The Editors of the Proceedings of the Nutrition Society *accept no responsibility for the abstracts of papers read at the Society's meetings for original communications. These are published as received from the authors.*

ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Forty-ninth Scientific Meeting of the Nutrition Society was held in the Atkins Building, Queen Elizabeth College, Campden Hill, London W8 7AH, on Friday, 29 September 1972, at 15.00 hours, when the following papers were read:

The protein cost of pregnancy in the rat. By D. J. NAISMITH and CAROLYN RITCHIE, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Experiments with rats have clearly shown that, during pregnancy, protein is used with greater economy. This results from a reduction in the capacity of the liver to deaminate amino acids and to synthesize urea (Naismith & Fears, 1972). The aim of the present study was to discover the extent to which the requirement for protein for growth of the products of conception could be met by adjustments in maternal protein utilization when the diet provided a minimum proportion of protein.

Six litter-mate pairs of rats were accustomed, for at least 5 d, to a diet providing 7% of the total energy as protein. One of each pair was mated, and pregnant and control rats were pair-fed on the low-protein diet throughout gestation. From the 5th day of gestation, pregnant rats were offered, in addition, a protein-free supplement in order to satisfy their increased need for energy. Urine and faeces were collected daily and nitrogen balance was measured. On the 21st day of gestation, the rats were killed, and the products of conception (foetuses, placentas and amniotic fluid) were dissected and analysed for N. The results for five pairs of rats are given in Table 1.

Table 1. *Mean values for nitrogen balance (20 d) in pregnant and non-pregnant rats*

Physiological status	N intake (mg)	N excretion (mg)	N balance (mg)	Additional N retained (mg)	N in concepta (mg)
Non-pregnant	4471	3677	+ 794	—	—
Pregnant	4399	3144	+1255*	461	1028

*Value significantly different from control value ($P=0.02$).

All pregnant rats showed a greater positive N balance than that of their controls, the difference in N retention becoming more marked with the advance of pregnancy. This was due entirely to a reduction in urinary N excretion. By the end of pregnancy, apparent N retention was 58% higher than that of the non-pregnant rats, and the additional N that was spared could account for 45% of the protein cost of the products of conception.

The sixth pregnant rat, which had one pup only, showed a gain of 313 mg N. The N content of her foetus, placenta and amniotic fluid was 112 mg. Thus, by suppressing the catabolism of amino acids, she retained an additional amount of N

that was about three times as much as was found in the concepta. This observation suggests that, when the total amount of new protein to be synthesized is small in comparison with the turnover of protein in the maternal organism, as in a pregnant woman, then physiological adjustments in the metabolism of protein might easily cover the entire protein cost of pregnancy.

This work was supported by a grant from the Gerber Group, which is gratefully acknowledged.

REFERENCE

Naismith, D. J. & Fears, R. B. (1972). *Proc. Nutr. Soc.* 31, 8A.

Relationships between zinc deficiency and folic acid status of the rat.

By R. B. WILLIAMS and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and R. J. L. DAVIDSON, *City Hospital, Aberdeen*

Interrelationships between zinc and B-vitamin metabolism are suggested by the observations of Eggleton (1939) with thiamin, of Hsu (1965) and Gershoff (1968) with pyridoxine and of Chu, Schlicker & Cox (1970) with biotin. This communication presents evidence that Zn deficiency adversely affects the folic acid status of the rat.

Thirty-six male weanling rats were allocated in groups of six to dietary treatments (Table 1). Their diets were based on that of Williams & Mills (1970) with Zn, biotin or folic acid omitted. Zn-deficient diets were offered *ad lib.*; animals receiving Zn-supplemented diets were pair-fed, each with an animal in the Zn-deficient group receiving the same pattern of vitamin supplements. Automatic feeders were used (Quarterman, Williams & Humphries, 1970). Animals were killed after 6 weeks, livers of each group were pooled and their biotin and folic acid contents determined by microbiological assay. All Zn-deficient groups had lower liver concentrations

Table 1. *Influence of diets differing in biotin, folic acid and zinc contents on liver vitamin and Zn concentrations* (*μg/g dry matter*)

Treatment group		A	B	C	D	E	F
Supplements	Zn	—	—	—	+	+	+
	folate	—	+	+	—	+	+
	biotin	+	—	+	+	—	+
Liver biotin		1·2	0·5	1·0	1·0	0·5	1·5
Liver folic acid		18·0	35·5	31·7	58·8	58·0	47·0
Liver Zn		93	84	90	118	119	116

of folic acid than Zn-supplemented groups. No evidence of a Zn–biotin interaction was obtained, suggesting that the effect of Zn deficiency on folate level is not biotin-mediated.

Sequential changes in serum folate concentration in response to Zn depletion were studied separately. No significant differences attributable to Zn were detected during 19 d between groups C and F. A slow increase from the initially low serum folate concentration of group D (+Zn, −folate) did not occur in group A (−Zn, −folate).

The mechanism responsible for depletion of liver folate observed in the rat depleted of Zn for 6 weeks is unknown. There are no clear indications that earlier clinical effects of Zn deficiency are attributable to the same lesion.

