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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Eighty-fifth meeting of The Nutrition Society, was held in the New Meeting Rooms of the Zoological Society, Regent's Park, London, NW1, on Friday, 2 December, 1966 at 10.30 am, when the following papers were read:

The food intake of the vitamin A–deficient calf. By J. T. ABRAMS, K. C. BARNETT, P. S. BRIDGE, A. C. PALMER and F. R. SPRATLING, *School of Veterinary Medicine, University of Cambridge*, and I. M. SHARMAN, *Dunn Nutritional Laboratory, Cambridge, and Medical Research Council*

In experiments to determine the effects of vitamin A deficiency on the food intake of calves, nine sets of twins, three of them monozygotic, were fed on the same basal vitamin A-free diet. All calves received fortnightly supplements of ergocalciferol and of tocopheryl acetate, the former by intramuscular injection of a solution in ethyl oleate, and the latter orally. Dosage at these times was at the rate of 10^4 i.u. of calciferol and 400 mg of tocopheryl acetate per 100 lb live weight. On these occasions one animal of each set of twins also received an intramuscular injection of an aqueous emulsion of retinol palmitate at the rate of 10^5 i.u. per 100 lb live weight.

Twin calves occupied adjoining loose boxes so as to minimize environmental differences. All animals were bedded on peat moss, so that the usual straw bedding could not contribute to their vitamin A intake. At the beginning of the period of vitamin A deprivation the average age of the calves was 83 days (range 50–107). For purposes of plasma retinol determinations the calves were bled regularly but not for at least 7 days after retinol injections.

Twin calves were pair-fed, with the pelleted food being freely available to both animals on a sufficient number of days (ideally 50%) to show how the voluntary intake of the deprived calf differed from that of its twin.

Initially the control calves had a mean plasma retinol level of 80 i.u./100 ml. For the deprived animals the initial mean was 87 i.u., the difference between the two groups not being significant ($P>0{\cdot}05$).

After a short period during which there were no consistent differences of food intake between paired calves, the appetites of the deprived animals relative to their controls fell regularly, with only brief and spasmodic remissions. The effect became quite marked when the plasma retinol levels of the deprived calves were still in the range of 40–50 i.u./100 ml. It was the first apparent sign of deficiency.

In the case of two pairs of calves the treatments were reversed after the initially deprived animal had begun to show signs of deficiency: appetite then increased quite markedly within about 5 days. In the case of a third pair of calves paired

feeding was ended after 150 days: the food intake of the control calf then increased markedly until the end of the experiment 90 days later.

It is believed that the accepted 'normal' plasma retinol levels for calves may have been set at too low a value and that there may be economic loss even though the plasma levels are in the range of 40–50 i.u./100 ml.

The authors gratefully acknowledge the help given by Vitamins Ltd, and Roche Products Ltd, in support of this work.

Variations in the composition of the skin surface lipid associated with dietary carbohydrates. By A. F. LLEWELLYN, *Department of Biochemistry, Guy's Hospital Medical School, London, SE1*

Low-fat diets consisting of 18% protein and either maize starch or sucrose as the carbohydrate were given to eight healthy young men for 16 days. Skin surface lipid was collected from the back with ether–methanol 2:1 as the solvent using a modification of the cup method (Boughton, Hodgson-Jones, MacKenna & Wheatley, 1955). The lipid was then analysed by qualitative thin-layer chromatography after the method of Wheatley (1964). When the dietary carbohydrate was starch the quantity in the triglyceride fraction of the skin surface lipid decreased whereas it increased when the dietary carbohydrate was sucrose.

The author wishes to thank Dr I. Macdonald for useful discussion of this work and the Dunhill Trust for a Research Fellowship and financial support of this work.

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Some long-term effects of dietary sucrose on the serum lipids of male baboons. By T. M. COLTART, *Department of Physiology, Guy's Hospital Medical School, London, SE1*

When healthy adult men eat a high-sucrose: low-fat diet for up to 4 weeks, there is a continuous rise in the concentration of fasting serum glycerides (Macdonald & Braithwaite, 1964). Because of the inconvenience of this diet to the volunteers, it was decided to transfer the work to baboons. This had the advantage of enabling the diet not only to be fat-free but also to be administered for a longer period of time.

