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-fifth Scientific Meeting of the Nutrition Society was held in the Barnes Lecture Theatre, The Royal Society of Medicine, 1 Wimpole Street, London W1M 8AF, on Friday, 19 May 1972, at 10.30 hours, when the following papers were read:

Losses of vitamin C during machine-peeling and soaking of peeled potatoes.

By T. P. Eddy and Anne Stock*, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

Experimental losses of vitamin C from mechanical peeling and soaking described by Platt, Eddy & Pellett (1963) were higher than losses found in similar experiments by Zarnegar & Bender (1971). Our experiments were undertaken following the observation, reported by Platt *et al.* (1963), that in two hospitals potatoes soaked in sinks overnight lost 45–60% of vitamin C. One hospital sink contained a dilute solution of sodium metabisulphite, water in the other was a deep-brown colour; such waters probably contained little dissolved oxygen. Feldheim & Hjelm (1963) described vitamin C losses by leaching from peeled and cut potatoes in unoxygenated water, but in oxygenated water vitamin synthesis in the actively metabolizing potato more than compensated for losses. Biosynthesis of vitamin C could occur in laboratory conditions and might account for experimental differences and for our finding that in three of a total of four experiments, in which peeled potatoes were soaked for 42–48 h, vitamin C content increased.

Below are shown the results from eighty-two analyses of reduced vitamin C in potatoes, hand-peeled or mechanically peeled for 0·5–6 min followed by periods of soaking for 14–24 h; and those peeled by a domestic peeler (soaked 4–24 h). There were no significant differences in results in relation to the duration of these procedures and they are pooled. The values in fresh hand-peeled potatoes on which percentage values are based varied between 8 and 30 mg/100 g.

The small domestic peeler was driven by a hose attached to an ordinary tap. It took 4·5 min to peel three or four potatoes completely, as contrasted to 1 min

Treatment		No. of analyses	% of (1) ±SE	Significance of *P* value
Hand-peeled	(1) analysed immediately	25	100± 4·4	
	(2) soaked 14–24 h	5	79·6±10·8	(1) *v.* (2)>0·10
Canteen and hospital mechanical peelers, 0·5–6 min	(3) analysed immediately	16	87·7± 4·1	(1) *v.* (3)=0·05
	(4) soaked 14–24 h	25	73·8± 4·6	(3) *v.* (4)<0·05>0·02
				(1) *v.* (4)<0·001
Small domestic low-powered hydraulic peeler, 4–9 min	(5) analysed immediately	3	95·5± 3·1	(1) *v.* [(5)+(6)], not significant
	(6) soaked 4–24 h	8		

*Present address: Department of Nutrition, Queen Elizabeth College, London W8 7AH.

https://doi.org/10.1079/PNS19720066 Published online by Cambridge University Press

for 0·7 kg potatoes in the industrial peelers. In the small peeler, potatoes were subjected to less pressure and friction; this may account for the similarity of results to those with hand-peeling, and the superiority of both methods to industrial machine-peeling with long soaking. Similar losses of 60–80% of thiamin from peeled potatoes, soaked overnight, have been reported by Lowe (1969).

REFERENCES

Feldheim, W. & Hjelm, G. (1963). Ernähr.-Umsch. **10**, 145.
Lowe, S. (1969). The determination of thiamine in school meals. PhD Thesis, University of Leeds. Quoted in *Vitamins* (1971), p. 94 [M. Stein, editor]. London: Churchill Livingstone.
Platt, B. S., Eddy, T. P. & Pellett, P. L. (1963). *Food in Hospitals* p. 70. London: Oxford University Press.
Zarnegar, L. & Bender, A. E. (1971). *Proc. Nutr. Soc.* **30**, 94A.

Changes in the lens epithelium of the rat in vitamin A deficiency. By ANTOINETTE PIRIE and MARY OVERALL, *Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW*

One of the profound effects of vitamin A deficiency on the eye is keratinization of the epithelium of the cornea. This becomes so dense that intraocular examination is impossible. No changes have been reported in the lenses of animals or men but, since the epithelium of the lens is derived from the same ectodermal layer as the corneal epithelium, we have now examined the effect upon it of prolonged vitamin A deficiency. Pirie & Overall (1972) found that, in pair-fed Lister rats, vitamin A deficiency depressed the number of mitoses, caused abnormalities of nuclear shape and a thickening of the lens epithelium at the anterior pole. The thickened area showed abnormalities of cell division and possible keratinization of some cells.

This work was done with flat preparations—whole mounts—of lens epithelium which is a single layer of cuboidal cells covering only the front surface of the lens. When antero–posterior sections were made, keratinized cells at the anterior pole of the lens could be seen more clearly and they were found to lie on the inner, rather than the outer, side of the epithelial layer. This accords with the embryological origin of the mammalian lens (Mann, 1949) which is an invagination of ectoderm. The cells thus become inverted so that the basal layer of the ectodermal cell is on the outer surface of the lens. Corneal epithelium keratinizes on the outer surface, but it seems that the lens epithelium keratinizes inwards as an abnormal inner layer. We suggest that this correlates with its embryological origin.

The lesion of the cornea is termed xerophthalmia, and keratinization is sometimes thought to be connected with a general drying up of the eye secretions. Such drying cannot cause keratinization of lens epithelium as the lens is constantly bathed by aqueous humour.

When vitamin A is given, the area of reduplicated epithelium gets gradually smaller. Cell nuclei and then individual cells disappear. Complete disappearance may take several months. Clearing of the cornea enables the lesion in the lens to be watched biomicroscopically.

REFERENCES

Mann, I. (1949). *The Development of the Human Eye.* London: British Medical Association.
Pirie, A. & Overall, M. (1972). *Expl Eye Res.* **13**, 105.

Riboflavin status of the elderly. By G. B. THACKRAY, *Department of the Public Analyst, City of Portsmouth,* I. M. SHARMAN, *Dunn Nutritional Laboratory, Milton Road, Cambridge,* and D. E. HYAMS*, *Chesterton Hospital, Cambridge*

As part of a nutritional survey of the elderly, initiated by the Committee on Medical Aspects of Food Policy of the Department of Health and Social Security (Department of Health and Social Security, 1972), riboflavin was measured in the urine of elderly men and women in Cambridge and Portsmouth.

In Cambridge, subjects received, after emptying their bladder, a standard breakfast low in riboflavin. They were then given a 1 mg dose of the vitamin, and urine was collected during the ensuing 4 h. In Portsmouth, subjects were not dosed; they remained on their usual diet and 24 h specimens of urine were collected. In both centres the specimens were examined for riboflavin by the fluorimetric method of Kodicek & Wang (1949) and for creatinine. Daily intakes of riboflavin were calculated from dietary surveys using tables of food composition compiled by the Department of Health and Social Security (unpublished).

All subjects were aged 65 or over. In Cambridge seventeen men and fifteen women, and in Portsmouth forty-six men and forty women were investigated and the riboflavin excretions during the respective collection periods were calculated.

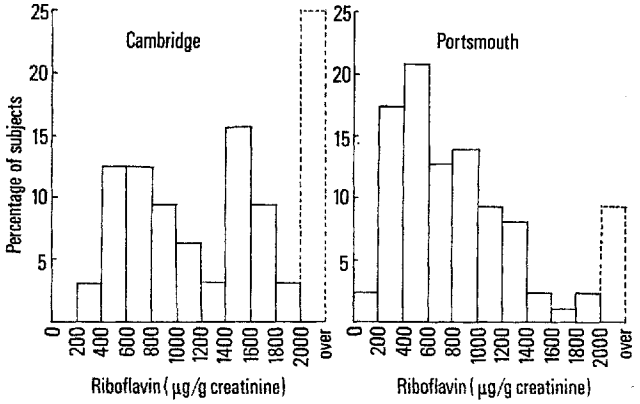

Fig. 1. Urinary excretion of riboflavin in elderly subjects at Cambridge (thirty-two subjects) and Portsmouth (eighty-six subjects).

Fig. 1 shows values for μg riboflavin/g creatinine. Intakes of riboflavin at Cambridge ranged from 1·00 to 2·84 mg and at Portsmouth from 0·87 to 2·77. Three subjects at Cambridge and seventeen at Portsmouth had intakes of less than 1·1 mg.

In further trials at Cambridge eight healthy young control subjects were investigated. All showed marked increases in their riboflavin excretion, during the 4 h

*Present address: Guy's Hospital Department of Geriatric Medicine, New Cross Hospital, London SE14.

period immediately after a 1 mg dose, above their normal excretion measured over the previous 24 h, the average rise being 265 μg riboflavin/g creatinine above their normal level of 150. Since the majority of elderly subjects in the Cambridge study excreted over 400 μg riboflavin/g creatinine after the 1 mg dose (Fig. 1), it would appear that they all had a satisfactory riboflavin status, with one possible exception.

Wide variations in individual intakes and excretion rates were found. Correlations appeared uncertain—only the extremes of high and low intakes were reflected in the excretion rate. No clinical manifestations of deficiency were observed.

REFERENCES

Kodicek, E. & Wang, Y. L. (1949). *Biochem. J.* **44**, 340.
Department of Health and Social Security (1972). *Reports on Health and Social Subjects* no. 3 (In the Press.)

Feeding patterns in 'resistant' obesity. By G. L. S. PAWAN, *Metabolic Division, Department of Medicine, The Middlesex Hospital Medical School, London W1P 7PN*

There is some evidence that the feeding pattern ('meal eating' *v.* 'nibbling') of the daily energy intake may affect body composition—particularly body-fat content—in man and laboratory animals (Cohn, 1963; Kekwick & Pawan, 1966; Fábry & Braun, 1967).

A study was made over several years on the feeding patterns in (a) a group of subjects with 'resistant' obesity (ninety women, 140–205% ideal body-weight, aged 17–69, mean age 31 years; twenty-eight men, 140–198% ideal body-weight, aged 18–57, mean age 37 years) who were referred for failing to respond to diet and drug therapy, and (b) a group of lean, but otherwise healthy, individuals (seventy-four women, aged 18–70, mean age 35 years, less than 95% ideal body-weight; fifty-three men, aged 19–65, mean age 37 years, less than 95% ideal body-weight). In the obese group 60% of the women and 54% of the men were found to have a feeding pattern of two meals daily, the larger meal being consumed in the evening or at night, whereas only 16% of the women and 32% of the men consumed more than three meals daily. In this group of obese subjects 91% of the women and 68% of the men consumed more than 55% of their daily food energy in the form of carbohydrate.

