The One Hundred and Ninety-fourth meeting of The Nutrition Society was held in St George's Hospital Medical School, London, SW1, on Friday, 1 *December* 1967 *at* 10.30 *h, when the following papers were read:*

Effects of obesity and weight reduction on serum insulin levels. By STEPHEN SZANTO, *Department of Nutrition, Queen Elizabeth College, London, W8*

It is known that a high level of insulin-like activity (ILA) in the blood is a feature of obesity (Rabinowitz & Zierler, 1962; Karam, Grodsky, Pavlatos & Forsham, 1965; Samaan & Fraser, 1963; Balsano, Di Noto & Pappalardo, 1964). Moreover, there is a rise in plasma ILA during the process of weight loss in the obese (Hales & Randle, 1963; Rudnick & Taylor, 1965). There has however been no report of insulin levels after the loss of excessive body-weight.

The insulin response to an oral glucose tolerance test was measured in fifteen patients attending the obesity clinics of three hospitals, before they began their treatment for weight reduction. This consisted of a low-calorie diet, together in some cases with appetite-depressive drugs. Serum insulin was measured by iodine-125 radioisotope immunoassay.

Of the fifteen patients four reached a weight within 15% of the 'ideal', as classified by the Metropolitan Life Insurance Company (1962), between 5 and 8 months from the beginning of treatment. When this happened, the levels of insulin were again measured after an oral glucose tolerance test. The results show that in all four patients the insulin levels had fallen to within normal limits (Table 1).

Table 1. *Effects of weight reduction on blood sugar and serum insulin levels during glucose tolerance tests*

Patient no.	Stage of trial	Glucose (mg/100 ml blood) 0	30 min	60 min	120 min	Insulin (μ-units/ml serum) 0	30 min	60 min	120 min
1	Before weight reduction	106	174	175	60	51	99	157	118
	After weight reduction	72	96	89	84	10	79	46	18
2	Before weight reduction	108	174	159	100	52	116	184	49
	After weight reduction	95	132	138	77	23	50	63	22
3	Before weight reduction	87	128	155	88	42	40	134	115
	After weight reduction	89	132	130	74	5	13	43	14
4	Before weight reduction	116	181	186	111	67	248	215	79
	After weight reduction	83	152	132	80	25	84	80	36

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Dietary intake and urinary loss of iron in the human. By Y. K. MAN and G. R. WADSWORTH (introduced by B. S. PLATT), *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London, WC1*

Very few detailed investigations of the normal loss of iron in human urine, especially in relation to the diet, have been made. Analysis of urine may provide useful information about dietary intake of nutrients (Wadsworth, 1963) and we have been exploring this possibility in the case of Fe.

Urine is collected directly into polythene bottles over periods of 24 h. The concentration of Fe is determined by a modification of a method described by Bothwell & Mallett (1955) for use on serum. In the final stage colour is developed with bathophenanthroline (4,7-diphenyl-phenanthroline). The reaction is highly sensitive.

The mean quantities of Fe excreted daily by two young adults on different levels of Fe are shown in the table. Throughout each test period the diet remained exactly the same each day, and no Fe other than that in the foods was provided. The female subject was kept for 1 month on each of the dietary levels of Fe, but only twenty-eight urine samples were analysed for each test period as none were collected during menstruation. The male subject was kept for 11 days on each dietary Fe level and urine samples were collected daily throughout the trial.

Daily loss of iron in the urine by two subjects on known dietary intakes

	Female			Male		
Fe intake (mg/24 h)	5·3	11·7	23·9	10·8	21·5	33·8
Fe in urine (μg/24 h)						
Mean	81·3	101·3	118·7	51·8	79·1	138·5
Standard deviation	±14·1	±17·4	±15·0	±7·2	±5·4	±7·1

The results are compatible with those found by Leverton (1941); and they lend support to the proposal that urine analysis may be used to assess dietary intake of Fe when other methods of doing so would be impracticable or insufficiently accurate.

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Availability of iron from hen's egg. By K. K. NARULA and G. R. WADSWORTH, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London, WC1*

In 1871 Subbotin (quoted by Stockman, 1893) described how, in spite of a high content of iron, egg yolk fed to pigeons resulted in relatively low haemoglobin levels. Recently attention has again been given to the availability of Fe from egg. The results of an experiment which we have made on mice may therefore be of interest.

Three groups of young mice were fed respectively on diets in which the main source of Fe was wheat flour, whole egg or a mixture of wheat flour and egg. The protein content of each diet was adjusted to 20% by weight by addition of casein, and vitamins and minerals, except Fe, were included in appropriate amounts.

After 2 weeks on the diets the animals were killed and the total amount of Fe in the carcass was measured. The amounts found were compared with those in a control group of animals which had been killed at the commencement of the test. The results are given in the table.