Five healthy fully grown male baboons weighing 14–20 kg, were given a diet containing 75% sucrose, 18% calcium caseinate, and 7% yeast. Salts and vitamins were added and the whole diet was made up as a liquid. The amounts given were adjusted to keep the weights of the animals stable.

Fasting blood samples were taken at intervals throughout the 17 weeks on the diet and the serum glycerides estimated.

A 4 g/kg body-weight sucrose meal was given to each animal by stomach tube before the start of the diet and after 13 weeks on the diet. The sucrose was uniformly labelled with ^{14}C. Blood samples were then taken up to 5 h after the meal.

Results

(1) All five animals showed a rise in the concentration of fasting serum glycerides, maximum levels for each animal being reached between 3 and 5 weeks after the start of the diet. The peak value occurred at 3 weeks, with a mean level of 69 mg/100 ml serum. This represented a rise of 27 mg/100 ml serum on the mean fasting level.

(2) At 17 weeks the fasting serum glyceride level was still raised in all animals. The mean value of the samples from each animal taken at 15 and 17 weeks after the commencement of the diet was significantly elevated above the original mean value for all animals ($\bar{x} = 7$ mg/100 ml; $P = 0{\cdot}025$–$0{\cdot}01$).

(3) The ^{14}C counts from the sucrose meal after 13 weeks on the diet showed that there was three times as much incorporation of sucrose into glycerides as compared with the pre-diet value. This would indicate that in these male baboons that have been exposed to dietary sucrose over a long period of time, proportionally more of the sucrose ingested is converted to glyceride.

I am grateful to Cadbury Brothers Ltd, Bournville for a research grant.

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Sucrose tolerance and fasting serum glyceride concentrations in young men and women. By J. N. Crossley, *Department of Physiology, Guy's Hospital Medical School, London, SE*1

When healthy young men were given low-fat diets enriched with sucrose, the concentration of their fasting glycerides increased. Healthy young women eating the same diet for the same length of time showed a fall in their fasting serum glyceride levels (Macdonald, 1965). The end-products of the digestion of sucrose, glucose and fructose, enter the blood and are both converted in part to glycerides in the liver and adipose tissue. The contrasting effects of sucrose on fasting serum glyceride levels which were found in men and women could therefore be explained in two ways. Either there were differences in the rates of entry of glucose and fructose into the blood in men and women or there were differences in the metabolic conversion of glucose and fructose to glycerides. This second alternative would be supported if those men who developed high concentrations of glucose and fructose after sucrose meals had high fasting serum glyceride concentrations and those women who developed the same high concentrations of these hexoses after the same sucrose meals did not.

The fasting serum glycerides of twelve healthy young men (aged 21–33 years) and eleven healthy young women (aged 21–32 years) were estimated by a semi-automated technique. Four estimations of fasting serum glycerides were obtained for each subject at weekly intervals. After a 10 h fast each subject took a meal of 2 g sucrose/kg body-weight and the same meal was repeated 1 week later. In the women the meals were taken on days 1 and 8 of the menstrual cycle. Venous blood was taken before, and then at 15, 30, 60, 90 and 120 min after the meal. Serum fructose was estimated by an automated Roe's method (Roe, 1934) and serum glucose by a glucose oxidase method.

It was found that those healthy young men who had high fasting serum glyceride concentrations were those who developed high levels of serum glucose and fructose after sucrose meals. A comparable group of healthy young women who developed the same blood sugar changes in response to the same meals and who had the same range of fasting glyceride concentrations as the men showed no correlation between fasting serum glyceride concentrations and the changes in their serum glucose and fructose. These findings would be consistent with the view that there is a difference between men and women in the metabolic conversion of glucose and fructose to glycerides.

This work was done with the aid of a grant from the Medical Research Council.

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The daily food consumption of laying hens in relation to egg production.