In the lean group of subjects, 16% of the women and 6% of the men consumed two meals daily, whereas 70% of the women and 71% of the men had a feeding pattern of four or more meals daily. In the lean group less than 50% of the daily energy was consumed in the form of carbohydrate by 81% of the women and 83% of the men. A history of juvenile-onset obesity was found in 72% of the obese men and 77% of the obese women. In agreement with the findings of Widdowson & McCance (1936), Widdowson (1947), and Harries, Hobson & Hollingsworth (1962), interindividual differences in mean daily energy intake were large in both groups of

subjects. Some lean individuals consistently consumed over 500 kcal (2·09 MJ) more than some of the obese subjects.

I am grateful to my dietetic colleagues for help and to several physicians for referring patients to me.

REFERENCES

Cohn, C. (1963). *Ann. N.Y. Acad. Sci.* **110**, 395.
Fábry, P. & Braun, T. (1967). *Proc. Nutr. Soc.* **26**, 144.
Harries, J. M., Hobson, E. A. & Hollingsworth, D. F. (1962). *Proc. Nutr. Soc.* **21**, 157.
Kekwick, A. & Pawan, G. L. S. (1966). *Metabolism* **15**, 173.
Widdowson, E. M. (1947). *Spec. Rep. Ser. med. Res. Coun.* no. 257.
Widdowson, E. M. & McCance, R. A. (1936). *J. Hyg., Camb.* **36**, 293.

A comparison of soft-tissue radiography, reflected ultrasound, skinfold calipers and thigh circumference for estimating the thickness of fat overlying the iliac crest and greater trochanter. By SUSAN F. HAWES, A. ALBERT*, M. J. R. HEALY and J. S. GARROW, *Clinical Research Centre, Watford Road, Harrow*

Clinical observation suggests that subcutaneous fat over the iliac crest (IC) and greater trochanter of the femur (GT) is more resistant to dietary reduction than fat at other sites. Fat thickness can be accurately measured from radiographs (Garn, 1957) but radiation dose limits serial measurements on normal subjects. Skinfold calipers are useful in skilled hands (Tanner, 1959) but are very inaccurate in measuring pelvic fat in obese subjects. Thigh circumference is a simple but indirect measure of fatness. We have compared the above methods with estimates obtained with an Ultrasonoscope Flaw Detector Mark 2C, which was used to measure the distance between the skin and reflecting surface of IC or GT in thirty-two normal volunteers of average build.

At the iliac crest, the mean values and within-subject SD on repeated measurement were by radiography 20·4 mm $\pm$ 2·2, by calipers (mean of triplicate measurements) 9·8 mm $\pm$ 1·5, and by ultrasound 21·4 mm $\pm$ 2·5. At the GT the values were by radiography 26·3 mm $\pm$ 1·9, thigh circumference 583 mm $\pm$ 7·6 and by ultrasound 33·6 mm $\pm$ 2·5. The discrepancy between mean estimates by radiography and ultrasound arises because the latter includes muscle thickness which averages about 6 mm over the GT.

Between-subject correlation analysis showed that the relationship between subcutaneous fat thickness at the two sites was poor, but 'error-free' correlation between estimates by radiography and ultrasound was good (r=0·97 at IC, r=0·83 at GT). 'Error-free' correlations of the caliper measurements with radiographic measurements at the two sites were 0·82 and 0·47, and for thigh circumference 0·60 and 0·77 for IC and GT respectively.

*Permanent address: University of Liege, Laboratoire de Chemie Medicale, 1 Rue des Bonnes Villes, Liege, Belgium.

We conclude that ultrasound provides a simple and acceptable method for measuring fat thickness over bony points with an accuracy comparable to that of radiography but without the radiation hazard.

REFERENCES

Garn, S. M. (1957). *Hum. Biol.* **29**, 337.
Tanner, J. M. (1959). *Proc. Nutr. Soc.* **18**, 148.

Electrical resistance and skin reaction to eight electrode jellies. By A. DE LOOY and S. JAMES (introduced by J. S. GARROW), *Clinical Research Centre, Watford Road, Harrow*

By means of the socially acceptable monitoring instruments (SAMI) integrated heart-rate recorder (Baker, Humphrey & Wolff, 1967) it is possible to estimate 24 h energy expenditure (Goldsmith, Miller, Mumford & Stock, 1967). However, it is essential that the resistance of the skin electrodes of the SAMI should be less than 10 kΩ throughout the recording period, which could be more than 24 h, if accurate estimates of heart rate are required (Hanish, Neustein, Van Cott & Sanders, 1971).

The electrolyte composition, pH and water content of seven commercially available electrode jellies have been determined before and after prolonged use, (greater than 48 h), and these results have been correlated with electrical resistance and skin irritation. A thin layer of plastic skin applied before skin abrasion was found to reduce skin irritation due to the gel or the adhesive annulus. Generally, the higher the salt concentration the better the conduction but the worse the skin irritation, especially of the abraded site. A jelly was therefore prepared with a salt concentration which maintained a low skin impedance and was comparatively non-sensitizing. Methyl *p*-hydroxybenzoate was used as the preservative and tragacanth as the thickening agent.

For about 100 g of paste, 0·15 g methyl *p*-hydroxybenzoate was dissolved in 100 ml hot water. To this solution 5 g NaCl and 3·5 g tragacanth were added, dissolved and cooled. Twenty-five drops of glycerine were then added to prevent the gel from drying out. Air-bubbles present in the gel were removed under reduced pressure and the gel was stored in airtight polyethylene containers.

REFERENCES

Baker, J. A., Humphrey, S. J. E. & Wolff, H. S. (1967). *J. Physiol., Lond.* **188**, 4P.
Goldsmith, R., Miller, D. S., Mumford, P. & Stock, M. J. (1967). *J. Physiol., Lond.* **189**, 35P.
Hanish, H. M., Neustein, R. A., Van Cott, C. C. & Sanders, R. T. (1971). *Am. J. clin. Nutr.* **24**, 1155.

Sleep after a bedtime beverage. By VLASTA BREZINOVA and I. OSWALD, (introduced by MAURICE BROOK), *Department of Psychiatry, University of Edinburgh, Edinburgh EH10 5HF*

Little is known about the influence of nutrition on sleep. We have compared sleep after a placebo capsule with sleep after a hot, concentrated milk and cereal

beverage (Horlicks) had been taken before sleep. Each subject had five nights under each condition, in a balanced order. Subjects were run in matched pairs of opposite conditions. The first two nights (one placebo, one the beverage) for each person were treated as adaptation nights. All-night electrophysiological recordings were made and the records later scored blind. Friedman's two-way analysis of variance was used as a non-parametric test.

In a group of ten young adults we confirmed a recent report that sleep after the bedtime beverage is less restless at the end of the night.

In a group of eight subjects aged 42–66 years (mean age 55) mean total sleep time after the beverage was increased by 11 min ($P<0{\cdot}05$). Periods of intervening wakefulness between first sleep onset and the attainment of a total of 6 h of sleep averaged 29·7 min after placebo and 12·0 min after the beverage ($P<0{\cdot}02$). In the second 3 h of sleep considered alone, the mean duration of intervening wakefulness was reduced after the beverage ($P<0{\cdot}02$). The advantage in favour of the bedtime beverage increased significantly across successive experiences ($P<0{\cdot}025$).

The time taken to fall asleep was not altered in either group. No relation could be found between the findings and (1) attitudes expressed in a questionnaire, nor (2) professional/non-professional background.

Nutrition during severe prolonged exercise in trained cyclists. By J. D. Brooke and G. J. Davies, *Human Performance Laboratory, University of Salford*, and L. F. Green, *Beecham Products (UK) Ltd, Beecham House, Great West Road, Brentford, Middlesex*

In prolonged sports performance, depletion of carbohydrate stores can cause exhaustion. Prolonged maintenance of human physical power output in the laboratory has been assessed on four dietary treatments: T1 (glucose syrup); T2 ('normal' diet—canned rice pudding, canned fruit salad and sucrose); T3 (low-energy drink with electrolytes as in T1); T4 (no dietary supplement). Subjects were permitted to eat their normal diet before test.

Eight male racing cyclists each cycled to exhaustion on four occasions (one per treatment) on Ergowheels (Worthwhile Designs Limited, Manchester) set to approximate their racing load, 65–70% of each individual's maximum physical work capacity (Brooke & Davies, 1970). Treatments were allocated to subjects by randomized Latin square design.

In T1 and T2, the energy expended was replaced every 20 min; in T3 a volume of drink equal to that used in T1 was given. In all treatments, an additional 150 ml of an electrolyte solution, approximating to the concentration of sweat, was given every 20 min. Exhaustion was defined as the inability of the subject to maintain voluntarily the power output required.

Mean work times (min) to exhaustion were: T1, 216; T2, 201; T3, 180; T4, 148. There were statistically significant differences ($P<0{\cdot}05$) between any two pairs of mean work times. Subjects who took the glucose syrup drink worked longer than

when they took any of the other treatments. The respiratory quotient (RQ) differentiated high- from low-energy treatments, but did not differentiate within the high-energy group. Mean RQ at exhaustion were: T1, 0·85; T2, 0·84; T3, 0·81; T4, 0·76. Throughout the ride, blood-glucose values were higher for T1 and T2 compared with T3 and T4.

At 120 min, mean blood-glucose values (mg/100 ml) were: T1, 72; T2, 82; T3, 66; T4, 55. But, when the subjects took the glucose syrup drink, before exhaustion, the mean blood-glucose values rose to 96 mg/100 ml.

These results are an advance upon the earlier research of Christensen & Hansen (1939) and Hermansen & Saltin (1967) in that, for the maintenance of physical work, they differentiate between a high-energy diet in the form of a glucose syrup drink, and the diet, which includes fat and protein, normally used by cyclists.

When a glucose syrup drink is taken during cycling under the conditions outlined, the most efficient work of longest duration is performed before exhaustion occurs.

This research was supported by Beechams Products. We acknowledge gratefully the analyses of blood glucose by Mr Hooper, Chief Biochemist, Crumpsall Hospital, Manchester.

