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ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Seventy-fourth Scientific Meeting of the Nutrition Society was held in the Physiology Lecture Theatre, Guy's Hospital Medical School, St Thomas Street, London SE1 9RT, on Thursday, 5 December 1974, *at* 11.00 *hours, when the following papers were read:*

Serum biochemical changes in rats deprived of food or water for 24 h. By F. P. JENKINS and J. A. ROBINSON, *Environmental Safety Division, Unilever Limited, Colworth House, Sharnbrook, Bedford*

The finding of abnormalities in the serum biochemistry of a rat which had been unintentionally deprived of water prompted an investigation of the effects of food or water deprivation upon serum biochemistry. Colworth-Wistar rats, 4 weeks old, were divided into three groups of eight males and eight females and given a purified diet. After 3 d, one group was deprived of food for 24 h and another group was deprived of water for 24 h. Blood samples were then obtained from one-half of the rats in each of the three groups and, after 24 h refeeding, from the remaining rats.

The blood serums were analysed for sodium, potassium and chloride and, in addition, aspartate aminotransferase (*EC* 2.6.1.1) (ASA), alanine aminotransferase (*EC* 2.6.1.2) (ALA), lactate dehydrogenase (*EC* 1.1.1.27) (LDH), 1-hydroxybutyrate dehydrogenase (HBDH) and alkaline phosphatase (*EC* 3.1.3.1) (AP) were assayed.

The experiment was repeated using 14-week-old rats and on this occasion the serum samples were also analysed for creatinine, total protein, albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin; in addition, isocitrate dehydrogenase (*EC* 1.1.1.41) (ICD) and creatine kinase (*EC* 2.7.3.2) (CK) were assayed.

We observed no effect of food or water deprivation upon serum Na, K, Cl and creatinine or upon CK.

In 4-week-old rats ASA, LDH, AP and HBDH increased in animals deprived of food, while AP and HBDH increased in those deprived of water; after 24 h refeeding, these differences from control values disappeared. A different pattern was observed for ALA, which decreased in rats deprived of food or water, and then increased in comparison with the control group following 24 h refeeding.

In the 14-week-old rats deprived of food, serum total protein and serum albumin decreased, while in animals deprived of water, serum total protein and serum α_2-globulin increased; after 24 h refeeding, values returned to normal.

In 14-week-old rats deprived of food or water, we observed an increased ICD in male rats and, in both males and females, a decreased AP. Other serum enzyme changes were similar to, though less marked than, those described for 4-week-old rats.

The results may be relevant in human nutrition and in experiments in which rats are intentionally deprived of food or water, or trained to become meal eaters.

The activity of ornithine decarboxylase in the quadriceps and gastrocnemius muscles of rats recovering from undernutrition. By P. A. MCANULTY and J. P. G. WILLIAMS, *Department of Growth and Development, Institute of Child Health, Guilford Street, London WC1N 1EH*

The polyamines, spermidine and spermine, occur in high concentrations in rapidly growing tissues, and their concentrations appear to be correlated with the rate of growth. This relationship with growth appears to derive from a facilitation of protein synthesis resulting from their influence on RNA synthesis (Raina & Jänne, 1970). The rate-limiting enzyme in the synthesis of the polyamines is ornithine decarboxylase (*EC* 4.1.1.17) (ODC), and the activity of this enzyme is a good indicator of the changes in polyamine concentrations that occur in growing tissues. We have previously shown that the activity of ODC is rapidly elevated in the livers of rats that have been rehabilitated after a period of nutritionally induced growth restriction (McAnulty & Williams, 1975).

Dickerson & McAnulty (1975), using the same undernutrition and rehabilitation regimen, showed that during rehabilitation muscle grew rapidly, and that the rate of growth was different in various muscles of the hind-limb. They found that the different rates of growth were related to different rates of RNA accumulation. We have examined ODC in the quadriceps and gastrocnemius muscles of rats during rehabilitation to determine whether the activity of this enzyme also exhibits differences between muscles.

Male and female weanling rats were maintained at constant body-weight for 28 d by restricting their normal diet, and then rehabilitated by allowing them access to unlimited food. ODC activity was measured in the muscles at various times during the first 25 d of rehabilitation.

During the period of undernutrition ODC activity was undetectable in both muscles. On rehabilitation, enzyme activity began to increase within 8 h in both muscles in both sexes, but more rapidly in the gastrocnemius. The increase continued, and in the males a peak occurred at 3 d in the quadriceps and at 5 d in the gastrocnemius, the peak in the gastrocnemius being 30% higher than in the quadriceps. In the females a peak occurred at 3 d in both muscles, and throughout rehabilitation the activity was lower than that of the males.

Thus during rehabilitation ODC activity did vary between muscles. However, unlike the enzyme in the liver (McAnulty & Williams, 1975), the activity of muscle ODC during rehabilitation showed no correlations with rate of increase in muscle weight or rate of RNA accumulation.

This research was supported by the Medical Research Council through a long-term grant to Professor J. M. Tanner.

REFERENCES

Dickerson, J. W. T. & McAnulty, P.A. (1975). *Br. J. Nutr.* **33**, 171.
McAnulty, P. A. & Williams, J. P. G. (1975). *Proc. Nutr. Soc.* **34**, 32A.
Raina, A. & Jänne, J. (1970). *Fedn Proc. Fedn Am. Socs exp. Biol.* **29**, 1568.

A non-destructive method for the measurement of muscle growth in the rat. By J. P. G. WILLIAMS and P. C. R. HUGHES (introduced by P. A. MCANULTY), *Department of Growth and Development, Institute of Child Health, Guilford Street, London WC1N 1EH*

Studies of muscle growth in laboratory animals normally require the death of the animal to enable the muscles to be measured and weighed. In man, growth and development of soft tissues can be followed by radiographic methods. A radiographic method has been developed to measure skeletal development in the rat (Hughes & Tanner, 1970), and this method has now been extended to measure muscle.

Rats are measured under diethyl ether anaesthesia on a specially designed measuring board, nose–rump length and tail length being measured separately. The error of these measurements in skilled hands is less than 1%. The animals are then placed, still anaesthetized, onto fine-grain non-screen X-ray film. The animal is carefully positioned so that the femur is at right angles to the long axis of the body, the tibia at right angles to the femur and the foot at right angles to the tibia; this ensures that the same tension is placed on the calf muscle of each animal at each observation time. With the animals positioned the film is exposed, and after processing the shadow of the calf muscle can be clearly seen. The maximum width of the muscle is measured using special calipers reading to 0·1 mm. The error of measurement of the muscle was found to be less than 2%.

A group of rats was measured and X-rayed thirteen times between 3 d and 228 d of age. A curve similar to those shown by others for muscle weight was obtained. A sex difference was apparent, and the results of brief periods of undernutrition were evident. Although these results were self-sufficient, meaningful comparisons with those of other workers could not be made as we had only linear measurements and others had only measurements of weight.

A cross-sectional study of a group of 165 rats of different ages was therefore carried out. After being measured and X-rayed the animals were killed. The calf muscle was carefully removed and weighed. The correlation coefficient of muscle width with muscle weight (r) was 0·91, but the relationship was not linear. We conclude that although muscle weight cannot be derived directly from muscle width, measurement of the width of the calf muscle of the rat is a measure of muscle growth which can be followed longitudinally, allowing a more efficient use of experimental animals.

This work was supported by a long-term grant to Professor J. M. Tanner from the Medical Research Council.

REFERENCE

Hughes, P. C. R. & Tanner, J. M. (1970). *J. Anat.* **106**, 349.