REFERENCES

Chu, R. C., Schlicker, S. A. & Cox, D. H. (1970). *Nutr. Rep. int.* **1**, 11.
Eggleton, W. G. E. (1939). *Biochem. J.* **33**, 403.
Gershoff, S. N. (1968). *Proc. Soc. exp. Biol. Med.* **127**, 1207.
Hsu, J. M. (1965). *Proc. Soc. exp. Biol. Med.* **119**, 177.
Quarterman, J., Williams, R. B. & Humphries, W. R. (1970). *Br. J. Nutr.* **24**, 1049.
Williams, R. B. & Mills, C. F. (1970). *Br. J. Nutr.* **24**, 989.

The effects of dietary zinc concentration on reproduction in the rat.

By R. B. WILLIAMS, P. DEMERTZIS* and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Weanling female rats were raised to maturity on the semi-synthetic basal diet described by Williams & Mills (1970), supplemented with 40 μg zinc/g. This regimen was maintained during mating with stock colony males, during the subsequent pregnancy, and until the offspring were weaned at 22 d. Female weanlings were then randomly assigned in three groups of eight to the basal diet supplemented with 6, 9 or 12 μg Zn/g (groups A, B and C respectively). Animals were mated after oestrus cycles had been observed in all groups; gestation and weaning of the litters continued at the same dietary Zn concentrations.

Table 1. *Effects of differing concentrations of dietary zinc on reproductive performance in the rat*

(Mean values with their standard errors; figures in parentheses refer to number of litters)

Group	A	B	C
Dietary Zn concentration (μg/g)	6	9	12
Proportion of females which produced live young	5/8*	8/8	8/8
No. of litters surviving to weaning	3	7	8
Litter size at birth	9·0±1·3 (7)	9·9± 0·9 (8)	11·3± 0·4 (8)
No. in litters surviving to weaning	8·7±0·3 (3)	8·1± 1·1 (7)	10·4± 0·5 (8)
Litter wt at birth (living young only) (g)	41·3±8·7 (5)	48·9± 5·9 (8)	57·8± 1·7 (7) a
Birth wt of individual young (g)	4·91	4·95	5·06
Wt of surviving litters at weaning (g)	248·4±9·5 (3)	268·3±40·4 (7)	356·5±18·2 (8) b,c
Wt of individual young at weaning (g)	28.7 (3)	33.0 (7)	34·5 (8)
Percentage of young surviving to weaning	41·3 (7)	72·2 (8)	92·2 (8)

a: significantly different from Group A ($P<0·1$)
b: significantly different from Group A ($P<0·01$)
c: significantly different from Group B ($P<0·1$)
*In Group A one female ate its litter at birth.

Most females in group A were severely affected by the low Zn intake (Table 1) and five of them either gave birth to dead young or consumed their litters within 2 d

*On leave of absence from the Laboratory of Physiopathology and Animal Reproduction, Aghia Paraskevi-Attikis, Athens.

of birth. All females in groups B and C gave birth to viable young, but survival of the offspring to 22 d was markedly improved at the highest Zn concentration.

A concentration of 12 μg Zn/g of a diet based on egg albumen has been shown to be sufficient for maximum growth of weanling rats (Forbes & Yohe, 1960; Williams & Mills, 1970). The present results suggest that at least this concentration is required for satisfactory maintenance of pregnancy and postnatal survival of the rat.

REFERENCES

Forbes, R. M. & Yohe, M. (1960). *J. Nutr.* **70**, 53.
Williams, R. B. & Mills, C. F. (1970). *Br. J. Nutr.* **24**, 989.

The response of pre-term low-birth-weight newborn infants to supplements of pteroylmonoglutamic acid and pteroylpolyglutamate. By W. L. BURLAND* and PAMELA D. SAMUEL*, *Glaxo Laboratories, Greenford, Middlesex* and K. SIMPSON, *The General Hospital, Leicester*

Folic acid by injection prevents the development of low serum and red cell folate concentrations in low-birth-weight pre-term infants (Burland, Simpson & Lord, 1971).

The pre-term newborn infant's dietary requirements for folate are thought to be greater than those of a full-term infant (Vanier & Tyas, 1967). The total folate content of proprietary milks prepared for infant feeding in the UK is from 30 to 70 ng/ml (Ford & Scott, 1968; Burland *et al.* 1971), thus low-birth-weight, pre-term infants are unlikely to receive an adequate intake of folate in the early weeks of life.

Forty-five newborn pre-term low-birth-weight infants were divided into three groups. Group A received a daily supplement of 50 μg pteroylmonoglutamic acid by mouth, group B 50 μg pteroylpolyglutamate daily and group C remained untreated. All the infants were fed with a standard milk preparation containing 49 ng total folate/ml. Serum and red cell folate concentrations were determined (before feeding) on day 1 and day 14 (Table 1).

Pteroylpolyglutamate and pteroylmonoglutamic acid were absorbed equally well. This indicates that there is no deficiency of jejunal pteroylpolyglutamate hydrolase in pre-term infants. The administration of 50 μg pteroylmonoglutamic acid each day resulted in treated infants having significantly higher serum folate concentrations at 14 d than untreated infants.