REFERENCES

Brooke, J. D. & Davies, C. J. (1970). *Ergonomics* **13**, 529.
Christensen, E. H. & Hansen, O. (1939). *Skand. Arch. Physiol.* **81**, 137.
Hermansen, L. & Saltin, B. (1967). In *Symposium of the Swedish Nutrition Foundation* Vol. 5. Uppsala: Almqvist & Wiksell.

Is there a specific requirement for carbohydrate in the diet? By D. J. NAISMITH and MARY C. CURSITER, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

The major function of dietary fats and carbohydrates is that of providers of energy, in which capacity they are believed to be interchangeable. Though it is recognized that a minimum amount of fat is essential in the diet to satisfy the need for linoleic acid (1% of the total energy), a minimum requirement for carbohydrate has not been defined. A diet devoid of carbohydrate, although adequate in energy, might nevertheless induce the wastage of some dietary protein in order to synthesize enough glucose to meet the specific requirements of the brain and nervous system.

To test this hypothesis, ten litter-mate pairs of weanling rats weighing, on average, 55 g were used. One of each pair was given a carbohydrate-rich diet containing 9% by weight of casein supplemented with methionine, 75% maize starch and 10% margarine. The other was given a diet which provided the same proportion of the total energy as protein, but in which all the remaining calories were contributed by margarine. The animals were pair-fed with respect to energy intake. On day 22, the rats were killed by stunning. The livers were removed, weighed, and the activities of two enzymes—phosphoenolpyruvate carboxykinase (PEPCK),

which is believed to be a rate-limiting enzyme in gluconeogenesis, and glucose-6-phosphatase (G-6-Pase)—were measured. The carcasses were analysed for water, protein and fat. The summarized results were:

Diet	Final body-weight (g)	Carcass composition			Hepatic enzyme activity	
		Water (g)	Protein (g)	Fat (g)	PEPCK (i.u./liver)	G-6-Pase (i.u./liver)
High-carbohydrate	137	86·2	20·5	13·0	5·36	172·8
Carbohydrate-free	121	74·0	17·0	16·4	14·02	163·4

The mean total energy intakes of the two experimental groups were identical (838 kcal) (3·51 MJ). The intakes of nitrogen by the animals fed on the carbohydrate-rich and carbohydrate-free diets were 2·73 and 2·79 g respectively. The rats maintained on the carbohydrate-free diet grew less well and showed a total reduction of 11·7% in body-weight. The lack of carbohydrate in the diet caused a significant reduction of 17% in carcass protein and a rise of 26% in body fat. A threefold rise in the activity of PEPCK was found in the animals which had been deprived of carbohydrate, indicating that gluconeogenesis had been greatly increased. Thus, when carbohydrate was excluded from the diet, protein, which would normally have been used for growth, had to be diverted for the manufacture of glucose.

Under these experimental conditions, fat and carbohydrate given in isocaloric amounts clearly did not exert an equal protein-sparing effect.

Effects of the route of inoculation and of the period of experimental feeding on the survival of chicks given diets containing fish- or meat-meal and inoculated with *Salmonella gallinarum*. By I. M. Smith and R. Hill, *Royal Veterinary College, London NW1 0TU*

Diets based on meat and wheat meals, or fish and wheat meals, had previously given survival differences in birds inoculated orally with *Salmonella gallinarum* (Hill & Smith, 1969; Smith & Hill, 1972). Similar diets were given to birds that were inoculated either orally, subcutaneously, intramuscularly or intraperitoneally with *S. gallinarum*. With all routes of inoculation meat-meal diets gave greater survival than fish-meal diets. There was a tendency for survival difference to be greatest with oral inoculation but there was no significant interaction between diet and route.

Diets containing meat- or fish-meal were given from 1 d old or a standard semi-purified diet (26W) was given to 12 d of age and the meat and fish diets thereafter; all birds were inoculated at 15 d of age with *S. gallinarum*. The meat-meal diet gave greater survival than the fish-meal diet and the difference was similar whether the diets were given from 1 d or 12 d of age.

When test diets containing meat- or fish-meal were given for varying periods and changed to a standard diet before inoculation, the meat diet, surprisingly, gave

greater survival than the fish diet. Moreover, when the test meat and fish diets were given from 1 d to 13 d of age (2 d before inoculation) survival values were about the same as when the same test diets were introduced at 13 d of age and continued for the remainder of the experiment, 18 d after exposure to infection.

In further experiments meat and fish diets were given and reversed at selected times between 3 d before and 18 d after inoculation. From the limited number of reversed patterns studied it was apparent that the dietary effect on survival was greatest in the first few days after inoculation, and a change of diet 8 d or more after inoculation was almost without effect on survival.

REFERENCES

Hill, R. & Smith, I. M. (1969). *J. comp. Path.* **79**, 469.
Smith, I. M. & Hill, R. (1972). *J. comp. Path.* **82**, 209.

The survival of chicks inoculated with *Salmonella gallinarum* and given diets containing extracted meat or fish meals, and meat or fish protein.

By R. HILL and I. M. SMITH, *Royal Veterinary College, London NW1 0TU*

Meat meal has consistently given greater survival than fish meal when each was used as the sole dietary protein supplement (Smith & Hill, 1972). These commercial meat (about 60% crude protein) and fish (about 66% crude protein) meals were extracted with water or with a buffered salt solution (0·30 M-KCl; 0·09 M-KH_2PO_4; 0·06 M-K_2HPO_4), the residue was dried at 70° and incorporated, in place of the untreated meals, into a wheat-based diet with appropriate supplements. The untreated and extracted meals were included at 20% of the diet. In six experiments, with a total of 168 birds per treatment, mean percentage survival values were: for meat, untreated 42, water-extracted 36 and salt-extracted 32; and for fish, untreated 6, water-extracted 21 and salt-extracted 13. The water- and salt-extracted meat meals gave slightly poorer survival than the untreated meal; the water- and salt-extracted fish meals gave slightly greater survival than the untreated meal. However, for all three comparisons of meat with the corresponding fish preparation, meat gave significantly greater survival than fish.

In further experiments meat and fish preparations of very high-protein content (about 80%) and containing practically no bone were used in a semi-purified, starch-based diet in place of casein and gelatin. Each protein source (beef powder, fish flour or casein plus gelatin), included at 26% of the diet, was used in six experiments with a total of 252 birds on each dietary treatment inoculated with *Salmonella gallinarum*. Mean percentage survival values were 32 for beef powder, 8 for fish flour and 28 for casein plus gelatin. These results support the view that the protein fractions of the original meat and fish meals were responsible for the difference in survival, but survival with both meat and fish diets was unexpectedly low by comparison with earlier results (Smith & Hill, 1972). In those experiments meat and fish meals were included in wheat-based diets, and this may have caused the generally higher level of survival. In three further experiments this was tested: beef powder

and fish flour in the starch-based diet gave lower survival (20 and 6% respectively) than these products in the wheat-based diet (35 and 10% respectively). Thus it appears that the general level of survival is probably influenced by factors other than the meat and fish products.

REFERENCE

Smith, I. M. & Hill, R. (1972). *J. comp. Path.* **82**, 209.

Digestion of proteins in the duodenum and the jejunum of growing pigs.

By R. BRAUDE, A. G. LOW and J. W. G. PORTER, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Ash re-entrant cannulas (Ash, 1962) were inserted into the duodenum or jejunum (at 10–15 cm and 250–260 cm respectively, beyond the pylorus) of 30 kg male Large White pigs, housed in modified Shinfield metabolism cages and fed on the Shinfield scale (Braude & Mitchell, 1951) at 09·00 and 15·00 hours. Samples of digesta were taken hourly during 24 h collection periods from a sealed apparatus for the collection and return of digesta at equal rates, temperature and pressure. The progress of digestion of three isonitrogenous diets, a practical-type cereal-based diet supplemented with fish meal (A) and two purified diets based on starch, sucrose and maize oil with groundnut (B) or casein (C) as the only protein sources has been followed by analysis of digesta using Kjehldahl-nitrogen, protease, amino acid and gel filtration analysis.

The mean total amounts of N in the digesta passing through the duodenum and jejunum in 24 h as a ratio of the amounts eaten in the diet during 24 h were:

	Duodenum			Jejunum		
	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C
Total digesta N	0·95	1·07	0·74	0·77	0·80	0·53
Soluble digesta N	0·52	0·44	0·52	0·66	0·62	0·51
Non-protein soluble digesta	0·44	0·37	0·37	0·42	0·37	0·33

The amounts of pepsin secreted in 24 h (measured in the duodenum) were in the ratio 6·5 : 2·9 : 3·0 for diets A, B and C respectively. The ratios of chymotrypsin to trypsin secretion levels in 24 h were 0·59, 0·98 and 0·74 for diets A, B and C respectively.

The release of free amino acids from the diets during digestion corresponded more closely with the specificities and relative proportions of the proteases than with their content in the dietary protein.

REFERENCES

Ash, R. W. (1962). *Anim. Prod.* **4**, 309.
Braude, R. & Mitchell, K. G. (1950–1). *Agriculture, Lond.* **57**, 501.

Sites of disappearance of apparently digestible energy and apparently digestible nitrogen in the digestive tract of cows receiving dried grass–concentrate diets. By M. J. WATSON, G. P. SAVAGE and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

A cow (M) equipped with a rumen fistula and with re-entrant cannulas into the proximal duodenum and terminal ileum was given a ration comprising (by weight, dry-matter basis) equal parts of chopped dried grass and concentrate, when dry and pregnant, and when lactating. The ration was also given to a second dry, pregnant cow (B) equipped only with re-entrant cannulas at the duodenum.

The concentrate comprised dry, rolled barley (at a low or high level), soya-bean meal and mineral–vitamin mixture. Comparable rations in which the rolled barley was replaced by rolled, high-moisture barley (propionic acid-treated) or by ground and pelleted maize were also given to cow M when lactating. Each diet was offered in three feeds/24 h for at least 4 weeks before 5 d faecal collections, after which 24 h ileal and separate 24 h duodenal collections, were made. Chromic oxide-impregnated paper (30 g/24 h) was given through the rumen fistula with the first and third of the daily feeds, and all duodenal and ileal values were adjusted to 100% recovery of the marker.

