. DONE, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, London, N.W.7*

Tritium-labelled pig serum albumin was prepared by injecting 5 mc DL-[^{3}H]-leucine into a 6-day-old piglet weighing 2·4 kg. After 20 h the animal was anaesthetized and killed by bleeding from the neck veins. Samples of serum (1 ml.) were fractionated by electrophoresis on paper (Done & Payne, 1956), and the albumin, about 0·25 μc in 11·0 mg, injected into recipient animals. Albumin concentration and radioactivity per ml. serum were determined at intervals up to 15 days. Tritium was assayed by the procedures described (Payne & Done, 1958). The radioactivity of the albumin in some 1 ml. samples was found to be at least 95% of that in the total serum.

The size of the intravascular albumin pool was calculated from the dilution of activity 5 min after injection. Total exchangeable albumin was calculated from the dilution after equilibrium appeared to be established, a correction being made for katabolic loss during this period.

The equilibration of water was studied after doses of THO had been given by stomach tube and by intravenous and intraperitoneal injection. Equilibration after oral administration was markedly slower in animals fed on low-protein than those on normal diet. Total body water was calculated from the dilution after equilibrium had been reached.

Pig no.	Body-weight (kg)	Serum-albumin concentration (g/100 ml.)	Intravascular albumin (P) (g)	Extravascular albumin (L) (g)	$\frac{L}{P}$	Total body water (%)
80	6·0–10·4	2·1–2·7	11·5	25·5	2·2	62
83	6·0	1·5	5·6	4·7	0·8	68
75	7·6	1·1	5·4	3·7	0·7	80

Pig T. 80: labelled albumin given at 30 days; normal animal, fed on a commercial pig feed. Pig T. 83: labelled albumin at 65 days; a litter-mate of T.80, had been fed a low-protein diet (LP) (Heard, Platt & Stewart, 1958). Pig T. 75: labelled albumin at 172 days; had been fed a low-protein diet with carbohydrate supplement (LP + CH) (Heard *et al.* 1958) till 156 days. This pig showed signs of oedema.

The table shows striking differences between the animal on the control diet and those on LP and LP + CH diets. There are also notable differences between the

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animal on LP and that on LP + CH. From other data (Heard, Platt & Stewart, 1958; Platt, 1958) these differences are probably due to the extra carbohydrate in the ration and not the difference in the length of time on the diets.

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The net dietary-protein value (N.D-p.V.) of mixtures of foods—its definition, determination and application. By B. S. PLATT and D. S. MILLER, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, London, N.W.7*

The factors involved in the evaluation of protein for human nutrition have recently been examined by Platt & Miller (1958), who drew attention to the scarcity of measurements of the quantity and quality of protein in mixtures—dishes, meals and dietaries as ordinarily eaten by man. There is no appropriate way of assaying or of expressing the nutritional value for man of protein in these mixtures. Assays on rats, however, are easily made on mixtures prepared as for human consumption if the material is freeze-dried.

Miller & Bender (1955) have developed a technique for determining net protein utilization (N.P.U.); assays are made under standardized conditions with respect to level of protein fed and the amounts of fat, minerals and vitamins in the diet; we call this N.P.U. (standardized). Using this technique, but feeding the freeze-dried mixtures, i.e. dishes, meals or diets without modification, a value for N.P.U. (operative) is determined. N.P.U.(operative) = biological value × digestibility and is a measure of protein quality. N.P.U. (operative) × (nitrogen in the mixture assayed × 6·25) is called net dietary-protein value (N.D-p.V.). This term represents the utilizable protein in the mixture and is a function of both quality and quantity; it may be translated into French as *valeur protéique nette de la ration* (V.P.N.R.).

Mitchell (1922) proposed a term net protein value (N.P.V.—in French V.P.N.), which is obtained by multiplying N.P.U. (standardized) by the crude protein content; this is a useful basis for comparing materials rich in protein. The supplementary effect of protein concentrates on the N.D.V. of food mixtures can and should be determined experimentally.

FAO (1957*a*) express protein requirements in terms of 'reference protein' which theoretically should contain 16% nitrogen and be completely utilized; protein values of diets are calculated in terms of reference protein by 'scoring' their amino-acid composition against a provisional pattern of amino-acid requirements for man. There are, in the present state of knowledge, certain advantages in the method we use over that proposed by the FAO committee.