Changes in total carcass iron in mice fed for 2 weeks on egg and wheat diets

Dietary source of Fe	No. of animals	Mean body-weight (g)		Mean total carcass Fe (μg)		Mean change in total carcass Fe (μg)
		Initial	Final	Control	Test	
Wheat	10	15·9	23·4	813	915	+102
Egg	9	15·9	24·1	816	594	−222
Egg+wheat	20	16·9	27·8	853	814	− 39

The results of the experiment suggest that the Fe in egg is not available to the mouse and furthermore the presence of egg in the diet may inhibit the absorption of Fe present in another food. When egg was the only source of dietary Fe there was apparently an actual loss of Fe from the body. These effects must be substantiated by further experiments, but they are compatible with the presence in egg of protein with a high affinity for Fe (Bunge, 1885; Halkett, Peters & Ross, 1958; Greengard, Sentenac & Mendelsohn, 1964).

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The determination of the true digestibility of interesterified fats in young pigs. By C. P. FREEMAN, D. W. HOLME and E. F. ANNISON, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Measurement of the digestibility of fats by conventional methods is made difficult by the contribution of endogenous fat to faecal fat excretion. Corrections for endogenous fat production using the output of fat when a fat-free diet is fed, are based

on the assumption that the production of endogenous fat is unrelated to the level or composition of dietary fat. The validity of this assumption has been checked by examining the digestibilities of labelled and unlabelled fats, where the proportion of labelled fat retained is a direct measure of true digestibility.

The digestibilities of natural and interesterified lard, soya-bean oil and coconut oil were measured in young pigs (age, about 9 weeks) and the same fats were examined after the interesterification of each fat with ^{14}C-labelled lauric, palmitic, stearic and oleic acids. The level of inclusion of dietary fat was 10% (w/w) in each treatment. The experiment followed a randomized incomplete block design with four litter-mate pigs per block. A total of twenty-eight pigs were on trial in seven such blocks. Records of food intake and faeces output were made during two consecutive periods of 10 days in the case of the unlabelled fats. Each unlabelled interesterified fat was replaced by the appropriate labelled fat for 1 day at the end of the second period and collection of faeces continued until radioactivity had disappeared. Control diets of minimal fat content were fed to provide values for endogenous fat production, which allowed correction of the apparent digestibilities.

The results summarized in Table 1 show that apparent digestibilities, when corrected for endogenous fat production, provide valid estimates of the true digestibilities of the fats fed under the conditions of these experiments. There were no significant differences between the digestibilities of the fats before and after interesterification.

Table 1. *Digestibility coefficients (%) of natural and interesterified soya-bean oil, lard and coconut oil in young pigs*

Dietary fat (10% level)	Digestibility (%) Apparent	Corrected	True
Natural soya-bean oil	79·4	89·9	—
Interesterified soya-bean oil	81·5	93·1	92·8
Natural lard	73·8	84·9	—
Interesterified lard	68·1	82·6	85·2
Natural coconut oil	77·5	91·4	—
Interesterified coconut oil	83·6	92·4	92·8
No of determinations	8	4	2
Coefficient of variation (%)	10·0	5·2	—
Significant difference ($P=0·05$)	7·4	7·9	—

The effect, on nitrogen retention by growing heifers, of protein and non-protein nitrogen fed in isocaloric rations. By C. C. BALCH and J. A. BINES, *National Institute for Research in Dairying, Shinfield, Reading*

In experiments to assess the nutritive value of non-protein nitrogen it is as important to control the energy intake as to ensure that the levels of protein N and non-protein N in the rations are the same.

Six Friesian heifers with an initial average live weight of 388 kg were used in a 6×6 Latin square experiment to assess the effect on N balance of three levels of N fed either as groundnut or as urea. The heifers received 8 kg of a low-N basal diet

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containing barley straw, barley, sugar-beet pulp and molassine meal with additions of minerals and vitamins. The three control treatments were 124, 372 and 930 g groundnut meal, with appropriate additions of maize starch at the two lower levels to ensure that the net energy intake remained constant. The amounts of N supplied were, respectively, 9·2, 27·6 and 69·0 g. The three experimental treatments were 20, 60 and 150 g urea daily each given with 680 g starch, supplying the same amount of energy and respectively the same amounts of N, as the control treatments.

Each experimental period consisted of a 16-day preliminary period followed by a 12-day period in which faeces and urine were collected, and stored under acid with mercuric chloride as a preservative.

The results shown in Table 1 suggest that, in this experiment, urea N was used as efficiently as protein N. The decrease in apparent biological value with increasing levels of N suggests that energy was limiting at all, except possibly the lowest, levels of N input.

Table 1. *Mean nitrogen inputs, outputs and balances (g/day) of six heifers fed three levels of nitrogen, either as urea or as groundnut, in isocaloric rations*

Treatment	N input	N output			N balance ± SE	Apparent biological value
		Faeces	Urine	Total		
Low urea	91·07	55·20	27·69	82·89	8·18 ± 1·45	75·4
Medium urea	109·93	54·16	44·21	98·37	11·56 ± 3·45	62·9
High urea	151·86	56·26	77·32	133·58	18·28 ± 1·25	49·3
Low protein	91·85	55·61	27·01	82·62	9·23 ± 1·24	76·6
Medium protein	110·17	57·52	43·37	100·89	9·28 ± 1·93	62·2
High protein	155·86	62·26	74·58	136·84	19·02 ± 2·68	50·7

There was no evidence of any divergence from a linear response to additional increments of N in either form over the range studied. With energy intake limiting, a curvilinear diminishing response to additional protein had been expected, as dietary protein was used to supply energy. To clarify this point it is intended to supply N at higher levels than were used in this experiment, in an attempt to obtain a diminishing response to additional N, and to compare retention of N from the two sources at these higher levels.

