The Two Hundred and Fifth meeting of The Nutrition Society was held in the School of Agricultural Sciences, University of Leeds, on Thursday, 5 December 1968 at 13.30 h, when the following papers were read:

A simplified radioisotopic procedure for the determination of calcium and phosphorus availability. By C. T. WHITTEMORE and A. THOMPSON, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

The principle disadvantage of the conventional isotopic procedures for the determination of the availability of dietary calcium and phosphorus is their dependence upon a chemical or radiochemical balance study. The proposed carcass ratio procedure allows availability to be calculated using radionuclide recovery data from a pair of animals, one dosed orally, the other intramuscularly with indentical amounts of ^{45}Ca and ^{32}P. It may be shown that the ratio so obtained will measure directly the proportional absorption of radionuclide through the intestinal wall.

In a series of three trials fifty-two inbred albino rats of some 50 days of age and of approximately 125 g live weight were used. Litter-mate pairs of rats of similar live weight were housed throughout the experimental period in individual metabolism cages and were fed restricted quantities of P.I.D.A. No. 1 Sib diet; a separate batch of diet was used for each trial, the mean calcium and phosphorus contents being 0·87 and 0·84% respectively. In trial 1, balance data were collected over a 10-day period following administration of the radioactivity; in all experiments animals were slaughtered 10 days after dosing.

The application of the ratio method to larger animals, such as the pig, would raise problems of handling and subsampling of large quantities of material. Therefore the use of femur and femur diaphysis ratios has also been investigated.

Table 1. *The availability of calcium and of phosphorus calculated using four different procedures*

Expt no.	Procedure	Availability (%) Calcium Mean ± SE	Phosphorus Mean ± SE
1	Carcass ratio	33·8 ± 4·0	61·0 ± 3·1
	Comparative balance	32·0 ± 3·5	62·0 ± 4·4
2	Carcass ratio	34·4 ± 1·4	59·0 ± 1·7
	Whole femur ratio	34·6 ± 1·5	58·9 ± 2·5
3	Carcass ratio	33·6 ± 1·8	70·9 ± 2·2
	Femur diaphysis ratio	36·6 ± 1·8	70·0 ± 1·3

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It may be seen from the results summarized in the table that the carcass ratio procedure compares favourably with the comparative balance, and that the whole femur and femur diaphysis are equally useful samples for the estimation of availability. However, further work has shown that the femur diaphysis has a lower rate of deposition of radionuclide than the epiphyses; problems concerned with the separation of the diaphysis from the epiphyses may reduce the accuracy of this method.

The ratio procedures appear to offer satisfactory alternatives to the comparative balance, choice of the carcass or the whole bone ratio being governed largely by the size of the animal involved.

Levels of fat mobilizing substance (FMS) in urine of human subjects in physiological and pathological conditions of increased lipid metabolism. By G. L. S. PAWAN, *Department of Medicine and Institute of Clinical Research, Middlesex Hospital Medical School, London W1*

A fat mobilizing substance (FMS) has been extracted from urine of man and some mammalian species during conditions of fasting and active fat catabolism. This material has been shown to produce marked effects on fat metabolism when

Table 1. *FMS activity of 24 h urine, assayed by the post-injection increase in 'ketones' and free fatty acids in mice*

Subjects	No. of subjects	Diet	Mouse plasma (% increase) 'Ketones' Mean ± SD		FFA Mean ± SD	
Normal (85–115% ideal body-weight)	72	*Ad lib.*	Nil		Nil	
	72	1-day fast	122	82	110	74
	12	*Ad lib.* (strenuous physical exercise)	45	29	38	18
Obese (150–210% ideal body-weight)	28	*Ad lib.*	Nil		Nil	
	28	1-day fast	65	48	40	28
	18	5-day fast	92	46	66	24
	12	10-day fast	18	10	12	8
	18	Day-5 of 1000 kcal 90% fat diet	63	34	30	22
	15	Day-5 of 1000 kcal 90% protein diet	42	28	23	16
	15	Day-5 of 1000 kcal 90% carbohydrate diet	Nil		Nil	
Anorexia nervosa	11	Variable	22	16	18	10
Diffuse lipoatrophy	6	*Ad lib.*	101	34	70	28
	6	1-day fast	164	26	131	35
Diabetic ketosis	8	?	48	15	33	18
Alcoholic hyperlipaemia	2	? Normal	42		35	
			54		40	
Post-surgical	10	? Normal	32	14	24	8
Pituitary ablation	6	*Ad lib.*	Nil		Nil	
	6	1-day fast	Nil		Nil	

administered to certain animals, both in vivo and in vitro (Chalmers, Pawan & Kekwick, 1960; Cahill, Pawan & Chalmers, 1961), and to human subjects (Kekwick & Pawan, 1968).

FMS extracts were prepared from 24 h urine collections by the method outlined by Kekwick & Pawan (1967) with certain special precautions, and assayed by injection into groups of mice. The index of activity was the percentage increase of free fatty acids (Dole & Meinertz, 1960) and 'ketones' (Pawan, 1958) in blood plasma of the mice, 6 h after injection of 5% of the 24 h urine extract, as compared with the levels of these substances in plasma of control groups of litter-mate animals similarly injected with 0·9% aqueous sodium chloride solution. The results are shown in Table 1.