The 'safe practical allowance' (F.A.O. 1957*a*) can be expressed in terms of N.D-p.V. as follows (figures in parentheses—protein calories % total calories (approxi-

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mately protein per cent by weight of mixture, FAO, 1957*b*)): for an adult 34 g N.D-p.v./day (4·6%), a child (8 years) 29 g (5·9%), a toddler (2 years) 24 g (7·8%), an adolescent (14 years) 61 g (8·4%), and for a lactating mother 76 g (9·5%).

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The estimation of net dietary-protein value (N.D-p.v.) of meals and diets from the total sulphur content. By D. S. MILLER and D. J. NAISMITH, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, London, N.W.7*

Many attempts have been made to interpret the nutritive value of proteins in terms of their content of specific essential amino acids. Of the chemical methods currently employed, the most promising is the measurement of 'available lysine' in protein concentrates used in animal feeding (Carpenter & Ellinger, 1955), but from inspection of the composition of human diets, containing proteins from many sources, the sulphur-containing amino acids, methionine and cysteine together, rather than lysine, appear to be limiting. This view is supported by experimental evidence (Miller & Dema, 1958); the biological values of 70% of the human diets tested were shown to be improved by supplementation with methionine.

The relationship of N.D-p.v. (Platt & Miller, 1959) to content of S-containing amino acids, as measured by total S determination, has been investigated by Miller & Naismith (1958). Biological measurements on sixteen freeze-dried human diets from various parts of the world were made by the method of Miller & Bender (1955). Total S was determined by combustion of the material in a Berthelot–Mahler bomb, precipitation of the sulphuric acid, so formed, with benzidene hydrochloride (Young, Edson & McCarter, 1949), and titration of the precipitate with 0·01 N-NaOH. A highly significant correlation between N.D-p.v. and total S content was found ($r = 0{\cdot}967$; $P < 0{\cdot}001$). The regression equation was N.D-p.v. $= 0{\cdot}0521$ S $- 1{\cdot}06$.

In order to test the validity of this equation, the S content and N.D-p.v. of an additional twelve diets were determined experimentally. The values for N.D-p.v. were then compared with values calculated from the regression equation. None differed by more than 1·0 N.D-p.v. units; ten differed by less than 0·6, and six by less than 0·3 units.

On the other hand, the correlation found between total S content and net protein value of protein concentrates, although statistically significant was of little practical value. S content, however, may be of use in predicting the value of a concentrate as a dietary supplement. For example, the addition of 10% sesame flour (S content 730 mg %) to a Nyasaland cassava diet (S content 87 mg %) raised its N.D-p.v. from 3·5 to 6·3; the figure calculated from the regression equation was 6·5.

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A ballistic bomb calorimeter for the rapid determination of some nutritive values of foodstuffs. By H. C. Fox, D. S. Miller and P. R. Payne, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, London, N.W.7*

A method has been developed for the rapid determination of caloric values of foods. A measure of the heat of combustion is obtained by recording the maximum deflection of a galvanometer connected to a thermocouple affixed to the lid of a Berthelot–Mahler bomb.

Féry (1912) has described a method based on the measurement of the equilibrium temperature of the bomb casing by means of a thermocouple; the present method differs from this in that no attempt is made to achieve temperature equilibrium throughout the material of the bomb. However, advantage is taken of the fact that on combustion of a sample most of the heat is transmitted to the lid and upper parts of the bomb casing, which rapidly rise to a high temperature and as rapidly fall. The galvanometer reading reaches the peak within 55 sec of firing.

The relationship between peak galvanometer readings and energy released on combustion of known weights of benzoic acid is linear over the range 4–10 Cal.; and the reproducibility is good (S.E. = 0·01 Cal.).

A calorie balance was carried out with the calorimeter. Eight weanling rats (litter-mates) were divided into two groups of equal weight. One group was killed, the four carcasses dried at 100° and ground together. Five samples (8 g in all) were combusted and the total energy content of the pooled carcasses calculated (Rat Bodies I). After feeding for 7 days on a Jamaican diet the remaining group were treated in the same way (Rat Bodies II). The caloric value of food eaten, faeces, urinary solids and brushings was determined. The difference between Rat Bodies II

Calorie balance

Dry weight (g)		Calories (Cal.) In	Calories (Cal.) Out	Energy values (Cal.)
133·0	Diet (Jamaican)	612·3		Gross = 612·3
11·8	Faeces		62·9	Digestible = 549·4 (612·3 − 62·9)
5·6	Urinary solids		17·3	Metabolizable* = 530·4 (549·4 − 17·3 − 1·7)
3·0	Brushings		1·7	
	Gain { Rat bodies II–I 369·8–335·9		33·9	Stored = 33·9
	Heat emitted		496·5	
	Total	612·3	612·3	

* Metabolizable energy/g food = 3·98 Cal.