By B. A. MORRIS and T. G. TAYLOR,* *Department of Physiology and Biochemistry, University of Reading*

It had been observed previously in balance experiments with colostomized hens that daily food consumption showed a definite pattern in relation to egg production and an experiment was performed to find whether or not the same phenomenon occurred in unoperated birds.

Thirteen pullets (Shaver 288) which had been in lay for about 3 months were housed singly in metabolism cages and fed a commercial layer's diet *ad lib.* for a period of 24 days. Food consumption was measured from 09.30–09.30 h and from 14.30–14.30 h each day and times of oviposition were recorded.

Table 1 shows that the mean food intake was reduced by approximately 25% during 24 h periods in which egg formation was in progress for only a few hours ('non-egg-forming' days). Of the thirteen birds, two failed to show the reduction in food intake on the one day when they did not lay.

Egg formation was not entirely absent on days designated as 'non-egg-forming' days, since when 14.30 h was taken as zero-hour the day covered the early stages of formation of the succeeding egg, and when 09.30 h was taken, it covered the

*Present address: Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh 9.

Table 1. *Mean food consumption (g/bird day ± SE) on 275 'egg-forming' and 31 'non-egg-forming' days by thirteen pullets observed over a period of 24 days with zero-hour at 09.30 h and 14.30 h*

Zero-hour, 09.30 h		Zero-hour, 14.30 h	
Egg-forming	Non-egg-forming	Egg-forming	Non-egg-forming
125·8 ± 1·3	93·9 ± 4·4***	125·1 ± 1·2	101·4 ± 3·7***

***Significantly different from mean of egg-forming days ($P < 0.001$, t test).

final stage of formation (shell calcification) of the preceding egg. With the 09.30 zero-hour mean food consumption was significantly less ($P < 0.01$, paired t test) on 'non-egg-forming' days than with 14.30 h. This suggests that the need for calcium on egg-forming days is not likely to be the main factor responsible for controlling food intake. Other possible regulators are the requirements for energy and protein.

The effect of undernutrition early in life on the brain and spinal cord of pigs. By J. W. T. Dickerson and J. Dobbing, *Department of Growth and Development, Institute of Child Health, London, WC1*

We have reported (Dickerson, Dobbing & McCance, 1967) that pigs undernourished from 2 weeks of age till they were 12 months old had a body-weight of 5–6 kg and a brain and spinal cord weight characteristic of normal animals at 10 weeks of age. The concentration of cholesterol in the forebrain and spinal cord of the undernourished animals was lower than normal for their age. On rehabilitation the weight of the body, and of the brain and spinal cord increased to about 80% of that expected. Though the concentration of cholesterol in the central nervous system increased during rehabilitation it remained below normal for the animals' age in the forebrain and spinal cord.

The present paper concerns the effect of undernutrition, and of rehabilitation, on the concentrations of cerebrosides, and sphingomyelin and on those of the phosphatides of ethanolamine, choline and serine + inositol. These substances were separated from chloroform: methanol (2:1, v/v) extracts of tissues from three pigs of each kind by thin-layer chromatography on silica gel. Cerebrosides were determined as sphingosine (Lauter & Tram, 1962) and the phospholipids as phosphorus (King, 1932).

In the cerebrum and spinal cord of the undernourished and rehabilitated animals the concentrations of cerebrosides, like those of cholesterol, were lower than they were in normal animals of the same age.

In normal animals the concentrations of total phospholipids, and of each of the fractions separated, with the possible exception of cord sphingomyelin, appeared to reach their adult values by 12 months of age. In the cerebrum, choline phosphatides did not further increase after 4 weeks of age. In the undernourished animals 1 year old the concentrations in the cerebrum were similar to those in normal

animals of the same brain weight (10 weeks old). The concentrations in the spinal cord were, however, closer to those of normal animals of the same body-weight (4 weeks old).

On rehabilitation the phospholipid concentrations reached levels characteristic of normal animals of the same age.

The results were discussed in the light of current concepts of myelin composition.