I thank Drs T. M. Chalmers, J. D. N. Nabarro, J. D. H. Slater, Professors J. L. Codaccioni, A. H. Crisp, L. P. LeQuesne and A. Kekwick for specimens from their patients, and students, colleagues and others for urine samples.

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Feeding pattern and glucose tolerance: a species difference. By E. FLORENCE and J. QUARTERMAN, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Human subjects who have been adapted to several meals a day have a better oral glucose tolerance than those who eat the same number of calories in one or two meals a day (Fábry, Fodor, Hejl, Braun & Zvolankova, 1964; Gwinup, Byron, Roush, Kruger & Hamwi, 1963; Young, 1968).

We have found that rats showed the opposite response. Animals given their food *ad lib.* or a restricted amount by means of a device delivering food evenly and continuously over 24 h had a greater rise in plasma glucose levels after oral or intraperitoneal administration of glucose than rats allowed to eat for only once a day ('meal-eaters'). The rats eating for a short time only were either pair-fed with the continuously fed rats or were given food *ad lib.* for the restricted period.

Poor glucose tolerance is often associated with elevated plasma free fatty acid (FFA) levels. Continuously fed rats had fasting FFA levels of about 650 μ-equiv./l. whereas meal-eating rats had about 350 μ-equiv./l. The difference of the glucose tolerance between the two groups of rats showed a tendency to increase with increasing time on the experimental feeding patterns. Young (1968) found that the fasting FFA levels were lower in human subjects given two feeds a day than in those given eight feeds a day. These results are similar to our findings with rats.

Adaptation to meal-eating involves changes in several processes. These include hypertrophy and increased activity of the alimentary tract, an increase in endogenous respiration, and hepatic and adipose tissue lipogenesis and pentose pathway activity (Fábry & Braun, 1967). The difference of glucose tolerance in rats reported here is not likely to be a consequence of gut changes since it was shown by both oral and intraperitoneal administration of glucose to the rat. The difference of glucose tolerance between man and the rat may arise from a difference in glucose uptake by the tissues and by a process which is not influenced primarily by plasma FFA levels.

Other effects of frequency of feeding beside glucose tolerance are known to vary with species (Veum, Pond & Walker, 1966).

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Some effects of early experience of carbohydrates upon subsequent carbohydrate preference. By HEATHER GREENFIELD, *Department of Nutrition, Queen Elizabeth College, London, W8*

The effects of early experience are now widely accepted as having lasting consequences for the development and behaviour of the individual (Beach & Jaynes, 1954). It seemed pertinent to inquire whether or not early experience of only one type of carbohydrate affects the adult preference for different forms of carbohydrate.

Groups of six female rats of a hooded Lister strain were weaned at 23 days of age and maintained for various periods (8, 16, 24, 32 or 40 days) on a complete purified diet in which the carbohydrate moiety was either 60% sucrose ('sucrose diet') or 60% maize starch ('starch diet'). At the conclusion of the period on either diet, the rats were allowed to choose between the two diets until the end of the experiment. A further group of six weanlings was maintained on choice throughout the experiment.

The experiment continued for 64 days, i.e. until sexual maturity. Effects due to food pot position preference and oestrus cycle variations were eliminated by a standardized experimental technique. Environmental and husbandry conditions were constant throughout. Food intakes were measured daily, totalled for 4-day periods, and the preference for the sucrose diet was calculated as the proportion of the total diet eaten over each 4-day period.

The following results were obtained:

(1) In the immediate post-weaning period rats showed a strong preference for starch followed by a rapid swing to sucrose; this was established as a steady sucrose preference after 24 days.

(2) Both the 8-day sucrose group and the 8-day starch group were conservative in their immediate selection when offered a choice; the original diet was preferred.

(3) All other groups showed a neophilic reaction preferring the other diet when offered a choice. This was followed by a trend, similar in all groups, that finally reached a steady sucrose preference after 24 days.

(4) Thus all groups, regardless of initial diet, ended by selecting more sucrose than starch.

(5) By the end of the experiment, the sucrose groups considered together were choosing less sucrose in the diet than were the starch groups ($P<0.05$).

These results indicate that under these conditions, recent experience rather than early experience is the determining factor in forming carbohydrate preference.

Further work is in progress which indicates that the difference found between the immediate reaction of the 8-day groups, compared with that of the later groups, cannot be explained simply in terms of degree of habituation.

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Amino acid patterns in the small intestine. By D. W. T. CROMPTON and M. C. NESHEIM, *Department of Poultry Science, New York State College of Agriculture, Cornell University*

Nasset (1968) concluded that during digestion exogenous protein is diluted by sufficient endogenous protein to produce a relatively constant mixture of amino acids in the lumen of the intestine irrespective of the nature of the exogenous protein. The aim of the work reported in this communication was to test Nasset's conclusion using ducks allowed to feed *ad lib.* on water and rations of known composition. Intestinal contents were collected as required from various sections of the small intestine and free amino acids and percentage nitrogen were estimated by means of a Technicon Autoanalyzer and a micro-Kjeldahl procedure respectively.