Since no further appreciable fall in serum folate occurs in untreated infants after the 3rd week of life (Burland *et al.* 1971), it is suggested that a daily supplement of 50 μg pteroylmonoglutamic acid is sufficient to protect pre-term newborn infants from risk of folate deficiency. It may be justified to advise a daily supplement of 50 μg for the first 3 months of life or alternatively to fortify infants' milks to ensure a total daily intake of 70 μg folate or more.

*Present address: Servier Laboratories, Harrow, Middlesex HA1 4HQ.

Table 1. *Serum and red cell folate concentrations in three groups of pre-term infants on the 1st and 14th days of life*

Group	A	B	C
Mean gestational age (weeks)	34	35	34
Sex:			
♂	8	6	6
♀	7	9	9
Mean birth weight (kg)	1·67	1·78	1·85
Red cell folate concentration (mean±SE) (ng/ml):			
Day 1	525±86 (9)*	596±62 (10)*	615±38 (4)*
Day 14	449±24	445±59	475±52
P, day 1:day 14	NS	NS	NS
Serum folate concentration (mean±SE) (ng/ml):			
Day 1	30·0±3·0 (9)*	37·7±7·7 (15)	36·4±5·1 (12)*
Day 14	16·9±1·8	15·5±1·6	6·6±0·07
P, day 1:day 14	<0·01	<0·02	<0·001
% day 14:day 1 (mean±SE)	58·9±21·6	54·1±24·0	21·4±13·1
P between groups			
A:B	NS		
A:C	<0·001		
B:C	<0·01		

NS, not significant.
*The numbers of infants providing 'paired' samples were fewer than 15 because of withdrawals due to intercurrent illness or inability to obtain adequate blood samples.

REFERENCES

Burland, W. L., Simpson, K. & Lord, J. (1971). *Archs Dis. Childh.* **46**, 189.
Ford, J. E. & Scott, K. J. (1968). *J. Dairy Res.* **35**, 85.
Vanier, T. M. & Tyas, J. F. (1967). *Archs Dis. Childh.* **42**, 57.

Estimation of maintenance requirements of amino acids in the rat by measurement of the rate of ^{14}C oxidation in vivo. By R. J. NEALE and J. C. WATERLOW, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, WC1E 7HT*

Said & Hegsted (1970) found that when rats were given diets deficient in leucine or lysine the net protein utilization (NPU) values were much above those predicted from the amino acid score. They pointed out that it is difficult to estimate requirements accurately when the dose-response curve is flat, as it is with these two amino acids. This is to be expected, since isotopic results show that these essential amino acids are very highly reutilized, and conserved with great efficiency (Garlick, Waterlow & Millward, 1972).

We therefore tried another approach—to see whether it is possible to determine maintenance requirements from the loss of radioactivity from the body after a single dose of [^{14}C]leucine or [^{14}C]lysine.

Rats weighing about 100 g were put on a diet which provided 0·45 NDP:E as protein, so that they maintained constant weight; 6 μCi ^{14}C-labelled amino acid were given by intragastric tube. Respiratory carbon dioxide was collected for 3 h to determine the extent of the initial loss of activity. Some rats were killed immediately, others after 15, 20 and 30 d, and the remaining radioactivity was measured.

The loss of radioactivity from the body was two to three times greater in the first than in the second 15 d period. The rate of loss of activity only reflects the rate of oxidation, i.e. net loss of amino acid from the body, when the specific activities in all tissues are equal. This condition is never fulfilled, but it is more nearly fulfilled at the end than at the beginning of the experiment. During the second 15 d period, radioactivity was lost from the whole body at the rate of about 2% daily. In five rats the total amounts of leucine or lysine (protein-bound+free) were measured. From these results the following daily rates of loss were calculated, per kg body-weight: lysine 28 mg; leucine 20 mg. In terms of metabolic body size ($kg^{0·75}$) the value for lysine is 157 mg/d, for leucine 110 mg/d. These are much higher than the values for daily maintenance requirements obtained by Said (lysine 34, leucine 44 mg/$kg^{0·75}$). It must be emphasized, however, that the aim of the experiments reported here was to find suitable conditions for determining maintenance requirements by isotopic methods.

REFERENCES

Garlick, P. J., Waterlow, J. C. & Millward, D. J. (1972). *Physiol. Rev.* (In the Press.)
Said, A. K. & Hegsted, D. M. (1970). *J. Nutr.* **100**, 1363.

Effects on brain free amino acids of low- and high-protein diets given in amounts of equal energy value to weanling rats. By J. W. T. DICKERSON and S. K. PAO, *Department of Biochemistry, University of Surrey, Guildford, Surrey*

The free amino acid pool contributes to brain function and plays a significant part in the metabolism of brain protein (Lajtha, 1964). The concentrations of γ-aminobutyric acid, glutamic acid, alanine and aspartic acid have been reported to be reduced in the cerebrum of young and adult rats fed on a 90 g protein/kg diet for 12–16 weeks (Rajalakshmi, Govindarajan & Ramakrishnan, 1965). The present study was undertaken to compare the effects of giving low- and high-protein diets in amounts that were of equal energy value to weanling rats for 8 weeks.

