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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Forty-sixth Meeting of The Nutrition Society was held at the Royal Free Hospital School of Medicine, London, W.C.1, on Saturday, 2 December 1961, at 10.30 a.m., when the following papers were read:

Fractionation of human faecal lipids. By P. D. S. Wood and F. Aylward, *Department of Chemistry and Food Technology, Borough Polytechnic, London, S.E.1*

Cook, Edwards & Riddell (1956) have examined the unsaponifiable and acidic fractions of human faecal lipids, but little work has been reported on the separation of fractions from unsaponified faecal lipids.

We have devised techniques for the separation of human faecal lipids, the three main steps being (a) extraction of stool homogenate with ethanol-ether and partition of the extract between 15% ethanol in water and light petroleum (b) fractionation of the 15% ethanol extract, and (c) fractionation of the light petroleum extract by ion exchange and silicic acid chromatography. The components separated include soaps and fatty acids, mono-, di- and tri-glycerides, phospholipids, sterols and sterol esters, ester waxes, fatty alcohols, hydrocarbons and bile acids.

Quantitatively, free fatty acids and soaps are the most important components. Steroids are found in a number of fractions: bile acids; free sterols (represented by coprostanol, cholesterol and sterols of similar polarity, as well as by significant amounts of more polar sterols); and variable amounts of sterol esters (Schön, 1959; Aylward & Wills, 1961). All of these classes are of potential importance in studies of cholesterol metabolism.

The triglycerides present in faeces from a normal subject are quite highly unsaturated in contrast to the relatively saturated free fatty acids; this suggests that the triglycerides are partly of metabolic origin. Mono- and di-glycerides were also found in significant quantities. Other esters present include phospholipids and long-chain waxes, the latter occurring in the sterol ester region on silicic acid chromatography. Free fatty alcohols are also found, forming part of the triglyceride region. Clearly caution must be exercised in interpreting the results of silicic acid chromatography of faecal lipids.

Surprisingly high amounts of hydrocarbons are often present. These consist in part of straight-chain paraffins, probably of dietary or bacterial origin, but cyclic material is found which appears to be derived from liquid paraffin present in processed foods. The results emphasize the complexity of faecal lipid mixtures; this complexity must be borne in mind in developing routine laboratory methods for nutritional studies on lipids.

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Lipid excretion in the rat. By T. R. E. PILKINGTON, *St. George's Hospital, London, S.W.*1, and F. AYLWARD and P. D. S. WOOD, *Department of Chemistry and Food Technology, Borough Polytechnic, London, S.E.*1

The methods for the fractionation of human faecal lipids outlined by Wood & Aylward (1962) have been applied to rats and have been combined with radiotracer techniques in order to follow the excretion of cholesterol and its degradation products.

Rats were fed on Oxoid diet 41 with soya-bean oil (25% by weight) for 10 days and then injected intraperitoneally with [2-^{14}C]mevalonate. Two days later serum lipoprotein (containing labelled cholesterol and its esters) was isolated (Lewis, Pilkington & Hodd, 1961). This lipoprotein was injected through the tail vein into a second group of rats which had been maintained for 10 days on the Oxoid–oil diet and then for 2 days on a low-fat diet. Faeces were collected following injection.

Our results (based on the first 2 days' collection) showed that the pattern of lipid distribution was similar to that described for human faeces. Quantitative differences were found, notably much lower amounts of soaps and pigments in the rat. Activity was concentrated in the bile acid and sterol fractions, including coprostanol, cholesterol and similar unsaturated sterols, more polar sterols and sterol esters. No significant activity was found in the other fractions.

The specific activity of the sterol isolated from the sterol esters was only one-third that of the major free sterol fractions, suggesting that sterol esters are in part derived (via the bile (see Phillips, 1960) or the intestinal mucosa) from serum cholesterol but that activity from this source has been considerably diluted with dietary sterol esters. The ratio of activity (total bile acid fractions): (total sterol fractions) was 2·4 : 1, indicating the importance of bile acid excretion as the major pathway for the elimination of cholesterol from the rat (cf. Wilson & Siperstein, 1959).

Parallel experiments using rats fed on diets containing butter in place of soya-bean oil have given similar ratios and confirmed the importance of bile acids.

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The distribution of nitrogen in young rats which had lost equal amounts of weight after being fed on diets (a) low in protein and (b) high in protein but limited in calories. By J. W. T. Dickerson and Vera Cabak (British Council Scholar) (introduced by Elsie M. Widdowson), *Medical Research Council Department of Experimental Medicine, University of Cambridge*

Young male rats weighing initially about 90 g were given diets containing (a) a low, and (b) a high protein: calorie ratio (Widdowson & McCance, 1957). One group was killed at the start of the experiment; a second group was fed unlimited amounts of the low-protein diet, and a third was fed the high-protein diet in amounts limited to cause the animals to lose weight at the same rate as those in the low-protein group.

