The Two Hundred and Sixty-fourth Scientific Meeting of the Nutrition Society was held in the Edward Lewis Lecture Theatre of the Middlesex Hospital Medical School, Cleveland Street, London W1P 7PN, on Friday, 15 March 1974, at 14.00 hours, when the following papers were read:

Studies on the growth and composition of rats during realimentation on a restricted intake regime. By A. M. STEWART*, *Department of Agriculture, University of Rhodesia, Salisbury, Rhodesia*

Animals which exhibit compensatory growth appear to use their dietary energy more efficiently than during normal growth from the same body mass (Allden, 1970). If this is true it may be expected that, during the early stages of recovery from undernutrition, animals will synthesize more body material than will their normal counterparts when both groups are given the same amount of food. This experiment was conducted to test the hypothesis.

Male Sprague-Dawley rat pups were reared to 50 g in litters of sixteen (Group C) or eight (Group B) or to 75 g in litters of three, after which they were reduced to 50 g live weight (Group D). Once they had reached 50 g body-weight by the selected patterns, they were given 6 g food/d for 1 week. During the 2nd and 3rd weeks they received 8 and 10 g/d respectively. At the end of the experiment, body-weight was significantly highest in group D animals. The main reasons for this were the high increases in water and fat in these animals (Table 1).

Table 1. *Mean gain in empty body-weight and in carcase components (g) of groups of four rats given the same restricted intake for 3 weeks from 50 g body-weight*

	Group			LSD	
Measurement	B	C	D	$P=0.05$	$P=0.01$
Body-weight	74·0	74·9	93·8	3·9	5·7
Water	49·8	48·6	62·6	2·5	3·6
Protein	13·7	13·5	13·5	1·5	2·2
Fat	6·3	10·2	13·7	1·2	1·8
Ash	4·3	2·9	4·0	0·7	1·1

LSD, least significant difference.

The results will be discussed in relation to muscle parameters and to the severity of nutritional deprivation experienced by each group.

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Thanks are due to Mr E. Kumbula for technical assistance.

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Developmental changes in muscle protein metabolism in congenitally malnourished rats. By D. J. MILLWARD, D. O. NNANYELUGO and P. J. GARLICK, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

We have shown previously that in muscle, protein deficiency reduces both the capacity for protein synthesis (RNA: protein ratio) and the efficiency of synthesis (synthesis rate: RNA) (Millward, Garlick, James, Nnanyelugo & Ryatt, 1973). We have now measured the capacity and efficiency of protein synthesis and calculated the rate of protein breakdown in colonies of hooded rats fed on either a good, or a marginally inadequate diet (energy supplied by protein : total metabolizable energy, 0·068) for ten generations (Stewart, 1973). The results are shown in the table.

Table 1. *Protein synthesis and breakdown rates in rats given either a good or an inadequate diet*

Age (d)	Growth rate of muscle protein* (/d)	RNA: protein ratio ($\times 10^3$)	Protein synthesis rate* (/d)	Synthetic efficiency (g protein/g RNA per d)	Protein breakdown rate*† (/d)	Half-life (d)
			Well-fed			
23	0·061	15·00±0·73	0·286±0·026	19·15±1·69	0·225	3·1
46	0·030	11·32±0·80	0·161±0·023	14·20±1·09	0·131	5·3
65	0·017	8·39±0·65	0·115±0·020	13·80±3·00	0·098	7·1
130	0·007	5·20±0·46	0·053±0·004	10·16±0·19	0·046	15·1
330	0·003	4·33±0·55	0·049±0·006	11·45±1·02	0·046	15·1
			Malnourished			
30	0·056	12·71±0·61	0·138±0·023	11·40±1·89	0·082	8·5
60	0·041	10·25±0·22	0·109±0·014	11·00±1·2	0·068	10·2
120	0·009	5·58±0·21	0·065±0·004	11·83±0·61	0·056	12·4
190	0·003	4·89±0·33	0·063±0·002	9·70±1·04	0·060	11·6
330	0·001	4·60±0·53	0·042±0·007	9·10±0·52	0·041	16·7

*Fractional rate constant.

†Calculated as synthesis rate − growth rate.