Wistar male rats, 24 d of age, were randomly divided into three groups of five and were caged individually. One group (LP) was given a low-protein diet, containing (g/kg): casein 30 (with 2 g methionine/kg) and starch 780. A second group (HP) was given a high-protein diet, containing (g/kg): casein 250 (with 2 g methionine/kg) and starch 560, the amount given being such that it was of equal energy value to that eaten by the LP rats. The third group, the controls, were given the

high-protein diet *ad lib.* All diets were supplemented with vitamins and minerals. Water was available *ad lib.* throughout the experiments.

After 8 weeks, the rats were killed by decapitation and their heads dropped immediately into liquid nitrogen; the frozen brains were dissected into forebrain cerebellum and brain stem (Dickerson & McAnulty, 1972). Brain free amino acids were measured with an autoanalyser (Thomas, 1970).

The body-weight of the LP rats was 25% of that of the controls, whereas that of the HP rats was 70% of that of the controls. The weight of the brain of the LP rats was significantly lower than that of the controls, whereas that of the HP rats was not.

Compared with the controls, the LP rats had higher concentrations of certain amino acids in one or more parts of the brain (glycine in the forebrain, phenylalanine in the brain stem, and histidine and methionine in all three parts), but the concentration of lysine was lower in the forebrain. In contrast, the HP rats had lower concentrations of γ-aminobutyric acid and leucine in the brain stem, of lysine in the cerebellum, of alanine in the forebrain and brain stem, and of glycine in all three parts of the brain.

Possible mechanisms for these changes were discussed.

We are grateful to Dr Alan Thomas for the amino acid determinations.

REFERENCES

Dickerson, J. W. T. & McAnulty, P. A. (1972). *Resuscitation* **1**, 61.
Lajtha, A. (1964). *Int. Rev. Neurobiol.* **6**, 1.
Rajalakshmi, R., Govindarajan, K. R. & Ramakrishnan, C. V. (1965). *J. Neurochem.* **12**, 261.
Thomas, A. J. (1970). *In Automation, Mechanization and Data Handling in Microbiology* p. 107 [A. Baillie and R. J. Gilbert, editors]. London: Academic Press Inc.

Effects of low-protein diets containing sucrose or starch in amounts of equal energy value on serum albumin concentration and liver composition of weanling rats. By R. F. GRIMBLE* and J. W. T. DICKERSON, *Department of Biochemistry, University of Surrey, Guildford, Surrey*

In the rat, a low-protein diet decreases the concentration of serum albumin whether the dietary carbohydrate is sucrose (Grimble, Sawyer & Whitehead, 1969) or starch (Kirsch, Brock & Saunders, 1968). Sucrose causes a fall in the serum albumin concentration in young men on a normal protein intake (Brice, Coles, Jourdan & Macdonald, 1969).

The present longitudinal study compares the effects of feeding low-protein diets, containing sucrose or starch in amounts of equal energy value, on liver growth and serum albumin concentration.

Wistar weanling rats were caged individually and equal numbers (thirty) were randomly assigned to one of three experimental groups. The controls received a normal pellet diet (Spillers, 210 g/kg protein) *ad lib.*, group 3 ('starch') a 60 g/kg protein

*Present address: Department of Physiology & Biochemistry, University of Southampton.

diet prepared by diluting a powder form of this diet with starch, and group 2 a similar diet containing sucrose instead of starch. The low-protein diets were supplemented with vitamins, minerals and maize oil. Groups 2 and 3 were pair-fed. Animals of each group were killed at intervals up to 28 d, and samples of serum and the livers were stored at $-20°$ until analysed.

Serum albumin (Doumas, Watson & Biggs, 1971), liver DNA (Zamenhof, Bursztyn, Rich & Zamenhof, 1964) and total protein (micro-Kjeldahl) were determined.

The body-weights of the two low-protein groups were similar throughout and both groups were lighter than the controls from day 3. The livers of the 'sucrose' rats weighed more than those of the 'starch' rats from day 3, and from day 14 contained more protein and DNA. The liver protein concentration of the 'sucrose' rats was lower than that of the 'starch' group from day 7. The protein:DNA ratio in the liver of the 'sucrose' rats was lower than that of the starch-fed rats at day 28 only.

Serum albumin concentrations in the controls rose throughout the experiment. In both low-protein groups, the concentration fell after 3 d and remained lower than those of the controls throughout the experiment. The concentrations in the 'sucrose' group continued to fall until day 14, whereas in the starch-fed group they rose after day 14. At day 28, the serum albumin concentration in the 'sucrose' animals was lower than in those given starch.

REFERENCES

Brice, J., Coles, B. L., Jourdan, M. H. & Macdonald, I. (1969). *Proc. Nutr. Soc.* **28**, 62A.
Doumas, B. T., Watson, W. A. & Biggs, H. G. (1971). *Clinica chim. Acta* **31**, 87.
Grimble, R. F., Sawyer, M. B. & Whitehead, R. G. (1969). *Br. J. Nutr.* **23**, 879.
Kirsch, R. E., Brock, J. F. & Saunders, S. J. (1968). *Am. J. clin. Nutr.* **21**, 820.
Zamenhof, S., Bursztyn, H., Rich, K. & Zamenhof, P. J. (1964). *J. Neurochem.* **11**, 505.