If these results were confirmed, they would suggest that urea can be used by the growing animal as efficiently as groundnut protein provided starch is supplied with urea in amounts equivalent to the net energy of the groundnut.

Variation, in relation to feeding, in the levels of certain energy-yielding metabolites in the blood of a cow receiving an all-concentrate or all-hay diet. By J. A. Bines, *National Institute for Research in Dairying, Shinfield, Reading*

As a preliminary trial in a series of experiments designed to examine possible chemostatic regulators of intake in ruminants, variations in plasma concentrations

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of acetate, propionate, β-hydroxybutyrate, lactate and glucose were measured at intervals after feeding a Friesian cow a diet of 100% hay or 100% concentrates. The cow was well-adapted to the diet and had been trained to eat one meal daily in a short time. The meal was offered at 10·00 h, with *ad lib.* access for 2 h. Blood samples were obtained from a cannula previously established in the jugular vein. Protein-free filtrates prepared from plasma were analysed for glucose by the glucose oxidase method and for organic acids by the method of Ramsey (1963).

The results for the all-hay diet were:

Time after feeding (min)	Level of metabolite (mg/100 ml)				
	Acetate	Propionate	β-hydroxy-butyrate	Lactate	Glucose
−45	4·03	0·33	1·86	4·27	65·3
−15	4·08	0·26	1·55	3·33	67·1
30	4·06	0·25	1·12	4·04	60·8
70	4·38	0·41	1·15	3·45	55·1
120	4·50	0·42	1·10	2·64	53·3
170	3·79	0·25	1·09	3·05	56·1
225	3·84	0·21	1·64	3·05	60·4
285	4·18	0·41	1·02	4·07	62·8
345	4·56	0·23	1·52	2·44	62·3
405	3·91	0·15	1·43	3·19	63·0

Values shown are means of determinations made on 4 different days.

The results reveal a remarkable constancy in concentrations of all substrates examined except glucose, where there was a marked depression after feeding. Since there was no discernible rise in the level of any of these compounds in the blood, it seems unlikely that variations in any of those examined can have provided signals bringing about the end of periods of eating. This conclusion is in line with the view (Balch & Campling, 1962) that physical mechanisms are of greatest importance in regulating the voluntary intake of roughage diets. On the other hand, when the all-concentrate diet was fed to the cow, there were marked rises in the levels of all the organic acids measured together with a simultaneous fall in plasma glucose level. The values were:

Time after feeding (min)	Level of metabolites (mg/100 ml)				
	Acetate	Propionate	β-hydroxy-butyrate	Lactate	Glucose
−45	3·39	0·06	0·60	2·05	69·3
−15	2·75	0·05	0·59	1·78	69·7
30	2·01	0·07	1·19	4·32	63·1
70	4·47	0·71	5·23	6·68	58·4
120	6·33	1·51	6·59	9·82	47·5
170	6·79	2·04	8·05	13·40	44·1
225	6·00	1·21	7·57	12·40	47·8
285	5·26	0·98	7·65	6·53	54·3
345	4·19	0·48	6·55	5·00	65·1
405	4·08	0·31	6·01	5·14	68·7

Values shown are means of values determined on 3 consecutive days.

Under these conditions, chemostatic regulators of intake may well have operated, mediated by any of the acids examined, either singly or in combination. Of particular

interest are the levels of propionate and lactate reached after feeding. Propionate amounted to nearly one-third as much as the peak acetate level; lactate showed the greatest concentration change of any of the substrates examined. On the all-concentrate diet it appears that in the cow studied, the amount of propionate absorbed from the rumen was not completely removed by the liver. If this were shown to be the case generally with high-concentrate diets, it would not be out of place, contrary to previous ideas, to examine the effects of intravenous infusions of propionate on the intake of high-concentrate diets by ruminants.

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Rumen fermentation of soluble carbohydrates in cows receiving diets high in flaked maize. By J. D. SUTTON, *National Institute for Research in Dairying, Shinfield, Reading*

Clear differences were found in the fermentation of six soluble carbohydrates incubated in vivo and in vitro with rumen contents from two cows fed 70% hay and 30% dairy cubes (Sutton & Balch, 1966). To see whether differences persisted on a very different basal fermentation, the experiments were repeated when the same two cows were given about 20% hay and 80% flaked maize. Five monosaccharides were infused into the rumen for 8 h at 200 g/h on isolated occasions. In the in vitro experiments, 896 mg of the same monosaccharides or 851 mg sucrose were added continuously during a 2 h incubation to 150 g rumen contents from the same two cows; this rate of addition approximated that used in vivo. Each carbohydrate was incubated on three occasions with each cow.

The range in molar proportions of the major volatile fatty acids (VFA) before the start of the carbohydrate additions was for acetic 45–64%, propionic 19–38%, n-butyric 7–20% and n-valeric 1–4%. These ratios differed markedly from those found when the hay diet was fed. Despite the variability in the basal fermentation, clear differences were detected amongst the carbohydrates. The in vitro results are presented in Table 1.