There was an age-dependant fall in both the capacity and efficiency for protein synthesis in the well-fed rats. The reduced rates of synthesis in the young malnourished rats resulted from a low efficiency of synthesis. The rate of protein breakdown was considerably slower in the young malnourished rats than in the well-fed animals.

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Protein and amino acid requirements of the growing meat rabbit. By D. SPREADBURY (introduced by J. DAVIDSON), *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The level of crude protein required in the diet of growing rabbits has been given by various workers as between 140 and 220 g/kg. The US National Research Council (1966) concluded that the rabbits' sensitivity to protein quality was unknown. More recently, evidence obtained with synthetic amino acid mixtures indicates that despite the contribution of bacterial protein from coprophagy, high-quality dietary proteins are essential.

Experiments to estimate the protein and amino acid requirements have been carried out with growing rabbits of the New Zealand White breed, reared under barrier-maintained conditions until weaning at 28 d. The growth period studied was from 35 d until slaughter at 56 d.

When the crude protein (N×6·25) level in a mixed-cereal–fish-meal diet was raised from 120 to 300 g/kg, no increase in growth occurred above that found with 140–150 g crude protein/kg. The mean maximum growth rate over the 21 d period was 45 g/d. Nitrogen balance data and body analyses substantiated the results of these growth experiments.

To investigate the effect of protein quality, a variety of protein concentrates were used to supply approximately half the crude protein in an oat-based diet. The resultant growth rates divided the diets into two groups, giving significantly different responses in growth: those containing fish meal, soya-bean meal or casein (>40 g/d), and those including groundnut meal, gelatin or maize gluten (<30 g/d).

The addition of lysine and methionine to the diet containing groundnut meal restored growth rate to that of the higher group. The optimum levels for lysine and total sulphur amino acids were found to be 9 and 5·5 g/kg diet respectively. Adamson & Fisher (1973) found the requirements to be 7 and 6 g/kg diet respectively.

The use of a purified diet based on dextrin and casein permitted the feeding of a lower level of arginine than could have been achieved with natural diets. Such a diet allowed normal growth, and suggested that the requirement for this amino acid was no more than 6 g/kg diet. This contrasts with the findings of Adamson & Fisher (1973), who concluded that the growing rabbit required 10 g arginine/kg diet, an unusually high figure for a mammal.

This work was supported by a grant from the Agricultural Research Council.

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Effects of age and diet on the secretion of insulin and growth hormone in rabbits. By M. R. TURNER, K. A. ALLEN and K. A. MUNDAY, *Department of Physiology and Biochemistry, The University, Southampton SO9 3TU*

Effects of protein-energy malnutrition (PEM) on the secretion and actions of hormones have implications not only in the aetiology and treatment of nutritional

disorders, but also for metabolic diseases such as diabetes mellitus. This paper describes work on rabbits subjected from weaning to a severe protein deficiency achieved without the usual concomitant decrease in total energy intake. Results are compared with those from control animals in their first day of life (newborn).

The plasma insulin level in newborn rabbits (21 μU/ml) was more than twice that of older control animals (mean 8 μU/ml). Although the newborn had not been fasted prior to sampling, their plasma glucose concentration was only 3·22 mmol/l, significantly less than in older animals (mean 8·15 mmol/l). Therefore the higher insulin level at birth may be due to age difference. Following a glucose challenge (2 g/kg body-weight intraperitoneally) the mean plasma glucose increment was 10·54 mmol/l in the newborn, but in adults was much greater (mean 23·31 mmol/l). The use of body-weight in animals of different age to determine the dose required to produce a standard increment in plasma concentration appears unsatisfactory, a problem which is discussed more fully for diabetogenic drugs elsewhere (Turner & Heard, 1974). However, despite the large differences in the maximal glucose increment, variations in the maximal insulin response were much less pronounced, ranging from 15 μU/ml in the newborn to 36 μU/ml in 18-week-old animals.