Table 1. *Ratios of selected amino acids to leucine (unity) in a section of the intestine extending from 41 to 60% of the intestinal length*

	Soya-bean diet		Maize gluten diet	
Amino acid	Intestinal lumen	Diet	Intestinal lumen	Diet
Arginine	0·85	0·76	0·34	0·22
Glycine	0·53	0·64	0·15	0·27
Histidine	0·35	0·27	0·11	0·14
Isoleucine	0·47	0·58	0·23	0·30
Lysine	0·72	0·59	0·19	0·13
Methionine	0·24	0·18	0·15	0·15
Phenylalanine	0·64	0·57	0·47	0·38
Threonine	0·86	0·45	0·42	0·22
Valine	0·57	0·59	0·27	0·19

The data in the table show that the composition of the diet does affect the amino acid mixture in the intestinal tract. Other evidence was also obtained to suggest that the secretion of endogenous protein is probably too small to cause the formation of a constant mixture of amino acids during the digestion of exogenous protein. There is no reason to suppose that protein digestion in other animals will be very different from that in birds provided that normal feeding is permitted.

We thank Mrs Margaret DeGraff for excellent technical help and D.W.T.C. gratefully acknowledges grants from the P. A. Molteno Fund and Travelling Expenses Fund, University of Cambridge.

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Digestion of heather by red deer. By B. W. STAINES, *Nature Conservancy Unit of Grouse and Moorland Ecology, Natural History Department, University of Aberdeen*

Red deer (*Cervus elaphus L*) in Scotland have been assumed to be primarily grass feeders. However in a study of wild deer shot in Glen Dye, Kincardineshire, a substantial fraction of the winter rumen contents, rising to as much as 88% on a dry-weight basis, was found to consist of ling heather (*Calluna vulgaris*). In the same area, puberty is 1 year earlier than is usual in the Highlands, fertility is high, and initial growth rates are faster than in other areas studied (Mitchell, 1967; Staines, 1967). All this suggests better nutrition. In these field studies it is not possible to assess the digestibility of the heather, or how well the proportion of recognizable heather fragments in the rumen reflects the actual proportion eaten.

Two tame, adult hinds were used to study these questions at the Rowett Research Institute. Both were already fitted with rumen cannulas and were housed indoors. All heather present on an area of 15 m^2 was cut to ground level in February–March before any growth had started, and was trimmed to remove old wood. Such heather may not be representative of what deer might select in the wild. In the first experiment they were given dried grass, to which were added increasing proportions of heather. The proportions of grass to heather in samples from the rumen were related linearly to the proportions eaten.

In the second experiment the deer were housed in metabolism cages and were first offered 1500 g dried grass daily, and later 1500 g cut heather. On proximate analysis the grass and heather had 24% and 22% crude fibre and 10% and 5% of crude protein respectively. The intake and digestibility of the two foods is given in Table 1. The dry-matter digestibility of this heather by the deer is similar to that (36%) by red grouse (*Lagopus lagopus scoticus*) which depend almost entirely on heather for their food (Moss, 1967). Urinary and faecal nitrogen exceeded intake by about 4 g daily.

Table 1. *Apparent digestibility of dried grass and heather*

	Dried grass				Heather			
Deer	Dry matter (%)	Cellulose (%)	Nitrogen (%)	Dry-matter intake (g)	Dry matter (%)	Cellulose (%)	Nitrogen (%)	Dry-matter intake (g)
Adija	67	63	47	1325	42	30	14	792
Hamisi	64	61	49	1246	41	29	19	781

The heather given in this trial was clearly of poor nutritive value to the deer, which is contrary to what one might expect from the condition of the wild deer shot. Presumably this was because of their known selective feeding abilities (Taber & Dasmann, 1958).

I would like to thank Dr R. N. B. Kay, Dr A. Watson and Mr E. D. Goodall for their help with this experiment and paper.

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Effects of dietary tannic acid on the solubility of polyethylene glycol.

By R. N. B. KAY, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

In the course of trials to measure the digestibility of heather in red deer (Staines, 1969) an attempt was made to measure the volume of fluid in the rumen by use of the reference substance, polyethylene glycol (PEG) (Hydén, 1955). It was found, however, that 1·5 h after introducing 15 g PEG in solution into the rumen, the concentration in rumen fluid had fallen to between 0·0 and 0·3 g/l. When PEG solution was incubated in vitro at 30° with rumen contents from the deer receiving heather (0·2 g PEG/100 ml contents) little PEG remained in solution after 30–90 min, whether or not the contents had previously been boiled. This indicates that the effect was not due to enzymic or microbial action. PEG was also precipitated by an aqueous extract of milled heather, but most PEG remained in solution when it was incubated with centrifuged rumen fluid from the deer, or with whole rumen contents from deer or sheep receiving dried grass.

It is suspected that tannins are responsible for this effect. On analysis (Pro, 1952) the heather yielded about 5 g tannic acid/100 g dry matter. A solution of tannic acid precipitated PEG from solution, 3·5 g tannic acid being enough to precipitate 1·7 g PEG. Tannins are present in appreciable amounts in many plants, including leguminous forages. Therefore it seems that PEG is unsuitable for measurement

of rumen volume unless it can be shown that such interfering substances are absent from the diet.