The effect of postnatal undernutrition on the activities of enzymes involved in the synthesis of brain lipids in the rat. By B. L. G. MORGAN and D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

It is now believed that glycoproteins and gangliosides are localized in dendritic and axonal membranes, and in the synaptic connections in the brain (Lapetina, Soto & De Robertis, 1967). Roukema, Van den Eijnden, Heijlman & Van der Bergh (1970) have suggested, therefore, that a correlation exists between the development of axons and dendrites and the synthesis of glycoproteins and gangliosides. The enzyme which activates N-acetylneuraminic acid (NANA) for incorporation into glycoproteins and gangliosides (CMP-NANA synthetase), and sialidase, which splits off bound NANA from these compounds, show different patterns of activity during brain development. Sialidase exhibits a profile similar to the production of gangliosides and sialoglycoproteins, whereas CMP-NANA synthetase shows a peak activity at day 6 post partum, which precedes the maximal development of these compounds, and thereafter falls to a constant level at day 16, persisting to maturity (Roukema *et al.* 1970).

Postnatal undernutrition after the 3rd day of life has little effect on the complement of neurons in the brain (Dobbing, 1970), but such treatment could have a profound effect on further development of the nervous system by retarding the rate of growth of axons and dendrites, and their synaptic connections. To test this hypothesis, litters of eight pups were suckled by dams fed on a high-protein diet during days 3–16 of lactation (group A) or by litter-mate dams fed on a low-protein diet during the same period (group B).

On day 17 the pups were killed, their brains were pooled in litters for analysis for gangliosidic NANA and glycoprotein NANA, and the activities of CMP-NANA synthetase and sialidase were measured. The results are shown in the Table:

(Mean values for ten matched litters/group)

Group	Mean brain wt (g)	Gangliosides (μmol NANA /brain)	Glycoprotein (μmol NANA /brain)	Sialidase activity (μmol NANA released /brain per h)	CMP-NANA synthetase activity (μmol CMP-NANA formed /brain per h)
A	1·318	2·322	1·140	0·405	10·88
B	1·160	2·049	0·880	0·577	13·55

The brains of rats in group A contained significantly more gangliosides and glycoprotein than those of rats in group B ($P<0.001$). However, the activities of both enzymes were lower in the well-nourished pups of group A ($P<0.001$).

These findings suggest that undernourishment of the pups of group B had retarded the attainment of peak activity in the enzymes controlling ganglioside and glycoprotein synthesis, and hence the development of axons and dendrites.

We gratefully acknowledge a grant from the Gerber Company.

REFERENCES

Dobbing, J. (1970). *Am. J. Dis. Child.* **20**, 411.
Lapetina, E. G., Soto, E. F. & De Robertis, E. (1967). *Biochim. biophys. Acta* **135**, 33.
Roukema, P.A., Van den Eijnden, D. H., Heijlman, J. & Van der Bergh, G. (1970). *FEBS Lett.* **9**, 267.

Maternal protein deficiency and the immune response of the progeny in the rat. By KATRINE I. MCLEOD* and F. Y. LIEW†, *Departments of Clinical Science and Microbiology, John Curtin School of Medical Research, Australian National University, Canberra, Australia*

Offspring of protein-deficient rat dams have been shown to exhibit permanent stunting of growth and reduced organ weights associated with a reduced cell number (McLeod, Goldrick & Whyte, 1972*a*, *b*). These animals also exhibit anomalies in protein and lipid metabolism in adult life, despite adequate postnatal feeding (McLeod, Nestel & Goldrick, 1973*a*, *b*).

It has been suggested that severe malnutrition in early life may have a similar long-term effect on the immune system (Jose, Welch & Doherty, 1970) resulting from 'nutritional thymectomy' occurring at a critical stage of development. We have studied some of the morphological and functional aspects of the immune system in offspring of protein-deficient dams (test progeny).

Test and control progeny, 8 weeks old, all of which were fed *ad lib.* on commercial laboratory chow from weaning, were injected intraperitoneally with the antigens flagellin, acetoacetylated flagellin or sheep erythrocytes. After 4 weeks, a secondary injection of antigen was given into the left hind-foot pad and a saline injection into the right pad. The relative swelling of the foot pads (a measure of cell-mediated immunity) was determined and a sample of blood was taken for differential and total white blood cell counts. Antibody titres were measured weekly for 6 weeks following the primary injection (the titres being a measure of humoural immunity) and at the end of this period plasma proteins were determined. The numbers and sizes of cells in the spleen and thymus were also estimated.

There were no significant differences in antibody titres between test and control progeny following the primary injection, but after the secondary injection the titres of test progeny were significantly lower than those in control animals (Table 1). Foot-pad swelling was also significantly reduced in the test animals. The increase in total white cell count, and in particular the lymphocyte count, following the secondary injection was not as great in test progeny as in controls, and a similar effect was seen in the plasma protein response, especially with regard to γ-globulins. In test progeny the thymus and spleen were not only reduced in weight, compared with controls, but the ratios, thymus or spleen weight: body-weight were significantly smaller. Weight reductions were associated with both reduced cell size and number in these organs.

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Table 1. *Antibody titres and foot-pad swelling in offspring of protein-deficient and control rat dams*

(Mean values and standard deviations for groups of twenty rats)

	Antibody titre		Relative foot-pad swelling (mm)	
Antigen	Control	Deficient	Control	Deficient
Flagellin	6028±1421	1280±303*	1·71±0·1	0·50±0·06*
Acetoacetylated flagellin	40±15·5	10±3·5*	4·07±0·2	1·41±0·12*
Sheep erythrocytes	320±85	80±22*	1·06±0·05	0·23±0·04*

Value significantly different from control value: *$P<0.001$.

Thus maternal protein deficiency would appear to have permanent effects on some aspects of the immune response in these animals.

REFERENCES

Jose, D.G., Welch, J.S. & Doherty, R. L. (1970). *Aust. pediat. J.* **6**, 192.
McLeod, K. I., Goldrick, R. B. & Whyte, H. M. (1972*a*). *Aust. J. exp. Biol. med. Sci.* **50**, 435.
McLeod, K. I., Goldrick, R. B. & Whyte, H. M. (1972*b*). *Aust. J. exp. Biol. med. Sci.* **50**, 731.
McLeod, K. I., Nestel, P. J. & Goldrick, R. B. (1973*a*). *Aust. J. exp. Biol. med. Sci.* **51**, 811.
McLeod, K. I., Nestel, P. J. & Goldrick, R. B. (1973*b*). *Aust. J. exp. Biol. med. Sci.* **51**, 825.

Biochemical 'tests' of marginal protein deficiency in the rat. By T. A. DUODU and J. C. WATERLOW, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

In most experimental models of human protein-energy malnutrition, animals have been subjected to severe deficiencies of energy or protein or both. From the point of view of public health, moderate or marginal malnutrition represents a much more wide-spread problem than severe kwashiorkor or marasmus. Not very much information is available about the value of biochemical indices for assessing marginal protein deficiency. The work reported here was an attempt to fill this gap by using a more appropriate animal model. Another consideration was that measurements to be used in the field in developing countries must be simple, involving the minimum of equipment.

Groups of rats weighing initially about 70 g were given for 5 weeks diets containing (/kg): 80, 120, 150, 180 or 240 g casein. Measurements were made at weekly intervals of body-weight, serum total protein, albumin, transferrin, cholinesterase (*EC* 3.1.1.8) creatine kinase (*EC* 2.7.3.2) and ribonuclease (*EC* 2.7.7.16). The sum of the three branched-chain amino acids was determined by thin-layer chromatography in 5 μl serum. The recovery was checked with radioactive valine and averaged 85%. Not all measurements were made on all groups of rats. In some experiments muscle mass was measured at the end of the 5-week feeding period.

The most sensitive indicators, apart from body-weight, were serum albumin and the branched-chain amino acids. Results in two dietary groups are shown in the Table.

Group	Casein content of diet (g/kg)	Rate of weight gain (g/rat per d)	Serum albumin concentration at 5th week (g/l)		Serum branched-chain amino acid concentration* (μmol/l)	
			Mean	SE	Mean	SE
B	120	2·66	42·5	2·1 (20)	591	69 (8)
D	180	3·51	48·2	1·7 (20)	714	103 (8)
Ratio, B:D		0·76	0·88		0·83	

No. of samples in parentheses.

*Sum of valine, leucine and isoleucine.