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The flow of intestinal digesta in the pig. By D. E. NOAKES, K. J. HILL, C. P. FREEMAN and E. F. ANNISON, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Six Large White or Large White × Wessex pigs, 17–40 kg body-weight, with intestinal re-entrant fistulas were used to measure the flow of digesta in different regions of the digestive tract. Flow was measured by disconnecting the re-entrant cannulas and collecting the material which flowed from the cranial cannula in 50–100 ml quantities. After sampling, the digesta were immediately returned to the intestine through the caudal cannula.

A standard diet (17·6% protein, 2·7% fat, 3·3% fibre) and water were available *ad lib.* and in pigs which ate about 1 kg and drank 1·5 l. water during an experiment (8 h) the mean hourly flows in different parts of the tract were: anterior jejunum—400 ml (17 kg body-weight); 460 ml (27 kg); posterior jejunum—197 ml (28·5 kg); ileum—120 ml (17 kg). There were little variations in the hourly flow rate although in longer experiments there were reduced flows during the night period when the pigs ate little. Flow rates in pigs which ate greedily and consumed greater amounts of food and water were correspondingly greater.

During the first 1–2 h collection after a fast of 12–24 h, 200–250 ml/h of a mixture of water, saliva, gastric juice and bile of low dry matter was collected from the duodenum. Subsequently, the flow decreased but increased markedly when a meal was consumed. Thus pig 102 (33 kg), which ate a single meal (350 g) and drank 2 l. of water over 8 h, had a maximal flow of 1500 ml/h and a mean flow of 893 ml/h from the duodenum. Collection from the same pig 2 weeks later (40 kg) over an 8 h period during which it ate 2 kg and drank 3·0 l. water gave a maximal flow of 2250 ml/h and a mean flow of 1240 ml/h. Similar large flows in response to feeding after a period of starvation were obtained from other regions of the tract, although they were smaller than those obtained from the duodenum. The introduction of 10% lard into the diet had no apparent effect on flow rate in the jejunum.

Application of the flow values has been made to analytical data on intestinal content and some of this quantitative information was discussed.

The absorption of micellar fat in pigs. By C. P. FREEMAN, E. F. ANNISON, D. E. NOAKES and K. J. HILL, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

The importance of micelle formation during the digestion and uptake of fats in man in now well established (Hofmann & Borgström, 1962; Borgström, 1967) but there are little data on other species. We have, therefore, examined the process of fat digestion in the pig, in which a wide range of dietary fats is digested, and have demonstrated the occurrence of micellar lipid in the contents of the small intestine after the ingestion of fat.

The approximate composition of micellar lipid (free fatty acid, 45%; monoglyceride, 25%; diglyceride, 10%; triglyceride, 5%; cholesterol, 5%; cholesterol esters, 5%; phospholipid, 5%) was closely similar to that reported in man (Hofmann & Borgström, 1964). The proportions of individual lipid classes in micellar lipid were independent of the composition of dietary fat, and of the level of fat intake. The absorption of micellar fat in vivo was examined using pigs prepared with double re-entrant cannulas in the jejunum. Micellar solutions of ^{14}C-labelled oleic acid (0·1%) in sodium taurocholate (0·5%) were circulated through the jejunal loop, and samples examined at 10 min intervals. The radioactivity of the solution declined steadily (50% disappearance in 70 min) and the total volume remained largely unchanged. In experiments with mixed micellar solutions of ^{14}C-labelled stearic acid and ^{3}H-labelled oleic acid no differences were observed in the relative rates of uptake of these acids. Similar results were obtained when the uptake of stearic and oleic acids in micellar solution was examined in vitro using isolated rings (Crane & Mandlestam, 1960) of jejunum from pigs (age 3–4 weeks), and no effect of chain length of fatty acid in the range C-12 to C-18 on the uptake of fatty acids in micellar form could be demonstrated using this preparation.

These results suggest that the mechanism of fat absorption in pigs is closely similar to that in man. Differences in the digestibilities of fatty acids of varying chain length and degree of unsaturation must be ascribed to differences in the rates of lipolysis and of micelle formation, and not to the effects of fatty acid structure on rates of micelle absorption.