Large numbers of pale needle-shaped crystals, tentatively identified as hippuric acid, appeared in the deer's urine during storage in acid at about pH 3. These did not appear when dried grass was fed. Some 16 g of the crystals were excreted daily by deer consuming about 1 kg heather, representing a loss of 1·2 g nitrogen to the animal. However, as much material, behaving as hippuric acid by the analytical method of Hampton (1948), remained in solution in the acidified urine as crystallized from it. This fraction may include free and conjugated hydroxybenzoic acids and further work is in progress on its composition.

Much of this work was carried out by Miss Maureen Reid for whose technical assistance I am most grateful.

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A comparison of methods for measurement of starch in food and gut contents. By J. H. Topps, *School of Agriculture, University of Aberdeen* and R. N. B. Kay, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Analysis of the gut contents of sheep for starch has produced divergent results. MacRae & Armstrong (1966) and Topps, Kay & Goodall (1968) reported that the amount of starch passing to the duodenum of fistulated sheep was less than 10% of that consumed when barley-rich rations were given and Sutton & Nicholson (1968) recorded similar results in sheep fed on large amounts of flaked maize. Yet when sheep were given cracked or ground maize, Wright, Grainger & Marco (1966) found high concentrations of starch in the abomasum and Tucker, Mitchell & Little (1968) found that starch reaching the intestine may exceed 30% of that consumed. Although some of the divergence between laboratories may be attributed to differences in the form in which the grain is fed, as found by Ørskov, Fraser & Kay (1969), a part may also be due to the use of different analytical methods.

To compare these methods directly, samples of food and duodenal contents from sheep were analysed by each of the laboratories referred to above. The diets and animal techniques used are described by Topps *et al.* (1968). The results are summarized in Table 1.

Table 1. *Starch content of the samples (glucose polymer, mg/g dry matter)*

Laboratory ...	A	B	C	D	E	E	F
Method ...	Enzymic					Anthrone	
Pelleted hay diet	57	56	46	43	43	83	78
Pelleted concentrate diet (85% barley)	560	610	500	530	480	520	810
Duodenal contents (hay diet)	10	10	6	12	6	40	19
Duodenal contents (concentrate diet)	50	58	49	48	46	77	96
Starch recovery from standard	98%	*	94%	*	98%	98%	*

*Read from standard curve.

Laboratories A, B, C and E analysed the samples by a method utilizing enzyme hydrolysis (MacRae & Armstrong, 1968), and laboratory D by another enzymic method (Wright *et al.* 1966). Laboratories E and F used methods employing acid hydrolysis and reaction with anthrone. The two laboratories using the anthrone methods produced inconsistent results; possibly carbohydrates other than α-linked glucose polymers yielded to hydrolysis. The enzymic methods gave much more consistent and credible results, although even here differences between laboratories were apparent.

We thank Professors D. G. Armstrong and G. E. Mitchell and Drs E. R. Ørskov, J. D. Sutton and P. L. Wright for their friendly co-operation and for permission to publish their results.

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Sites of digestion in sheep of a dried lucerne fed in three different physical forms. By D. J. THOMSON, D. E. BEEVER, J. F. COEHLO DA SILVA and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

Lucerne was dried commercially (120°) and used to prepare material of three different physical forms: (1) chopped, (2) wafered—prepared by compressing the chopped material into cylindrical cubes of approximately 2·5 cm diameter and 2·5 cm long and (3) pelleted—prepared by grinding the chopped lucerne through a 1·96 mm sieve prior to pelleting. Each form was fed to each of three sheep in two feeds/day at the same level of intake (910 dry matter/day) for periods of 4 weeks. The sheep also received at each feed a constant weight of chromic oxide-impregnated paper. Seven-day collections of faeces in the 3rd week of feeding were followed by 24 h collections of ileal and of duodenal digesta. The values for duodenal and ileal

digesta were corrected to give 100% recovery of chromic oxide. Overall digestibilities and sites of digestion for gross energy and for cellulose determined by the method of Crampton & Maynard (1938) are given in the Table.

Table 1

Form of dried lucerne . . .	Chopped	Wafered	Pelleted	SE of mean
Apparent digestibility of gross energy (%)	56·5	59·8	57·8	±0·65
Disappearance of apparently digested energy (%)				
(i) prior to duodenum	38·6	42·5	23·3	±1·42
(ii) in small intestine	33·8	34·0	51·1	±2·67
(iii) in caecum and colon	27·6	23·5	25·6	±1·64
Digestibility of cellulose	58·0	59·5	59·0	±0·44
Disappearance of digestible cellulose (%)				
(i) prior to duodenum	85·4	79·8	63·3	±1·85
(ii) in small intestine	−3·7	−2·9	9·3	±3·29
(iii) in caecum and colon	18·3	23·2	27·5	±0·93

Each value is the mean for three sheep.

At the level of intake fed there was no effect of physical form on the overall apparent digestibility of gross energy, nor for the chopped and wafered materials were there any significant differences in the amounts of the apparently digested energy disappearing in the different parts of the tract. However, grinding and pelleting of the dried lucerne induced marked changes in the relative proportions of the digested energy disappearing prior to, and within, the small intestine. On this ration disappearance of apparently digested energy in the stomach was significantly depressed while in the small intestine it was markedly increased. Pelleting did not cause any change in the contribution of the caecum and colon to overall energy digestion as compared with the other two forms.