In group D the protein intake was at a level considered to be fully adequate for the rat; in group B it was sub-optimal, but still enough for substantial growth. In the albumin test the group means showed differences which were significant ($P<0·05$) and in the serum branched-chain amino acid test the differences were significant in samples obtained in 4 of 5 weeks from fasted rats, but it would not be possible to distinguish between individuals.

The metabolism of [U-^{14}C]valine in adult rats given high- or low-protein diets. By R. J. NEALE, *Department of Applied Biochemistry & Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

Protein-energy malnutrition in weanling rats induced by feeding protein-free or very-low-protein diets causes no reduction in the catabolism of [U-^{14}C]valine to $^{14}CO_2$ whether administered by single injection (Neale, 1971; Reeds, 1974) or by continuous infusion (Neale & Waterlow, 1974). There is, however, evidence of a reduction in [^{14}C]leucine metabolism to $^{14}CO_2$ in rats maintained on diets low in protein (20–30 g casein/kg) but sufficiently high to prevent continual loss of body-weight such as occurs with a protein-free diet (McFarlane & von Holt, 1969; Sketcher, Fern & James, 1974). The catabolism of [U-^{14}C]valine has therefore been re-examined in adult male Wistar rats (250–300 g) given high-casein (HC) or low-casein (LC) diets (250 and 50 g/kg respectively) such that body-weight was maintained for periods of 7–9 d. Without prior overnight fasting, rats were infused in the morning with 0·5 μCi [U-^{14}C]valine/h for periods up to 7 h, with continuous collection of $^{14}CO_2$ at hourly or half-hourly intervals.

The specific radioactivity (SR) of CO_2 and the percentage dose of ^{14}C excreted as $^{14}CO_2$ rose to a constant plateau value in both HC and LC groups between 4·5 and 5 h and was maintained constant up to 6·5–7 h. The absolute hourly output of CO_2, although significantly higher in the LC group in the first 3 h, declined in both groups in the next 3 h so that values became similar. The SR of CO_2 and the percentage of dose excreted in the HC group at plateau was significantly greater than that in the LC group (Table 1).

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Table 1. *Mean specific radioactivity (SR) of CO_2, percentage excretion of $^{14}CO_2$ and absolute CO_2 excretion in adult rats on high-casein (HC) and low-casein (LC) diets 4·5–6·5 h after the start of an intravenous infusion of* L-[*U*-^{14}*C*]*valine*

(Values are means with their standard errors; no. of 30 min intervals in parentheses)

Diet	SR of CO_2 (counts/min per mmol CO_2 per kg body-wt)	$^{14}CO_2$ excreted (% dose of ^{14}C given)	CO_2 excreted (mmol CO_2/kg body-wt per 30 min)
HC	63 410±1150 (5)	16·3 ±0·47 (5)	23·9±0·4
LC	21 680± 590 (5)	6·38±0·16 (5)	25·3±0·7

The reduction in over-all oxidative catabolism of [U-^{14}C]valine to $^{14}CO_2$ under these conditions of isotopic equilibrium in the LC group can be viewed as a beneficial adaptation to a low-protein diet. A protein-free diet, however, causes a breakdown in this adaptation process, resulting in a high, indiscriminate loss of essential amino acids from the body (Neale & Waterlow, 1974), and aggravates the low intake of protein. The importance of achieving this minimum dietary intake of protein at different ages so that adaptation processes can function without breaking down is therefore immensely important, and outlines the dangers of trauma and infection in causing acute protein loss from the body in conditions of marginal protein intake.

REFERENCES

McFarlane, I. G. & von Holt, C. (1969). *Biochem. J.* **111**, 557.
Neale, R. J. (1971). *Nature New Biol.* **231**, 117.
Neale, R. J. & Waterlow, J. C. (1974). *Br. J. Nutr.* **32**, 11.
Reeds, P. J. (1974). *Br. J. Nutr.* **31**, 259.
Sketcher, R. D., Fern, E. B. & James, W. P. T. (1974). *Br. J. Nutr.* **31**, 333.

Fatal self-medication with retinol and carrot juice. By *Z.* A. LEITNER, *52 Welbeck Street, London W1M 7H3*, T. MOORE *Strangeways Research Laboratory, Wort's Causeway, Cambridge CB1 4RN*, and I. M. SHARMAN, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council, Milton Road, Cambridge CB4 1XJ*

The death of a man (BRB), aged 48, who had held high qualifications in chemistry was widely reported in the press some months ago. The coroner attributed death to carrot juice addiction.

The dietary regimen that eventually proved fatal was started in 1968 when the patient conceived a high regard for the efficacy of retinol to cure various minor ailments from which he was suffering. He took 2–6 tablets retinyl acetate (up to 90 000 μg retinol) daily, increasing this amount gradually during the ensuing 3 months up to 1 500 000 μg daily. A total intake for the 3 months period may, therefore, have been of the order of 60 000 000 μg. This amount may be compared with a daily recommended intake of 750 μg, and a typical 'good' liver reserve of 150 000 μg. Sufficient retinol was taken, therefore, to fill, empty and refill the liver, at an ordinary level of storage, about 400 times.

The patient had been warned of the danger of hypervitaminosis and, on noticing itching of the skin, he temporarily discontinued taking the tablets but consumed large doses of carrot juice. On medical examination in 1969 we found no evidence of skeletal lesions typical of hypervitaminosis A. However, a specimen of blood contained 13 000 μg retinol/l, a value about 20 times the normal average.

In 1972 BRB had viral pneumonia and in 1973 prolonged ill health with ascites, oedema and anasarca. He died in January 1974, when autopsy revealed severe jaundice with liver enlargement. In a specimen of the liver made available to us by Dr Martin Israel 184 g fat/kg was found. Even after fixation, a process which is normally destructive to retinol, a high concentration remained.

The case of BRB was unusual in involving overdosage with both retinol and carrots. How far the carrots should be incriminated, as in the coroner's verdict, seems uncertain. Possibly carrot addiction may have lowered the patient's condition through a reduced intake of more nourishing food. Since the pathological cause of death was stated to be liver cirrhosis, however, it may be relevant to recall that Russell, Boyer, Bagheri & Hruban (1974) reported two cases of non-fatal liver injury, again without bone lesions, in patients who took massive doses of retinol over long periods but without carrot juice. The case of BRB must serve as a further example of the danger of carrying individual ideas on nutrition to extremes.

REFERENCE

Russell, R. M., Boyer, J. L., Bagheri, S. A. & Hruban, Z. (1974). *New Engl. J. Med.* **291**, 435.

The effect of feeding magnesium-enriched diets to laying hens on the quality of the albumen of stored eggs. By D. S. ROBINSON and J. B. MONSEY, *ARC Food Research Institute, Colney Lane, Norwich NOR 7OF*, and W. S. MILLER and M. CLARKE, *Kennett Nutritional Centre, Spillers Limited, Bury Road, Kennett, Newmarket, Suffolk CB8 8QU*

It has been demonstrated that magnesium ion plays an important role in stabilization of the thick egg-white gel in the domestic hen's egg after it is laid and hence the maintenance of good internal quality in eggs for human consumption (Monsey & Robinson, 1974; Robinson, 1972). Accordingly, we have undertaken an experiment to determine whether it is possible to enhance the stabilization of egg-white gel by feeding hens with Mg-enriched diets.

From 1-d-old to point of lay, different groups of fifty pullets of a white egg strain were given diets with and without supplements of 4 and 8 g Mg/kg, added as an equimolar mixture of the chloride and carbonate salts. As determined by chemical analysis, the amounts of Mg in different mixes of the unsupplemented diets ranged from 1·5 to 1·8 g/kg.

From 21 weeks eighteen birds randomly selected from each of these three groups were given a 'layers' diet with and without added Mg in the same amounts as supplied previously. Another eighteen birds selected from those which hitherto had received no supplementary Mg were given the 'layers' diet with 9 g added Mg/kg.

Eggs laid on 3 consecutive d by the four groups of hens were collected at intervals of 3 weeks for assessment of internal egg-white quality by measurement of the proportional weight of thick egg white by the method of Monsey & Robinson (1974). Measurements were made on different eggs laid by individual hens in each 3 d period to assess internal egg quality initially within 24 h of oviposition and after storage for 20 d at 20°.