Both groups of animals on the experimental diets lost about 40% of their initial empty live weight, and a quarter of the total weight lost, on a fat-free basis, was accounted for by the loss in weight of the skin. In both groups the loss of non-collagen nitrogen from the skin accounted for 18% of the total amount lost from the body. The amount of collagen lost from the skin accounted for 55% of the total loss in the animals on the low-protein diet, and 77% of the total in those on the high-protein diet.

The quadriceps muscles of the animals in both groups lost over 50% of their initial weight. In both groups the muscles lost about 60% of their sarcoplasmic and fibrillar proteins, and 40% of their non-protein nitrogen. The absolute amount of extracellular protein tended to increase during malnutrition. The ratio intracellular potassium: intracellular protein nitrogen was reduced in the muscles of both groups of malnourished animals, but more severely in those on the high-protein diet.

On the whole the effects of the two diets were similar, and the accompanying undernutrition, rather than the nature of the diet, was the more important factor in bringing about the changes in body composition.

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Electroencephalographic observations during induced hypoglycaemia in young dogs and pigs. By G. Pampiglione, C. J. Friend and C. R. C. Heard, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

The intravenous administration of insulin may be followed in man and many animals by a number of clinical phenomena believed to be due to hypoglycaemia. The electrical activity of the brain as recorded from the scalp gives continuous information of some cerebral events which cannot be studied by direct biochemical means.

Continuous electroencephalograms (EEG) were taken in twelve young pigs and four dogs (2 months old) before, during and after administration of insulin. Blood

sugar estimations were carried out at appropriate intervals. The EEG technique employed has been described (Pampiglione, Platt & Stewart, 1960).

In the young pig a small dose of insulin (0·1 i.u./kg body-weight) was sufficient to induce a fairly marked hypoglycaemia sometimes lasting for over 1 h. The fall in blood sugar to below 20 mg/100 ml was accompanied only by mild changes in the electrical activity of the brain and in that of the heart. The clinical changes were minimal in all cases (either slight restlessness or slight somnolence) in spite of the marked fall in blood sugar. No distress, seizures or coma were seen even when the hypoglycaemia lasted over 1 h.

In the young dogs a prolonged fall in blood sugar could be achieved only by a much larger dose of insulin and a variety of clinical phenomena and of changes in the EEG occurred. After preliminary recording, 10 i.u. of insulin were injected intravenously. Twenty minutes later a further dose of 30 i.u. of insulin was injected. This prevented a significant rise in blood sugar concentration and maintained it at a low level (between 20 and 40 mg/100 ml) for over 1 h. The clinical and electroencephalographic changes following insulin administration did not appear to be closely related either to the blood sugar fall or to the minimal level reached, though the duration of the hypoglycaemia appeared to play a part. Seizures occurred in two dogs over 1 h after the initial blood sugar fall when the blood sugar level was already tending to rise. The clinical recovery was obvious by the next morning, but the alterations in the EEG patterns persisted for a few days.

From our observations, it appears that following insulin administration, the changes in the electrical activity of the brain in dogs and pigs were more closely related to the occurrence of some clinical events than to the actual blood sugar level at any given time. It seems, therefore, probable that the changes in cerebral function may be more variable and complex than would be expected from any simple relationship to the hypoglycaemia.

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Correlation of albumin and vitamin A in human serum. By A. W. WOODRUFF, *London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.*1, and R. J. C. STEWART, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.*7

Vitamin A is present in serum mainly as the alcohol and it is transported as a complex with protein. The protein has been stated by Garbers, Gillman & Peisach (1960) to be α_1-globulin, and Friend, Heard, Platt, Stewart & Turner (1961) demonstrated a correlation between the levels of serum 'albumin' and serum vitamin A. The latter investigation was, however, carried out on pig serum in which, with the technique used, the albumin and α_1-globulin fractions are not separated. The

present work describes tests on human serum in which complete separation is obtained.

Blood samples (fifty-seven) were collected in the Bugoye Region of Western Uganda during an investigation into the relationship between onchocerciasis and ocular lesions. Total proteins were estimated by the Johnston & Gibson (1938) modification of the method of Wu (1922) and the fractions were separated electrophoretically by the method of Flynn & de Mayo (1951). Vitamin A was estimated as described previously (Friend *et al.* 1961) and there was again no attempt to differentiate between the alcohol and ester fractions.