Overall cellulose digestibility was not affected by processing. Pelleting significantly depressed the extent of cellulose digestion in the stomach, a depression which was partially compensated for by increased digestion of this constituent in the caecum and colon (pelleted *v.* chopped, $P<0{\cdot}01$; pelleted *v.* wafered, $P<0{\cdot}05$; chopped *v.* wafered, $P<0{\cdot}05$). The small negative values recorded in the table for digestion of cellulose by animals fed chopped or wafered material clearly reflect the virtual absence of its digestion in this part of the alimentary tract. The apparent, though small (9·3%) contribution to overall cellulose digestion occurring within the small intestine on the pelleted diet is surprising; however, none of these differences in the small intestine were significant.

One of us (D.J.T.) was on secondment from the Grassland Research Institute, Hurley.

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Effects of drying grass on sites of its digestion in sheep. By D. E. BEEVER, D. J. THOMSON, E. PFEFFER and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

Perennial ryegrass (S24) was cut, chopped immediately (approx. 6 cm lengths), and weighed amounts containing a given dry-matter content were subjected either to blast freezing for 48 h at −21°, or to drying in a forced-draught oven for 18 h at 100°, before low-temperature storage (−15°) in paper bags. Each of the diets was fed to the same three sheep for 4-week periods; the daily dry matter offered was 923 g, given in two equal feeds. Feed refusals were recorded daily and subsequently analysed. The use of chromic oxide-impregnated paper and collections of duodenal, and ileal digesta and faeces were as described by Thomson, Beever, Coehlo da Silva & Armstrong (1969).

The results (see table) showed there was no effect of drying on overall apparent digestibility of gross energy, but that significantly less of this fraction disappeared prior to the duodenum in the sheep fed dried grass. This smaller loss was compensated for by a marked increase in the amount of apparently digestible energy disappearing in the small intestine of these sheep, although this increase did not reach statistical significance.

Table 1. *Sites of digestion in sheep of the apparently digested energy and of the apparently digested nitrogen in a chopped grass, fed fresh or dried*

	Fresh (Mean ± SE)	Dried (Mean ± SE)	
Apparent digestibility of gross energy (%)	67·5 ± 1·69	68·2 ± 1·29	NS
Disappearance of apparently digestible gross energy (%)			
(i) prior to duodenum	63·1 ± 0·26	53·4 ± 2·12	*
(ii) in small intestine	23·5 ± 1·97	32·6 ± 5·05†	NS
(iii) in caecum and colon	13·4 ± 2·17	14·0 ± 2·65†	NS
Apparent digestibility of N (%)	75·2 ± 2·27	71·0 ± 2·56	NS
Disappearance of apparently digestible N (%)			
(i) prior to duodenum	3·5 ± 4·48	−28·6 ± 4·23	**
(ii) in small intestine	80·3 ± 6·43	105·4 ± 3·85†	*
(iii) in caecum and colon	16·2 ± 2·21	23·2 ± 2·80†	NS

†Values relate only to two animals.

While the mean daily nitrogen intakes for the sheep on both feeds were very similar (dried 27·0 ± 0·63 g; fresh 25·6 ± 0·67) and there were no significant differences in overall N digestibility, the amounts of N (g/24 h) passing at the proximal duodenum were markedly higher on dried than on fresh grass (32·4 ± 1·44 and 25·0 ± 1·47 respectively). This difference is reflected in the proportion of the apparently digested N lost prior to the duodenum. In the absence of information concerning the nature of the N moieties one cannot be sure that the values in the table reflect an increased passage of protein to, and digestion in, the small intestine as a result of drying the grass. Nevertheless the energy data suggest that this might be the case, particularly as analyses of all samples showed no significant quantitative differences in sites of digestion of cellulose, hemicellulose, starch or water-soluble carbohydrate on either ration.

One of us (D.J.T.) was on secondment from the Grassland Research Institute, Hurley.

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Thomson, D. J., Beever, D. E., Coehlo da Silva, J. F. & Armstrong, D. G. (1969). *Proc. Nutr. Soc.* **28**, 24A.

Absorption of volatile fatty acids in sheep. By B. N. Perry and D. G. Armstrong, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

Sheep fitted with a cannula into the rumen and a re-entrant cannula into the proximal duodenum have been used to study the absorption of volatile fatty acids (VFA) prior to the duodenum. The rumen was emptied, washed out with 0·9% saline and refilled with 3 l. of artificial saliva. Solutions of VFA of known molar composition and strength were then pumped in at constant rates (approx. 5 l./24 h) for periods of 72–96 h. All effluent from the abomasum was collected during the infusion period, during which time approximately 9 l./24 h of duodenal material from a donor animal were pumped continuously into the distal re-entrant cannula. Rumen liquor samples were removed every 2 h throughout the infusion and together with samples of infusion mixtures and of the daily effluent from the abomasum, were analysed for composition of VFA and pH.