For the period of lay up to 45 weeks of age, the amount of thick egg white in eggs at oviposition was 560 mg/g and was independent of the Mg content of the diet and age of the hens. In comparison the amount present in eggs after storage was less for birds receiving the unsupplemented diet. The amount of thick egg-white gel which liquified during storage increased as the birds grew older. When the hens were 45 weeks old, approximately 63% (w/w) of the thick egg white present at oviposition was lost during storage. In contrast, in eggs from birds receiving 9 g/kg diet supplementary Mg, the loss during storage was approximately 26% (w/w). The dietary supplements of 4 and 8 g Mg/kg supplied throughout from 1-d-old to 45 weeks of age also resulted in improved stabilization of the thick egg-white gel during storage of whole eggs.

REFERENCES

Monsey, J. B. & Robinson, D. S. (1974). *Br. Poult. Sci.* **15**, 369.
Robinson, D. S. (1972). In *Egg Formation and Egg Production* [B. M. Freeman and P. E. Lake, editors]. Edinburgh: British Poultry Science Ltd.

A comparison of apparent digestibility values for nutrients from measurements in the ileum and faeces of growing pigs. By R. BRAUDE, A. G. LOW, I. G. PARTRIDGE and I. E. SAMBROOK, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The suggestion that the apparent digestibility of some nutrients may be more satisfactorily estimated by measuring their disappearance anterior to the terminal ileum, rather than in the whole gut, was investigated. Six pigs, of 30 kg live weight, were fitted with Ash-type re-entrant cannulas in the terminal ileum. The animals were fed in turn on a diet containing barley meal, weatings and fish meal (diet A) and a diet containing starch, sucrose, maize oil, cellulose and casein (diet B). Both diets, supplemented with minerals and vitamins, were given according to the Shinfield scale based on live weight (Barber, Braude, Mitchell & Pitman, 1972) after being mixed with 2·5 times their weight of water immediately before feeding, twice daily. Ileal digesta were collected during four 24 h periods from each pig on each diet. The same diets were fed in a similar manner to twelve other pigs (six per diet) without cannulas in a balance trial. Faeces were collected from each pig during four 5 d balance periods.

Mean apparent digestibility coefficients from analyses of ileal digesta and faeces, with their standard errors, are given in the Table:

	Diet A			Diet B		
	Content (g/kg in diet)	Apparent digestibility coefficient		Content (g/kg in diet)	Apparent digestibility coefficient	
		Ileal digesta	Faeces		Ileal digesta	Faeces
Dry matter	868·2	0·72±0·022	0·79±0·017	911·8	0·92±0·002	0·96±0·006
Ash	49·9	0·17±0·088	0·46±0·042	32·3	0·52±0·018	0·74±0·048
Water	131·8	0·17±0·074	0·86±0·007	88·2	0·77±0·010	0·99±0·002
Nitrogen	24·9	0·75±0·020	0·78±0·015	24·5	0·91±0·003	0·97±0·006
Acid detergent fibre	73·5	0·26±0·063	0·26±0·015	30·4	0·10±0·031	0·36±0·096
Lipids	40·1	0·75±0·030	0·63±0·024	30·7	0·89±0·010	0·90±0·014
Carbohydrates (by difference)	622·6	0·77±0·018	0·84±0·003	692·5	0·95±0·002	0·97±0·003

Most of the apparent digestion of dry matter and total nitrogen was completed in the stomach and small intestine. There was evidence of both digestion and secretion or synthesis of lipid, further digestion of fibre and major absorption of ash and water in the large intestines.

REFERENCE

Barber, R. S., Braude, R., Mitchell, K. G. & Pitman, R. J. (1972). *Anim. Prod.* **14**, 199.

The apparent absorption of minerals in the small and large intestines of growing pigs. By I. G. PARTRIDGE (introduced by R. BRAUDE), *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Some aspects of mineral absorption were studied in conjunction with the work described in the previous communication (Braude, Low, Partridge & Sambrook, 1975). The cereal diet (A) and semisynthetic diet (B) were formulated primarily for studies on protein digestion and differed in mineral composition. The amounts of each element supplied by the diets and the apparent absorption coefficients from analyses of ileal digesta and faeces (mean values and their standard errors for six pigs) are given in the Table:

	Diet A			Diet B		
	Content (g/kg diet)	Apparent absorption coefficient		Content (g/kg diet)	Apparent absorption coefficient	
		Ileal digesta	Faeces		Ileal digesta	Faeces
Calcium	9·5	0·25±0·072	0·33±0·010	8·3	0·50±0·023	0·75±0·036
Phosphorus	8·1	0·49±0·072	0·45±0·009	6·2	0·64±0·027	0·74±0·033
Sodium	2·4	−1·72±0·172	0·81±0·022	2·7	0·31±0·010	0·98±0·003
Potassium	7·5	0·72±0·064	0·68±0·013	2·8	0·85±0·026	0·95±0·010
Magnesium	1·7	0·01±0·084	0·24±0·019	0·4	0·07±0·077	0·57±0·065

The importance of the large intestine in mineral absorption was clearly demonstrated. While a large proportion of the total apparent absorption of P, K and to a

lesser extent Ca occurred anterior to the cannula, there was little or no net absorption of Mg, and Na exhibited either a large net secretion with diet A or only limited net absorption with diet B in this region. Faecal estimations, although made in different animals, indicated that the principal site of net absorption of Na and Mg was the large intestine.

The large difference between diets in ileal apparent absorption coefficients for Na were associated with a similar difference in the volume of digesta passing this site, the rate of flow being 3·5 times greater for diet A than for diet B. Different rates of Na recycling were necessary to maintain tonicity. The relationship between dietary factors affecting the rate and volume of ileal flow and intestinal Na flux is being further investigated.

REFERENCE

Braude, R., Low, A. G., Partridge, I. G. & Sambrook, I. E. (1975). *Proc. Nutr. Soc.* **34**, 46A.

A secondary interaction between gossypol and cottonseed protein. By S. DAMATY and B. J. F. HUDSON, *University of Reading, Department of Food Science, London Road, Reading RG1 5AQ*

Reaction between gossypol and protein during the processing of cottonseed adversely affects protein quality. This is primarily due to the reduced availability of certain amino acids, owing to the formation of Schiff bases from the ∈-amino groups interacting with the formyl groups of gossypol. This reaction cannot, however, account fully for the over-all nutritional effect. The complex reaction between gossypol and cottonseed protein has been studied by evaluating the affect of bound gossypol, free gossypol and heat treatment on both structural and functional protein isolates derived from the same original sample.

The effect of bound gossypol on solubility was illustrated by the reduction of yield from 34·2% to 31·5% for structural protein and from 10·1% to 6·3% for functional protein when high-gossypol cottonseed flour was compared with its gossypol-free equivalent.

Selective cleavage of the peptide chain by enzymic hydrolysis, followed by Sephadex fractionation of the components of the protein hydrolysate, afforded a comparison of the amounts of indigestible residues. For the structural protein, a rise from 0 to 9 g/kg in gossypol content led to an increase in the proportion of nitrogen (from 12·7 to 20·5%) appearing in the residue. For functional protein, a rise in gossypol content from 0·7 to 4·9 g/kg led to an increase from 14·7 to 24·9% in the proportion of undigestible N.

Bound gossypol had a selective effect in modifying the pattern of amino acids released both by enzymic and by strong acid hydrolysis. In the instance of structural protein, lysine fell from 52 to 40 mg and methionine from 24 to 16 mg/g total amino acids: in the functional protein lysine fell from 101 to 61 mg and methionine from 34 to 23 mg/g. Falls were also significant with some of the other essential amino acids.

Further study of the insoluble residues showed that enzymic digestion was

without effect, and that even under the conditions of strong acid hydrolysis, a substantial part of the bound-gossypol residues was not hydrolysable.