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Interdependence in amino acid allowances. By J. P. F. D'MELLO, D. HEWITT and D. LEWIS, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics.*

It is no longer satisfactory merely to specify minimum levels of essential amino acids in defining adequate diets for animals since growth may be retarded by excesses, as severely as by deficiencies of specific amino acids. The ill effects of feeding such imbalanced diets can generally be prevented by including greater levels of other amino acids in the diet. A concept of an agent and target interaction has in fact been proposed (see Lewis, 1965) to account for the relationship. The agent is the amino acid addition of which leads to the phenomenon, and the target is the amino acid which must be added to counteract the growth depression.

It can be considered that the consequence of the addition of the agent amino acid is that the requirement for the target amino acid is increased. A specific interaction of this type has been described by Smith & Lewis (1966) for lysine (as agent) and arginine (as target). This interrelationship in terms of allowances has been defined in quantitative terms. Using young chicks it has been demonstrated that a satisfactory growth rate is obtained when the dietary lysine level is 1·10% at an arginine content of 1·0%. When the lysine level is raised to 1·5% of the diet the arginine requirement is around 1·3% whilst at a lysine content of 1·9% the arginine need is 1·6%. This can be expressed in the form of a linear relationship.

A similar interdependence in amino acid allowances has been examined for the tryptophan–threonine interaction (see Florentino & Pearson, 1962) and for leucine–isoleucine (see Spolter & Harper, 1961).

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Relationships between the composition of dietary proteins and the changes in blood plasma amino acid levels after a single meal containing a protein. By S. BURACZEWSKI, J. W. G. PORTER, D. R. WESTGARTH and A. P. WILLIAMS, *National Institute for Research in Dairying, Shinfield, Reading*

The levels of amino acids in pooled samples of deproteinized blood plasma from groups of six adult male hooded Norwegian rats were determined with an automatic amino acid analyser (EEL, Halstead, Essex, England) after single meals of 500 mg of casein, α-protein, ADM assay protein, cod muscle protein (unheated and heat-damaged) and zein given alone or with 500 mg of sucrose or lactose. Rats weighing 300–400 g maintained on a commercial basal diet were kept in anti-coprophagy cages for 2 days and were then starved for 18 h before receiving a test meal. Samples of portal and systemic blood were taken from anaesthetized rats after starving or 1, 2 or 3 h after the meal. The contents of the stomach and small intestine were also collected for analysis of dry matter and of content and distribution of nitrogenous substances.

The results for the measured content of plasma amino acids were interpreted either by comparing the levels in portal blood after feeding with those in fasted rats,

or by comparing the levels in portal and systemic blood of the same rat. Linear regression analysis of the results considered in these alternative ways showed that when native proteins were fed alone or with carbohydrate either procedure gave significant or highly significant correlations between the increases in individual essential amino acids after feeding and the amino acid composition of the protein fed. When heat-damaged cod muscle proteins having lowered contents of available amino acids were fed, the levels of amino acids in portal and systemic blood in fed rats were often lower than those in fasted rats. However in such cases direct comparison of the levels in portal and systemic blood yielded results that again showed statistically significant correlations between these differences and the content of amino acids in the protein fed.

Feeding carbohydrate with a protein increased the slope of the regression line relating the amino acid composition of the protein to the differences in amino acid levels in portal and systemic blood. The effect occurred with each protein, except zein, and tended to be more pronounced with added lactose than sucrose. This accorded with the slower rate of stomach emptying found when proteins were fed with carbohydrates, and the exceptional behaviour of zein was due to the better digestion and utilization of this protein when it was given with carbohydrate.

This work was in part supported by Grant No. AM-06771 from the National Institutes of Health, Bethesda, Md, USA.

Amino acid digestibility as a measure of amino acid availability. By J. E. Ford, K. M. Henry and J. W. G. Porter, *National Institute for Research in Dairying, Shinfield, Reading*

Kuiken & Lyman (1948) proposed a rat assay to measure the biological availability of all the amino acids in food proteins, in which the net faecal excretion of amino acids is measured and compared with the intake of amino acids in the diet. We have applied this method to the measurement of available lysine, methionine, leucine, isoleucine, arginine, histidine and valine in four whale meat meals which had similar amino acid composition, but represented a wide range of nutritive quality, and in three preparations of dried skim milk—one of good quality, another damaged by heating, and the third damaged by storage under warm, humid conditions. For each sample the measured percentage availabilities of all the amino acids were approximately equal to the percentage true digestibility of the nitrogen of the protein.