Alteration of dietary fatty acid by human intestinal bacteria. By JOY R. PEARSON (introduced by SIR FRANCIS AVERY JONES), *Medical Research Council Gastroenterology Unit, Central Middlesex Hospital, London NW10 7NS*

One of the striking differences between the fatty acid compositions of dietary and faecal fat is the presence in faecal fat of 10-hydroxystearic acid, a fatty acid absent from dietary fats (James, Webb & Kellock, 1961). Small amounts (less than 3% of the total faecal fatty acids) of hydroxystearic acid were found in all twelve normal subjects studied by Kellock, Pearson, Russell, Walker & Wiggins (1969). Greatly increased amounts were found in all patients with gross steatorrhoea and in over 50% of patients under investigation for malabsorption with milder or no steatorrhoea. The maximum amount was 23% of faecal fatty acids.

The formation of hydroxyacids from unsaturated fatty acids by a *Pseudomonas* species and by eight strains of enteric bacteria has been reported (Davis, Wallen, Goodwin, Rohwedder & Rhodes, 1969; Thomas, 1972).

We have studied the ability of bacteria isolated from the faeces of normal human subjects and patients with high and low faecal hydroxystearic acid to produce hydroxyacids from unsaturated fatty acids. Most of the bacteria were grown in peptone-yeast broth (Cato, Cummins, Holdeman, Johnson, Moore, Smibert & Smith, 1970) supplemented with 1 g oleic acid/l finely dispersed by ultrasonication. Cultures of anaerobes were incubated in an atmosphere of hydrogen–carbon dioxide (9:1). Facultative bacteria were grown under aerobic conditions. Some strict anaerobes were grown in Robertson's Cooked Meat medium (obtainable from Southern Group Laboratories), the batch used contained about 3·5 g/l oleic acid. Some strains were also tested with linoleic and palmitoleic acids. After incubation for 3 d cultures were saponified and the fatty acids were extracted and examined by thin-layer chromatography of the free acids or gas–liquid chromatography of their methyl esters.

Two hundred and twenty-eight strains from five genera were tested; 103 strains were able to convert oleic acid into hydroxystearic acid. The results are given in Table 1. There was no significant difference in the proportion of active strains among bacteria isolated from normal subjects or patients with high or low faecal hydroxystearic acid, except among the enterobacteria. None of fourteen strains tested converted linoleic acid into an unsaturated hydroxyacid. Five of eleven strains tested converted palmitoleic acid into hydroxypalmitic acid.

Table 1. *Bacteria isolated from faeces which produce hydroxystearic acid*

Organism	No. tested	% forming OH-stearic acid
Bacteroides	34	18
Bifidobacteria	47	32
Clostridia	22	50
Enterobacteria	52	21
Enterococci	52	98
Strict anaerobes (not further classified)	21	33

REFERENCES

Cato, E. P., Cummins, C. S., Holdeman, L. V., Johnson, J. L., Moore, W. E. C., Smibert, R. M. & Smith, L. D. S. (1970). *Outline of Clinical Methods in Anaerobic Bacteriology* p. 100. Blacksburg, Virginia: Anaerobic Laboratory, Virginia Polytechnic Institute & State University.
Davis, E. N., Wallen, L. L., Goodwin, J. C., Rohwedder, W. K. & Rhodes, R. A. (1969). *Lipids* **4**, 356.
James, A. T., Webb, J. P. W. & Kellock, T. D. (1961). *Biochem. J.* **78**, 333.
Kellock, T. D., Pearson, J. R., Russell, R. I., Walker, J. G. & Wiggins, H. S. (1969). *Gut* **10**, 1055.
Thomas, P. J. (1972). *Gastroenterology* **62**, 430.

Chemical composition of rumen bacteria. By R. H. SMITH and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Although rumen bacteria provide an important part of the nutrients passing into the duodenum of the ruminant, information on their chemical composition is, in many respects, sparse. For example, though much is known of the proportions of

different amino acids that they contain, the total amount of protein in rumen bacterial cellular matter is uncertain. The value (65% based on dry weight) given by Hungate (1966), which is frequently quoted, is higher than some more recently reported values (Milwid, Oliver & Topps, 1968), probably because Hungate recorded values for a protein-containing fraction from rumen bacterial cells (Weller, 1957) as values for the whole cells.

Samples of mixed rumen bacteria were separated from the rumen contents of different animals by suitable centrifuging and washing (Smith & McAllan, 1972). Some of their components are shown in Table 1.