Vitamin A and protein fractions of serum : concentrations and correlations

	Mean value/ 100 ml serum	S.D.	Correlation coefficient (r)	P
Vitamin A	145 i.u.	78·8		
Total protein	7·90 g	0·752	0·0575	$>0·10$
Total globulin	4·89 g	0·732	0·1976	$>0·10$
α_1-globulin	0·26 g	0·108	0·0898	$>0·10$
α_2-globulin	0·53 g	0·216	0·1531	$>0·10$
β-globulin	0·84 g	0·224	0·0810	$>0·10$
γ-globulin	3·24 g	0·768	0·2182	$>0·10$
Albumin	3·04 g	0·475	0·3045	$<0·03$

The only statistically significant correlation (Table 6, Fisher & Yates, 1943) is between the concentration of serum albumin and that of serum vitamin A.

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The thyroid glands of protein-malnourished pigs. By B. S. PLATT and R. J. C. STEWART, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.*7

Pigs were maintained on low-protein (LP), low-protein with additional carbohydrate (LP+CH), low-protein in which 5 or 20 g casein replaced equal weights of starch in each 100 g of diet (5 CLP or 20 CLP) or normal (Nl) diets (Friend, Heard, Platt, Stewart & Turner, 1961).

The changes found in the thyroid glands of the animals fed the low-protein diets included: (a) increase in length and decrease in height of the follicular cells, (b) reduction in the number of Aaron vacuoles and (c) intensified staining of the colloid with eosin.

The microscopical appearance of the thyroid glands of the malnourished pigs was similar to that seen in simple colloid goitre but the size was not increased. There were differences between animals on the same diets and even within the gland of a single animal, but these were small compared with those found between animals on diets with different protein values. The length : height ratios (L : H) of 500 cells of each of four animals of one litter were 1·6, 2·2, 4·1 and 3·8 for animals maintained on the Nl, 5 CLP, LP and LP+CH diets respectively. The thyroid glands of animals which, after a period of protein depletion, were offered food with a higher protein value showed evidence of colloid discharge. The L : H ratio of the cells after 4 weeks of the 20 CLP diet was 1·2 or, after a similar period on unlimited quantities of the Nl diet 0·96.

The results might be considered as secondary to the pituitary changes (Godwin & Platt, 1960) but Aschkenasy (1961) suggests that protein deficiency has a direct effect on the thyroid gland, for whereas thyroxine had approximately equal effects on the metabolism of normal and deficient rats, the response to thyrotropin was lower in the latter. Plotnikova (1959) reported that in rats on a 3·5% protein diet thyroid function (^{131}I uptake) was reduced and the glands showed morphological changes.

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Pathology of acute experimental protein malnutrition in the force-fed rat.

By B. S. PLATT, K. HALDER and B. H. DOELL, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Protein malnutrition, similar to that found in the human was produced in pigs by feeding low-protein, or low-protein plus carbohydrate diets (Platt, 1958*a*; Heard, Platt & Stewart, 1958). Sidransky (1960) produced some kwashiorkor-like changes in rats force-fed diets in which the nitrogen was supplied by either (a) a mixture of amino acids from which one essential amino acid was omitted or (b) plant protein. Forcible feeding of infants with excessive amount of carbohydrate is considered to be a factor in producing protein malnutrition (Platt, 1958*b*).

Rats are known to reduce their food consumption when offered protein-deficient diets. To ensure adequate food intake, rats were fed non-protein diet by stomach tube, at the rate of 1·3 g/10 g body-weight daily—the intake of a complete diet when fed *ad lib*. The animals were killed after 6 days; on examination the livers were found to be heavily infiltrated with fat and the pancreas, salivary glands and testis showed gross damage. The stomach, small intestine, adrenal cortex, anterior pituitary, thymus and spleen showed less severe changes. When the same amounts of diet, in

which carbohydrate was replaced by 2, 5, 10 and 15% casein, were fed, progressively less severe and extensive changes were noted at the 2% and 5% levels until at the 10% and 15% casein levels, no histological abnormalities were seen. Animals offered the non-protein diet *ad lib.*, ate 0·7 g/10 g body-weight daily; neither these nor rats force-fed this amount of diet, showed any histological change.

Each forced-feeding was followed by a period of hyperglycaemia reaching a higher peak and falling more slowly in those fed the non-protein diet than in the group fed a diet containing 15% casein. A number of animals force-fed the non-protein diet died in a hyperglycaemic state. However, in surviving animals of all groups fasting blood sugar levels were within the normal range. With decreasing intake of casein plasma proteins progressively fell while fasting blood lactic acid and liver lipid concentration increased.