In comparison to the results with the hay diet, the most notable features were the large increase in the proportion of propionic acid and decrease in the proportion of n-butyric acid produced from galactose and the pentoses. The mean proportions of VFA produced from sucrose, glucose and fructose were similar on the two diets but, with the flaked maize diet, there was wide variability in the proportions of propionic and n-valeric acids produced; the proportions of these two acids tended to vary inversely. Mean recovery in VFA of carbon from metabolized carbohydrates ranged from 37 to 43% compared to 27 to 35% with the hay diet. The results of the in vivo experiments agreed in general with those of in vitro incubations and will be presented elsewhere.

Table 1. *Mean percentage of carbohydrate metabolized and products of fermentation when six carbohydrates were incubated with rumen contents from two cows fed diets containing a large proportion of flaked maize*

		Net production					
Carbohydrate	Carbohydrate metabolized (%)	Lactic acid (mM)	Total volatile fatty acids (mM)	Acetic acid (molar %)	Propionic acid (molar %)	n-Butyric acid (molar %)	n-Valeric acid (molar %)
Sucrose	86·5	0·35	3·47	45·3	32·2	16·3	6·2
Glucose	85·9	0·19	3·79	39·2	43·7	13·1	4·0
Fructose	73·1	0·41	2·76	42·2	30·9	20·2	6·2
Galactose	70·0	0·00	3·40	43·1	51·0	5·0	0·9
Xylose	58·0	0·00	2·67	41·3	54·9	2·4	1·4
Arabinose	63·8	0·00	2·99	42·1	55·0	1·8	1·1
5% LSD	8·8	0·37	0·95	7·4	16·4	6·0	8·0

LSD, least significant difference.

These studies were supported in part by a grant from the Sugar Research Foundation, New York.

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Intestinal disaccharidases in the calf. By R. C. SIDDONS (introduced by J. W. G. PORTER), *Department of Nutrition, National Institute for Research in Dairying, Shinfield, Reading*

On the assumption that the rate of hydrolysis is the limiting factor in the utilization of disaccharides, a quantitative study of the disaccharidase activities in homogenates of intestinal mucosa is useful in assessing the ability of an animal to utilize various disaccharides. In the present study, the lactase, cellobiase, maltase, trehalase, sucrase and palatinase activities have been measured in homogenates prepared from mucosa of the stomach, small intestine and large intestine of calves ranging in age from 4 days to 4 months.

No sucrase or palatinase activity was detected in any of the calves. The other disaccharidase activities were located mainly in the small intestine and showed a non-uniform pattern of distribution along the small intestine: trehalase activity was highest in the proximal part and decreased distally, lactase and cellobiase activities were highest in the middle and proximal parts and the maltase activity was highest in the distal part. The lactase and cellobiase activities showed a constant ratio at all levels of the small intestine suggesting that the two activities might be exerted by the same enzyme. Chain, Mansford & Pocchiari (1960) and Miller & Crane (1961) have shown that the disaccharidases exert their physiological activity intracellularly and therefore it is possible that the location of optimal activity along the small intestine may reflect the site of absorption of the various disaccharides.

The effect of age on the disaccharidase activities was investigated by studying the intestinal disaccharidase activities of calves of different ages which had been maintained on whole milk diets. The lactase and cellobiase activities (expressed per mg of protein) were highest in the youngest calves and decreased with age while the maltase and trehalase activities did not change with age but were lower than the lactase activity even in the oldest calves. No marked differences were observed in the carbohydrase activities of the intestinal mucosa of 4-month-old calves fed solely on milk and calves given a concentrate–hay diet from 6 weeks of age.

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Plasma amino acid levels in the cow. By A. F. HALFPENNY and J. A. F. ROOK, *Division of Agricultural Chemistry, School of Agricultural Sciences, The University, Leeds 2*

According to Christensen (1964), the plasma level of each of the amino acids is controlled by the balance between the entry and exit from the plasma. Lactation imposes an exceptional drain on the amino acids of the blood plasma and its effect on plasma levels has been investigated in the cow.

Six cows were used, three of the Friesian and three of the Jersey breed. Samples of blood were taken, by catheter, from the jugular vein at about 10·00, 13·00, 16·00 and 19·00 h, on 2 successive days at the end of the 8th month of pregnancy and again on 2 successive days in the 6th week of lactation. The plasma was separated immediately, the proteins precipitated with picric acid and the supernatant stored at −20°. Samples were analysed by the method of Moore & Stein (1958), using an EEL automatic multi-channel amino acid analyser.

Mean values, with SEM, for the concentrations (mg/100 ml blood plasma) of individual acids for the Friesian and Jersey cows respectively, in pregnancy, were: lysine, 1·31 ± 0·07 and 1·75 ± 0·13; histidine, 0·72 ± 0·03 and 0·68 ± 0·06; arginine, 0·84 ± 0·05 and 0·94 ± 0·08; threonine, 0·76 ± 0·06 and 0·69 ± 0·04; serine, 0·85 ± 0·06 and 1·36 ± 0·14; glutamic acid, 0·65 ± 0·04 and 0·67 ± 0·06; proline, 0·50 ± 0·06 and 0·62 ± 0·10; glycine, 2·14 ± 0·11 and 1·89 ± 0·19; alanine, 1·15 ± 0·09 and 1·20 ± 0·08; valine, 1·46 ± 0·11 and 2·09 ± 0·13; isoleucine, 0·98 ± 0·07 and 1·18 ± 0·08; leucine, 1·07 ± 0·08 and 1·22 ± 0·07; tyrosine, 0·41 ± 0·05 and 0·38 ± 0·05; phenylalanine, 0·44 ± 0·05 and 0·43 ± 0·04.