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and I represents the stored energy. In classical procedures the heat stored is obtained by difference; here it is heat emission which is ascertained by difference.

By the use of this method measurements of energy, calcium and sulphur content of foods and of mixed diets can be made simultaneously.

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Pancreatic islet cells and blood-sugar regulation in pigs maintained on low-protein diets. By R. J. C. STEWART and C. R. C. HEARD, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, London, N.W.7*

In pigs fed on low-protein diets the individual cells of the pancreatic islets are reduced in size (Heard, Platt & Stewart, 1958; Platt, 1958). These changes led us to test the animals' ability to regulate blood-sugar levels. Pigs were fed on normal (N), low-protein (LP), and low-protein plus carbohydrate (LP + CH) diets as before. Others received a diet (CLP) in which casein (5% w/w) replaced an equal weight of starch in the LP diet. LP and CLP were fed at isocaloric levels. The animals were fasted for 18 h before each test. Glucose was measured colorimetrically (Asatoor & King, 1954).

Decreasing the protein and increasing the carbohydrate content of the diet had the following effects: (a) a tendency towards slightly higher blood-sugar levels (total range 40–140 mg/100 ml.) but with neither glucosuria nor ketosis; (b) increased liver glycogen; (c) increased tolerance to oral glucose (0·8 g/kg) and starchy food (4 g LP/kg); (d) increased sensitivity to insulin (de Bodo & Altszuler, 1958).

Insulin (0·1 i.u./kg) rapidly produced hypoglycaemia ($\leqslant$20 mg/100 ml.) in all pigs. This was corrected in normal animals within 1 h, but in protein-deficient pigs only after 2 h. When oral glucose was administered 30 min after insulin, the blood-sugar levels were usually restored at near normal rates. The protein-deficient pigs can convert excess blood glucose to liver glycogen, but are unable to release the glucose again at normal rates when subjected to insulin hypoglycaemia.

Blood-sugar and tissue-glycogen levels are regulated by a number of hormones and enzymes. In protein malnutrition production of all hormones is likely to be modified but not necessarily to equal extents. This was shown in our pigs by unequal changes in the α and β cells of the pancreas. The β cells appear to be relatively inactive, while the proportion of α cells is increased.

Diet	N	CLP	LP	LP + CH
Islet cell sizes as percentage of normal cell size	100	88	65	59
No. of α cells (phloxin stained) as percentage of total no. of cells	25	26	38	32
No. of β cells (aldehyde fuchsin stained) as percentage of total no. of cells	71	68	58	59

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All the phenomena reported here support the concept of a precariously balanced endocrine system resulting from prolonged deficiency of dietary protein. This system is easily disturbed and slow to recover as demonstrated by sensitivity to insulin.

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Protein metabolism in the rat with malaria (*Plasmodium berghei*). By I. S. Dema, D. S. Miller and B. S. Platt, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, Mill Hill, London, N.W.7*

Several times in recent years the possibility that zymotic disease contributes to malnutrition of the host has been envisaged (see for example Platt, 1948-9, 1949-50, 1957). The results of work reported in this communication show that infection of rats with *Plasmodium berghei* produces some changes similar to, but more marked than those encountered in protein malnutrition.

Three groups each of eighteen weanling rats (Mill Hill black-hooded strain) aged 30 days (a) malaria infected, (b) pair-fed and (c) fed *ad lib.*—all on diet 41 (Bruce & Parkes, 1949)—were studied for 13 days. After 1 week the infected animals failed to gain weight; those in group (b) continued to gain weight though not so much as those in group (c). Animals in group (a) showed a low efficiency of utilization of food, reduced apparent digestibility of nitrogen and markedly reduced intestinal proteolytic activity. The infected animals also had a low urea N : total urinary N; a statistically significant (1% level) increase in total body water; a low haematocrit value; a greatly reduced haemoglobin concentration, a marked increase in erythrocyte sedimentation rate and reduction in total serum proteins. The spleens and livers of the infected animals were heavier than those of groups (b) and (c); the livers of rats in (a) contained about twice the concentration of fat found in groups (b) and (c). The amounts of N in the livers of the malarious rats tended to be higher, but the N of the carcass + liver was lower than for rats (b) and (c).