Histological and some biochemical changes, typical of protein malnutrition can be rapidly produced in rats by force-feeding. The degree of these changes is correlated with the net dietary-protein values.

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The influence of a low-protein diet on the level of zinc and iron in the serum and liver of the pig. By B. S. PLATT and W. FRANKUL, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

The zinc contents of the whole blood, the finger nails and the skin of Chinese suffering from protein deficiency and subacute and chronic beriberi were reduced to half the normal value (Eggleton, 1938, 1940). About one-third of the zinc in the serum is firmly bound to globulin and the remainder loosely bound to albumin (Vikbladh, 1951). However, the effect of a low-protein diet on the level of zinc and iron in the serum of any experimental animal has not been investigated hitherto.

Nine pigs were maintained on diets similar to those described by Friend, Heard, Platt, Stewart & Turner (1961); two had the 20 CLP diet, three the LP, two the LP+CH, and the remaining two had the LP and LP+CH diets respectively for 13 weeks followed by the 20 CLP for 7 weeks. All the animals received isocaloric quantities of the basal diet, except those on the LP+CH which received additional calories as carbohydrate. The intakes of minerals were the same for all the animals of 7 mg zinc and 75 mg iron daily. All the tissues were ashed at 500° before estimation of zinc by the dithizone method (Vallee & Gibson, 1948) and iron by $\alpha\alpha$-dipyridyl (Ramsay, 1954). The DNA phosphorus was determined according to Schmidt & Thannhauser (1945). The results obtained are shown in the table.

Diet	No. of pigs	Mean content/100 ml serum: Zinc (μg)	Iron (μg)	Total protein (g)	Mean ratio to DNA phosphorus in the liver: Zinc	Iron
20 CLP	2	105	163	8·4	0·28	0·78
LP	3	36	140	4·66	0·10	0·61
LP+CH	2	50	143	3·63	0·16	0·70
LP→20 CLP	1	70	155	6·59	0·25	0·35
LP+CH→20 CLP	1	108	185	6·01	0·20	0·39

The zinc and total protein content of the serum and the zinc : DNA P in the liver of the animals on LP and LP+CH are low. No corresponding changes are found for iron. However, the iron: DNA P falls in the liver of the animals on LP and LP+CH during recovery on the 20 CLP diet.

It is suggested (1) that protein malnutrition might induce a secondary zinc deficiency, possibly due (a) to lack of a transport medium such as serum albumin, which is markedly reduced in pigs on the LP and LP+CH diets (Heard, Platt & Stewart, 1958), or (b) to impaired absorption, or (c) to both factors; (2) that the anaemia in these animals is due to lack of protein rather than to lack of iron.

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The importance of dietary fat for the utilization of protein in the very young. By D. J. NAISMITH, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Dietary fat is important not only for the provision of calories, but also for the efficient utilization of calories: a deficiency of the essential fatty acids (EFA) has been shown to impair the metabolism of energy in young rats (Wesson & Burr, 1931) and in human infants (Adam, Hansen & Wiese, 1958). Any increase or reduction in available energy leads to a corresponding change in nitrogen balance (Munro & Naismith, 1953); EFA deficiency might therefore be expected to impair the utilization of nitrogen, i.e. of protein.

Into a basal diet containing fat-free casein (18%), cholesterol (1%), sucrose, vitamins, minerals and cellulose powder, either hydrogenated coconut oil (1%) or sunflower-seed oil (7%) was introduced at the expense of an isocaloric amount of sucrose. The diets were fed to weanling albino rats, paired with respect to weight, sex and litter, in such a way that, at the end of 5 weeks, body-weights within each

pair were identical. The group fed hydrogenated coconut oil showed characteristic but not severe signs of EFA deficiency, and were consuming approximately 40% more food than their controls. Food intakes were then equalized on a caloric basis, and nitrogen balances measured over a 5-day period.

Mean gains in body-weight and nitrogen balances, of groups of twenty-four rats fed diets containing or lacking the essential fatty acids

Nature of dietary fat	Gain in body-weight (g)	Nitrogen intake (mg)	Urine nitrogen (mg)	Faecal nitrogen (mg)	Nitrogen balance (mg)
Sunflower-seed oil	21	1581	1034	100	+447
Hydrogenated coconut oil	8	1583	1186	114	+283

The table shows that exclusion of EFA from the diet produced a marked change in protein utilization. The increases in urinary and faecal nitrogen excretion (14·6% and 14·2%) and the decrease in nitrogen retention (36·6%) were all statistically highly significant ($P<0.001$). No difference was found in the digestibility of energy-containing components by the two groups. In an experiment conducted under identical conditions, the presence of cholesterol in the diet was shown to have no effect, *per se*, on protein utilization.