The mean recovery of chromic oxide in the 24 h duodenal collections was 83·0% (SE ± 2·2) and in the 24 h ileal collections 82·6% (SE ± 7·5). Apparent digestibility of gross energy was not affected by the higher levels of food intake associated with lactation or by the nature of the cereal (Table 1). Disappearance of apparently

Table 1. *Sites of disappearance of apparently digestible energy (ADE) and apparently digestible nitrogen (ADN) in cows receiving dried grass–concentrate diets*

Ration	Dried grass–rolled barley				Dried grass–ground and pelleted maize
Cow	B	M	M	M	M
No. of 24 h duodenal collections	3	1	2	2	2
No. of 24 h ileal collections	0	1	2	2	2
Dry-matter intake (kg/24 h)	5·19	5·08	8·59	9·02*	8·99
ADE (%)	73·6	72·8	73·7	72·2	74·8
Disappearance of ADE (%):					
Before duodenum	65·3 ± 2·6	66·7	64·5	58·4	53·4
In small intestine	34·7	17·4	19·1	33·0	34·7
In caecum and colon		15·8	16·5	8·7	12·0
ADN (%)	76·1	69·7	71·7	72·7	72·6
Disappearance of ADN (%):					
Before duodenum	24·4 ± 6·2	22·0	9·4	−7·7	−11·2
In small intestine	75·6	62·8	79·2	99·6	98·6
In caecum and colon		15·2	11·4	8·1	12·6

*High-moisture barley.

digested energy (ADE) before the duodenum in the two dry cows showed good agreement. When cow M was given the higher level of the diet containing dry, rolled barley, there was little difference in the sites of energy digestion. For the diets

containing high-moisture barley or pelleted maize, disappearance of ADE before the duodenum was less and disappearance in the small intestine greater—the latter most probably reflecting the greater proportion of apparently digested nitrogen (ADN) disappearing in the small intestine when high-moisture barley or pelleted maize was included in the diet. It can be seen from the results that when dry, rolled barley was given, appreciable amounts of ADN disappeared before the small intestine.

Sites of disappearance of apparently digestible cellulose and apparently digestible α-linked glucose polymers in the digestive tract of a cow receiving dried grass–concentrate diets. By M. J. WATSON, G. P. SAVAGE, I. BROWN and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

In the previous communication (Watson, Savage & Armstrong, 1972) sites of disappearance of apparently digested energy and apparently digested nitrogen were reported for cows fed on dried grass–concentrate diets. In this communication similar results are reported for cellulose determined by the method of Crampton & Maynard (1938) and for α-linked glucose polymers determined by the method of MacRae & Armstrong (1968), when cows were given dried grass–concentrate diets containing dry, rolled barley at two levels, and ground and pelleted maize (for composition, see Watson *et al.* 1972).

With reference to the sites of disappearance of cellulose, it can be seen that approximately 90–98% of the digested cellulose disappeared in the rumen (see below).

Digestibility values for α-linked glucose polymers indicated that with both rolled barley, and ground and pelleted maize the starch was virtually completely digested (see below). When the ration (5·08 kg dry matter/24 h) containing the lower level of rolled barley was given, 91% of the α-linked glucose polymers disappeared before the duodenum and the remainder in the small intestine, in agreement with the findings for sheep (MacRae & Armstrong, 1969). Whereas with the ration (8·59

Ration	Dried grass–rolled barley		Dried grass–ground and pelleted maize
Dry-matter intake (kg/24 h)	5·08	8·59	8·99
Apparent digestibility of cellulose (%)	76·3	67·1	73·8
Disappearance of apparently digestible cellulose (%):			
Before duodenum	90·5	98·3	88·9
In small intestine	−2·7	−2·7	3·9
In caecum and colon	12·2	4·4	7·2
Apparent digestibility of α-linked glucose polymers (%)	97·4	99·7	97·5
Disappearance of apparently digestible α-linked glucose polymers (%):			
Before duodenum	91·4	83·9	76·6
In small intestine	9·0	12·4	16·1
In caecum and colon	−0·5	3·7	7·3

https://doi.org/10.1079/PNS19720066 Published online by Cambridge University Press

kg dry matter/24 h) containing the higher level of rolled barley, appreciable amounts of α-linked glucose polymers escaped fermentation and entered the small intestine, wherein 75·8% of it was apparently digested, 22·5% was fermented in the caecum and colon, and 1·7% appeared in the faeces. With the ration containing ground and pelleted maize an appreciably greater quantity of α-linked glucose polymers entered the small intestine, which is in agreement with the findings of other workers (see Armstrong & Beever, 1969). Of this quantity, 62% disappeared in the small intestine, 28% was fermented in the caecum and colon, and 10% appeared in the faeces.

REFERENCES

Armstrong, D. G. & Beever, D. E. (1969). *Proc. Nutr. Soc.* **28**, 121.
Crampton, E. W. & Maynard, L. A. (1938). *J. Nutr.* **15**, 383.
MacRae, J. C. & Armstrong, D. G. (1968). *J. Sci. Fd Agric.* **19**, 568.
MacRae, J. C. & Armstrong, D. G. (1969). *Br. J. Nutr.* **23**, 377.
Watson, M. J., Savage, G. P. & Armstrong, D. G. (1972). *Proc. Nutr. Soc.* **31**, 98A.

Binding of magnesium ions by isolated cell walls of rumen bacteria and the possible relation to hypomagnesaemia. By T. J. FITT, K. HUTTON, A. THOMPSON and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

Cell walls prepared from *Staphylococcus aureus* H and *Micrococcus* sp. 24 are capable of binding magnesium, and the extent of binding is increased by degradation of the cell wall (Heptinstall, Archibald & Baddiley, 1970). For example, removal of alanine ester residues from the teichoic acid in the walls results in a significant increase in the ability of the walls to bind Mg. The presence of bound Mg in the cell wall is believed to be essential for maintenance of membrane function (Hughes, Stow, Hancock & Baddiley, 1971). Availability of dietary Mg for ruminants is considerably less than that for monogastric animals (see Agricultural Research Council, 1965, 1967). It is possible that the intervention of the rumen microbial population is largely responsible for this reduced availability owing to binding of Mg by bacterial cells in the reticulo-rumen. If the alanine residues are subsequently cleaved from the teichoic acids in their passage through the small intestine, binding capacity would be further enhanced. At the present time no mammalian enzyme has been isolated which can destroy residual glycerol or ribotol phosphate chains of teichoic acids and these may be excreted intact together with a substantial amount of bound Mg. However, teichoic acid hydrolase activity has recently been demonstrated in soil bacteria (Wise, Glickman & Teimer, 1972). Only Gram-positive bacteria contain teichoic acids, but it is probable that the heptosephosphate and ethanolamine components of the lipopolysaccharides of Gram-negative organisms may perform a similar function.

In the present study the effects of pH and Mg ion concentration on the binding of ions by isolated cell walls of rumen bacteria are reported. Bacteria were isolated by differential centrifugation from the rumen fluid of sheep which had been fed on dried-grass pellets. Cell walls were prepared from the isolate, and Mg ion uptake was measured by the methods of Heptinstall *et al.* (1970). The results are shown

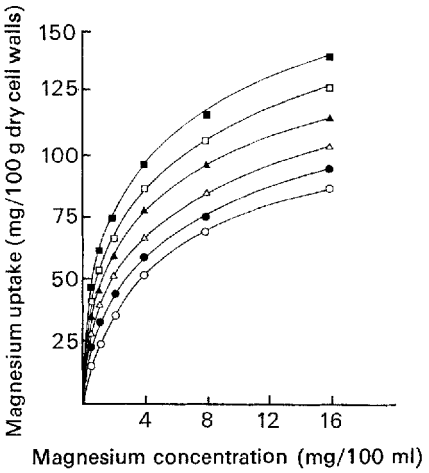

Fig. 1. Uptake of magnesium by isolated cell walls of rumen bacteria. ○—○, pH 5·0; ●—●, pH 5·5; △—△, pH 6·0; ▲—▲, pH 6·5; □—□, pH 7·0; ■—■, pH 7·5.

in Fig. 1. It can be seen that Mg uptake increases with increased Mg concentration and with increased pH, and that at low concentrations (when the levels may be critical with reference to the animal's requirement) Mg uptake is substantial and directly proportional to the Mg concentration.

In conclusion, it appears that further investigations into Mg binding by rumen microbial cell walls, and studies on the subsequent fate of such Mg, are necessary and may lead to a better understanding of the aetiology of hypomagnesaemia in ruminants.

REFERENCES

Agricultural Research Council (1965). *Nutrient Requirements of Farm Livestock : Ruminants.* London: Agricultural Research Council.
Agricultural Research Council (1967). *Nutrient Requirements of Farm Livestock : Pigs.* London: Agricultural Research Council.
Heptinstall, S., Archibald, A. R. & Baddiley, J. (1970). *Nature, Lond.* **225**, 519.
Hughes, A. H., Stow, M., Hancock, I. C. & Baddiley, J. (1971). *Nature, New Biology* **229**, 53.
Wise, E. M. Jr, Glickman, R. S. & Teimer, E. (1972). *Proc. natn. Acad. Sci. U.S.A.* **69**, 233.

Effect on type of rumen fermentation and digestibility of feeding whole as opposed to processed barley to sheep. By E. R. ØRSKOV and C. FRASER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The effect of processing of roughages on rumen fermentation has been known for some time (see Rhodes & Woods, 1962), and it has also been shown that the quantity and method of feeding may markedly change the pattern of rumen fermentation in barley-fed steers, apparently due to the survival of rumen protozoa (Whitelaw, Eadie, Mann & Reid, 1972).

To investigate whether the rolling of barley before pelleting is required for the most efficient utilization of the grain by lambs, diets consisting of 91% barley,

7·5% white fish meal and 1·5% limestone were given. We found to our surprise that dry-matter digestibility decreased from 77·8 to 75·3 ($P<0·01$) and the dry matter required per kg live-weight gain increased from 2·56 to 2·80 kg ($P<0·05$) as a result of rolling.

This work has now been extended in a trial in which twelve lambs were given either whole unprocessed barley or whole barley pelleted through a 7·8 mm die. The other ingredients were similar to those mentioned above. Digestibility of dry matter, organic matter and acid-detergent fibre was measured twice during the fattening period when the diets were offered at near maximal intake. At slaughter, when the live weight of the lambs was about 38 kg, and on the day before slaughter rumen pH was measured and samples of rumen contents were obtained for volatile fatty acid analysis. The results are given in Table 1.