The malarious rats showed more profound changes in protein nutrition than found in the pair-fed controls, i.e. the changes in the former cannot be accounted for by reduced intake of food consequent on anorexia and it must be concluded that zymotic factors in disease contribute in other ways to the development of malnutrition in the host subject, e.g. by causing 'toxic destruction of protein'.

We are grateful to Dr J. D. Fulton, Chemotherapy Division, National Institute for Medical Research, Mill Hill, for a culture of *Plasmodium berghei*, and for advice on the technique of experimental malarial infections.

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Repeat individual weighed dietary surveys. By JEAN W. MARR, J. A. HEADY and J. N. MORRIS, *Social Medicine Research Unit of the Medical Research Council, London Hospital, London, E.1*

The study being reported is part of an investigation of the health of middle-aged men. We are trying to obtain reliable, individual weighed dietary information to be used as a yardstick, or 'gold standard', for the validation of simpler methods of assessing individual diets that can be applied in large-scale epidemiological surveys.

Individual weighed surveys for 7 consecutive days were carried out in Brighton on bank officers of three of the 'big five'. All the men taking part were aged 40–55 inclusive. Ninety-nine men were eligible for the survey. Of these eighty-four co-operated; but the records of seven were inadequate, leaving seventy-seven reliable individual weighed surveys. In all cases the men were asked to describe carefully food eaten away from home and unweighed.

In order to test the reliability of the standard itself, men in one of the banks were asked to co-operate a second time. All the twenty-six men (of the twenty-nine total eligible) who carried out the first survey in this bank repeated it for another week; and all but one produced a reliable book again.

The first surveys were carried out between January and September 1957, and the second were arranged to take place as nearly as possible 6 months later to test also for seasonal variations. The results suggest that there is considerable stability in the nutritional habits of these men, though differences were of course found in the actual foods eaten by individuals in their two surveys. For example, much more soup and porridge were taken in December, January and February, more packet cereals and salads by the same individuals during June, July and August.

Twelve % of the main meals in each survey were eaten away from home. The nature of these meals, and their distribution among the men, were very similar in the two surveys.

Twenty-five individual repeat weeks weighed surveys
(Bank officers, males, 40–55 years inclusive)

	1957-8		
	First survey	Second survey	Correlation coefficient
	Average daily consumption		
Calories (Cal.)	2769	2819	+ 0·84
Protein (g)	82	83	+ 0·72
Carbohydrate (g)	326	338	+ 0·82
Fat: total (g)	127	126	+ 0·85
animal (g)	93	92	+ 0·80
marine (g)	6	7	+ 0·81
vegetable (g)	28	27	+ 0·87
Calories from fat (%)	41	40	+ 0·83

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The rate of secretion of mixed saliva in the cow. By C. B. BAILEY* (introduced by C. C. BALCH), *National Institute for Research in Dairying, Shinfield, Reading*

In four dry, fistulated Shorthorn cows the rate of secretion of saliva during eating was calculated from the rate of eating and the difference in the mean moisture content of the offered food and of the swallowed food sampled at the cardia. More fibrous foods were eaten slower than less fibrous foods and stimulated a larger flow of saliva per unit weight of food consumed. This resulted in mean rates of saliva secretion which were similar for all foods (Table 1).

Table 1. *Saliva production during eating*

Diet	Saliva		Eating rate
	g/g food	ml./min	(g food/min)
Dairy cubes	0·68	243	357
Fresh grass	0·94	266	283
Silage	1·13	280	248
Dried grass	3·25	270	83
Hay	3·63	254	70

In cows which were resting (neither eating nor ruminating) the rate of secretion of saliva has been measured with a variety of rations at four different times following feeding. Saliva was collected in a small rubber bag held over the cardial orifice. In all experiments the rate of secretion was lowest shortly after eating, highest shortly before eating and intermediate during the times between. On any one ration large differences in mean rates of secretion were noted between animals, but within given animals the rates of secretion were always consistent relative to different rations. The differences in secretory rates between rations were not obviously related to the pH or the volatile fatty-acid, ammonia and inorganic-ion composition of the rumen fluid.