It can be concluded that the interaction between gossypol and cottonseed protein starts through the reaction of the formyl groups of gossypol with the ε-amino groups of lysine and arginine, and that gossypol may also react with the thiol group of cysteine. Such compounds as are first formed, and have been isolated by fractionation, may then undergo intramolecular changes, and ultimately the formation of insoluble, indigestible polymerization products may take place.

Effect of processing temperature on some indices of nutritional significance for micronized soya beans. By K. HUTTON and P. D. FOXCROFT, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Whole soya beans may be fed to cattle with the minimum of processing, e.g. cracked or kibbled, with beneficial results (Morrison, 1956). However, for feeding pigs and poultry, the beans are usually ground and, for optimal results, must also be strongly heated to destroy anti-nutritional factors which impede performance of monogastric livestock fed raw, fresh soya beans. Even for ruminant feeding the heating of soya-bean protein has been shown to be advantageous. Particular care is required during heating to destroy the anti-nutritional factors with minimal damage to the protein.

Steam-heating and toasting are the techniques commonly used for soya-bean meal processing, but the micronization process, a recently developed technique for preparing cereals for feeding to farm livestock (Lawrence, 1972) offers a rapid alternative method for processing whole soya beans. The effect of processing temperature on the nutritional value of micronized soya beans may be estimated using simple laboratory tests. Urease activity, protein solubility and trypsin inhibitor capacity indicate whether or not under-heating has occurred during processing and lysine availability can indicate overheating of a sample; only a dye-binding test using cresol red indicates whether or not heat-processing has been sufficient to destroy anti-nutritional factors without seriously reducing the protein quality (Olomucki & Bornstein, 1960). This paper describes the evaluation of samples of micronized flaked soya beans processed under different conditions. The time during which the whole beans were subjected to radiant heat (the 'dwell time') was varied from 25 to 95 s and the resultant temperatures of processing measured immediately prior to rolling of the heated beans are shown in Table 1.

The results obtained show that the urease activity, trypsin inhibitor capacity and protein solubility may be reduced to a minimum level without a serious loss in availability of lysine; the results of the dye absorption test are consistent with these observations, as a value of 3·8–4·3 is considered acceptable, and it is concluded that a micronizing temperature of between 200 and 225° is required for optimal processing of whole soya beans.

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Table 1. *Effect of processing temperature on some indices of nutritional significance for micronized soya beans*

	Heating time (s)	Temperature (°)	Urease activity (mg nitrogen released/g N per min)	Trypsin inhibitor capacity (mg trypsin inhibited/g N)	Protein solubility (g soluble N/g total N)	Available lysine (g/kg crude protein)	Dye absorption (mg dye absorbed/g oil-free meal)
Control 1: whole beans	0	—	81·3	106·0	0·712	52	2·0
Control 2: cold-rolled beans	0	—	78·7	105·8	0·706	53	2·0
Micronized flaked beans	25	180	32·9	108·4	0·515	52	3·0
	30	180	29·3	106·0	0·422	52	3·1
	45	185	16·6	87·6	0·355	50	3·3
	60	190	8·2	73·3	0·234	51	3·5
	75	200	6·1	39·3	0·164	50	3·9
	85	215	1·7	25·5	0·143	50	4·0
	95	225	0·8	15·4	0·135	49	4·2

REFERENCES

Lawrence, T. L. J. (1972). *Cereal processing and digestion*, p.77. London: US Feed Grains Council.
Morrison, F. B. (1956). *Feeds and Feeding*, 22nd ed. Clinton, Iowa: The Morrison Publishing Co.
Olomucki, E. & Bornstein, S. (1960). *J. Ass. off. agric. Chem.* **43**, 440.

Effect of micronizing on the utilization of soya beans by growing rats.

By K. HUTTON and A. THOMPSON, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

In an investigation of the effects of micronizing on the utilization of soya beans, micronized soya-bean samples previously evaluated in vitro (Hutton & Foxcroft, 1975) were fed as a source of protein to growing rats.

Thirty-six Wistar male albino rats of approximately 150 g initial live weight were distributed between the respective test diets such that seven diets had four replicates each; two additional diets containing respectively 80 and 10 g albumen/kg were similarly replicated as a reference standard and for estimation of endogenous and metabolic losses. All animals were housed in individual metabolism cages fitted with urine and faeces separators (Thompson, 1970). After weighing and allocation to treatments, rats were fed 8 g/d of their respective diets for a preliminary period of 5 d. Total outputs of faeces and urine were measured over a 7 d collection period during which all animals still received 8 g/d of their diet. Experimental diets were

formulated on a dry matter basis to contain an estimated 80 g digestible crude protein, 80 g fibre and 100 g oil/kg; the remainder of the diets was made up of starch and glucose and a supplement of vitamins and minerals.

Apparent digestible energy (ADE), true digestibility of nitrogen (TD of N), biological value (BV) and net protein utilization (NPU) were calculated for the different soya-bean treatments and the results are summarized in Table 1, together with results for the albumen diets.

Table 1. *Effect of processing temperature on the utilization of micronized soya beans by growing rats*

Heating time (s)	Temp. (°)	Dry matter (DM) intake (g/week per rat)	Apparent digestible energy content (MJ/kg DM)	True digestibility of dietary nitrogen	Biological value	Net protein utilization
0	—	51·8	17·3[a]	0·906[a]	0·526[a]	0·476[a]
30	180	52·0	17·4[a]	0·914[a]	0·616[b]	0·564[b]
45	185	51·9	17·3[a]	0·926[a]	0·623[b]	0·577[b]
60	190	52·1	17·3[a]	0·912[a]	0·643[b]	0·586[b]
75	200	51·5	17·7[a]	0·899[b]	0·732[c]	0·657[c]
85	215	52·5	17·4[a]	0·883[b]	0·727[c]	0·642[c]
95	225	52·4	17·3[a]	0·874[b]	0·758[c]	0·663[c]
Control Diet 1— 10 g albumen/kg		51·8	17·3	—	—	—
Control Diet 2— 80 g albumen/kg		51·7	17·9	0·975	0·941	0·918

Different superscripts denote significant differences.

Increasing processing temperature had no significant effect on ADE. However, the BV of the protein in the diets containing soya beans processed at 200° or above were significantly greater than those of the other four soya-bean-containing diets ($P<0·01$) and the BV of the raw soya-bean protein was significantly less than all other soya-bean-containing diets. Although the increased processing temperature resulted in a reduced TD of N, the NPU values for those diets containing soya beans processed at 200° and above were significantly greater ($P<0·01$) than those for the other four diets and the raw soya-bean protein had the lowest NPU of all. These results are consistent with those of Hutton & Foxcroft (1975) and confirm that a micronizing temperature of 200–225° is required for optimal processing of whole soya beans.

REFERENCES

Hutton, K. & Foxcroft, P. D. (1975). *Proc. Nutr. Soc.* **34**, 49A.
Thompson, A. (1970). *J. Inst. Anim. Tech.* **21**, 15.

A new source of protein for growing pigs. By R. BRAUDE and K. G. MITCHELL, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*, and D. N. RHODES, *ARC Meat Research Institute, Langford, Bristol BS18 7DY*

The nutritive value of a new source of protein developed by the Imperial Chemical Industries Ltd (Agricultural Division, Billingham, Cleveland) was studied in tests

with growing pigs. The ICI protein, which is not yet on the market, is of bacterial origin and is produced in a continuous fermentation process by growing the organism currently classified as *Pseudomonas methylotropa* on methanol as the source of energy. The proximate analysis of one of the consignments used was as follows (g/kg): dry matter 964, crude protein 740 (118·4 nitrogen × 6·25), diethyl ether extract 5, crude fibre 4, ash 112 (Ca 0·4, P 26, NaCl 0). The nucleic acid N content was 23 g/kg. The amino acid composition was similar to that of high-quality white fish meal. The ICI protein was used to replace white fish meal as the sole protein supplement in cereal-based diets on the basis of its content of non-nucleic acid N × 6·25. The following four treatments were used: (1) 'standard protein level' control diet containing 70 g white fish meal/kg fed up to 54 kg live weight and 35 g/kg thereafter; (2) as (1) but with all the fish meal replaced with ICI protein; (3) 'marginal protein level' control diet containing 30 g white fish meal/kg (reduced to 15 g/kg after the pigs reached 54 kg live weight); (4) as (3) with all the fish meal being replaced with ICI protein on the same basis as in (2).