The values were in general higher than, but correlated closely with, those predicted from chemical and microbiological tests, with the marked exception that for lysine in the damaged milks the rat assay values were considerably higher than those given by the chemical assay of Carpenter (1960). The interaction of lysine and lactose in heated milk is well known and our results seem to indicate that the resulting lysine–lactose complex must be metabolized or absorbed in the intestine, for no corresponding amount of lysine was recovered from the faeces. Growth tests with

rats showed that the protein efficiency ratio of the damaged milks was very low, but increased almost to equal that of the good-quality preparation when they were supplemented with lysine in the amounts calculated from the chemical estimation of the loss.

Thus, our tests using the Kuiken & Lyman method indicate that in this procedure the availability of amino acids is determined mainly by the digestibility of the protein. However, it is clear that other factors may also exert an influence. Bunyan & Price (1960) measured NPU in a series of whale meat meals that included the four that we have examined and found that amino acids absorbed from the poorer meals of lower digestibility were relatively poorly retained by the rat, possibly reflecting differences in the pattern of available amino acids released from the different meals during digestion.

We must conclude that amino acid digestibilities may give misleading indications of amino acid availability.

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Protein utilization in germ-free and conventional chicks given a purified diet. By W. S. MILLER, *National Institute for Research in Dairying, Shinfield, Reading*

In each of two experiments chicks in germ-free and conventional environments were given a practical diet from hatching to 21 days of age. For the next 7 days selected pairs of chicks in each environment were offered *ad lib.* a nitrogen-free diet or a diet containing casein, gelatin and L-cystine providing 26·4% protein (N × 6·25). One pair of male and one pair of female chicks received each diet in each environment. For the last 4 days the excreta of each pair of birds were collected daily.

The combined results of the two experiments are given below. In both experiments the chicks reared in the germ-free environment were heavier at the start of the test period. On the N-free diet but not on the casein–gelatin diet the N excretion per g food eaten was significantly lower ($P<0.05$) in the conventional than in the germ-free chicks. However, the NPU of the casein + gelatin diet, calculated by dividing the difference in the N balance of chicks receiving the two diets by the difference in N intake, was no greater in the conventional than in the germ-free environment. In these experiments there was no difference in efficiency of protein utilization between germ-free and conventional chickens.

	Mean body-wt (g)		mg N excreted/g food eaten		NPU
	N-free	Casein–gelatin	N-free	Casein–gelatin	Casein–gelatin
Germ-free	200	293	9·0	19·9	62
Conventional	164	258	5·6	18·2	63

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A re-appraisal of the Miller–Payne equations at high protein levels. 1. The proportion of dietary energy coming from protein. By K. J. CARPENTER and K. ANANTHARAMAN, *School of Agriculture, University of Cambridge*

Chicks fed on diets containing high levels of groundnut flour as the protein source (Carpenter & de Muelenaere, 1965) grew faster than expected from the formulas of Miller & Payne (1963). The formulas were developed primarily, as regards poor proteins, from the response of rats to wheat gluten (Miller & Payne, 1961) but were claimed as having general significance. We have tried to analyse the cause of the differences between predicted and actual results.

The formulas require metabolizable energy (ME) values for the diets to which they are applied. Miller & Payne (1959) recommended that this be estimated, where necessary, as '0·95 × gross energy (kcal/g) − 0·075 × dietary N (%)'. This can be a considerable overestimate for a mixture based on vegetable protein concentrates such as diet E (groundnut flour 58, L-lysine hydrochloride 0·43, arachis oil 5, minerals 6, vitamins and starch *ad* 100). The following results were obtained with young rats using chromic oxide as an inert marker substance:

Diet (protein source)	E (groundnut plus lysine)		G (whole egg)	
Type of value	Determined	Calculated	Determined	Calculated
Digestible protein (N × 6·25) (%)	25·2	26·7*	23·5	23·8*
Metabolizable energy (corrected to N equilibrium) (kcal/g)	2·63	3·46	3·49	3·70
P (% of dietary ME from protein†)	40·6	32·4	28·4	27·0
Protein score (FAO, 1957)	—	56	—	100
NDpCal% (protein calories retained per 100 metabolizable calories eaten†)	14·5	8·1 (6·1)‡	13·3	14·2 (14·1)‡
Live-weight gain (g/day)	4·00	2·16 (0·73)‡	3·57	3·98 (3·67)‡

*Assuming a constant 95% digestibility of the determined dietary N.

†Following the conventions implicit in the formula of Miller & Payne (1963), of assigning 26·6 kcal/g digested dietary N and 25 kcal/g retained N.

‡Using the determined *P* values in the formulas.

For the egg diet G the prediction of growth rate proved accurate. However, with diet E, although quite different predictions were obtained according to whether 'calculated' or 'determined' *P* values were used in the formulas, neither prediction corresponded to the results actually obtained with the rats.

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A re-appraisal of the Miller–Payne equations at high protein levels. 2. Nitrogen retention from poor proteins. By K. ANANTHARAMAN and K. J. CARPENTER, *School of Agriculture, University of Cambridge*

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Our experiment was designed to test the formulas of Miller & Payne (1961, 1963) for predicting nitrogen retention on the assumption that NPU falls linearly with *P* (see table). Six diets of the type already described (Carpenter & Anantharaman, 1967) and a N-free diet were fed *ad lib.* for 10 days to both young rats and chicks. Net N retention was determined by direct carcass analysis. The ME values of the diets were determined from the gross energy of food and faeces. The results, in comparison with the predictions from the formulas were:

Diet code	B	C	D	E	G	H	(SE of
Protein source (score, FAO (1957))	Wheat gluten (40)		Groundnut+lysine (56)		Egg (100)	Mixed (73)	diet means)
P (calculated % of dietary ME from protein)	21·0	32·8	20·2	32·4	27·0	31·1	—
NDpCal % (protein calories retained per 100 diet calories eaten):							
(a) Predicted for all species	6·3	6·4	8·1	8·1	14·2	10·7	—
(b) Determined for rats	5·4	8·4	9·2	14·5	13·3	13·4	±0·5
(c) Determined for chicks	8·2	11·4	11·0	17·9	17·5	19·1	±0·2

Three of the consequences of the Miller & Payne (1961, 1963) formulas and our own relevant findings in each case, are:

(1) 'For a particular protein source increasing the *P* value above 27 gives a falling off in NDpCals at a rate equal to that obtained with decreasing the value below 27'. In both species diet C has given better retention than diet B, and diet E better than D. (If the formulas had been applied to actual *P* values the discrepancies would have been greater, since C and E would then have been predicted as actually inferior to B and D respectively.)

(2) 'Maximum NDpCals obtainable with any diet for any species is 14·6%'. Our chicks have given higher values than this. The highest value was obtained with the practical mixture H.

(3) 'Different species retain the same NDpCals for any given diet.' Our chicks, males from a strain selected for rapid growth, have given higher values than did the rats.

We believe that published data generally indicate that poor proteins at high levels give N retentions greater than predicted and that Miller & Payne (1961) misinterpreted their own results in this respect. Miller & Payne (1964) and Payne (1966) have already considered some possible modifications to their formulas.

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Protein depletion and toxic liver injury due to carbon tetrachloride and aflatoxin. By A. E. M. McLEAN, *Medical Research Council Laboratories, Toxicology Research Unit, Woodmansterne Road, Carshalton, Surrey*, and ELIZABETH K. McLEAN, *Department of Pathology, Royal Free Hospital, London*

Rats fed a protein-free diet for 4 days or more become highly resistant to the lethal and liver-damaging effects of carbon tetrachloride (CCl_4).