In the newborn, insulin secretion was less than would have been expected from the size of the glucose increment, which is compatible with the poor glucose-stimulated insulin secretion observed in vitro in pancreas from newborn rabbits (Turner, Allen & Munday, 1974). The insulin increment in response to glucose increased with age to a maximum of 30 μU/ml in the adult, similar to that shown previously to be the maximal possible response to oral glucose. Diet had a clear-cut effect on insulin secretion. After 6 weeks on the protein-deficient (LP) diet the insulin secretory response to glucose was virtually absent. Apart from inefficient food utilization, and retarded growth, the general health of the animals at this time was apparently good. After 18 weeks on the diet, and in one animal after only 12 weeks on the diet, the LP rabbits abruptly became apathetic and began to appear outwardly less 'healthy'. In these animals the fasting plasma growth hormone (GH) level was elevated from the mean control value of 7 ng/ml to a mean of 24 ng/ml, which is characteristic of PEM in man. Except in the newborn, there was no secretion of GH in response to an intraperitoneal challenge with arginine (2·8 mmol/kg) or lysine (5·5 mmol/kg) nor was there a reduction in the GH concentration following glucose administration in either dietary group at any age.

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Effects of varying the dietary protein source on reproduction in rats.

By M. R. TURNER and K. A. MUNDAY, *Department of Physiology and Biochemistry, The University, Southampton SO9 3TU*

Rats of the Wistar Albino strain were mated at 200±10 g body-weight and fed throughout the reproductive cycle on unrestricted amounts of diets containing

100 g/kg dry weight as protein derived from one of ten different protein foods, namely: (1) accelerated freeze-dried egg, (2) casein, (3) white fish meal, (4) soya-bean meal, (5) extracted soya-bean protein, (6) oatmeal, (7) barley meal, (8) a mould (mycoprotein), (9) whole wheat flour, (10) maize gluten (prairie meal). The concentration of protein in the diet was selected as being just able to support reproduction and therefore likely to allow differences in dietary quality to be demonstrated as variations in reproductive ability.

The protein quality of these protein sources was measured by net protein utilization (NPU) assay. Reproductive performance was assessed both in terms of litter size, body-weight and viability of the offspring, and as a single figure intended to incorporate all of these variables designated the Reproduction Index.

$$\text{Reproduction Index (RI)} = \frac{\text{the total weight of offspring weaned}}{\text{the number of litters brought to term.}}$$

It has been shown that reproductive performance under the conditions described is not merely a function of protein quality as measured by NPU, but that the total food consumption by the mother is of prime importance. It has been demonstrated previously that rats consuming marginally protein-deficient diets based on casein are unable to increase their food consumption sufficiently to meet the nutrient needs for optimal lactation (Turner, 1973). This observation is confirmed in these experiments, both food intake and reproduction performance being poor in the casein group (RI 33). At the other extreme, the 'palatability' of the barley meal and oatmeal diets was high, and reproductive performance in these groups was comparable with that of the egg group (RI: egg, 182; oatmeal, 179; barley meal, 175). Rats eating the mould diet (RI 142) and the soya-bean meal diet (RI 136) also reproduced very well, but the other dietary protein sources, namely fish meal (RI 101), casein (RI 33), extracted soya-bean protein (RI 24), whole wheat (RI 66), and maize gluten (RI 18), were found to be inadequate.

It has been shown that 'palatability', which determines the total food intake, is more important for optimal reproduction than is protein 'quality'. The unsuitability of casein as a dietary protein for nutrition experiments of this type is particularly emphasized.

We are grateful to Miss F. Foord for her skilled technical assistance throughout this project.

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The effect of protein supplementation in early pregnancy on the growth of the rat foetus and placenta. By D. J. NAISMITH and B. L. G. MORGAN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

During the first 2 weeks of pregnancy, when competition by the foetuses for nutrients is minimal, the rat dam builds up a substantial store of protein in her tissues.

This is achieved by raising food intake and by increasing the efficiency with which she uses protein (Naismith, 1969, 1973). During the last week of pregnancy, when the foetuses are growing rapidly, the protein store is catabolized, irrespective of the concentration of protein in the maternal diet. If the transient protein store is used to augment the supply of amino acids for foetal growth, then a supplement of protein given during the early 'anabolic phase' to undernourished dams should have an appreciable influence on the growth of the products of conception.

To test this hypothesis, eleven rats were fed, from conception, on a low-protein diet containing 60 g casein/kg. The low-protein diet was given to another eleven rats, litter-mates of the control group, but from days 6–11 inclusive, the concentration of protein in the diet was raised to 250 g casein +2·5 g DL-methionine/kg. On day 22 the mothers were killed, and the foetuses and placentas were weighed and analysed for protein and DNA. The results are summarized in Table 1.