Table 1. *Composition of mixed bacterial samples from rumen contents taken 4–6 h after giving diets with 80–120 g crude protein/kg in dry matter and containing 500–1000 g roughage (hay, dried grass or straw)/kg and 0–500 g concentrates/kg. Results are mean values with their standard errors for mg dry bacterial cells/g*

	No. of animals	No. of samples	Crude protein*	Nucleic acid	'True' protein†	Total carbohydrate‡	'Starch'§	Ash‖
Sheep	13	20	438±13	55	383±11	62± 7	26± 5	255±17
Cows	3	4	475±31	52	423±26	86±10	48±10	180±11
Calves	8	32	315±14	55	260±11	165± 9	117± 9	270± 7

*Total nitrogen × 6·25.
†Total N − nucleic acid N × 6·25.
‡Total sugars released on refluxing for 4 h with 0·5 M-H_2SO_4.
§Glucose released on refluxing for 4 h with 0·5 M-H_2SO_4.
‖After heating at 600° for 24 h.

Differences in composition between bacteria from calves and adult animals were probably due, at least in part, to the calves having been reared in a segregated environment (Smith & McAllan, 1972). It is likely that calves reared in contact with older animals would not show these differences.

In giving mean values in Table 1, no attempt has been made to show variations in microbial composition which occurred with diet variations. For example, with conditions other than those shown in Table 1, mean values ±SEM for amounts of crude protein per kg in calf bacterial dry matter were 238±10 (eleven samples from five calves) and 442±27 (six samples from three calves) for diets containing 80 and 160–220 g crude protein/kg respectively. However, irrespective of such variations, the results showed that rumen bacteria produced under normal conditions contained less protein than is often supposed and a surprisingly large amount of inorganic material. These constituents, together with carbohydrate, accounted for about 750 g of the total dry matter/kg.

REFERENCES

Hungate, R. E. (1966). *The Rumen and its Microbes* p. 307. New York: Academic Press.
Milwid, M. S., Oliver, J. & Topps, J. H. (1968). *S. Afr. J. agric. Sci.* **11**, 493.
Smith, R. H. & McAllan, A. B. (1972). *Science, N.Y.* (In the Press.)
Weller, R. A. (1957). *Aust. J. biol. Sci.* **10**, 384.

Variations in available carbohydrate and physical work ability with repeated prolonged severe exercise. By J. D. BROOKE, *Human Performance Laboratory, Physical Education Section, University of Salford*, and L. F. GREEN, *Beecham Products, Great West Road, Brentford, Middlesex*

Severe prolonged exercise in the human subject depletes the glycogen stores. With adequate availability of carbohydrate there is over-compensation to remedy this depletion, seen, for example, in abnormally increased muscle glycogen concentrations (Saltin & Hermansen, 1967). While studying the effect of repeatedly obtaining such exhaustion of human subjects we have observed systematic variations.

Following the procedures previously described (Brooke, Davies & Green, 1972), cycle ergometer exercise at approximately 60% maximum physical work capacity was performed to close to exhaustion of available carbohydrate stores by three habituated male racing cyclists. The criterion of exhaustion was a non-protein metabolic respiratory quotient (RQ) of 0·73, or incapacity. During work, subjects were given 150 ml of an electrolyte solution every 20 min. Following the procedure of Saltin & Hermansen (1967), three trials were made with 70 h between each. Subjects ate normally (approximately 14·65 MJ with 8·80 MJ as carbohydrate/d) except for 20 h fasting before exercise.

Over the period of the trials, systematic adaptation occurred with respect to the carbohydrate available for work and the time taken for it to be used up. The first trial resulted in a mean RQ of 0·78 after 40 min work, and a work time to exhaustion of 1·71 h. There was a marked reduction in capacity in the second trial with a mean RQ of 0·74 after 40 min work, and a mean work time of 1·01 h. The trend was reversed in the third trial, which took place 140 h after the first and resulted in a mean RQ of 0·80 after 40 min, and a raised mean work time of 3·80 h, a duration of over twice that of the first trial.

The experiment was repeated with three different subjects, a 9 h fast before test, and work cut-off point at RQ 0·75. Similar results were obtained, but with greater variation between subjects. Statistically significant differences ($P<0·05$) were obtained by comparing the work times to exhaustion or the RQ levels override. The mean weight of carbohydrate utilized per subject during work fell in the second trial to 17% of the value in the first trial, with the third trial showing a 150% increase over the first.

With a constant power output during trials more efficient work was performed for the first 4 h of the third trial, since the mean oxygen uptake was approximately 250 ml lower for this period. This increased efficiency, with internal availability of carbohydrate, corresponds to that found for the external provision through glucose syrup supplements, as reported previously (Brooke *et al.* 1972).

Such carbohydrate adaptation was not seen for trials more than 14 d apart. The adaptation curve and regulatory mechanisms are not clear, but note is taken of muscle biopsy studies which draw attention to the significance of increased glycogen synthetase activity in local absence of available carbohydrate (Hultman & Bergström, 1967). The rhythm of adaptation reported has significance for sports performance preparation and for the design of experiments in nutrition and work.

This research was supported by Beecham Products.

REFERENCES

Brooke, J. D., Davies, G. J. & Green, L. F. (1972). *Proc. Nutr. Soc.* **31**, 93A.
Hultman, E. & Bergström, J. (1967). *Acta med. scand.* **182**, 109.
Saltin, B. & Hermansen, L. (1967). In *Nutrition and Physical Activity* p. 32 [G. Blix, editor]. Uppsala: Almqvist & Wiksell.