Table 1. *Effect on digestibility and rumen fermentation of feeding lambs on whole barley as opposed to barley processed into pellets*

		Molar proportion (%) of:			Digestibility (%) of:		
Treatment	pH of rumen liquor	Acetic acid	Propionic acid	Butyric and higher acids	Dry matter	Organic matter	Acid-detergent fibre
Whole barley	6·2	53·1	33·6	13·4	80·2	83·5	27·9
Whole barley, pelleted	5·2	39·4	43·9	16·8	78·9	81·6	20·9
SE of means	0·14	2·63	2·21	2·96	0·33	0·40	2·87

The lambs given whole barley had a higher pH in the rumen ($P<0·01$), and a higher proportion of acetic acid ($P<0·01$) and a lower proportion of propionic acid ($P<0·05$) in rumen liquor than those receiving pelleted barley. The proportion of butyric and higher acids was not significantly changed. The digestibility of dry ($P<0·05$) and organic matter ($P<0·01$) was higher for the whole unprocessed barley diet. This effect may well have been due in part to an increase in the fibre digestibility and to a presumed survival of cellulolytic bacteria at the higher rumen pH.

The results show that it may be possible to manipulate rumen fermentation by processing concentrates without reducing digestibility. This method is now being used to study the effects of type of rumen fermentation on growth and on the partitioning of dietary energy.

REFERENCES

Rhodes, R. W. & Woods, W. (1962). *J. Anim. Sci.* **21**, 483.
Whitelaw, F. G., Eadie, J. M., Mann, S. O. & Reid, R. S. (1972). *Br. J. Nutr.* **27**, 425.

The Two Hundred and Forty-seventh Meeting of the Nutrition Society (Ninety-eighth of the Scottish Group) was held in the School of Agriculture, University of Aberdeen, 581 King Street, Aberdeen, on Friday, 2 June 1972, at 09.30 hours. This was a joint meeting with the Biochemical Society. The following papers were read but the abstracts are published thus: those without reference marks in the Proceedings of the Nutrition Society; *those marked with an asterisk in the* Biochemical Journal *and those marked with a dagger in both journals:*

***Introduction of ^{15}N into protein of rats.** By P. FÜRST, B. JOSEPHSON and B. PHILLIPSON, *St Erik's Hospital, Stockholm, Sweden*

***Concentration of different amino acids in the muscle cells of man in relation to food and amino acid infusions.** By J. BERGSTRÖM, P. FÜRST and E. VIMNARS, *St Erik's Hospital, Stockholm, Sweden*

Amino acid metabolism by protein-depleted rats. By R. J. NEALE, *Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7, Jamaica.*

The metabolism of [^{14}C]-phenylalanine, -valine, -leucine, and -lysine by rats given high-protein (HP) and protein-free (PF) diets was investigated by infusing the amino acids by both an intragastric and an intravenous route. Expired $^{14}CO_2$ was collected during the infusion period (4 h) and at the end of this time the rat was killed and the liver, gastro-intestinal tract, skin and remaining carcass were dissolved in 2 M-potassium hydroxide. Samples of these solutions were then assayed for ^{14}C radioactivity and the total counts contained in the various organs and tissues were expressed as percentages of the counts infused.

In general, for all the amino acids infused intragastrically the counts in the gastro-intestinal tract were the same in both HP and PF groups of rats, that is approximately 20 %. Counts in the skin of the HP group (7%) were double those in the PF group. Counts in the livers of the PF group (14%) were significantly greater than those in the livers of the HP group (10%). Counts in the carcass of both groups was 30–35%, with little difference between either group.

Counts expired as $^{14}CO_2$ were the same in both groups for valine, leucine and phenylalanine (approximately 20%), indicating no economy of these amino acids by protein-depleted rats. The specific activity of $^{14}CO_2$ expired rose continuously in both groups over 4 h with no plateau being reached.

These results confirm previous findings, which showed no economy of branched-chain amino acid oxidation in protein-depleted rats (Neale, 1971), but go further in showing no real major change in amino acid distribution between the various tissues in the different nutritional states.

REFERENCE

Neale, R. J. (1971). *Nature, New Biology* **231**, 117.

***Effect of plane of nutrition of maternal and foetal plasma amino acid concentrations in the sheep.** By JOHN S. SLATER and DAVID J. MELLOR, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

***Turnover rates of myocardial proteins in the rat.** By E. B. CHAIN and P. M. SENDER, *Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY*

***Insulin and protein synthesis in the perfused rat heart.** By E. B. CHAIN and P. M. SENDER, *Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY*

***Aminoacyl–tRNA synthetases in myocardial tissue.** By KEITH GIBSON and PETER HARRIS, *Institute of Cardiology, University of London, 2 Beaumont Street, London W1N 2DX*

The effect of dietary carbohydrate on the flow of nitrogen through the duodenum of the sheep. By N. W. OFFER, R. A. EVANS and R. F. E. AXFORD, *Department of Biochemistry and Soil Science, Memorial Buildings, Bangor, Caerns.*

The nature of the energy source may be the limiting factor in determining the rate of microbial synthesis in the rumen (Hogan & Weston, 1970). We examined the effect of different carbohydrate supplements on the passage of nitrogenous materials to the duodenum.

A Welsh mountain wether, fitted with a pair or re-entrant cannulas in the proximal duodenum, was restrained in a metabolism cage and connected to an automatic device which took continuous representative samples of the digesta passing through the cannulas (Axford, Evans & Offer, 1971; Evans, Axford & Offer, 1971). The sheep was given a pelleted ration for eight consecutive periods, each of 6 d. During periods 1 and 8 the ration consisted of soya-bean meal (200 g/d), dried grass (100 g/d), molasses (30 g/d) and a mineral–vitamin mixture (20 g/d). For the other experi-

mental periods, supplements as indicated in Table 1 were incorporated into the ration in addition to the components of the basal ration. The amounts of total nitrogen, ammonia N, total amino acid N and gross energy in the diet and digesta for every 24 h of the 48 d of the experiment were determined.

Table 1

	Diet			Nitrogen flowing through intestine (g/d)		
Period	Energy supplement	Digestible energy (cal/d)	(kJ/d)	Total N	Amino acid N	Ammonia N
1	Basal	1142	4·78	13·1	7·0	1·20
2	150 g starch	1714	7·17	17·2	10·1	0·80
3	300 g starch	2015	8·43	20·3	10·5	0·28
4	150 g paper	1638	6·85	16·3	9·8	0·89
5	300 g paper	1963	8·21	16·1	9·8	0·95
6	150 g paper+ 150 g starch	1825	7·64	20·2	13·4	0·82
7	300 g paper+ 300 g starch	2516	10·52	25·5	16·9	0·42
8	Basal	1196	5·00	12·2	6·7	1·14

Mean daily total N intake for the eight periods = 20·0 ± 0·2 g N/d.

Values given in Table 1 are the means for the last 3 d of each dietary period. There was considerable loss of N across the rumen during periods 1 and 8. This loss was prevented by supplementation with 300 g of starch or 150 g starch + 150 g paper. A net gain of N across the rumen was recorded during the period of highest energy supplementation. The flow of amino acid N was greatest when the dietary supplement contained both starch and paper. It was further noted that starch and paper, given together, exerted a synergistic effect. The resulting flow of amino acid N was greater on the mixed supplement than on equi-energetic rations made up of either of the components. Under all dietary regimens the amino acid composition of the hydrolysed digesta was constant and similar to that reported by Purser & Buechler (1966) for microbial protein. The postabomasal digestibility of the nitrogenous components of the digesta was constant throughout the experiment, suggesting that the amino acids gained by supplementation were available to the animal.

We are grateful to the Lord Rank Research Centre for providing the starch.

REFERENCES

Axford, R. F. E., Evans, R. A. & Offer, N. W. (1971). *Res. vet. Sci.* **12**, 128.
Evans, R. A., Axford, R. F. E. & Offer, N. W. (1971). *Proc. Nutr. Soc.* **30**, 40A.
Hogan, J. P. & Weston, R. H. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* p. 474 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.
Purser, D. B. & Buechler, S. M. (1966). *J. Dairy Sci.* **49**, 81.

Utilization of dietary nucleic acids by sheep. By M. A. Razzaque and J. H. Topps, *School of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD*

Two lambs which were 5–6 months of age and provided with abomasal cannulas, were used in measuring both the apparent digestibility of RNA or DNA added to a

diet with a high content of starch and the effect of each nucleic acid on the digestibility of certain dietary constituents. Each lamb received daily 600 g flaked maize, 200 g dried grass, 200 g hay and 70 g fish meal followed by the same diet plus 5 g of either RNA or DNA. Faeces and abomasal digesta were collected and sampled during the last week on each diet, and these samples, along with the foods, were analysed for RNA and DNA by the method of McAllan & Smith (1969) (Table 1).

Table 1. *Daily intake and excretion of total, RNA and DNA nitrogen and the amount of RNA-N and DNA-N expressed as percentage of total N in abomasal digesta (mean values for two lambs)*

	Total N (g)		RNA-N (mg)		DNA-N (mg)		In abomasal digesta	
Diet	Intake	Faecal	Intake	Faecal	Intake	Faecal	RNA-N	DNA-N
Basal (see text)	21·6	7·35	400	211	152	127	3·47	1·73
Basal + 5 g RNA	19·6	6·40	1133	183	162	125	4·11	2·10
Basal + 5 g DNA	22·6	6·48	428	234	896	130	4·52	2·54

Despite large differences between the three diets in intake of RNA and DNA, the faecal excretions and contents of abomasal digesta were relatively constant. These results support previous findings that dietary nucleic acids are degraded in the reticulo-rumen and the microbial nucleic acids synthesized in that part of the gut are highly digestible (Ellis & Bleichner, 1969).

Table 2. *Apparent digestibility (%) of organic matter, nitrogen, crude fibre and cellulose in the three diets (mean values for two lambs)*

Diet	Organic matter	Nitrogen	Crude fibre	Cellulose
Basal (see text)	73·4	66·1	38·7	48·8
Basal + 5 g RNA	80·0	72·1	60·8	70·2
Basal + 5 g DNA	78·2	71·3	51·4	59·9

The addition of either RNA or DNA to the basal diet apparently caused a large increase in the proportion of the fibrous constituents digested (Table 2). Similar results have been obtained when the digestibility of cellulose in a hay sample was measured in vitro over 24 h. The addition of very small amounts of either RNA or DNA to the system increased digestibility from 28·4% to either 48·4 or 44·4% respectively. The addition of either RNA or DNA to the diet appears to stimulate the activity of cellulolytic micro-organisms in the reticulo-rumen, an effect which may be useful in overcoming the depression in activity that is frequently found with diets rich in starch.