Table 1. *Mean weights and compositions of individual placentas and foetuses of rats fed on a low-protein diet, supplemented or unsupplemented in early pregnancy*

(Eleven litter-matched dams in each group.)

Diet	No. in litter	Placenta			Foetus		
		Weight (mg)	Protein (mg)	DNA (μg)	Weight (g)	Protein (mg)	DNA (mg)
Low-protein	10·7	406	18·8	132	3·310	203	65
Low-protein + supplement	11·9	448	23·8	187	4·794	296	85

Litter size was not influenced by the change in diet. The protein supplement had the effect of raising the body-weight of the individual foetus by 45%, and of increasing tissue protein and tissue cellularity. The weight of the individual placenta was increased by 10%, and the number of cells in the placenta, as measured by DNA content, by 40%. Thus additional protein supplied to the mother in early pregnancy was clearly used to promote the growth of the conceptus after withdrawal of the supplement. Improved transport of nutrients by a larger placenta may also have contributed to the better nourishment of the foetus.

The nutritional implications of this observation will be discussed.

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The influence of the composition of the diet on the digestion of organic matter and nitrogen in sheep receiving diets containing hay, barley and flaked maize. By D. G. CHAMBERLAIN and P. C. THOMAS, *The Hannah Research Institute, Ayr*

Three wether sheep fitted with ruminal and duodenal re-entrant cannulas received a series of diets based on chopped hay and a cereal mixture, containing 50 parts rolled barley and 50 parts flaked maize, in five successive periods. Details of the

diets and the order of the treatments are given in Table 1. All diets were given in two equal meals at 10.00 and 22.00 hours each day and at each meal a capsule containing 1 g chromic oxide powder was given through the rumen cannula. Water and mineral blocks were available *ad lib.* Each period consisted of 2 weeks controlled feeding followed by a 7 d period of faecal collection and a 5 d period during which samples of rumen and duodenal digesta were taken for analysis. The procedure for the collection of duodenal digesta was similar to that of Nicholson & Sutton (1969).

The intakes, duodenal flows and faecal losses of organic matter and nitrogen (adjusted to give 100% recovery of chromic oxide) are given in Table 1.

Table 1. *The intake, duodenal flow and faecal loss of organic matter and nitrogen in sheep receiving diets consisting of chopped hay, rolled barley and flaked maize*

		Organic matter (g/d)			N (g/d)		
Period	Diet (g/d)	Intake	Duodenal flow	Faecal loss	Intake	Duodenal flow	Faecal loss
1	700 hay	535	239	162	11·9	12·5	4·2
2	500 hay, 100 barley and 100 flaked maize	557	271	131	11·5	14·4	3·7
3	300 hay, 200 barley and 200 flaked maize	573	236	101	10·8	13·5	3·8
4	100 hay, 300 barley and 300 flaked maize	586	190	58	10·2	11·8	2·3
5	350 barley and 350 flaked maize	593	191	52	9·8	11·6	2·2
	SE of the difference between two means		8·7	3·1		0·78	0·13

As the diet became richer in cereals there were increases in the percentage of dietary organic matter fermented in the rumen and decreases in the organic matter lost in the faeces. For all diets the flow of N to the duodenum was higher than the N intake, but the 'ruminal addition' of N was greater for the diets containing 500 and 300 g hay/d than for those containing either higher or lower amounts. The percentage of duodenal N lost in the faeces decreased from 34% in period 1 to 27% in periods 2 and 3 and to 19% in periods 4 and 5.

Two main findings emerge from the experiment. First, in animals receiving the cereal-rich diets, synthesis of protein in the rumen associated with the high fermentation of organic matter was insufficient to offset the low protein intake. Secondly, there were important synergistic effects between dietary constituents in promoting the flow of N to the duodenum. In this respect it is interesting that Offer, Evans & Axford (1972) reported that ruminal protein synthesis was stimulated more by the addition of a 50:50 mixture of starch and paper to the diet than by an isoenergetic addition of either constituent alone.