Only very small amounts of VFA reached the duodenum irrespective of the amount or composition of the VFA mixture infused. Since for any level of infusion there was no significant day-to-day increase in the concentration of VFA in the rumen liquor and in the absence of any significant microbial population, the amounts given daily can be considered as representing the amounts of VFA absorbed. For any one VFA mixture the relationship between concentration of total VFA in the rumen liquor and the amounts given (absorbed) was linear, thus indicating that under these conditions absorption of VFA was directly proportional to concentration. Using the mean daily values for amounts of VFA infused (kcal/24 h=X), and the mean daily concentration of VFA in the rumen liquir (m-equiv./100 ml=Y), the following regressions were obtained. For an infusion mixture of molar composition acetic:propionic:n-butyric of 50:40:10, $Y=0{\cdot}7720+0{\cdot}003562\ X$ ($r=0{\cdot}94$). For a molar composition of 70:20:10, $Y=0{\cdot}8660+0{\cdot}004655\ X$ ($r=0{\cdot}97$). With either mixture, although the small changes in the molar proportions of acetic, propionic and n-butyric acids in the infusion mixture and rumen liquor were significantly different ($P<0{\cdot}001$), the mean molar proportions of VFA in the rumen liquor throughout each infusion period closely reflected the composition of the VFA mixture infused and were independent of the amount of VFA given (kcal/24 h). These data suggest that under these conditions the relative concentrations of the individual VFA in the rumen liquor closely reflect the relative amounts absorbed.

Nucleic acids in bovine nutrition. 3. Fate of nucleic acids presented to the small intestine. By R. H. SMITH, A. B. MCALLAN and W. B. HILL, *National Institute for Research in Dairying, Shinfield, Reading, RG2 9AT*

The ileal contents of a milk-fed calf contained appreciable amounts of DNA and RNA but these were virtually unaffected by the addition of DNA or RNA to the diet and were presumably of endogenous origin. Digesta presented to the duodenum of ruminating calves aged 4–10 months and fed a variety of diets contained about 8–12·5% of their total nitrogen as nucleic acids of microbial origin (Smith, McAllan & Hill, 1968). Amounts of nucleic acids entering the duodenum of similar calves were compared with those leaving the ileum. The latter amounts often exceeded those found for milk-fed calves and were significantly related to the amounts entering the duodenum. Nevertheless a majority was degraded and mean proportions (thirty-three experiments with four calves) lost in the small intestine were $75 \pm 2\%$ for DNA and $85 \pm 1\%$ for RNA. This compared with a net absorption for total N of $67 \pm 2\%$. In a preliminary investigation of the fate of ingested nucleic acids, experiments were carried out with rats kept in anti-coprophagy cages and fed high- and low-protein diets free of purines or pyrimidines. Some were given supplements of pure RNA or DNA and it was found that about 25% of the nucleic acid N appeared in the urine as allantoin. Additional urea N in the urine accounted for most of the remainder. A similar proportion of RNA N given to milk-fed calves was excreted as allantoin although urea excretion was not measured. It seems probable that some of the microbial nucleic acids in the ruminant gut meet a similar fate and the results of Topps & Elliot (1965) relating urine allantoin with nucleic acid concentrations in the rumen of sheep support this view. The quantitative aspects are, however, uncertain and the possible presence of partially degraded nucleic acids in the ileal contents of ruminants requires investigation.

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β-Hydroxybutyrate as a precursor of milk fat in the ruminant. By S. MCCARTHY and G. H. SMITH, *Division of Agricultural Chemistry, School of Agricultural Sciences, University of Leeds*

There is much indirect evidence that β-hydroxybutyrate (BHBA) is an important participant in milk fat synthesis in the ruminant. Arteriovenous-difference studies show that the mammary gland of the cow takes up large amounts of BHBA (Shaw & Knodt, 1941). Intraruminal infusion of butyrate results in an increased secretion of milk fat in association with a rise in the level of BHBA: the secretion of all acids from C_4 to C_{16} is increased. Intraruminal infusion of acetate results in a similar increase of fat secretion, but BHBA has a more marked effect on the shorter-chain fatty acids.

Tracer studies using the intact goat (Popják, French & Folley, 1951) and the isolated perfused cow udder (Kumar, Lakshmanan & Shaw, 1959) have provided evidence for the incorporation of BHBA into the fatty acids of milk fat mainly as an uncleaved C_4 unit, with a preferential incorporation into short-chain acids. In contrast, the results of Linzell, Annison, Fazakerley & Leng (1967), with the isolated perfused goat udder, indicated a more even incorporation into all acids from C_4 to C_{16} by at least two routes; the distribution of radioactivity in butyrate indicated about 40% cleavage of BHBA into C_2 units.

We have studied the incorporation of labelled BHBA, both as sole substrate and in the presence of (unlabelled) glucose and acetate, into milk fatty acids in slices of cow mammary tissue. Incorporation was one-half to three-quarters of that of labelled acetate. Differences in the mode of incorporation between BHBA and acetate were revealed when the slices were presented with a constant substrate mixture of BHBA, acetate and glucose. The mixture of fatty acids synthesized from BHBA had a relatively higher proportion of C_4–C_{10} than the mixture synthesized from acetate. Moreover, decarboxylation of the fatty acids indicated that only 20% of the BHBA was cleaved into C_2 units prior to incorporation. These results support the suggestion that a pathway for synthesis is involved different to the extramitochondrial 'malonyl' pathway as studied in most tissues.

The limited cleavage of BHBA and preferential incorporation into short-chain acids suggested by our results are difficult to reconcile with the increase in C_{16} in response to intraruminal infusion of butyrate, if this is the sole mechanism by which BHBA contributes to the production of fatty acids. Cleavage of BHBA may have assumed a greater significance in the experiments of Linzell *et al.* (1967) because of the low concentration of BHBA in the circulating plasma, and this could account for the more marked incorporation in the longer-chain acids.