REFERENCES

Ellis, W. C. & Bleichner, K. L. (1969). *Fedn Proc. Fedn Am. Socs exp. Biol.* **28**, 623.
McAllan, A. B. & Smith, R. H. (1969). *Br. J. Nutr.* **23**, 671.

Nucleic acid and protein concentrations in the livers of rats of different ages and on different diets. By R. DAWSON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Variations in the quantity or quality of dietary proteins, or in the supply of dietary amino acids, have been shown to affect the liver protein concentrations and the liver nucleic acid concentrations and metabolism (Munro & Clark, 1959; Allison, Wannemacher, Banks & Wunner, 1964; Munro, 1964) but, surprisingly, little information is available concerning possible variations in liver protein and nucleic acid concentrations with age. The present investigation was carried out to obtain further information on these points.

Male rats of the Hooded Lister strain from the Institute colony were used throughout the investigation. For the study of the effects of age on liver protein and nucleic acid concentrations the animals were maintained on the stock colony diet. At each age investigated several animals were killed by a blow on the head; the livers were quickly removed, washed, blotted and deep-frozen until required, when the frozen livers were thawed, weighed, minched and homogenized in a Potter-Elvejhem type homogenizer. Protein was determined by the Lowry, Rosebrough, Farr & Randall (1951) method and the nucleic acids by a modification of the Schmidt & Thannhauser (1945) procedure.

Table 1. *Protein and nucleic acid concentrations in rat livers (mg/g wet weight)*

	RNA		DNA		Protein	
Age	Value	SEM	Value	SEM	Value	SEM
1 month	11·9	0·34	2·29	0·11	211	6·4
6 weeks	11·5	0·24	2·13	0·08	214	4·5
2 months	10·3	0·24	1·87	0·08	212	4·5
3 months	9·9	0·24	1·14	0·08	217	4·5
Significance of age differences	$P<0·05$		$P<0·05$		Not significant	

From the results shown in Table 1 it will be seen that liver RNA and DNA showed a significant decrease in concentration with increasing age but liver protein concentration remained unchanged.

Weanling rats were used for the evaluation of diets by the protein efficiency ratio (PER) technique. These diets contained one of the following four proteins as the sole nitrogen source: (1) casein, (2) fish meal, (3) soya-bean meal, (4) meat meal. At the end of the PER experiment the rats were killed, the livers removed as described above and the concentrations of protein and nucleic acids were determined. The results did not substantiate the earlier work referred to. For RNA concentrations only the fish-meal diet gave a value significantly different from the others and for DNA concentration only the meat-meal diet gave a value significantly different from the others. Protein concentration showed no significant difference with any of the diets.

REFERENCES

Allison, J. B., Wannemacher, R. W. Jr, Banks, W. L. Jr, & Wunner, W. H. (1964). *J. Nutr.* **84**, 383.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Munro, H. N. (1964). In *Mammalian Protein Metabolism* Vol. 1, p. 381 [H. N. Munro and J. B. Allison, editors]. New York and London: Academic Press.
Munro, H. N. & Clark, C. M. (1959). *Biochim. biophys. Acta* **33**, 551.
Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.

The effect of dietary protein level on DNA metabolism in some tissues of the growing rat. By S. B. TELFER*, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Using chemical and isotopic techniques, Enesco & Le Blond (1962) and Winick & Noble (1965) have partly defined the patterns of hyperplastic and hypertrophic growth in several tissues of the young growing rat. The present contribution describes the influence of differing dietary protein concentrations upon these measurements of organ growth in the young rat.

Weanling Black-hooded Norwegian rats of the Rowett Institute strain were offered semi-synthetic diets containing 8 or 16% protein, and increases in the total protein, RNA and DNA contents of liver, kidneys, spleen, testes, gastrocnemius muscle and jejunal mucosa were determined at intervals up to 65 d of age. The incorporation of tritium-labelled thymidine into the DNA of each organ was simultaneously determined using labelling periods of from 2 h up to 21 d.

Determinations of total organ DNA showed that, with the exception of the testes and jejunal mucosa, consumption of the lower-protein diet adversely affected hyperplastic growth. No significant differences in DNA:RNA or DNA:protein ratios were detected.

The characteristics of the biological decay curves of tritium-labelled thymidine activity in the DNA of liver, kidneys and muscle of rats slaughtered at different intervals after thymidine injection suggested that there was recycling of this nucleoside with re-incorporation into newly formed DNA. This phenomenon was particularly evident in the liver of rats maintained on the 8% protein diet.

*Present address: Department of Animal Nutrition and Physiology, The University of Leeds.

REFERENCES

Enesco, M. & Le Blond, C. P. (1962). *J. Embryol. exp. Morph.* **10**, 530.
Winick, M. & Noble, A. (1965). *Devl Biol.* **12**, 451.

The digestion of chloroplasts in the rumen of sheep and the effect of disruption and glutaraldehyde treatment. By JANET WEST and J. L. MANGAN, *Biochemistry Department, Institute of Animal Physiology, Babraham, Cambridge*

Chloroplast soluble stroma and insoluble lamellar proteins make a large contribution to the diet of herbivores. Chloroplasts from 10 kg kale (*Brassica oleracea* L. var. *marrowstem*) were prepared from batches of 300 g leaves (West & Mangan,

1970) and used for each experiment. The sheep had a permanent rumen fistula, and rumen fluid was sampled continuously. Maintenance ration was 1000 g chaffed hay and 200 g crushed oats once daily, the oats being omitted on the day of experiment. Chloroplasts or casein were introduced through the rumen cannula together with the rumen volume markers, polyethylene glycol (12·5 g) and Li_2SO_4. H_2O (0·6 g) 4 h after the feed started. Rumen particulate matter was fractionated on sucrose density gradients (Mangan & Pryor, 1968), centrifuging at 300 *g* for 1 h at 0°.

(1) Casein was degraded rapidly (half-life 12·5 min) producing ammonia and δ-amino-valeric acid (DAVA) as typical degradation products.

(2) Chloroplasts (50–60% intact) were rapidly ingested by entodiniomorphid protozoa which contained 91% of the chlorophyll in the particulate matter after 36 min. Chlorophyll was rapidly degraded. No ammonia was produced but DAVA formation indicated protein degradation.

(3) Chloroplasts fixed with 100 mmol-glutaraldehyde (West & Mangan, 1970) were only taken up in the protozoal fractions to the extent of 22%, the remainder being free or clumped. Neither ammonia nor DAVA was produced.

(4) Chloroplasts disrupted by sonication had only 13% taken up in the protozoal fractions. Stromal protein was denatured by the treatment and no ammonia or DAVA was formed.

(5) Chloroplasts were disrupted by dialysis against water. The soluble protein released was rapidly degraded in the rumen, ammonia equivalent to 10% of the nitrogen and a considerable amount of DAVA were formed. Only 7·8% of the chloroplast lamellar fragments was ingested by protozoa after 36 min and chlorophyll was slowly degraded. Results are shown in Table 1.

Table 1. *Degradation products of chloroplasts and casein in the rumen*

	Added to rumen		Increase in rumen ammonia		Increase in rumen δ-amino-valeric acid		Maximum % chlorophyll in protozoa
	Total N (g)	Chloro-phyll (g)	mg N/ 100 ml	% of N added	μmol/l	μmol/ g N added	
Casein	3·11	—	14·4	27·8	23·6	45·2	—
Chloroplasts	2·09	3·97	0	0	13·6	40·2	90·8
Chloroplasts: glutaraldehyde-treated	2·53	2·67	0	0	0	0	21·9
sonicated	1·69	3·37	0	0	0	0	13·2
water-dialysed	2·46	1·825	4·5	10·0	16·2	36·0	7·8

REFERENCES

Mangan, J. L. & Pryor, M. J. (1968). *J. Physiol., Lond.* **200**, 18P.
West, J. & Mangan, J. L. (1970). *Nature, Lond.* **228**, 466.

***Some physical and kinetic properties of deoxycytidylate deaminase from normal and virus-infected mammalian cells.** By HILARY A. ROLTON, JOAN BATES and H. M. KEIR, *Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS*

https://doi.org/10.1079/PNS19720066 Published online by Cambridge University Press

***Comparison of UDP-glucuronyltransferase activities synthesizing *S*- and *o*-β-glucuronides.** By H. P. A. ILLING and G. J. DUTTON, *Biochemistry Department, The University, Dundee DD1 4HN*

†Desaturation of stearic acid by sheep tissue microsomes. By K. W. J. WAHLE and G. A. GARTON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Cook & Reiser (1968) showed that, in mitochondria-free preparations of sheep adipose tissue the triglycerides of which contained 34% stearic acid, desaturase activity towards stearic acid was considerably less than that in the adipose tissue of the pig and the rat, the triglycerides of which contain considerably less stearic acid. However, the proportion of stearic acid in the triglycerides of individual adult sheep varies greatly between different bodily sites, ranging from less than 5% in the pinnae of the ears, through 10–20% in subcutaneous adipose tissue, to more than 30% in perinephric adipose tissue (Duncan & Garton, 1967). Though the high proportion of stearic acid in perinephric triglycerides is probably associated with the incorporation of exogenous stearic acid derived from hydrogenation of dietary unsaturated C_{18} acids in the rumen (Duncan & Garton, 1967), some of the acid is evidently of endogenous origin, as evidenced by the observation that the perinephric triglycerides of sheep reared on a lipid-free diet contained 24% of stearic acid compared with 11% in their subcutaneous triglycerides (Duncan, Garton & Matrone, 1971), suggesting that desaturase activity may be greater in subcutaneous tissue.

Accordingly, the desaturation of [1-^{14}C]stearic acid was studied using microsomal preparations from both subcutaneous and perinephric adipose tissue of five individual sheep; the method used was that of Bickerstaffe & Annison (1969) except that stearic acid was added in solution in propylene glycol (Gellhorn & Benjamin, 1964). Desaturase specific activities, expressed as nmol stearic acid converted into oleic acid/30 min per mg microsomal protein, were 9·9 and 15·0 (mean values) respectively for perinephric and subcutaneous tissue; the difference was statistically significant ($P<0{\cdot}05$).