The values for serine, proline, alanine, valine, isoleucine, tyrosine and phenylalanine were not significantly altered by lactation. There was, however, a fall with lactation in all cows in the values for lysine (mean change, −33%), arginine (mean change, −30%) and threonine (mean change, −30%) and in all but one of the cows in histidine (mean change, −31%), glutamic acid (mean change, −26%) and leucine (mean change, −17%). Glycine levels tended to increase (mean change, +25%) especially in animals of the Jersey breed.

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The interpretation of plasma amino acid ratios in protein–calorie deficiency. By C. R. C. HEARD, SYLVIA M. KRIEGSMAN and B. S. PLATT, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London, WC1*

The ratio (N:E) of certain non-essential (N) to essential (E) plasma amino acids is increased in kwashiorkor but not in marasmus (Whitehead & Dean, 1964; Widdowson & Whitehead, 1966). We have measured N:E and serum protein concentration in fasting (18 h) blood samples taken at intervals up to 6 months of age, from fifty-three pups weaned on to diets of NDpCal = 10, 7, or 5%.

Over all (125 samples), there was a negative correlation between serum protein concentration and N:E ($r = -0{\cdot}32$; $P < 0{\cdot}001$). For any age and diet, protein concentration showed much less scatter than did N:E. Below 4 months of age, serum protein concentration always distinguished the group fed the diet of NDpCal = 10% from those fed diets of NDpCal = 7 or 5% ($P < 0{\cdot}001$), but N:E did not do so. In older pups, when the effects of dietary protein shortage were less critical, differences in serum protein concentration were not significant, but, in animals fed the diet of NDpCal = 10%, N:E fell to values significantly ($P < 0{\cdot}001$) below those for the other two groups.

Samples taken at 4 months of age from two litter-mates fed the diets of highest and lowest protein value were subjected to complete amino acid analysis. The deficient animal gave an aminogram 'typical of kwashiorkor' (L. E. Holt, personal communication), but the pup had none of the clinical signs. Most amino acids, including all but serine in the 'N' group, were markedly reduced in concentration, but N:E was elevated.

Failure to accept the diet of NDpCal = 5% led to marasmus in five pups of one litter. Their mean N:E ratio was $2{\cdot}7 \pm 0{\cdot}06$ (SE) compared with $3{\cdot}7 \pm 0{\cdot}24$ for five pups in a second litter, which accepted the diet. Serum protein showed no differences. Feeding the diet of NDpCal = 10% produced no change in N:E for the first group, but the ratio fell to $2{\cdot}65 \pm 0{\cdot}05$ in the second group. In both groups serum protein concentration increased.

Serum protein concentration proved to be the more accurate and specific index of protein-calorie deficiency. The N:E ratio related specifically neither to calories nor protein, since low N:E ratios were typical of falling protein requirements in normal animals, of marasmic animals, and of animals fed diets in which much of the carbohydrate was replaced by fat (Laksesvela & Payne, unpublished). N:E probably reflects the relative availability of carbohydrate and protein; carbohydrate because of its link with the glucogenic non-essential amino acids, and protein as the source of essential amino acids.

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Methionine sparing compounds. By D. S. MILLER and PAMELA SAMUEL, *Department of Nutrition, Queen Elizabeth College, London, W8*

The protein value of many diets is limited by the level of the sulphur amino acids (Miller & Donoso, 1963), and during heat processing these may be converted to other sulphur compounds of doubtful nutritional value. It is therefore of interest to ascertain which compounds can spare dietary methionine. Assays for net protein utilization (NPU) (Miller & Payne, 1961) were carried out with a diet limited by the sulphur amino acids, i.e. 10% casein, supplemented with various sulphur-containing compounds. In all instances the methyl donors choline and cyanocobalamin were present in the diet.

Table 1. *Net protein utilization of* 10% *casein diets supplemented with sulphur-containing compounds* (*mean values together with standard errors*)

Supplement	NPUst
None	76±2
DL-Methionine	95±2
Methionine hydroxy analogue	99±1
Homocysteine	95±2
Methionine sulphoxide	81±2
Methionine hydantoin	75±1
Methionine nitrile sulphate	70±1
4-Thiapentan-1-al sodium bisulphite	72±2
Methionine sulphonium hydrogen sulphate	66±1
Methionine ethyl ester hydrochloride	76±3
Methionine sulphone	68±1
Methionine sulphoximine	49±2
Cysteine	94±1
Cysteic acid	72±1
Sodium metabisulphite	75±2
Sodium sulphate	72±1
Ammonium sulphate	71±2
Sulphur*	74±2
Onions†	73±2

All supplements at the 0·1% level, except: * = 1%; † = 10%.