EFA deficiency is readily produced in human infants (Hansen, Haggard, Boelsche, Adam & Wiese, 1958). It is suggested that these observations may have considerable significance in relation to the nutrition of infants in communities where staple foodstuffs frequently contain negligible amounts of fat.

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A technique for the estimation of the ruminal production of volatile fatty acids (VFA) in the cow. By I. H. Bath, C. C. Balch and J. A. F. Rook, *National Institute for Research in Dairying, Shinfield, Reading*

Two dry cows, fitted with permanent rumen fistulas received daily 15 lb hay dry matter in two equal feeds at 12 h intervals. Water (100 lb/day) was infused continuously into the rumen and, after a control period of 2 weeks, additions to the infused water of acetic acid, propionic acid or butyric acid were made in successive periods, at a rate of 500 ml/day for 2 weeks and then at 1000 ml/day for a further week. Samples of rumen liquor were taken on the final day of each treatment, at approximately hourly intervals for 12 h. The mean values of pH, total VFA and the molar proportions of the individual VFA for both cows are given in the table.

Treatment		Ruminal pH A	Ruminal pH B	Total VFA (m-equiv./100 ml rumen liquor) A	Total VFA (m-equiv./100 ml rumen liquor) B	Individual acids in rumen liquor, molar percentages of total VFA: Acetic acid A	Acetic acid B	Propionic acid A	Propionic acid B	Butyric acid A	Butyric acid B	Valeric acid A	Valeric acid B
Water		6·39	6·59	8·17	8·19	67·7	65·9	19·1	19·9	11·0	12·2	2·2	2·0
Acetic acid:	500 ml	6·23	6·36	9·85	9·56	72·5	71·5	15·2	16·8	9·5	9·2	2·8	2·4
	1000 ml	5·86	6·50	11·1	9·02	79·1	75·4	12·1	14·4	7·2	8·3	1·6	1·9
Propionic acid:	500 ml	6·12	6·38	9·74	9·00	57·3	56·6	31·2	31·0	9·0	9·8	2·5	2·6
	1000 ml	6·24	6·37	9·89	9·78	47·9	46·8	42·9	42·1	7·3	8·5	1·9	2·6
Butyric acid:	500 ml	6·33	6·48	9·06	8·87	56·8	56·6	16·2	17·8	24·4	23·1	2·6	2·5
	1000 ml	6·41	6·56	8·87	8·66	51·9	47·9	15·5	15·4	30·1	34·7	2·5	2·0

On the assumption that the acid infusions did not alter the basal fermentation, the ruminal production of an acid was calculated from the change in its ruminal concentration, relative to that of the other acids, when the acid was infused. For example, with cow A the acetic acid concentration, on a molar basis, in the control period was 2·09 times that of the other acids whereas with the infusion of 500 ml acetic acid per day it was 2·63 times. This gives an estimate of the basal production of acetic acid of $(2{\cdot}09 \times 500) \div (2{\cdot}63 - 2{\cdot}09) = 1930$ ml/day.

The mean values with their standard errors for the estimates of the daily production of the individual VFA from 15 lb hay dry matter are 1710 g ± 152 g acetic acid, 530 g ± 33·2 g propionic acid and 359 g ± 25·4 g butyric acid. In terms of the carbon content, the estimated total acid production is equivalent to 41·2% of the hay organic matter, which may be compared with the observed rumen digestibility coefficient, by the lignin-ratio technique, of 33·2 and the overall digestibility coefficient of 69·4.

The mean estimated production of VFA was in the molar ratios of 71·8% acetic acid, 18·0% propionic acid and 10·2% butyric acid compared with the mean proportions observed in the rumen of 68·3% acetic acid, 19·9% propionic acid and 11·8% butyric acid.

Gastro-intestinal absorption of ^{28}Mg in sheep. By A. D. CARE* and D. B. ROSS, *Animal Health Trust, Farm Livestock Research Centre, Stock, Essex*

A decrease in plasma magnesium concentration was shown to accompany a change in diet from hay to young grass in a crossbred wether sheep. The grass was cut when it was growing rapidly and was stored in a frozen state until required. The dietary intake of hay and grass was arranged so that the total Mg ingested each day was constant, despite the change in diet. The minimum plasma Mg concentration was usually reached about 24–36 h after the dietary change: later, it gradually increased again to its normal range.