Plasma concentrations of insulin, corticosterone, lipids and sugars in rats fed on meals with glucose and fructose. By K. R. BRUCKDORFER, S. S. KANG and J. YUDKIN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Male Sprague–Dawley rats, weighing 150–200 g, were fed for 3 h daily on a purified diet with 680 g starch/kg. After 15 d acclimatization to this 'meal-feeding', half of the animals were given a meal in which starch was replaced by glucose, and half given one in which it was replaced by fructose.

From each group, four rats were killed just before the meal and another four at 1, 2, 3 and 5 h after the beginning of the meal. Pooled samples of plasma were taken for determination of triglycerides, free fatty acids, insulin, corticosterone, glucose and fructose (Table 1). The assays were carried out by conventional methods; for corticosterone we used the method of Corker, Naftolin & Richards (1971).

Table 1. *Plasma composition of rats fed on a meal with fructose or glucose*

Time after start of meal (h)	Triglyceride (mg/l)	Free fatty acid (μmol/l)	Insulin (IRI) (μU/l)	Cortico-sterone (μg/l)	Glucose (mg/l)	Fructose (mg/l)
			Glucose diet			
0	650	650	480	500+	1200	25
1	520	290	1970	170	1260	30
2	600	290	—	130	1240	25
3	770	270	2010	10	1180	5
5	770	250	1290	50	1200	35
			Fructose diet			
0	720	770	400	500+	1220	35
1	910	740	320	500+	1180	275
2	1370	690	400	400	1240	370
3	850	570	400	130	1120	225
5	950	570	480	150	1100	165

Compared with the meal of glucose, that with fructose produced a much greater increase in triglyceride, no increase in insulin and a smaller decrease in the free fatty acids. Fructose also produced a slower and smaller decrease in corticosterone, which is compatible with the finding that, in a proportion of human subjects, dietary sucrose increases the concentration of 11-hydroxycorticosteroids (Yudkin & Szanto, 1971).

REFERENCES

Corker, C. F., Naftolin, S. & Richards, M. R. (1971). *J. Physiol., Lond.* **218**, 28P.
Yudkin, J. & Szanto, S. (1971). *Br. med. J.* i, 349.

Plasma glucose and acetone concentrations in pregnant and lactating ewes. By P. J. DAVIES, *Essex Institute of Agriculture, Writtle, Chelmsford CM1 3RR*, and D. B. ROSS,* *The Animal Health Trust, Stock, Essex*

Blood concentrations of glucose and ketone bodies (usually expressed as acetone) have often been used as indices of inadequate energy intake by ruminant animals. Such indices are particularly useful at times of high energy and high glucose requirement, such as late pregnancy and early lactation. In the ewe, energy requirement at these times is chiefly influenced by the number of foetuses being carried and the number of lambs being suckled.

Results are presented for eleven individually fed ewes which proved to be carrying twin foetuses and subsequently suckled twin lambs for at least 21 d. Blood samples were taken at 19–23 d pre-partum (126 d pregnant) and again at 19–23 d post-partum. All pregnant ewes received the same daily food allowance (based on body-weight) for at least 7 d before blood sampling. After lambing, the ewes were split into two groups; five received the same daily food allowance as at 126 d pregnant and six received this allowance increased by 50%. Mean values for plasma glucose and acetone at both sampling dates are shown in Table 1.

Table 1. *Mean values with their standard errors for plasma glucose and acetone concentrations in pregnant ewes bearing twin foetuses and lactating ewes suckling twin lambs*

	21 d pre-partum	21 d post-partum	
Feeding scale (g/kg $W^{0.73}$ per d):			
Hay	40	40	60
Concentrate	20	20	30
No. of ewes	11	5	6
Level of energy intake (L*)	1·56	1·56	2·34
Plasma glucose (mg/l)	420±17	520±58	550±50
Plasma acetone (mg/l)	35±4·2	99±25·8	80±11·9

*Metabolizable energy intake expressed as a multiple of the maintenance requirement of the 70 kg non-pregnant ewe, which is 7·95 MJ/d.

The higher food intake during lactation resulted in a non-significant increase in milk production on the 21st day (2·01–2·27 kg). Over the 21 d lactation period, ewes on the higher energy intake lost 8·3 kg body-weight (68·3–60·0 kg) whereas ewes on the lower intake lost 11·2 kg (72·1–60·9 kg).

The results confirm that in the lactating ewe hyperketonaemia can exist in association with normal blood glucose concentrations (Reid & Hinks, 1962). By

*Present address: Huntingdon Research Centre, Huntingdon PE18 6ES.

contrast, in the undernourished pregnant ewe, plasma acetone concentrations above 80 mg/l are associated with hypoglycaemia (Russel, Doney & Reid, 1967; Davies, Johnston & Ross, 1971).

REFERENCES

Davies, P. J., Johnston, R. G. & Ross, D. B. (1971). *J. agric. Sci., Camb.* **77**, 261.
Reid, R. L. & Hinks, N. T. (1962). *Aust. J. agric. Res.* **13**, 1112.
Russel, A. J. E., Doney, J. M. & Reid, R. L. (1967). *J. agric. Sci., Camb.* **68**, 351.