It is well known that cysteine and cystine can replace dietary methionine to a large extent and that homocysteine is readily converted to methionine in the presence of methyl donors. Similarly, methionine hydroxy analogue, which is incidentally not an amino acid, can be aminated to methionine. None of the other compounds related to methionine had a sparing effect, and some even depressed growth rate. Despite occasional reports in the literature to the contrary, none of the inorganic forms of sulphur were effective in the rat.

We would like to thank Imperial Chemical Industries Ltd for supplying some of the supplements tested.

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The effect of diet on ocular refraction in the rat. By M. BARDIGER, D. S. MILLER and ANNE L. NICHOLSON, *Nutrition Department, Queen Elizabeth College, London, W8*

In a study of uniovular twins, Sorsby, Sheridan & Leary (1962) showed that factors relating to refraction of the eye must be genetically determined but they pointed out that the possible modifying influence of environmental factors such as diet, had not been adequately studied. Gardiner (1958) claimed to reduce myopic tendencies in poorly fed children by feeding additional protein but his work is strongly criticized (Anonymous, 1959); there is also some evidence that marasmic children tend to be more myopic (Halasa & McLaren, 1964). In experimental animals, Ward (1965), feeding rats at two protein levels, demonstrated retardation of the normal trend towards hypermetropia with the lower protein intake.

Group	Diet	Refraction* after 156 days Dioptres—SE(n)
1	10% wheat gluten	+2·55±0·20 (5)
2	5% casein	+3·17±0·40 (6)
3	20% casein	+2·25±0·33 (6)
4	50% lactose in Amvilac†	+1·81±0·19 (4)
5	50% sucrose in Amvilac†	+2·10±0·19 (5)
6	50% starch in Amvilac†	+2·64±0·26 (7)
7	0·5% ethionine in Amvilac†	+3·21±0·32 (6)
8	100% Amvilac†	+1·94±0·06 (8)

*Equivalent spherical error. †Stock diet.

In our experiment we fed eight groups of eight female Sprague–Dawley rats the diets shown in the table for 156 days, after initial screening for aberrant vision and cataract. The eyes were examined every fortnight by standard retinoscopy, under cycloplegia (1/80% hyoscine hydrobromide). At the end of the experiment fresh eyeball weights, and fresh and dry (105°, 48 h) lens weights were recorded. Final body-weights ranged from 80 to 300 g (initially 78±1 g) and the proportion eyeball weight:body-weight ranged from 53 to 160 mg/100 g; but the growth of the lens relative to that of the whole eyeball was almost constant and the weights of eyeballs of the smallest groups were only 20% less than the largest.

All animals showed the same trend towards hypermetropia which levelled off at around+2·5 dioptres after 6 weeks. The maximum increase in refraction was 2·7 dioptres (group 7), as opposed to changes in the order of 7·0 dioptres in Ward's experiments. Also in contradiction of Ward's experiments, the low-protein group (2) had slightly longer sight than the Amvilac group (8) ($P<0·01$). The addition of ethionine, an antimetabolite to methionine, which is the limiting amino

acid to Amvilac, gave results similar to the low-protein group. We are thus unable to confirm Ward's observations.

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Avian liver L-amino acid oxidase activity and dietary amino acid imbalance. By M. A. SHINWARI and D. LEWIS, *Department of Applied Biochemistry and Nutrition, University of Nottingham*

Amino acid supplementation of diets has been shown to result in higher L-amino acid oxidase activity in rat kidney (Scheffner & Bergeim, 1953). Rat liver L-amino acid oxidase activity is also known to fall in riboflavine deficiency and to increase on restoring the riboflavine intake (Bunch, Lowry, Padilla & Combs, 1956). The system therefore appears to respond in terms of activity to change in dietary circumstances. It has also recently been shown (Shinwari & Falconer, 1967) that there are, in the liver, natural activators and inhibitors of the activity of the enzyme.

It is possible that variations in liver L-amino acid oxidase activity in response to changes in amino acid intake can account for some of the consequences of consuming diets imbalanced in terms of amino acid supply (Lewis & D'Mello, 1968). An experiment has therefore been carried out to assess the activity of the enzyme in avian liver preparations of birds fed diets differing in the levels of lysine and arginine intake. Four groups of birds were fed from day-old to 4 weeks of age on different diets: slightly suboptimal levels of both lysine and arginine; an increased lysine content; an increased arginine content; and an increase in both arginine and lysine.

After 4 weeks the birds were killed and livers from each group assayed for L-amino acid oxidase activity using L-leucine as substrate and acetone powder of the liver microsomes as the source of enzyme. The relative rates of oxygen uptake under standard conditions were respectively: 100, 96, 134 and 191. The results indicate a significant increase in oxidase activity as the dietary level of a particular amino acid is raised. This could result in the bird in an increased catabolism of other amino acids; a situation that might contribute to the known ill-effects of dietary amino acid imbalance.

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Methionine, homocystine, choline, and betaine in the nutrition of 4-day chick embryos. By C. R. GRAU* (introduced by K. J. CARPENTER), *Department of Poultry Husbandry, University of California, Davis, California, USA*

It has been known for several years that the young chick can utilize homocystine effectively in place of methionine provided a source of labile methyl groups such as choline or betaine is included in the diet, but to what extent this replacement can occur during embryonic life has not been investigated.