Tracer quantities of ^{28}Mg as $MgCl_2$ were introduced via a fistula to label the rumenal contents of a sheep fed each diet in turn. From the radioactivity of the faeces the proportion of ^{28}Mg absorbed under each dietary regime was estimated by a method which minimized errors introduced by re-secretion of ^{28}Mg into the digestive tract. It also accounted for differences in the rate of faecal excretion of Mg.

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The estimation was repeated on each diet using the same sheep. In this way the average percentage efficiency of absorption of rumenal $MgCl_2$ from the digestive tract of the sheep was measured. Its value for the hay diet was 16·6 ± 0·1% and for the grass diet was 10·0 ± 0·5%. This marked difference in efficiency of absorption suggests that hypomagnesaemia is not caused by the presence in young grass of a high proportion of Mg which is bound so strongly that it cannot be released during digestion. The decrease in efficiency of absorption of ionic Mg is presumably the result of processes taking place in parts of the alimentary tract by which either the permeability of the intestinal wall to Mg^{2+} is reduced or the concentration of Mg^{2+} within the lumen is diminished.

An experiment in dietary survey methodology. By J. V. G. A. DURNIN and ELAINE C. BLAKE, *Institute of Physiology, University of Glasgow, Glasgow, W.2*

Several differing methods are at present in use for assessing the food intake of individuals or groups of individuals. The intrinsic accuracy of these methods, inevitably, varies considerably since some of them aim at obtaining rough guides to the values pertaining to population groups, large or small, whereas others attempt to obtain accurate results on relatively small numbers of individuals. However, within these limitations, little work has been done on the experimental error associated with any one method, although some comparisons between methods have been made (Morrison, Russell & Stevenson, 1949; Trulson, 1954).

The method which is probably almost universally accepted as giving the most accurate assessment of food intake of people living in their normal home environment is that which is commonly referred to as the individual weighed inventory method, where the food eaten at each meal is concurrently weighed and recorded. Yet the precise accuracy of this method is unknown. One variable affecting the overall accuracy is dependent upon the particular investigator supervising the study. The present experiment is an attempt to determine the variability occurring in a study where four investigators took part.

Four dietitians, with varying amounts of experience in the method utilized, measured the total food intake of four elderly women during an 8-day period and these 8 days of measurement were subdivided into four sets, each of 2 consecutive days. Each dietitian was allocated to one subject for one set of 2 days, then changed over to another subject for the following 2 days, changed again and so on. The whole arrangement was randomized and planned in the form of a Latin square, the variables being the four individual subjects, the four dietitian-investigators and the four sets of 2-day periods. After a few days' interval, the complete experiment was repeated with a different randomization of the Latin square.

Statistical analysis of the results showed that there was no significant difference in the values obtained for the caloric intake by the four dieticians. There was also

no significant difference between the differing 2-day periods within each 8-day experiment. Similar findings resulted for the analysis for protein, fat, carbohydrate, calcium and iron. The values for the two consecutive experimental periods were very similar for the individual subjects.

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Sulphur and nitrogen balances in rats receiving commercially processed protein concentrates. By E. L. MILLER and K. J. CARPENTER, *School of Agriculture, University of Cambridge*

Miller & Naismith (1958) found that the sulphur content of mixed human diets was highly correlated with their net dietary-protein values for rats. This would be expected when (a) the S amino acids are limiting, (b) the amounts in the diets are equally available and (c) other S compounds are present in small or constant amounts. We have tested the relationship for commercially dried protein concentrates, using meat, fish and whale meals (coded MM, FM and WM) distributed by the Agricultural Research Council (Zuckerman, 1959).

Twenty-five materials were analysed for S by the procedure of Bertolacini & Barney (1957) as modified by D. S. Miller & Donoso (private communication). The values (g S/16 g nitrogen) showed no correlation with their published net protein utilization (NPU) values (Bunyan & Price, 1960). Seven meals were studied further with the results summarized in the table.

Protein concentrate	FM6	WM9	MM18	MM16	FM4	MM10	WM7	SE
NPU	80	69	56	53	48	41	33	±4·4
Limiting amino acid			Cystine	Cystine	Cystine	Cystine	Not cystine	
Sulphur (g/16 g N)	1·30	0·97	0·99	1·31	1·34	0·83	1·01	±0·014
Cystine (g/16 g N)	0·9	0·9	0·8	2·8	0·7	0·9	0·5	±0·07
Methionine (g/16 g N)	2·3	2·5	1·8	1·1	1·6	1·2	2·0	±0·09
Non (cystine + methionine) S (g/16 g N)	0·57	0·19	0·40	0·35	0·81	0·33	0·46	
True digestibility of S (%)	90	93	87	53	73	63	49	±6·0
True digestibility of N (%)	93	90	87	75	73	79	56	±2·0
Corrected urine S (g/16 g N fed)	0·09	0·10	0·15	0·19	0·31	0·23	0·08	±0·034
g (cystine + methionine) S/16 g N × percentage digestibility of S ÷ 100	0·66	0·72	0·52	0·51	0·39	0·31	0·27	