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The effect of energy nutrition on blood amino acid levels in the lactating cow. By A. F. Halfpenny, G. H. Smith and J. A. F. Rook, *Division of Agricultural Chemistry, School of Agricultural Sciences, University of Leeds*

An increase in the plane of energy nutrition of the lactating cow causes a specific increase in milk protein content, and this effect is known to be dependent on an increase in the uptake of propionic acid from the rumen (Rook & Balch, 1961). Experiments have been done to determine the effect of plane of energy nutrition and of intraruminal infusion of propionic acid on the concentrations of individual amino acids in the blood plasma.

Four Jersey cows, in early to mid-lactation, were subjected to alternate low and high planes of energy nutrition over four successive, 3-weekly periods. One Jersey cow in mid-lactation and one Friesian cow in late lactation, both of which were fitted with permanent rumen cannulas, were given a standard diet over a period of 9 weeks: during weeks 1–3 and 7–9 they were given a continuous intraruminal infusion of 10 kg water/day and during weeks 4–6 10 kg of water + 1 kg propionic acid/day. Samples of jugular venous blood were taken, via a catheter, at 08.30, 10.30, 12.30 and 14.30 h on the last 2 days of each period in both experiments.

An increase in the plane of energy nutrition was associated on average with an increase in the plasma level of the group of amino acids whose skeletons may be derived from the general pool of glucogenic materials, and with a depression in the plasma level of other amino acids. Intraruminal infusion of propionic acid was associated in both cows with a decrease in the plasma concentration of lysine, histidine, arginine, glycine, valine, isoleucine and leucine. Glutamic acid concentration increased markedly and there was also a small increase in alanine concentration.

In both experiments, the amino acids which showed the most marked decreases are 'essential' amino acids present in milk proteins in high concentration, and the decreases presumably reflected the increased drain on those amino acids arising from an increased milk protein synthesis. The cause of the increased protein synthesis is not clear, but it could result from the increase in the supply of certain of the 'non-essential' amino acids to the mammary gland, if there is within the gland a restriction on amino acid formation from glucose and on interconvertibility between 'non-essential' amino acids.

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Using the oesophageal groove reflex in ruminants as a means of bypassing rumen fermentation with high-quality protein and other nutrients.

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During an experiment with young lambs we observed (Ørskov & Andrews, 1968) an increased growth rate in response to increases in dietary protein concentration up to 20% of the dry matter. The percentage of the dietary protein retained was, however, low, possibly due to an extensive degradation of dietary protein to ammonia in the rumen (Chalmers & Synge, 1954). In the young animal sucking milk causes closure of the oesophageal groove thereby directing liquid to the abomasum, thus bypassing the rumen. Wester (1930) and Watson & Jarret (1941) showed that solutions contaning sodium, and copper sulphate, respectively, stimulated closure of the oesophageal groove. This led us to explore the use of the oesophageal groove reflex as a means of preventing rumen degradation of protein. In our

first experiment, liquid suspensions of proteins (casein, dried milk, albumin and soya protein) were used along with various amounts of sodium chloride (0, 2 and 5% in dry matter) given as drenches to 6-month-old sheep. The course of the liquid suspensions was determined radiographically by use of $BaSO_4$.

The results showed that most of the marker could be found in the reticulum and rumen and that there were no apparent differences between protein source, salt concentration or animals. In a second trial, four lambs weaned at 2–3 weeks of age and trained to drink from a bottle received a liquid suspension of soya protein until they were 3 months old. From 3 to 5 months, they received a liquid suspension of a protein mixture (P) consisting of fish meal, sunflower, and soya-bean meal; from 5 to 8 months, a suspension of sunflower meal; and from 8 to 12 months, a suspension of casein. At all times dry feeds were also given. Radiographs were taken every 2–3 weeks. The animals readily sucked all the suspensions offered and the marker was quantitatively found in the abomasum and small intestine.

In a third experiment, three methods of administration were tested: drench; involuntary from teat on bottle, i.e. animals not trained to suck; voluntary from teat on bottle, i.e. animals trained to suck. Three liquids were used: water; protein suspension (P); and milk. The marker could only be found quantitatively in the abomasum and lower tract when the animals voluntarily sucked the suspension, regardless of type of liquid. It is concluded that chemical stimuli are of minor importance in determining the oesophageal groove response, which is a response by those animals that show eagerness for sucking. This condition can be maintained in the mature animal.

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The influence of caecal starch infusion in sheep on faecal output of nitrogen, starch and dry matter. By E. R. ØRSKOV and MERYL H. FOOT, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Recent work (Ørskov, Fraser & Kay, 1969) showed that when high amounts of concentrates, particularly ground and cracked maize, were fed to lambs, substantial quantities of starch escaped rumen fermentation. Much of this starch passed the terminal ileum and disappeared in the large intestine, the type of caecal fermentation was also affected. Since fermentation in the caecum rather than the rumen might render less microbial nitrogen available to the host, we examined the capacity for starch digestion in the large intestine and its influence on the composition of the faeces.