Corresponding mean values for liver microsomes from sheep (10), rats (4) and hens (3) were 1·5, 13·0 and 26·5 respectively, showing that marked differences exist between species (cf. Cook & Reiser, 1968; Thompson & Allen, 1969). Whereas lactating mammary tissue of the rat (Lossow & Chaikoff, 1958) and the rabbit (Bu'lock & Smith, 1965) shows no capacity to desaturate stearic acid, that of the cow (Lauryssens, Verbeke & Peeters, 1961), goat and pig (Bickerstaffe & Annison, 1970) has considerable desaturase activity. Sheep mammary tissue obtained from animals in full lactation was found to be as active as subcutaneous adipose tissue though, as the gland regressed, desaturase specific activity values fell to $<1{\cdot}0$.

REFERENCES

Bickerstaffe, R. & Annison, E. F. (1969). *Comp. Biochem. Physiol.* **31**, 47.
Bickerstaffe, R. & Annison, E. F. (1970). *Comp. Biochem. Physiol.* **35**, 653.
Bu'lock, J. D. & Smith, G. N. (1965). *Biochem. J.* **96**, 495.

Cook, L. J. & Reiser, R. (1968). *Proc. Meet. Am. Oil Chem. Soc. 42nd, New York*, Abstr. 80.
Duncan, W. R. H. & Garton, G. A. (1967). *J. Sci. Fd Agric.* **18**, 99.
Duncan, W. R. H., Garton, G. A. & Matrone, G. (1971). *Proc. Nutr. Soc.* **30**, 48A.
Gellhorn, A. & Benjamin, W. (1964). *Biochim. biophys. Acta* **84**, 167.
Lauryssens, M., Verbeke, R. & Peeters, G. (1961). *J. Lipid Res.* **2**, 383.
Lossow, W. J. & Chaikoff, I. L. (1958). *J. biol. Chem.* **230**, 149.
Thompson, E. H. & Allen, E. (1969). *J. Anim. Sci.* **29**, 127.

***Uptake of vitamin E by rat small intestinal slices.** By COLIN K. PEARSON and ALISON M. LEGGE, *Department of Biochemistry, Marischal College, University of Aberdeen AB9 1AS*

***The intestinal absorption of 3-*sn*-phosphatidylethanolamine in the rat.** By M. S. HARVEY, J. S. OWEN, G. H. SCOTT and J. D. BILLIMORIA, *Biochemical Research Laboratory, Department of Chemical Pathology, Westminster School of Medicine, Udall Street, London SW1P 2PP*

***The effect of polyoxin D on morphogenesis in *Coprinus cinereus*.** By GRAHAM W. GOODAY, *Department of Biochemistry, University of Aberdeen AB9 1AS*

***Inhibition of electron transport in isolated turnip (*Brassica campestris* L.) mitochondria by inhibitors of protein synthesis.** By ANTHONY L. MOORE and S. BRIAN WILSON, *Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS*

***The enzymic hydrolysis of malonated flavone glycosides.** By HAROLD E. DAVENPORT and M. SUSAN DUPONT, *Agricultural Research Council, Food Research Institute, Colney Lane, Norwich NOR 70F*

***Effect of hydroxyproline on extension growth in pea root segments.** By DEREK VAUGHAN and EVELYN CUSENS, *Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ*

***Configuration of soil arabinose.** By MARTIN V. CHESHIRE and S. J. THOMPSON, *Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ*

Studies on the branched-chain amino acid antagonism in chicks. By K. N. BOORMAN and P. J. BUTTERY (introduced by D. LEWIS), *Department of Applied Biochemistry and Nutrition, University of Nottingham, Sutton Bonington, Loughborough, Leics. LE12 5RD*

Feeding with diets containing excess leucine increases the nutritional requirements for isoleucine and valine in rats (Spolter & Harper, 1961) and chicks (D'Mello & Lewis, 1970). In investigating possible causes for this antagonism, studies on the branched-chain amino acid, 2-oxoglutarate aminotransferase, activities of chick liver and kidney have been undertaken. Activities were routinely assayed either by measurement of L-glutamate production or by 2-oxoisocaproate or 2-oxoisovalerate production in incubation mixtures containing 2×10^{-2}M-2-oxoglutarate, $2{\cdot}5\times10^{-2}$M-sodium pyrophosphate buffer, pH 8·7, and 2×10^{-2}M-L-leucine or L-valine. Pyridoxal phosphate, $0{\cdot}7\times10^{-3}$M, was added to incubations of fractions from column chromatography.

Aminotransferase activity was found for leucine and valine in liver and kidney, but activity was approximately fivefold that for both amino acids in the latter tissue. Maximum activity occurred at pH 8·7 for both amino acids although only with valine was a distinct pH optimum observed. In both tissues activity was confined essentially to the 100 000 *g* supernatant fraction. Ammonium sulphate precipitation and subsequent DEAE-cellulose chromatography of the 100 000 *g* supernatant fraction from kidney showed that activity for both leucine and valine was associated with a single peak. Thus it appeared possible that the leucine antagonism in chicks is partly caused by an induction of kidney branched-chain aminotransferase, as it may be in the rat (Wohlhueter & Harper, 1970).

Table 1. *Effect of excess dietary leucine on branched-chain aminotransferase activity in chick kidney. Each value is the mean with its standard error for eight replicate groups of two chicks each*

Diet	Body-wt gain** (g per chick/ 13 d)	Food intake** (g per chick/ 13 d)	Aminotransferase activity (μmol L-glutamate produced/g tissue/h) Leucine as substrate	Valine as substrate
Control	371±41	769±90	110±6	127±7
Control+3% leucine	282±30	593±71	121±8	128±6

**Differences significant ($P<0{\cdot}01$).

To test the above hypothesis cockerels (11 d of age) were given either a 17% balanced protein diet or the same diet to which 3% L-leucine had been added. Kidney branched-chain aminotransferase activity was determined after 13 d. Despite the expected depressions in growth and food intake, aminotransferase activity for leucine and valine was not significantly increased by excess dietary leucine (Table 1). Thus, under these experimental conditions, enhanced activity of kidney aminotransferase does not appear to be a cause of this antagonism in the chick. Consistent

with these observations is the failure to demonstrate increased oxidation of [^{14}C]-valine in cockerels receiving excess dietary leucine (Boldizsar, Boorman and Buttery, unpublished results).

We wish to thank Miss L. Robinson for technical assistance.

REFERENCES

D'Mello, J. P. F. & Lewis, D. (1970). *Br. Poult. Sci.* **11**, 313.
Spolter, P. D. & Harper, A. E. (1961). *Am. J. Physiol.* **200**, 513.
Wohlhueter, R. M. & Harper, A. E. (1970). *J. biol. Chem.* **245**, 2391.

†Uric acid synthesis in the perfused chicken liver. By EILEEN LOCKE, PETER J. BUTTERY and K. NEIL BOORMAN, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

Livers of cockerels (3–6 weeks old) were perfused with 200 ml of a 2:1 mixture of Krebs–Ringer bicarbonate and whole chicken blood, to which 360 mg of D-glucose were added, according to the method of Bickerstaffe, West & Annison (1970).

After an initial high rate, endogenous uric acid output, measured by the method of Kalckar (1947), was found to decrease to a mean linear rate of $0{\cdot}78 \pm 0{\cdot}08$ (10) (mean $\pm$ SEM of ten samples) mg/g liver per h for periods of up to 150 min. Output and accumulation within the liver, measured by the method of Bokori (1965), were not markedly affected by the addition of 5 mmol-NH_4Cl, 5 mmol-L-glutamine or 5 mmol-glycine either individually or as a mixture. The addition of xanthine (1·65 mmol), however, was found to increase uric acid output by $298 \pm 34\%$ (4). Similarly, stimulations of $314 \pm 12\%$ (3), $303 \pm 6\%$ (5), $210 \pm 30\%$ (4) and $72 \pm 10\%$ (4) were caused by additions of inosine (1·00 mmol), inosine-5-monophosphate (0·60 mmol), 4-amino-5-imidazole-carboxamide-ribotide (0·75 mmol) and 5-phosphorylribose-1-pyrophosphate (0·55 mmol) respectively. All these stimulations were statistically significant.

Addition of $0{\cdot}5 \times 10^{-3}$ i.u. insulin/ml perfusion medium, every 30 min, increased uric acid output in the presence of 5 mmol-NH_4Cl by 21% (25% and 18%), and additions of 5 mmol-L-glutamine, 5 mmol-glycine and 5 mmol-L-glutamine plus 5 mmol-glycine to this system increased outputs by $62 \pm 4\%$ (4), 126% (130% and 122%) and $140 \pm 3\%$ (5) respectively. L-aspartate produced no stimulation of uric acid output.

The mean in vivo ATP:ADP ratio (Adam, 1965) of the chicken liver of $0{\cdot}25 \pm 0{\cdot}002$ (8) was changed little by perfusion and is apparently not markedly affected by insulin addition. This ratio is very low compared with that of $2{\cdot}50 \pm 0{\cdot}10$ (3) found for the rat liver in vivo. Lactate:pyruvate ratios (Hohorst, 1965; Bücher, Czok, Lamprecht & Latzko, 1965) in the perfused chicken liver were found to vary between 6·5 and 10·5. Water content of the liver increased by less than 1% during 150 min perfusion.

E.L. holds a Ministry of Agriculture, Fisheries and Food Studentship.

REFERENCES

Adam, H. (1965). In *Methods of Enzymatic Analysis* 2nd ed., pp. 539 and 573 [H. U. Bergmeyer, editor]. London and New York: Academic Press.
Bickerstaffe, R., West, C. E. & Annison, E. F. (1970). *Biochem. J.* **118**, 427.
Bokori, J. (1965). *Acta vet. hung.* **15**, 307.
Bücher, Th., Czok, R., Lamprecht, W. & Latzko, E. (1965). In *Methods of Enzymatic Analysis* 2nd ed., p. 253 [H. U. Bergmeyer, editor]. London and New York: Academic Press.
Hohorst, H. J. (1965). In *Methods of Enzymatic Analysis* 2nd ed., p. 266 [H. U. Bergmeyer, editor]. London and New York: Academic Press.
Kalckar, H. M. (1947). *J. biol. Chem.* **167**, 429.