The 'rat' NPU values by the 'balance' procedure (Mitchell, 1923–4) with a S-free basal diet differed from the published figures obtained by carcass analysis (a discrepancy to be discussed elsewhere), but the ranking was similar. The values did not correlate with values for 'cystine + methionine', determined as 'cysteic acid + methionine sulphone' after performic acid oxidation, acid hydrolysis and separation on a short column (modified from Lewis, 1960), although cystine was confirmed to be limiting for four samples.

In the same rat experiment digestibility of S was lower than of N with two meals. In MM16 particularly this might be due to a proportion of keratin, rich in cystine

but poorly digested. Non-'cystine + methionine' S/16 g N varied but always exceeded the urinary S/16 g N fed. 'Unavailability' of S amino acids could therefore be explained in this short series by indigestibility: NPU correlates closely ($r=0{\cdot}94$) with 'cystine + methionine' × percentage digestibility of S.

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The addition of herring and vegetable oils to the diet of cats. By PATRICIA P. SCOTT and E. R. HUMPHREYS, *Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.*1

Either unrefined herring oil (Herring Industries Board) or stabilized unsaturated vegetable oil (Violmul Chemical Corporation) was added, in increasing amounts over a period of 15 weeks, to the mixed diet of two pairs of young cats. The animals grew well and maintained good health even on fat intakes of 30 g daily. This represented 64% of the dry-food intake. No evidence was obtained of ketone bodies in the urine or of abnormally high levels of fat in the faeces. At post-mortem very considerable deposits of fat were observed both subcutaneously and surrounding viscera and muscles in all four cats. Only one animal, whose diet was supplemented with unrefined herring oil, had some yellowish discoloration of the abdominal fat deposits, in the other three the deposits were creamy-white. The cardiovascular system was normal, the inner lining of heart and blood vessels was smooth and shining.

Patterns of movement of particles of different size in the alimentary tract of the cat. By PATRICIA P. SCOTT and E. R. HUMPHREYS, *Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.*1

Chromium sesquioxide, either inactive or containing ^{51}Cr, of particle size less than 5 μ, and nylon ball bearings, $\frac{3}{32}$ in. in diameter, were fed to fasted cats with a standard meal of 50 g (wet weight) minced ox heart. In most of the trials these materials were enclosed in gelatin capsules, but in some they were intimately mixed into the food. The capsules were administered either before, during or after the meal and both markers were given at the same time.

The cats were killed with Nembutal 2–7 h after the meal. The parts of the alimentary tract were isolated immediately with artery forceps, removed, opened and the contents carefully washed out. Nylon balls were sieved off and the chromium sesquioxide determined colorimetrically after conversion into dichromate, or by measuring its radioactivity. Results were expressed as percentages of the dose.

Food tended to remain in the stomach for periods up to 7 h; Cr_2O_3 tended to leave before the nylon balls. Both large and small particles moved rapidly through the duodenum and the proximal three-quarters of the small intestine, although some tended to accumulate in the distal quarter of the small intestine where the Cr_2O_3 appeared first. Cr_2O_3 usually appeared in the colon within 3 h of the meal; following its arrival the green colour was seen for some time to be more concentrated in the proximal than in the distal half of the colon. A sharp line of demarcation was usually seen and the division of the colon into proximal and distal portions was made at this point. When formed faeces were present in the distal colon, the faecal pellets each consisted of an unmarked core surrounded by a green marked layer.

Separation of Cr_2O_3 from nylon balls was more obvious in those experiments in which the markers had been given in capsules, suggesting incomplete mixing in the stomach.

The patterns of movement of nylon balls and Cr_2O_3 along the alimentary tract appear to us to reflect different phases of gastric emptying; neither, individually, marks the passage of the whole meal.

Nylon balls, active and inactive Cr_2O_3, and Geiger–Müller tubes were provided by Westminster Laboratories Ltd, London, to whom our grateful thanks are due.