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Fat mobilization in Large White pigs. By J. D. Wood, *ARC Meat Research Institute, Bristol BS18 7DY*

Fat deposition in meat animals is the result of both lipogenic and lipolytic processes which occur mainly in adipose tissue itself. There appear to be considerable species differences in the sensitivity of adipose tissues to lipolytic influences in vitro, and pig adipose tissue has generally been considered to be insensitive (Rudman, 1965). However, in vivo studies (Cunningham & Friend, 1965) have shown that considerable fat mobilization can occur in pigs in response to influences which accelerate lipolytic mechanisms. The present studies were designed to investigate lipolysis in four Large White pigs (40–50 kg live weight, prepared with polyethylene catheters in both external jugular veins) in four physiological states: (1) up to 4 h after feeding; (2) during constant infusion of norepinephrine; (3) in semi-starvation (16–21 h after a 200 g test meal); and (4) after insulin stimulation 21 h after the test meal. These states were investigated concurrently over a 2 d period.

The concentration of free fatty acids (FFA) in plasma (mequiv./l, mean of four pigs $\pm$SEM) increased from $0{\cdot}123 \pm 0{\cdot}037$ in the fed state to $0{\cdot}425 \pm 0{\cdot}053$ and $1{\cdot}062 \pm 0{\cdot}099$ after 16 h and 21 h of starvation, respectively; from $0{\cdot}123 \pm 0{\cdot}037$ before a 1 h infusion of norepinephrine (5 μg/kg per min) to $3{\cdot}125 \pm 0{\cdot}005$ during the last 20 min of that infusion; and decreased from $1{\cdot}062 \pm 0{\cdot}099$ 21 h after the 200 g test meal to $0{\cdot}190 \pm 0{\cdot}032$, 50 min after intravenous administration of insulin (0·33 U/kg).

The fatty acid composition of plasma FFA closely resembled that of the backfat triglycerides in semi-starvation and particularly after norepinephrine infusion, but not in the fed state. In particular the concentration of C18:1 in FFA increased and that of C18:2 decreased as rapid fat mobilization from adipose tissue occurred. Since the concentration of FFA in plasma of animals at slaughter varies depending on the degree of starvation or excitement they have experienced, these observations may explain why reported values for the composition of FFA are so variable.

The results also indicate that pig adipose tissue is extremely sensitive to lipolytic influences in vivo. The possibility that the rate or extent of lipolysis differs between breeds or strains of pig, contributing to metabolic differences between them, will be discussed.

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The effects of free and protected coconut oil on intake of food by cows. By J. A. Bines and J. E. Storry, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Voluntary intake of food is a major factor limiting the output of the dairy cow. Intake of energy can be increased by increasing the energy concentration of the ration, which may be achieved by including lipid. At high levels and in the free form, however, lipid may depress food intake but this effect may be overcome by

protecting the lipid from metabolism in the rumen by encapsulation in formaldehyde-treated protein (Storry, Brumby, Hall & Johnson, 1974). Free and protected coconut oil added directly to the rumen or incorporated into the concentrate food of cows were therefore compared for their effects on food intake.

Direct addition of 600 g free oil to the rumen of a cow in the 7th month of lactation, which was receiving 8 kg hay and 6 kg concentrates/d, depressed energy intake from 258 MJ to 78 MJ. Addition of increasing amounts of protected oil, up to 700 g oil equivalent, increased energy intake to 286 MJ; further increases depressed total energy intake. With both forms of oil, intake of both hay and concentrates was depressed.

Incorporation of increasing amounts of the free oil into the concentrate food of a non-lactating cow given a ration of 25% hay and 75% concentrate *ad lib.* reduced energy intake from 271 MJ without oil to 151 MJ when 800 g oil was given. In the protected form, 400 g oil caused a small increase in intake to 279 MJ. Further additions of oil depressed intake to 244 MJ and 226 MJ respectively when 800 g and 1000 g oil were given. In both instances, intake of concentrate only was depressed.

Incorporation of up to 1200 g oil in the protected form into the concentrate given to a cow in the 6th month of lactation, which was given a ration of 25% hay and 75% concentrate *ad lib.*, did not change energy intake significantly from 415 MJ/d. Further increases in the amount of oil given depressed intake.

In these experiments no advantage, in terms of increased energy intake, was obtained by the inclusion of protected coconut oil in rations for dairy cows. The cows used were not near the peak of lactation; further work is required to establish whether protected oils will increase intake of cows near the peak of lactation when energy requirements are maximal. Coconut oil in the free form depressed intake severely.