***Studies on the relationship between concentration of hepatic metabolites and induction of glycolytic enzymes in vivo.** By J. M. GUNN and C. B. TAYLOR, *Department of Biochemistry, University of Sheffield, Sheffield S10 2TN*

***The effect on the activity of some hepatic enzymes of weaning rats on to a diet high in sucrose content.** By JENNIFER R. WEBB and E. BAILEY, *Department of Biochemistry, University of Sheffield, Sheffield S10 2TN*

***Changes in the activity of some kidney enzymes during development of the rat.** By CHRISTINE A. HAUSER, ELIZABETH A. LOCKWOOD and E. BAILEY, *Department of Biochemistry, University of Sheffield, Sheffield S10 2TN*

***Effects of protein deficiency on UDP-glucuronyltransferase activity and phospholipid composition of rat liver microsomal fraction.** By ALLAN B. GRAHAM, GEOFFREY C. WOOD and BARRY G. WOODCOCK, *Drug Metabolism Research Unit, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow G1 1XW*

***The synthesis of β-corticotrophin-(1–24)-tetracosapeptide (Synacthen) of high specific radioactivity.** By D. E. BRUNDISH and R. WADE (introduced by D. F. ELLIOTT), *CIBA Laboratories, Horsham, Sussex RH12 4AB*

***Observations on the fate of ACTH in isolated rat adrenal cell suspensions and the separation of an enriched ACTH-sensitive steroidogenic cell fraction.** By GILLIAN BULLOCK, E. A. GILLAM, P. J. LOWRY, C. MCMARTIN and J. PETERS, *CIBA Laboratories, Horsham, Sussex RH12 4AB*

***Structure–activity relations for the 20β-hydroxysteroid NAD+ oxido-reductase activity of cortisone reductase with special reference to ring B.** By IAN H. WHITE and JONATHAN JEFFERY, *Department of Chemical Pathology, University of Aberdeen, Aberdeen AB9 2ZD*

***Structure–activity relations for the 3-hydroxysteroid NAD+ oxido-reductase activity of cortisone reductase with special reference to the A/B junction and ring D.** By WILLIAM GIBB and JONATHAN JEFFERY, *Department of Obstetrics and Gynaecology and Department of Chemical Pathology, University of Aberdeen AB9 2ZD*

***An effect of prolactin on prostate adenyl cyclase.** By MERIEL P. GOLDER, A. R. BOYNS, MAUREEN E. HARPER and KEITH GRIFFITHS, *Tenovus Institute for Cancer Research and Department of Chemical Pathology, Welsh National School of Medicine, Cardiff*

***Enzymically catalysed binding of amino acids to the protein cyst coat of the ciliate, *Colpoda steinii*.** By JACK TIBBS, *Department of Biochemistry, University of Dundee, Dundee DD1 4HN*

***Nuclear deoxyribonucleic acid polymerases from landschutz ascites–tumour cells.** By VALERIE M. MARSHALL, JOAN BATES and H. M. KEIR, *Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS*

***Applications of a simple staining technique for steroids on thin-layer chromatograms.** By JONATHAN JEFFERY, *Department of Chemical Pathology, University of Aberdeen, Aberdeen AB9 2ZD*

†A method for the analysis of ^{15}N-labelled amino acids in the effluent from preparative amino acid analysis columns. By SUSAN E. HAWKES, PETER J. BUTTERY and DYFED LEWIS, *Department of Applied Biochemistry and Nutrition, University of Nottingham, Sutton Bonington, Loughborough, Leics. LE12 5RD*

Analysis of micromolar quantities of ^{15}N-labelled amino acids in effluents from preparative amino acid systems presents considerable problems. In conventional methods, which require about 70 μmol nitrogen, (Anonymous, 1970) the Kjeldahl technique is used with subsequent conversion of the ammonia produced into nitrogen gas using hypobromite before analysis in a mass spectrometer. A technique is reported here using emission spectrometry (Leicknam, Middelboe & Proksch, 1968) which is suitable for quantities as small as 0·5 μmol amino acid contained in large volumes of citric acid buffer.

The fraction, up to 50 ml, containing the amino acid is brought to pH 5·5 and heated with half its volume of ninhydrin reagent, (1 g ninhydrin, 0·15 g hydrindantin, 70 ml 2-methoxyethanol, 30 ml water) for 15 min at 100°. The diketohydrindylidine-diketohydrindamine, which contains the α-amino nitrogen from the amino acid, is then extracted from the citric acid buffer with chloroform. It is essential to adjust the pH of the solution to 3·3 to facilitate extraction. Below pH 3·0 the diketohydrindylidine-diketohydrindamine is destroyed (Kennedy, 1965). The chloroform is then evaporated to dryness under reduced pressure in a glass bubble, of approximately 5 ml capacity. The bubble is crushed and the powder added to a thick-walled Pyrex glass tube together with 1·5 g CuO and 1 g CaO. The tube is evacuated to a pressure of 133 mN m^{-2} sealed and heated at 550° overnight. The ^{15}N content of the nitrogen gas produced (Dumas reaction) is determined by emission spectrometry using the Dumas tube as an electrodeless discharge tube (Leicknam *et al.* 1968).

Phenol is not used in the analyser buffers since it is extracted into the chloroform and causes the Dumas tube to explode. It is not possible to use the column effluent direct since the high carbon content gives rise to too much gas within the tube.

During the manipulation of samples, a certain amount of contamination is unavoidable; for example the % excess of 1 μmol of amino acid can be underestimated by up to 25%. Preliminary experiments indicate that the contamination can be determined by assay of a [^{15}N]glycine sample of known % excess with each batch of samples.

S.E.H. holds a Ministry of Agriculture, Fisheries and Food Studentship.

REFERENCES

Anonymous (1970). *Tech. Rep. Ser. int. atom. Energy Ag.* no. 111, p. 19.
Kennedy, I. R. (1965). *Analyt. Biochem.* **11**, 105.
Leicknam, J. P., Middelboe, V. & Proksch, G. (1968). *Analytica chim. Acta* **40**, 487.

†Degradation of sclerotan, a β-(1→3)-glucan, by enzymes from fungi parasitic on sclerotia. By JOHN S. D. BACON*, A. H. GORDON and D. JONES, *Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ*

The resting bodies, sclerotia, of a number of fungi pathogenic towards plants contain a gel-forming polysaccharide, first found in *Sclerotinia libertiana*, named 'sclerotan', and shown by Kitahara & Takeuchi (1961) to be a β-(1→3)-linked glucan with single glucose residues attached at intervals by β-(→6)-linkage. Studies of its enzymic degradation and biosynthesis (Johnson, Kirkwood, Misaki, Nelson, Scaletti & Smith, 1963; Batra, Nordin & Kirkwood, 1969) have emphasized the regularity of its structure.

We have examined the composition of cell walls of several sclerotia-forming plant pathogens (Jones, 1970; Jones, Farmer, Bacon & Wilson, 1972). Culture fluids from two fungi parasitic upon *Sclerotinia sclerotiorum* will lyse cell-wall preparations

*Present address: Department of Carbohydrate Biochemistry, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB.

from this species, in which sclerotan is the predominant wall component (Jones & Watson, 1969). When the culture fluid was fractionated (cf. Bacon, Gordon, Jones, Taylor & Webley, 1970) several β-(1→3)-glucanases were found, but none would lyse cell walls when acting alone. In both parasitic fungi, *Coniothyrium minitans* and *Trichoderma viride*, the main component was an exo-β-(1→3)-glucanase (cf. Chesters & Bull, 1963). Since an exoglucanase from a basidiomycete had been shown by others to degrade sclerotan completely (Buck, Chen, Dickerson & Chain, 1968) we have tested the glucanases on glucan extracted from the fungus.

Sclerotia of *Sclerotinia sclerotiorum* or autoclaved mycelia of *Sclerotium rolfsii* were homogenized and centrifuged at 100 000 ***g***. A water-clear, colourless gel was decanted, which with 0·2 vol. ethanol could be compressed to a small volume at 100 000 ***g***. Resuspension and repetition of the process gave a product that could be freeze-dried without affecting its properties.

The purified exo-β-(1→3)-glucanase from *Con. minitans* acted upon it to give the expected products (glucose and gentiobiose in molar ratio 2:1) but degradation beyond 30–35% was very slow. After dissolution in dimethyl sulphoxide and recovery, the resistance was unchanged.

Purified endo-β-(1→3)-glucanase, from *Con. minitans* or *Cytophaga johnsonii*, had a very restricted action on the preparation, the products being mainly glucose and a suspected trisaccharide, but no gentiobiose; the viscosity was not affected.

Simultaneous action of the two enzymes brought about almost 100% conversion into glucose and gentiobiose, with small amounts of other oligosaccharides.

The resistance of the cell walls to the β-glucanases used singly is thus partly attributable to resistance by the sclerotan component. The resistance shown by our preparations (and by one from *Claviceps purpurea* kindly provided by Dr K. W. Buck) may be due to occasional structural 'errors' that impede the exoglucanase, or possibly to non-glycosidic linkages; treatment with hot 3% NaOH renders the glucan more susceptible to exoglucanase.

REFERENCES

Bacon, J. S. D., Gordon, A. H., Jones, D., Taylor, I. F. & Webley, D. M. (1970). *Biochem. J.* **120**, 67.
Batra, K. K., Nordin, J. H. & Kirkwood, S. (1969). *Carbohydrate Res.* **9**, 221.
Buck, K. W., Chen, A. W., Dickerson, A. G. & Chain, E. B. (1968). *J. gen. Microbiol.* **51**, 337.
Chesters, C. G. C. & Bull, A. T. (1963). *Biochem. J.* **86**, 31.
Johnson, J., Kirkwood, S., Misaki, A., Nelson, T. E., Scaletti, J. V. & Smith, F. (1963). *Chemy Ind.* 820.
Jones, D. (1970). *Trans. Br. mycol. Soc.* **54**, 351.
Jones, D., Farmer, V. C., Bacon, J. S. D. & Wilson, M. J. (1972). *Trans. Br. mycol. Soc.* **59**, 11.
Jones, D. & Watson, D. (1969). *Nature, Lond.*, **224**, 287.
Kitahara, M. & Takeuchi, Y. (1961). *J. agric. Chem. Soc. Japan* **35**, 468.

***Double-helical conformation of sulphated polysaccharides and their potential biological function.** By F. B. WILLIAMS, *Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS.*