Vitamin A in the kidney of the cat. By T. MOORE and I. M. SHARMAN, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council,* and PATRICIA P. SCOTT, *Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.*1

Lowe, Morton & Vernon (1957), in the course of a study of the unsaponifiable constituents of the kidney in various species, found that the kidney of the cat is remarkable for its high concentration of vitamin A. In several individual specimens, or batches, of cat kidney measurements by ultraviolet spectrophotometry indicated a mean concentration of *c.* 100 i.u. of vitamin A/g. We have estimated vitamin A in kidneys and livers of over eighty cats, by the antimony trichloride method. Most of the cats had been used in experiments which were not directly concerned with the present investigation, but the diets, and intakes of vitamin A, had been recorded. Our findings confirmed those of Lowe *et al.* Concentrations of 2–850, mean 150 i.u./g were found for the kidneys, and 1–10 000, mean 1000 i.u./g in the livers. No consistent relationship was found between the concentrations of vitamin A in the kidneys and liver, except that when the diet was inadequate in vitamin A the concentration in both organs was low. In many instances the concentration of vitamin A in the kidney greatly exceeded that in the liver. The kidneys sometimes contained

unusually high concentrations of fat, but no consistent correlation was found between the fat and vitamin contents. Observations by fluorescence microscopy suggested that the vitamin was located in the convoluted tubules.

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The effect of the status of the animal upon the oxygen consumption of the isolated diaphragm in the rat and the newborn cat. By R. E. Moore, H. Baum and H. A. El-Khanagry, *Departments of Physiology and Biochemistry, Royal Free Hospital School of Medicine, London, W.C.*1

Diaphragms rapidly isolated from infant cats and rats of all ages exhibit a biphasic pattern of oxygen consumption. There is an initial rapid phase lasting some 10–20 min followed by a slower near-linear phase of respiration which may last up to 3 h.

We believe (Baum, El-Khanagry & Moore, to be published) that the initial phase is a function of the extent of mobilization of free fatty acid in the animal prior to killing, and that the steady phase represents the oxidation of free fatty acid mobilized from the isolated tissue into the medium during incubation.

Acute pretreatment of the animal, e.g. by exposure to cold for 2 h, injection of catecholamines 1 h before killing, does not affect the steady phase of respiration. However, these and other procedures may markedly influence the initial fast phase of respiration of the isolated tissue. Pretreatments which would be expected to raise blood levels of free fatty acid tend to accentuate this initial phase, and those which depress free fatty acid mobilization tend to eliminate it.

Xanthine oxidase in human milk. By E. C. Owen and L. I. Hart, *Biochemistry Department, Hannah Dairy Research Institute, Kirkhill, Ayr*, and F. E. Hytten, *Obstetric Medicine Research Unit (Medical Research Council), University of Aberdeen*

Modi, Owen & Proudfoot (1959) reported that they could find no xanthine oxidase in human milk, sow's milk or mare's milk in spite of the fact that the milk of cows, sheep, goats and rabbits tested at the same time and by the same method contained the enzyme. Bradley & Gunther (1960) found small amounts of this enzyme in human milk and thereupon Owen & Hytten (1960) re-examined human milk and confirmed Bradley & Gunther's observations. It is thus obvious that the human-milk samples which we tested for the results reported in 1959 were different from those tested in 1960. The 1959 samples were deep-frozen, packed in insulated containers and flown from Dyce Airport, Aberdeen, to Abbotsinch Airport, Renfrewshire, collected immediately and transported by car to the Hannah where they

were at once replaced in a deep-freeze cabinet pending analysis. On arrival these samples were still frozen solid. The 1960 samples were transported from Aberdeen to Ayr by rail.

Increase or 'revelation' of xanthine oxidase in cow's milk by cooling, heating or adding surface-active substances, is well-documented (Dixon & Kodama, 1926; Macrae, 1930; Wieland & Macrae, 1930; Robert & Polonovski, 1955). One of us (L.I.H.) has recently, in these laboratories, confirmed the observations of Macrae (1930) and Worden (1943) that agitation of cow's milk is an effective method of increasing the titre of this enzyme. 'Revelation' of xanthine oxidase has not been reported in human milk but it seems a reasonable assumption that the difference between our 1959 and 1960 results may have been due to the agitation of the 1960 samples on the railway. Kiermeier & Vogt (1957) reported investigators who found no xanthine oxidase in human milk and who therefore suggested its use as a test for distinguishing between human and cow's milks in the setting up of human-milk banks. Kiermeier & Vogt, however, also reported others who found the enzyme in human milk. Kiermeier & Capellari (1958) reported that the concentration of xanthine oxidase in cow's milk reflects the concentration of molybdenum in the diet. Xanthine oxidase in cow's milk or rat's liver is a protein containing molybdenum, iron and riboflavin-adenine-dinucleotide and in the rat, protein, molybdenum and riboflavin have all been shown to affect its concentration in the liver. Only further experiments can decide whether variations of xanthine oxidase in human milk are due to 'revelation' or to dietary influence or to both of these causes acting together.

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