In the main test twenty-four quadruplets of Large White pigs were used, one pig from each being allocated at random to the four treatments. All ninety-six pigs were put on test at about 20 kg live weight, and forty-eight (twelve on each treatment) were slaughtered at 60 kg, and the other forty-eight at 90 kg live weight.

A further six quadruplets were used in a balance test covering the period from 20–60 kg live weight (six pigs/treatment).

Preliminary results indicated that there was no significant difference in daily weight gain and food conversion ratio or in total N retention between the pigs given the respective diets containing the ICI protein and those given the control diet containing the white fish meal, and slaughtered at either 60 or 90 kg live weight. The results suggested that the nucleic acid was not utilized by the pigs and that they had no difficulty in disposing of it. The utilization of amino acids was studied by measuring their digestibility and levels in blood plasma. The carcass quality, the acceptability of the meat and its quality appeared not to be affected by the ICI protein, but some of these studies have still to be completed.

The effects of coating mixtures of urea phosphate and urea on the concentrations of ammonia in the rumen liquor and blood of cows. By R. G. HEMINGWAY and L. MAY LAW, *Animal Husbandry Department, Glasgow University Veterinary School, Bearsden, Glasgow*

Oral administration of urea phosphate (UP, 165 g nitrogen and 185 g phosphorus/kg) resulted in lower concentrations of ammonia in the blood of sheep than equivalent amounts of N given as urea (460 g N/kg) and dicalcium phosphate (DCP, 175 g P/kg), even when UP provided only one-quarter of the total urea N given (Hemingway, Parkins & Ritchie, 1972). Coating urea pills with fats or waxes reduced the rate of ammonia production from urea in in vitro tests with Jackbean urease

(Johnson, Bentley & Hershberger, 1962). In the present experiment, mixtures of UP and urea were coated by a similar method with urea–formaldehyde to reduce their rate of water solubility.

Mixtures of UP and urea, physically coated and uncoated, were prepared containing either 270 g N and 116 g P/kg (about 1·5 UP:1 urea) or 338 g N and 72 g P/kg (about 1 UP:1·5 urea). Comparisons were made with mixtures of DCP and urea supplying the same total amounts of N and P.

Each product was administered at 09.00 hours to each of three 500 kg cows to provide 100 g urea equivalent. Only limited hay (3 kg) was given at 17.00 hours on the evening previous to each investigation. Concentrations of ammonia were determined in the rumen liquor and blood.

Table 1. *Mean concentrations of ammonia in the rumen liquor (mg/l) and blood (μg/l) of cows (three cows/treatment) given 100 g urea equivalent in three different forms*

		Rumen liquor			Blood		
Mixture composition (g/kg)	Time (h)	Coated UP+U	Uncoated UP+U	DCP +U	Coated UP+U	Uncoated UP+U	DCP +U
270 nitrogen, 116 phosphorus	0	90	140	100	nd	nd	nd
	0·5	390	510	560	350**	400*	1380
	1·0	470	490	490	590**	670**	2130
	2·0	370	500	480	710**	1030*	2360
	3·5	240	430	390	590	1050	1710
338 nitrogen, 72 phosphorus	0	60	90	90	nd	nd	nd
	0·5	300	480	430	570	380	950
	1·0	550	570	540	780**	770**	2070
	2·0	420	510	510	1040	870	1850
	3·5	310	400	350	730	910	1440

UP, urea phosphate; U, urea; DCP, dicalcium phosphate; nd, not determined.
Values significantly different from those for the DCP+U treatment: * $P<0.05$, ** $P<0.01$.

There were no significant differences due to treatment in the concentrations of ammonia in the rumen liquor, although administration of both the coated UP+urea mixtures generally resulted in rather lower values (Table 1). Blood ammonia concentrations were consistently, and frequently significantly, less for both the coated and uncoated UP+urea mixtures than for the DCP+urea treatment. The over-all mean concentrations of ammonia of 670 (coated UP+urea), 760 (uncoated UP+urea) and 1730 (DCP+urea) μg/l indicate that urea–formaldehyde coating does not further lower the marked reduction in blood ammonia concentration found when UP+urea is given compared with DCP+urea.

The experimental materials were supplied by Chemicals and Phosphates Ltd, Haifa, Israel.

REFERENCES

Hemingway, R. G., Parkins, J. J. & Ritchie, N. S. (1972). *Br. vet. J.* **128**, lxxxii.
Johnson, R. R., Bentley, O. G. & Hershberger, T. V. (1962). *Res. Bull. Ohio agric. Exp. Stn* no. 917.

Regulation of food intake during growth in the Zucker rat. By J. D. RADCLIFFE, A. J. F. WEBSTER, P. J. S. DEWEY and T. ATKINSON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Previous work (Pullar & Webster, 1974) suggested that both congenitally obese and lean Zucker rats regulate food intake during growth in such a way as to sustain comparable, optimal rates of growth of bone and lean body mass, which further implies that the rate of fat accretion is regulated relatively poorly. To test this hypothesis, three groups of four 34-d-old lean female rats and the same number of fat rats were used. One group from each phenotype was killed at the start of the experiment and each individual analysed for total body energy, protein and lipid. Each individual in the remaining fat and lean groups was offered, *ad lib.*, a semi-synthetic diet containing 200 g lipid/kg and either 150 (150 C) or 300 g casein/kg (300 C). The diets were approximately isoenergetic. Collections made of urine and faeces were analysed to determine the metabolizable energy (ME) in the diet and the digestibility of dietary protein. After receiving the diets for 64 d, the rats were killed and their carcasses analysed to determine retention of nitrogen, energy and lipid. The results were:

Pheno-type	Diet	ME intake (MJ)	N retention (g)		Energy retention (MJ)		Lipid retention (g)		N retention/ digestible N intake
			Mean	SE	Mean	SE	Mean	SE	
Lean	150 C	15·0	4·73	0·26	2·65	0·23	52·4	5·9	0·28
	300 C	16·4	5·41	0·21	3·27	0·33	65·4	8·0	0·15
Fat	150 C	25·0	5·11	0·45	10·53	0·58	253	19·3	0·18
	300 C	26·0	5·21	0·25	11·38	0·40	274	18·6	0·09

Intake of ME was about 60% greater in the fat rats, and they retained about four times as much energy and lipid, but cumulative N retention did not differ significantly between phenotypes at either level of protein in the diet.

Since Pullar & Webster (1974) showed that N retention in fat rats was severely reduced when they were restricted to a food intake comparable with that of lean rats, the present results strongly support the hypothesis that fat and lean Zucker rats regulate their intake of a diet containing adequate protein in order to sustain the same rate of growth of lean body mass.

This work was supported by a grant from the British Nutrition Foundation.

REFERENCE

Pullar, J. D. & Webster, A. J. F. (1974). *Br. J. Nutr.* **31**, 377.

A comparison of the effects of an oral fructose load in hypertriglyceridaemic and normolipidaemic men. By A. R. MACRAE and I. MACDONALD, *Physiology Department, Guy's Hospital Medical School, London SE1 9RT*

A raised concentration of triglyceride in the fasting serum and abnormal carbohydrate metabolism are associated (Fredrickson, Levy & Lees, 1961). Dietary

fructose raises the fasting serum triglyceride level more than does dietary glucose or its polymers in men with normal levels of serum triglyceride (Macdonald, 1965). It was therefore decided to study the effects of an acute oral load of fructose in men with raised serum triglyceride concentration and in men with normal lipid levels.

Six men (mean age 32·8 years) with raised fasting serum triglyceride levels (mean 1·96 g/l) and four men (mean age 26·5 years) with normal serum triglyceride levels (mean 0·85 g/l) were given, after a 14 h fast, 0·8 g fructose/kg body-weight, made up to 4 ml/kg body-wt with distilled water. Venous blood samples were taken at 15, 5 and 0 min prior to the ingestion of fructose, and at intervals up to 150 min afterwards. The serum fructose (Bergmeyer, Bernt, Schmidt & Stork, 1970) insulin (Hales & Randle, 1963) uric acid (Praetorius & Poulsen, 1953) and triglyceride (Eggstein & Kreutz, 1966) concentrations were measured.