Methionine deficiency has rapid and profound effects on chick embryos, resulting in reduced growth, degeneration of the neural retina, and delayed erythrocyte maturation. These effects are easily observed in embryos that have been subjected to yolk-sac perfusion for 2 days, starting at 4 days of incubation, and serve as excellent criteria for determining whether methionine can be replaced by homocystine.

Chick embryos were prepared by previously published methods (Grau, Fritz, Walker & Klein, 1962; Austic, Grau & Matteson, 1966) and 30 ml of the liquid medium were perfused through the yolk-sac cavity every 8 h. The complete medium consisted of salts; glucose 5 g (per l.), L-amino acids 4·8 g, including L-cystine 90 mg, and L-methionine 120 mg, and various B vitamins, including vitamin B_{12} 0·2 μg, folic acid 0·1 mg, and choline chloride 300 mg. The medium was supplemented with 2·5 ml/l. of fresh yolk. Omission of free methionine from the medium resulted in reduced survival time, smaller than normal size at time of death or killing, and gross degeneration of the neural retina and brain.

When L-homocystine was added on a molecular equivalent basis to the methionine-deficient medium, the deficiency developed in the same way as it did when methionine was omitted. The presence of choline chloride did not alter the response. Betaine hydrochloride improved growth somewhat, but did not prevent degeneration of the neural retina. In other experiments, methionine labelled with carbon-14 in the methyl group was found to contribute significant amounts of carbon to the lecithin, but not to the kephalin, of embryo lipids, thus indicating that the methyl group of methionine can be transmethylated.

The results presented here should be considered only as preliminary. The inability of the young embryo to convert homocystine to methionine may have parallels to its inability to hydroxylate phenylalanine. It now appears that tyrosine is an essential for the normal development of young chick embryos (Grau, Austic & Matteson, 1965).

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Influence of sucrose and protecting action of moisture and fat on the quality of the protein of groundnuts during toasting. By G. VARELA, OLGA MOREIRAS-VARELA, CONCEPCION VIDAL, A. MURILLO and J. A. LUQUE, *Laboratory of Animal Physiology, University of Granada, Spain*

A study was made of the influence of moisture on the nutritive quality of the protein of groundnuts during toasting. For this, diets with three types of groundnuts were prepared: (1) normal moisture, (2) dried in a vacuum at 50°. Both were toasted in a laboratory toaster at 180° for 25 min. The third type of groundnut employed was the same as the first, toasted under the same time and temperature conditions, but with the toaster saturated with steam. The nutritive quality of the three proteins toasted was tested by means of the techniques of Mitchell (1923) and of Cremer (1963), and by that of Carpenter (1960) for utilizable lysine. In order to test the influence of fat, both defatted and un-defatted groundnuts were used. As the action of reducing sugars is known, it was also of interest to test the influence of sucrose. We determined the nutritive value of a carbohydrate-free protein of groundnuts, and of a mixture of it with sucrose at 4·5%. Both were toasted at different temperatures.

		Mitchell			Cremer Protein productive value	Carpenter Available lysine
		True digestibility	Biological value	Net protein utilization		
180°, 25 min	Normal moisture	89·7	46·8	41·9	11·64	1·23
	Dried	83·4	40·5	31·4	Negative	1·02
	Toasted saturated	91·7	51·4	47·1	18·22	1·50
	Un-defatted	—	—	—	20·18	2·13
	Defatted	—	—	—	11·11	1·61
60°, 25 min	Isolated protein: alone	98·7	58·4	57·3	—	2·41
	with sucrose	97·7	59·4	57·9	—	2·11
120°, 25 min	Isolated protein: alone	99·7	52·1	51·9	—	2·46
	with sucrose	98·2	54·6	53·6	—	1·86
180°, 25 min	Isolated protein: alone	96·3	50·4	48·5	—	1·51
	with sucrose	89·9	42·9	38·7	—	1·06

The results, given in the table, indicate (1) that the moisture of the grain gives protection against the damage caused to the protein by toasting and that damage is much less when the toaster is saturated with steam, (2) that the presence of fat protects the protein from thermal attack, and (3) that the presence of sucrose causes greater damage to the protein during toasting, but that this effect only appears at the highest temperature tested.

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Observations on the stomach content of the sucking-pig. By P. D. CRANWELL, D. E. NOAKES and K. J. HILL, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Examination of the gastric content from twenty-four pigs (two litters) born and reared under conventional conditions indicated that little or no hydrochloric acid secretion occurred during the first 24 h of life and that the time of onset of hydrochloric acid secretion varied between pigs. The amount of lactic acid formed by bacterial fermentation in the stomach also varied between pigs and there were indications of an inverse relationship between the concentration of hydrochloric acid and of lactic acid in the stomach content.

These findings were extended by observations on nine pigs from four separate litters born and reared either in a conventional environment or in one which had only been used occasionally for pigs. Gastric fistulas were established within 6 h of birth and the piglets returned to the sow. Sequential samples of stomach content were obtained from these pigs and the pattern of fermentation and secretion in the stomach was followed for periods up to 24 h.