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Synthesis of milk fat from acetate and 3–hydroxybutyrate in goats. By D. S. PARKER* and G. H. SMITH, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

By intravenous infusion of tracer quantities of [1-^{14}C]acetate and [3-^{14}C]-3-hydroxybutyrate (3-HBA) into lactating goats, the contribution of these precursors to milk fat synthesis was studied under conditions in which the yield of fat was varied by intraruminal infusions of butyric acid. There was a variable response to butyric acid infusion in terms of milk fat yield: three out of the five goats used showed an increase. The yield of all individual fatty acids was not increased to the same extent: C_4-C_{12} showed a greater response than C_{14}-C_{18} and hence the proportion of short-chain acids in the milk was increased. In those goats which showed a response there were also changes in the amount and manner by which acetate and 3-HBA contributed to milk fat synthesis.

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In goats receiving no intraruminal infusion of butyric acid there was a substantial contribution by 3-HBA to the methyl-terminal 4-carbon unit of each fatty acid, together with a contribution to the remainder of the molecule as 2-carbon units. In the experiments where butyric acid infusion produced an increase in milk-fat yield, the amount of radioactivity found in the carboxyl group of the fatty acids following both [1-^{14}C]acetate and [3-^{14}C]-3-HBA infusion indicated that an increased proportion of the fatty acid has been synthesized from 2-carbon as opposed to 4-carbon units.

Determination of the 'transfer quotient' allowed the estimation of the yield of fatty acids from each precursor (Table 1).

Table 1. *Yield of fatty acids (g/24 h) in milk fat of goats following intravenous infusion of labelled acetate and 3-hydroxybutyrate (3-HBA)*

	Total yield	Yield from acetate	Yield from 3-HBA: Total	Yield from 3-HBA: As 4-carbon unit	Yield from 3-HBA: As 2-carbon unit
Control	31·8	12·56	3·37	2·33	0·77
+ Intraruminal butyrate infusion	36·2	17·76	5·36	2·98	2·2
Difference	+4·4	+5·2	+1·99	+0·65	+1·43

Although the contribution from 3-HBA increased as a result of the butyrate infusion, this only represented 30% of the total increase in yield, and the contribution from acetate showed a larger increase. An increased uptake from the general 2-carbon pool probably also accounted for the increased contribution from 3-HBA as 2-carbon units, although the factor by which the incorporation of 2-carbon units from 3-HBA is increased (× 2·85) exceeds the factor by which the incorporation of acetate is increased (× 1·40). This is thought to reflect a rise in the activity of the pathway for the cleavage of 3-HBA with higher levels of its substrate.

This work was supported by a grant from the Agricultural Research Council.

A re-assessment of the status of *myo*-inositol as a vitamin. By N. D. Shepherd and T. G. Taylor, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

Many workers include *myo*-inositol in purified diets for laboratory animals, although it is known that this compound is synthesized by most tissues of the body (Eisenberg, 1967; Imai, 1967), and the object of this work was to determine whether or not it is an essential dietary component.

Isoenergetic diets based on starch, glucose or maize oil and containing 240 g casein/kg, together with minerals, vitamins and cellulose, were fed to groups of

Table 1. *Mean weights of* myo-*inositol in the blood, urine, liver and carcases of male rats fed on the basal and supplemented* (1 *g* myo-*inositol/kg*) *diets for* 12 *d*

(Mean values for three or four rats)

	−Inositol		+Inositol	
	Mean	SE	Mean	SE
Blood (μg free/g)	4·12	0·39	7·72**	0·93
Urine (mg free/d)	0·12	0·007	0·15*	0·007
Liver (mg total/liver)	4·36	0·04	4·81	0·34
Carcase (mg/kg wet wt)	354	1	372	8

*Significantly greater than unsupplemented mean ($P<0·05$).
**Significantly greater than unsupplemented mean ($P<0·001$).

young animals with and without inositol (1 g/kg) for periods ranging from 21 to 42 d. Altogether ninety-six rats, 102 mice and thirty-four hamsters were used in these experiments, and no significant differences in growth due to the presence of inositol were observed in either sex.

When the starch diet with and without supplementary inositol was fed to young rats fitted with tail cups to prevent coprophagy, no differences in growth between the two dietary treatments were observed over an experimental period of 14 d.