The hypertriglyceridaemic men had a moderate fructose intolerance as shown by the significantly greater serum fructose response following the fructose load. The fasting serum uric acid concentration and the serum uric acid response following the fructose meal were increased in the hypertriglyceridaemic subjects. A similar pattern was seen with the serum insulin levels. Also there was a direct correlation, common to both groups, between the fasting serum triglyceride concentrations and the area under the fructose *v.* time curve.

We are very grateful to the volunteers in this study and to the Wellcome Trust for a grant. This work forms part of a PhD thesis (A.R.M.) in the University of London.

REFERENCES

Bergmeyer, H. U., Bernt, E., Schmidt, F. & Stork, H. (1970). *Methods in Enzymatic Analysis*, p. 1163. Weinheim: Verlag Chemie.
Eggstein, M. & Kreutz, F. H. (1966). *Klin. Wschr.* **44**, 262.
Fredrickson, D. S., Levy, R. I. & Lees, R. S. (1961). *New Engl. J. Med.* **276**, pp. 34, 94, 148, 215, 273.
Hales, C. N. & Randle, P. J. (1963). *Biochem. J.* **88**, 137.
Macdonald, I. (1965). *Clin. Sci.* **29**, 193.
Praetorius, E. & Poulsen, H. (1953). *Scand. J. clin. Lab. Invest.* **5**, 273.

Some effects of an oral contraceptive on dietary carbohydrate–lipid interrelationships in the baboon. By VALERIE STOVIN and I. MACDONALD, *Physiology Department, Guy's Hospital Medical School, London SE1 9RT*

There is evidence that the oestrogen-containing oral contraceptive affects lipid metabolism, as shown by the rise in the fasting serum triglyceride concentration (Stokes & Wynn, 1971). Dietary sucrose is associated with an increase in the fasting serum triglycerides of men and post-menopausal women but not of pre-menopausal women (Macdonald, 1967). It therefore seemed worthwhile to learn whether a sucrose diet given together with a hormonal contraceptive augmented the serum lipid rise associated with this method of contraception.

Six male and six female sexually mature baboons were subjected to dietary regimens lasting for 7 weeks. The composition (g/kg) of the experimental diets was:

sucrose or glucose 750, calcium caseinate 180, dried yeast 50, salts 20, and added vitamins. The dietary mixture was made up in water and the amounts given were sufficient to keep the weights of the animals constant. The diets, administered in random order, were given either with or without an oral contraceptive (OC) (1·0 mg norethisterone and 0·05 mg mestranol); another regimen consisted of giving the normal laboratory diet with the oral contraceptive.

Fasting venous blood samples were taken before each diet began and after 21 and 49 d on the diet. The serum was examined for triglyceride and cholesterol concentrations.

The level of triglyceride in the serum was significantly raised when the diets contained sucrose or glucose together with the OC but not when the OC was given with the chow diet. The significant fall in serum cholesterol concentration seen in both male and female baboons on the glucose diet did not occur when the OC accompanied the glucose diet. In male animals, sucrose plus OC resulted in an increase in serum cholesterol concentration, whereas with sucrose alone no such rise occurred.

Hence the addition of the OC to a high-carbohydrate diet in baboons seems to produce some serum lipid levels that are greater than those found with the diet alone.

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REFERENCES

Macdonald, I. (1967). *Wld Rev. Nutr. Diet.* **8**, 143.
Stokes, T. & Wynn, V. (1971). *Lancet* ii, 677.

Effects, in the rat, of early carbohydrate feeding on carbohydrate–lipid relationships. By I. MACDONALD, T. REBELLO and ANNE KEYSER, *Physiology Department, Guy's Hospital Medical School, London SE1 9RT*

It is now established that both the amount and the type of dietary carbohydrate influence the lipid levels in the liver and serum in man and experimental animals (Macdonald, 1973). It is therefore of interest to see whether an excess of carbohydrate in the young animal causes permanent modification in the carbohydrate–lipid metabolism of that animal.

A diet consisting of (by wt) 70 carbohydrate, 21 calcium caseinate, 4 dried yeast, 3·5 salt mixture, 3 methyl cellulose and 1 sunflower-seed oil was given to ten male Wistar rats at weaning. The carbohydrate used was either glucose, sucrose or fructose. Control animals were given rat cubes. When each animal reached 200 g body-weight and was presumably sexually mature, it was either killed or given a rat-cube diet for a further 4 weeks before being killed. Prior to killing and after a 4 h fast each animal was given by stomach tube 5 μCi U-^{14}C-labelled glucose, sucrose or fructose, according to its previous diet. After 2 h the animal was killed, and blood, liver and depot-fat samples removed. The triglyceride (Eggstein & Kreutz, 1966) concentration and the radioactivity in the triglyceride samples were measured.

The results showed that while the animals ate the high-carbohydrate diets, the specific activity in their triglycerides greatly increased, and some differences in response between carbohydrates were apparent. However, 4 weeks after they were returned to the rat-cube diet, these differences from control animals were no longer apparent.

The fatty acid profile of the adipose tissue showed a significant increase in the proportion of oleic acid in the depot fat of all animals given carbohydrate compared with the controls. The liver triglyceride also showed an increase in the oleic acid fraction. There was a fall in the proportion of linoleic acid in the liver triglyceride in all carbohydrate groups, being greatest in those given the fructose diet. The return to control levels lagged in the fructose group.

We are grateful to Beecham Products Ltd for a research grant.

REFERENCES

Eggstein, M. & Kreutz, F. H. (1966). *Klin. Wschr.* **44**, 267.
Macdonald, I. (editor) (1973). *Progr. Biochem. Pharmac.* **8**.

The thermic effect of food in man at high altitude. By M. J. Stock, *Department of Physiology, Queen Elizabeth College, London W8 7AH*, Anna Ferro-Luzzi, *Istituto Nazionale della Nutrizione, Rome*, and N. G. Norgan, *Department of Human Sciences, Loughborough University, Loughborough LE*11 *3TU*

In this study the heat production of fasting subjects was measured at rest and during the performance of a 20 min step-test. Following the consumption of a standard breakfast (Nutrament, 1·67 MJ (400 kcal)), heat production was again measured at rest for 60 min before the step-test was repeated. Measurements were made on six subjects (four male, two female) before, during and after a 3-week sojourn in the Capanna Gnifetti (altitude 3650 m) on the Monte Rosa.

Body-weight decreased on average by 2·9 kg ($P<0.001$) at altitude. Fasting heat production at rest had increased by 6·1 J/kg per min ($P<0.05$) by week 2 at altitude but subsequently returned to sea-level values. Thermic responses were calculated

Table 1. *Post-prandial heat increments in human subjects at different altitudes*

		Increment (ΔkJ/min)	
Location	Altitude (m)	Rest	Exercise
London	0	0·88	1·21
Aosta	580	1·00	1·30
Gnifetti	3650		
Week 1		0·84	0·63
Week 2		0·79	0·21**
Week 3		1·00	0·84
Aosta	580	0·96	1·38

Value significantly different from London value: ** $P<0.01$.

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from the post-prandial increases in heat production above the fasting rates (Δ kJ/min) and the mean values for rest and exercise at each location are shown in Table 1.

At high altitude there appeared to be a small, but not statistically significant, decrease in the thermic response at rest. This mild effect of hypoxia was apparently exacerbated by exercise and resulted in a significant decrease in the exercising thermic responses after 2 weeks at high altitude. This decrease, however, became less marked as the subjects acclimatized, so that by the 3rd week at high altitude the responses were not significantly different from those at low altitude. But these mean values obscure the marked inter-individual variation that occurred. For example, the exercising thermic responses recovered earliest in the subject with most high-altitude experience, whilst the subject with least experience of high altitudes showed a progressive decline, with negative responses in the 3rd week at high altitude.