During protein depletion, the activity of the liver microsomal enzymes that attack compounds such as pyramidon, barbiturates and CCl_4 is largely lost. The protein-depleted rat can be forced to synthesize these enzymes by a single injection of DDT or three injections of phenobarbitone (McLean & McLean, 1966). These animals are then sensitive to CCl_4 again.

In contrast, aflatoxin is more lethal to protein-depleted animals than to normal ones (Madhavan, Suryanarayana-Rao & Tulpule, 1965).

Protein-depleted animals can be protected against aflatoxin poisoning by a single dose of DDT.

Influence of diet and DDT on the lethal effects of carbon tetrachloride and aflatoxin

	CCl_4 (ml/kg)		Aflatoxin (mg/kg)	
Diet	LD_{50}	95% probability range	LD_{50}	95% probability range
Stock (41B)	6·4	5·4– 7·6	25	22–28
No protein	14·7	13·4–16·1	10	8–14
No protein+DDT	4·3	3·7– 5·0	19	15–23

Carbon tetrachloride was given orally as a 1–1 mixture with paraffin. Aflatoxin, consisting of mixed crystalline aflatoxins containing 18% aflatoxin B_1, was dissolved in dimethylsulphoxide and injected intraperitoneally. The LD_{50} was calculated using the deaths in 8 days after giving the poison. The protein-free diet was fed for 7 days before giving the poisons. DDT was injected subcutaneously in a dose of 100 mg/kg 7 days before poisoning.

The most likely explanation for these findings is that carbon tetrachloride is activated by microsomal enzymes to form the true toxic substance; aflatoxin on the other hand is inactivated by the same enzyme system.

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Thiamine status of lambs in relation to cerebrocortical necrosis. By GWYNETH LEWIS, S. TERLECKI, L. M. MARKSON and RUTH ALLCROFT, *Central Veterinary Laboratory, Weybridge*, and J. E. FORD, *National Institute for Research in Dairying, Shinfield, Reading*

Cerebrocortical necrosis (CCN) in ruminant calves responds dramatically to parenteral administration of thiamine (Davies, Pill, Collings, Venn & Bridges, 1965). This implies a failure of ruminal synthesis of the vitamin or of its absorption from the gut, or some aberration in its metabolism within the animal.

Draper & Johnson (1951) induced symptoms of thiamine deficiency in preruminant lambs by feeding a thiamine-low diet. Since the histopathology was not described it seemed necessary to ascertain whether the lesion of uncomplicated thiamine deficiency was similar to that found in CCN (Terlecki & Markson, 1961).

Lambs given a thiamine-deficient diet from 48 h of age reached a crisis after 18–30 days; some died, others were killed *in extremis*. Control lambs remained healthy and were killed at 4 months. Tissues taken for histological examination showed no gross lesions of CCN. Two of the deficient animals showed slight changes in the cortex and thalamus resembling those found in field cases of CCN. Samples of urine, rumen contents and liver were assayed for thiamine, and blood samples for pyruvate. Urinary thiamine levels were 3·2 and 4·9 μg/ml for two control lambs and 0·007–0·034 μg/ml for four deficient lambs. Corresponding values for liver were 7·3 and 5·0 μg/g wet weight and $<$0·5 μg/g, and for rumen contents 5·5 and 5·9 μg/g and 0·52–1·70 μg/g. Blood pyruvate levels in the deficient lambs were abnormally high.

CCN occurs in calves and sheep of 3–12 months on a variety of diets. We therefore determined thiamine levels in urine, liver and rumen contents of grazing and stall-fed normal sheep of this age group for comparison with affected sheep. Livers of six healthy grazing sheep contained 1·5–5·2 μg/g, and of five suffering from CCN 0·4–1·3 μg/g. Corresponding values for rumen contents were 0·5–1·0 μg/g and 0·3–0·7 μg/g. In sixteen healthy grazing sheep, urine concentrations were 0·19–0·76 μg/ml; in eight animals on a stall diet the values were from 1·3 to $>$10, and in three CCN animals 0·035, 0·035 and 0·053.

The few results for rumen contents indicate no failure in rumen synthesis of thiamine in CCN, although liver and urine values suggest a low thiamine status.

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