Alcoholic beverages; a neglected factor in dietetics. By T. P. EDDY, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

The importance of the contribution made by alcoholic beverages to normal diets and of ethanol as one of the principal energy-yielding constituents is neglected in dietary studies, although WHO (1954) recommended that the distribution of alcohol consumption should be included in any nutritional survey. This has been noticed in a study of the contribution made by alcoholic beverages to the ordinary diets of 155 'non alcoholics' in the USA by Bebb, Houser, Witschi, Littell & Fuller (1971), and by Eddy, Stock & Wheeler (1971) and Eddy, Wheeler & Stock (1971) in their study of the diet of British seamen.

The omission is particularly noticeable in studies of the relationship of different energy-yielding constituents to heart disease and obesity, in which ethanol may be a considerable factor. Richardson (1972) gives a comprehensive list of references to work on the interrelationships of diet—particularly dietary fat and sugar—with body-weight, heart disease, blood cholesterol and smoking habits. Many, particularly those concerned with sugar consumption, refer to one dietary constituent only, and those which assess 'total' energy intake, do not include energy derived from alcoholic beverages.

Eddy, stock *et al.* (1971) found average daily intakes attributable to ethanol of 2·00 MJ (12% total energy) in the crew of a British tanker carrying fifty-four men; and in Loire valley miners, Bresard & Gombervaux (1962) found averages of 3·1 MJ (16% total energy) in twenty-nine normal controls and 6·7 MJ (31% total energy) in forty-two heavy drinkers. The British and French subjects were all men working in hot environments. For 114 American men studied by Bebb *et al.* (1971), corresponding values were 0·6 MJ (6% total energy) but twenty-six, i.e. 23%, of the men, who were habitual drinkers, had a daily intake of 1·7 MJ (17% total energy).

Bebb *et al.* (1971), Eddy, stock *et al.* (1971), Eddy, Wheeler *et al.* (1971), Bresard & Gombervaux (1962) and Bresard & Chabert (1963) all present evidence suggesting an inverse relationship between the intake of ethanol and intakes of fat and sugar. There are complicated nutritional and social relationships between dietary sugar, alcoholic beverages, fat intake and smoking habits, and the omission in dietary surveys of 10–20% or more of total energy intake derived from ethanol by habitual drinkers may be serious.

REFERENCES

Bebb, H. T., Houser, H. B., Witschi, J. C., Littell, A. S. & Fuller, R. K. (1971). *Am. J. clin. Nutr.* **24**, 1042.
Bresard, M. & Chabert (1963). *Bull. Inst. natn. Hyg.* **18**, 639.
Bresard, M. & Gombervaux, C. (1962). *Bull. Inst. natn. Hyg.* **17**, 217.
Eddy, T. P., Stock, A. L. & Wheeler, E. F. (1971). *Br. J. ind. Med.* **28**, 330.
Eddy, T. P., Wheeler, E. F. & Stock, A. L. (1971). *Br. J. ind. Med.* **28**, 342.
Richardson, J. F. (1972). *Br. J. Nutr.* **27**, 449.
WHO (1954). *Tech. Rep. Ser. Wld Hlth Org.* no. 84.

Alcoholic drinks and hangover effects. By G. L. S. PAWAN, *Metabolic Division, Department of Medicine, The Middlesex Hospital Medical School, London W1P 7PN*

Liberal consumption of alcoholic drinks frequently produces 'hangover' effects in certain individuals in the morning after a drinking bout of the preceding night. Symptoms of hangover may extend from mild indisposition to tiredness, headache, and severe physiological, psychological, and even pathological disorder. Effects vary with the concentration, volume, type of drink, and other factors, including the amount of non-ethanolic congener-constituents of a particular drink. Because of the increasing use of alcoholic drinks in the diet of many individuals and the controversy as to the causes of hangover, a study was made on the hangover-producing effects of some commonly used popular alcoholic drinks.

The experimental subjects were twenty healthy male volunteers aged 21–51 (mean 30) years who were occasionally moderate drinkers. Each subject received, under standard conditions, each of the following drinks at intervals of 1 week: red wine, white wine, rum, whisky, gin, vodka, brandy, diluted pure ethanol (20%) in orange juice. The amount of each drink consumed provided on all occasions 1·5 ml ethanol/kg body-weight. The subjects recorded the nature and severity of any unpleasant after-effects experienced in the morning after each drinking experiment of the preceding evening. Reports varied with the subjects and drink consumed. Symptoms included tiredness, headache, nausea, gastro-intestinal disturbance and general indisposition. Hangover effects appeared to be proportional to the congener content of a drink. Hangovers were produced most frequently by high congener-container drinks: brandy caused most effects, followed by red wine, rum, whisky, white wine and gin in decreasing order of occurrence. Vodka, and diluted ethanol in orange juice produced mild hangover effects (increased thirst and slight tiredness) in only two of the subjects. These results indicate the importance of non-ethanolic constituents in alcoholic drinks as potent factors in producing hangover.