In two pigs in a conventional environment hydrochloric acid secretion was not apparent until they were about 30 days old whereas in six pigs in a clean environment hydrochloric acid secretion was evident within the 1st week of life. Lactic acid concentration on the other hand was high in the stomach of pigs from the conventional environment and comparatively low in the pigs from the clean environment, particularly after they had started to secrete hydrochloric acid. A further pig born in a conventional environment and transferred at 24 h of age to a clean environment had a high lactic acid concentration in the stomach for 11 days but thereafter started to secrete hydrochloric acid and then had a low concentration of lactic acid in the stomach.

Prior to the onset of hydrochloric acid secretion the pattern of lactic acid production and hence the acidity of the gastric content was largely governed by the frequency of sucking, the lactic acid concentration increasing as the duration of the sucking to sampling interval increased.

The nature of the milk clot in all the pigs was similar regardless of whether hydrochloric acid was secreted or not and it is possible that in the achlorhydric pig the lactic acid produced by the gastric flora was sufficient to cause clot formation or to render the gastric pH suitable for enzymic clot formation.

Food conversion ratios in albino rats. By W. LANE-PETTER and M. R. HACKING, *Carworth Europe, Alconbury, Huntingdon*

Food intake and weight gain were measured in groups of albino rats of CFE strain raised within a strict barrier against infection. Group A comprised ten female rats with standardized litters of fourteen pups, five litters all male and five all female. Group B comprised sixty rats, thirty male and thirty female, from 21 to 63 days of

age. The diet was the standard breeding and maintenance diet used in the colony (diet CDD), which contains wheat 33·75%, barley 20·00%, soya-bean meal 12·50%, white-fish meal 15·00%, yeast 1·25%, fat supplement (42% fat in groundnut meal) 15·00%, NaCl 0·80%, vitamin premix 0·45%, and an inert binding agent 1·25%.

The average daily food intake of the rats in group B varied from 6·5% to 10·0% (maximum) of body-weight. Water intake was 2–2·5 times food intake. Weights attained at 63 days were approximately 300 g for males and 210 g for females. Conversion ratios varied from 1·0:1 in rats from 21 to 35 days, to less favourable ratios in (a) females from 42 days onwards, corresponding to the onset of oestrus, (b) both sexes with increasing age, (c) lactating females, especially later in lactation. There is some evidence to suggest that the conversion ratio is also affected by ambient temperature and by density of rats in the cage.

Some effects of dietary excesses of vitamins A and D in chicks. By T. G. Taylor*, Jean Kirkley* and K. M. L. Morris, *Department of Physiology and Biochemistry, The University, Reading*

Both vitamins A and D are toxic when fed in excessive amounts, but there is some evidence, reviewed by Nieman & Obbink (1954), that vitamin D may exert a protective influence against the harmful effects of excess vitamin A.

Groups of twelve male chicks (medium hybrids) were fed diets containing 1, 10, 100 and 1000 times the requirements of vitamins A and D in all sixteen combinations from 1 day of age. (The requirements of vitamins A and D were taken to be 2600 and 500 i.u./kg respectively). Blood samples were taken at 4, 5 and 6 weeks of age and the following determinations were made: packed cell volume, total plasma calcium, inorganic phosphorus, total proteins, acid and alkaline phosphatases, β-glucuronidase, sulphatases A and B and *N*-acetyl glucosaminidase.

Only the diets containing 1000 times the requirements of one or both vitamins influenced growth or any of the parameters measured.

The main effects of the diets containing 1000 times the requirements of vitamins were:

Vitamin A

(1) Packed cell volumes were greatly reduced.

(2) Plasma activities of β-glucuronidase, acid phosphatase and sulphatases were elevated.

(3) Plasma calcium was reduced.

Vitamin D

(1) Plasma calcium was increased.

(2) Plasma acid phosphatase was decreased.

(3) Plasma inorganic P was reduced, except when 1000 times the requirements of vitamin A was also fed.

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A marked antagonism between the effects of the two vitamins fed at toxic levels was observed only in respect of the plasma levels of Ca, P and acid phosphatase. These effects were confirmed in a second experiment. No consistent changes in plasma proteins, alkaline phosphatase or *N*-acetyl glucosaminidase were observed.

Chicks of Japanese quail (*Coturnix*) suffering from hypervitaminosis A also showed a large fall in packed cell volume and their bone marrow exhibited marked hyperplasia. Marrow smears from these birds were dominated by early normoblasts and immature heterophils, as opposed to late normoblasts and mature heterophils in controls. These results are consistent with the suggestion that excess vitamin A causes an increased fragility of the red cells (as shown by Dingle & Lucy (1962) in vitro), thus reducing their life span.

The increase in the plasma activity of three acid hydrolases in the chicks fed 1000 times their requirements of vitamin A may have been caused by a release of these enzymes from the lyosomes, but no information is available as to the tissue from which they arose. Since there was an antagonism between excess vitamins A and D only in respect of their action on plasma Ca, P and acid phosphatase it is possible that the major source of the latter enzyme was the bone and that the other acid hydrolases originated elsewhere.

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