Analysis of the blood, urine, liver and carcases of male rats fed on the basal and inositol-supplemented diets for 12 d showed that most of the dietary supplement was catabolized (Table 1). Although the concentration of free inositol in the blood was significantly increased in the supplemented rats, there were no significant differences in the total inositol contents of the liver and carcase. The urinary excretion of inositol was significantly increased in the supplemented animals but the increase in absolute terms was minute in relation to the amount ingested (approx. 30 μg/d compared with an intake of 12 mg/d).

We conclude that it is not necessary to supplement diets with *myo*-inositol to achieve normal growth in the species studied, provided that the diets are otherwise complete.

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The lipotropic action of *myo*–inositol. By N. D. SHEPHERD and T. G. TAYLOR, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

Groups of five male weanling rats were fed on a high-fat diet supplemented with choline (2·1 g choline citrate/kg) or *myo*-inositol (1 g/kg) or both for 14 d. A fourth group was given the unsupplemented diet and the experiment was replicated four times. The basal diet contained (g/kg): casein 240, maize oil 311, cellulose powder 389, minerals 40, vitamins 20; it was known to support excellent growth when supplemented with choline. Total esterified fatty acids (TEFA), cholesterol and

Table 1. *The effect of supplements of choline and* myo-*inositol* (*I*) *on plasma and liver lipids and on liver weight in rats given a high-fat diet for* 14 *d*

(Plasma values represent means for ten animals, liver values for twenty animals)

	Choline-deficient				Choline-supplemented			
	−I		+I		−I		+I	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma (mg/l)								
Cholesterol	1562	42	1579	17	1807	25	1897	59
TEFA*	3585	121	3603	111	3637	232	3732	337
Phospholipids	1505	74	1479	43	1605	77	1632	47
Liver								
Fresh wt (g)	5·12	0·22	4·95	0·25	5·25	0·34	4·80	0·23
Total lipids (mg)	565	34	438	30	430	21	364	19
Lipid concn (mg/g)	109·7	5·1	87·9	3·9	82·2	2·6	79·7	2·8

*Total esterified fatty acids.

phospholipids were determined in the plasma: liver weights and total liver lipids were also determined (Table 1).

Results were analysed by analysis of variance. Growth was significantly depressed in the absence of choline ($P<0·05$) but inositol had no effect on growth. Inositol did not influence plasma lipid concentrations but choline significantly increased plasma cholesterol ($P<0·01$) and phospholipids ($P<0·05$). There were no significant effects on liver weight but both supplements reduced total lipids ($P<0·001$) and lipid concentration in the liver ($P<0·01$ for inositol and $P<0·001$ for choline).

Similar effects were observed in another experiment in which groups of eighteen rats were given the maize-oil diet for 3 d after 2 d starvation, but in this experiment inositol significantly increased the concentrations of all the plasma lipid fractions and the plasma lipoproteins both in the presence and absence of supplementary choline.

In a 14 d feeding experiment using a starch-based diet, the lipotropic action of inositol was observed only in the choline-deficient rats.

Although dietary inositol is not essential for growth in rats it appears that requirements for inositol can under certain conditions exceed the synthetic capacity of the body, and when this occurs lipid transport is impaired.

Effect of lignosulphonate on the colon of guinea-pigs. By J. WATT, *Department of Pathology, University of Liverpool* and R. MARCUS, *Clatterbridge Hospital, Bebington*

We have investigated the effects on the colon of guinea-pigs of lignosulphonate given in the drinking fluid. Lignosulphonate is composed of sulphonated phenylpropane units, has a high molecular weight (around 20 000) and is obtained during the manufacture of wood pulp from spruce trees. It is frequently used as a food binder in pellets and cubes supplied to laboratory animals.

Adult male albino guinea-pigs (average body-weight 529 g) were fed on a standard cube diet (SG1; Nutrients Ltd), the cubes containing no lignosulphonate binding agent; their diet was supplemented with fresh cabbage and hay. One group of eight animals received an aqueous solution of sodium lignosulphonate (10 g/l) as drinking fluid over a period of 5 weeks; two groups of four animals each were given aqueous solutions of the lignosulphonate containing 20 g/l or 40 g/l over the same period. The lignosulphonate solutions were freshly prepared each day. Allowing for spillage from drinking bottles, the average daily consumption per animal of sodium lignosulphonate was less than 1·9, 2·6 and 4·4 mg/g body-weight in the three experimental groups. Control animals (eight) received water without added lignosulphonate.