Lack of previous exposure to high altitudes could explain the continued absence of exercising thermic responses, after 5 weeks at altitude, that was observed in an earlier study (Miller & Stock, 1969). The absence of thermic responses in high-altitude natives found on that study could possibly have been caused by their low plane of nutrition.

We wish to thank our colleagues, Dr John Stirling, Dr Elizabeth Evans and Dr Iain Campbell, for their collaboration. This work was supported by the Italian Foreign Ministry.

REFERENCE

Miller, D. S. & Stock, M. J. (1969). *Proc. Nutr. Soc.* **28**, 74A.

Losses of energy associated with oven-drying. By MAHSHID LOTFI, I. A. MACDONALD and M. J. STOCK, *Department of Physiology, Queen Elizabeth College, London W8 7AH*

It is common practice in many laboratories to oven-dry diets and animal carcasses prior to analysis of the major constituents. Marked changes in the appearance of the samples often occur, and this investigation sought to establish whether these changes had an effect upon the heat of combustion.

Three materials were investigated: (a) glycerol trioleate, (b) freeze-dried homogenates of whole rat carcass, (c) a synthetic animal diet. Pre-weighed samples in crucibles were dried in a hot-air oven for time periods that would normally be required to produce constant weight. Control samples were left at room temperature in a dessicator. Heats of combustion were determined by ballistic bomb calorimetry (Miller & Payne, 1959) and the results shown in the table have been given as galvonometer deflection units/g original material.

The results clearly demonstrate significant losses of energy resulting from oven-drying. The losses for a rat carcass heated at 105° for 3 d amount to one-tenth of its original energy content. The work of Thomson (1965) suggests that similar losses could occur with oven-drying of sheep carcasses.

Material	Treatment	No. of expts	GDU/g Mean	SE	
Glycerol trioleate	20°, 18 h	6	90·4	0·7	
	44°, 18 h	6	89·2	0·8	$P<0.005$
	105°, 18 h	6	84·1	0·9	$P<0.001$
	105°, 64 h	6	82·1	0·9	$P<0.001$
Rat carcass	20°, 64 h	10	54·8	0·6	
	72°, 64 h	7	50·2	1·0	$P<0.005$
	105°, 64 h	10	49.2	0·5	$P<0.001$
Diet	20°, 48 h	6	47·4	0·3	
	70°, 48 h	6	45·9	0·6	$P<0.05$
	105°, 48 h	6	44·5	0·5	$P<0.005$

GDU, galvonometer deflection units: directly proportional to heat of combustion.

In addition to losses of energy, small but significant losses of nitrogen (30 mg/g N, $P<0.005$) resulted from the oven-drying of rat carcasses.

We would recommend that when drying of samples is essential, freeze-drying or vacuum-drying at temperatures below 50° be used.

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REFERENCES

Miller, D. S. & Payne, P. R. (1959). *Br. J. Nutr.* 13, 501.
Thomson, D. J. (1965). *Publs Eur. Ass. Anim. Prod.* no. 11, p. 319.

The binding of bile salts in vitro to fibre from wheat bran and a mould (*Fusarium*). By D. E. Owen, K. A. Munday, T. G. Taylor and M. R. Turner, *Department of Physiology and Biochemistry, The University of Southampton, Southampton SO9 3TU*

The rise in plasma cholesterol in rats brought about by giving diets supplemented with cholesterol and cholate can be prevented by the inclusion of wheat bran or a mould, *Fusarium* (Lord Rank Research Centre), in the diet. Furthermore, these dietary components have been shown to cause a reduction in the plasma cholesterol levels of hamsters, even without the addition of cholesterol and cholate to the diet (Owen, Munday, Taylor & Turner, 1975). In order to identify the active components of wheat bran and the mould, different fibre fractions have been prepared, and their ability to bind bile salts in vitro has been measured. The data obtained with sodium taurocholate and crude fibre (CF) (Association of Official Analytical Chemists, 1970), normal acid fibre (NAF) (ap Griffith & Jones, 1963) and acid detergent fibre (ADF) (Van Soest, 1963) from bran, and ADF from mould, were as in the Table.

Analysis of variance revealed a significant difference in binding due to pH ($P<0.01$) and to type of fibre ($P<0.001$).

The bran fibre fractions had a substantially higher binding affinity for sodium taurocholate than the mould ADF preparation, although wheat bran and the mould had similar hypocholesterolaemic actions in rats. Similar results, in vitro, were obtained with other conjugated and unconjugated bile salts.

	Bile salt bound (%)					
	pH 6		pH 7		pH 8	
Fibre type	Mean	SE	Mean	SE	Mean	SE
Bran CF	20·2	1·8	13·8	0·4	21·9	2·9
NAF	23·6	1·3	20·2	1·3	19·5	3·9
ADF	28·5	0·7	24·5	1·2	13·6	2·5
Mould ADF	8·5	0·9	6·4	0·7	4·5	0·3

(Values are means for five samples)

The results suggest that the hypocholesterolaemic action of the bran may be due to its fibrous components binding to bile salts in the gut, whereas it may be that the mould acts by some other mechanism.

REFERENCES

ap Griffith, G. & Jones, D. I. H. (1963). *J. Sci. Fd Agric.* **14**, 380.
Association of Official Analytical Chemists (1970). *Official Methods of Analysis*, 11th ed., p. 129. Washington, DC: Association of Official Analytical Chemists.
Owen, D. E., Munday, K. A., Taylor, T. G. & Turner, M. R. (1975). *Proc. Nutr. Soc.* **34**, 16A.
Van Soest, P. J. (1963). *J. Ass. off. agric. Chem.* **46**, 839.

Blood glucose profiles in man after ingestion of glucose syrup fractions.

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Glucose syrups of varying composition are manufactured by controlled hydrolysis of maize starch. All are digested to glucose. A typical composition (average molecular weight 477) is (mg/g): monosaccharide 194, disaccharide 142, trisaccharide 118, higher saccharides 546.

Ultra-filtration (Birch & Kearsley, 1974) of glucose syrup has yielded three fractions:

	Composition (mg/g)		
	Low mol. wt (272)	Medium mol. wt (509)	High mol. wt (1295)
Monosaccharide	478	153	3
Disaccharide	229	146	13
Trisaccharide	119	162	35
Higher saccharides	174	539	949

When glucose syrup itself is ingested, the initial rate of absorption is quicker than with dextrose or sucrose (Dodds, Fairweather, Miller & Rose, 1959). Governing the rate of glucose assimilation are stomach emptying and intestinal absorption, which are influenced by the molecular weight and, hence, osmolarity.

Blood glucose profiles induced by glucose syrup and dextrose monohydrate (DMH) do not significantly differ (Butterfield, 1964), but the effect of ingesting fractions is unknown.

Four male volunteers ingested 50 g of each fraction or DMH on each of 4 successive weeks according to a Latin-square design. The blood glucose profiles were plotted over a period of 3 h. Sampling was by finger-prick technique (Green, 1973) and glucose was determined by the GOD-PERID method (Werner, Rey & Weilinger, 1970).

In harmony with the findings of other workers (McDonald, Fisher & Burnham, 1965), between-subject variability on DMH was noted. However, no significant difference was shown by us between the profiles of any of the fractions and DMH. Thus, advantage may be taken of the differing physical, chemical and organoleptic properties of such fractions, whilst retaining the assimilation characteristics of glucose syrup.

REFERENCES

Birch, G. G. & Kearsley, M. (1974). *Die Stärke* **7**, 220.
Butterfield, W. J. H. (1964). *Proc. R. Soc. Med.* **57**, 196.
Dodds, C., Fairweather, F. A., Miller, A. L. & Rose, C. F. M. (1959). *Lancet* i, 485.
Green, L. F. (1973). *Br. J. Sports Med.* **6**, 116.
McDonald, G. W., Fisher, G. F. & Burnham, C. (1965). *Diabetes* **14**, 473.
Werner, W., Rey, H.-G. & Weilinger, H. (1970). *Z. Analyt. Chem.* **252**, 224.