When the daily ration of hay of three cows was stuffed into the rumen through the fistula, the rate of secretion was notably higher 1 h afterwards, relative to subsequent rates, than after normal feeding in the same animals. Neither the digestibility of the hay nor the daily pattern of change of pH or ammonia and volatile fatty-acid levels in their rumen contents was altered. The act of eating, therefore, appears to be a major determinant of the daily secretory pattern.

There have been no measurements of the secretion of mixed saliva during rumination.

Daily rates of saliva production in three animals calculated from the secretion rates found during eating and those found during the non-eating period (exclusive of possible increments due to rumination) would amount to 74 l., 82 l. and 96 l. with hay and 90 l., 108 l. and 110 l. with grass, respectively.

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DEMONSTRATIONS

A simple graphing method for assessing child growth. By M. W. GRANT, *Applied Nutrition Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1*

When following the growth and development of individual children, it is desirable to have a method of charting which will show at a glance three important attributes: (1) the developmental level (height at any given age compared with the average for that age), (2) physique type (whether fat or thin, and the degree of fatness or thinness), (3) the rate of growth.

The method used by the Applied Nutrition Unit (Wigley, 1952) makes it possible for the assessor to obtain an immediate mental picture of the child whose measurements have been plotted and deviations from the expected line of development are clearly shown.

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Staining of marrow iron. By 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*

Although the demonstration of iron in the bone marrow (Rath & Finch, 1948) is now well known, it is apparent that in clinical practice and nutrition studies of iron-deficiency anaemia insufficient attention is given to this test.

Most studies in the tropics show that anaemias are predominantly hypochromic. Such cases, however, cannot always be assumed to be due to iron deficiency. In a small series of cases examined recently in Singapore, eight out of twenty-one hypochromic anaemias had demonstrable iron in the bone marrow.

With the extensive use of oral and parenteral iron therapeutically (Davidson & Richmond, 1958), the possibility of causing accumulation in the tissues must exist: this may not be without harmful effects (Golberg & Smith, 1958).

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Hepatic lipids in kwashiorkor and infantile marasmus. By I. MACDONALD*, *Physiology Department, University of the Witwatersrand, Johannesburg*

In view of the increase in hepatic lipid seen in kwashiorkor and infantile marasmus, and as the diet of the children developing these conditions tends to be low in fat as well as protein, the liver fat of fourteen Bantu children aged 1–4 years inclusive who had died of these types of malnutrition was examined.

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The hepatic lipid and its phospholipid fraction, non-saponifiable portion and fatty acids were determined as previously described (Macdonald, 1958) and expressed as a percentage of the dry weight of the liver. The degree of unsaturation of the lipids expressed as the iodine number, was also determined. The results can be seen in the table.

Hepatic lipids in kwashiorkor and infantile marasmus

Total lipid (g/100 g)	9·0	11·2	13·7	14·9	35·6	40·2	42·4	54·9	55·7	55·9	67·9	71·0	73·8	78·3
Iodine number of total lipid	107	97	76	63	72	64	47	63	49	57	47	54	59	49
Phospholipid fraction (g/100 g dry liver)	3·1	3·6	6·8	5·1	2·7	5·5	1·7	2·5	9·5	3·2	9·8	2·4	3·0	2·3
Non-saponifiable fraction (g/100 g dry liver)	1·2	0·9	1·8	3·5	0·5	0·4	0·5	—	1·8	0·3	0·1	0·4	0·2	1·1
Fatty acids (g/100 g dry liver)	4·7	6·6	5·2	6·3	32·3	34·3	40·2	—	44·4	52·4	58·0	68·3	70·5	74·9
Clinical diagnosis	K	K	M	K	K	M	K	K	K	K	K	M	M	K

K, kwashiorkor; M, marasmus.

The table shows that the increase in hepatic lipid is not associated with an increase in the phospholipid or non-saponifiable portions, but with an increase in fatty acids which tended to become more saturated. This latter finding is probably not due to the accumulation of unaltered depot fat in the liver as the degrees of saturation of the hepatic fatty acids were in most cases greater than could be accounted for by simple accumulation of depot fat in the liver.

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