There was no disturbance of bowel function except in one animal. At the end of 5 weeks, the average weight gain ($\pm$SE) of the guinea-pigs in the group receiving sodium lignosulphonate at a concentration of 10 g/l was 129 ± 24 g, in the combined groups receiving the more concentrated solutions 53 ± 7 g, and in the control group 202 ± 24 g.

All animals were killed by diethyl ether anaesthesia. The large bowel was emptied of faeces and carefully examined using transmitted light. Multiple focal ulcers mainly in the caecum were found in eight of the sixteen animals given sodium lignosulphonate in the drinking fluid. The incidence of ulceration in each group was two out of eight, two out of four, and four out of four in the animals receiving 10 g, 20 g and 40 g/l respectively. No ulcers were found in the large bowel in any of the control animals which received water only as drinking fluid.

These results indicate that sodium lignosulphonate can interfere with weight gain and produce ulceration in the large bowel of guinea-pigs when given as an aqueous solution in the drinking fluid. These findings may be of significance in the design and planning of nutritional studies which involve the use of food binders in the formulation of cubes or pellets supplied to the laboratory animals.

The induction of testicular cysts in the chick by various dietary salts.

By W. A. DEWAR, W. G. SILLER and C. C. WHITEHEAD, *Agricultural Research Council's Poultry Research Centre, West Mains Road, Edinburgh EH9 3JS*

Previous studies (Dewar, Whitehead & Siller, 1972; Whitehead, Siller & Dewar, 1972) have shown that dietary levels of sodium chloride below those previously thought to be harmless can cause testicular cysts when fed to chicks younger than 3 weeks of age. An investigation was made to establish the individual contributions of Na^+ and Cl^- ions and to study the effects of other ionic compounds. The compounds, fed from 1-day-old, were sodium chloride, sodium phosphate, trisodium citrate, potassium chloride, tripotassium citrate, calcium chloride, ammonium chloride and triammonium citrate. Their levels and effects at 21 d are shown in Table 1.

Sodium chloride and citrate were most effective in cyst-formation but sodium phosphate, even at levels that caused 70% mortality, induced no cysts. Chloride was the most effective of the potassium salts, although none of the cysts it induced

Table 1. *Effect of various dietary additives on testicular cyst formation in 3-week-old male broiler chicks*

Additive*	Amount added (g/kg diet)	Conc. of Na, K or Cl (mmol/kg)	No. of birds at start	Mortality (%)	No. of survivors having cysts		Incidence of cysts (%)
					Macro†	Micro‡	
None	—	—	90	5	0	0	0
Sodium chloride	25·0	430	36	33	4	10	57
Sodium chloride	37·5	650	40	50	9	—	45≥
Sodium phosphate§	43·4	430	20	0	0	0	0
Sodium phosphate§	65·0	650	30	70	0	0	0
Trisodium citrate	42·6	430	28	18	0	3	13
Trisodium citrate	63·9	650	30	63	2	4	55
Potassium chloride	19·1	260	20	5	0	1	5
Potassium chloride	38·2	510	20	5	0	7	37
Potassium chloride	57·3	770	13	8	0	3	25
Potassium chloride	76·4	1030	13	30	0	4	44
Tripotassium citrate	55·4	510	36	0	0	0	0
Tripotassium citrate	83·1	770	26	4	0	2	8
Calcium chloride	24·1	430	40	3	0	0	0
Calcium chloride	36·2	650	40	0	0	1	2
Triammonium citrate	63·3	—	40	7	1	0	3
Ammonium chloride	34·2	650	45	7	0	0	0

*Compound added to standard Poultry Research Centre diet chick mash (1·3 g Na, 6·0 g K, 1·8 g Cl/kg).
†Macroscopically discernible.
‡Microscopically discernible (see Siller, Dewar & Whitehead, 1972).
§An equimolar mixture of Na_2HPO_4 and NaH_2PO_4.

was macroscopically discernible. Calcium chloride and ammonium citrate and chloride induced few cysts.

These results are difficult to interpret in terms of acid-base balance and do not suggest a ready explanation of the phenomenon.

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