Two sheep received 900 g daily of pelleted dried grass containing 15·6% crude protein in dry matter from a continuous feeding device. A catheter was introduced

through an ileal cannula so that its tip lay just within the caecum. During a control period 2 l. of 0·8% NaCl solution were infused through the catheter daily. A suspension of maize starch was then added to this infusate increasing in amount by about 17·3 g dry starch daily to a maximum infusion rate of 260 g daily after 15 days. Total faeces were collected daily, and a fresh sample was taken from the rectum three times daily and frozen immediately for analysis of starch (method of MacRae & Armstrong, 1966) and nitrogen.

When up to 138 g of dry starch were infused daily 6% was recovered in the faeces, but when 138-260 g were infused 60% was recovered. Over the whole period, faecal N $\times 6{\cdot}25$ increased from 36·5 to 60 g/day and faecal dry matter from 198 to 378 g/day.

The capacity for starch digestion in the large intestine is evidently substantial, but in the present experiment when more than 138 g/day were given it was inefficiently digested and most appeared in the faeces. If the increased N excreted is assumed to be microbial, the results agree with estimates from the literature summarized by Hungate (1966) that 11–12 g microbial protein are synthesized per 100 g carbohydrate fermented. Hamilton (1942) showed an increase in faceal N $\times 6{\cdot}25$ of 3–4 g/100 g of soluble carbohydrate when it was fed and presumably fermented in the rumen. The nutritional significance of apparent N digestibility in ruminants must be treated with caution; in our experiment this was reduced from 69·3% to 48·7% by the starch infusion, suggesting that dietary regimes which increase caecal fermentation of carbohydrates are likely to render less microbial protein available to the host in relation to total amount of carbohydrate fermented.

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An estimate of bacterial protein synthesis in sheep on a constant feeding regime. By SARAH A. ROBERTS and E. L. MILLER, *School of Agriculture, University of Cambridge*

Two sheep were fed 720 g daily of a high-energy diet (44% rolled barley, 20% crushed oats, 20% molassed sugat-beet pulp, 12% soya-bean meal, 4% vitamins and minerals: 13·3% crude protein) in twenty-four equal feeds delivered hourly by a 'constant feeding machine'. The consistency of the rumen digesta was checked by measurement of various metabolites over a period of 3 h in one animal, and a day in the other. Results are shown in the table.

^{35}Sulphur was used to label bacterial protein (Henderickx, 1961). Solutions of [^{35}S]sulphate and polyethylene glycol (PEG) were infused into the rumen together with the animal's total water requirement over a period of 4 days. Drinking water was withheld for this period. Bacterial and trichloroacetic acid precipitable fractions

Table 1. *Means with standard deviations of single observations of rumen metabolites*

Sheep no.	Dry matter (%, w/w)	Total N (mg/g)	Trichloroacetic acid precipitable N (mg/g)	Ammonia N (mg/ml)	Volatile fatty acids (m-moles/100 ml)
1	14·6 ± 1·63	6·1 ± 1·04	5·6 ± 0·50	0·26 ± 0·041	6·4 ± 0·94
2	10·6 ± 1·00	6·4 ± 0·38	5·3 ± 0·58	0·29 ± 0·020	8·3 ± 0·73

were prepared from samples taken during the last 2 days of the infusion. The bacterial fraction was separated by centrifugation at 2000 **g** to remove food particles and protozoa, followed by centrifugation of the supernatant at 22 000 **g**. This fraction contained 8·13 ± 0·44% (w/w) N. The counts:nitrogen ratio was calculated for each fraction, and used to estimate the amount of bacterial protein per ml of rumen fluid. PEG was measured by the method of Hydén (1955), and used to calculate the rate of flow of liquid from the rumen. If it is assumed that the bacteria move with the liquid phase, the rate of passage of bacterial protein from the rumen can be calculated (Hungate, 1966). It was found that this varied with the rate of water input, e.g. when water input was 76·8 ml/h, the outflow of bacterial protein represented 53% of the dietary protein intake, but when inflow was 115 ml/h, this figure was 73%.

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Methods for the direct estimation of undigested dietary nitrogen in sheep faeces. By V. C. Mason, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

The estimation of the true digestibility of dietary nitrogen or the excretion of metabolic faecal nitrogen to date has, of necessity, involved the use of indirect methods. In search of direct procedures, the ability of five techniques to extract nitrogen and the bacterial amino acid α-ε-diaminopimelic acid (DAPA), from faeces of sheep fed a variety of diets, was compared. These methods were a modification of the neutral detergent method of Van Soest & Wine (1967), a modification of the acid detergent method of Van Soest (1963), the ultrasonic probe technique of Hellström & Aamisepp (1965), the phenol–acetic acid–water (PAW) method of Jennings & Watt (1967), and an enzymatic procedure based on lysozyme and trypsin.

It was found that whereas the first three methods extracted virtually identical amounts of faecal nitrogen, the PAW and enzymatic techniques were far less effective. Of particular interest was the observation that virtually all the DAPA was extracted from the faecal samples by the acid detergent and probe techniques, whereas the PAW method extracted no DAPA. Intermediary positions were held by the neutral detergent and enzymatic procedures in this respect. In separate analyses,

the detergent methods and the probe technique effectively degraded cellular debris of succus entericus collected from the small intestine of a cannulated sheep. These methods thereby offer possibilities for the removal of nitrogen originating from the host animal and bacteria from undigested dietary residues.

When applied to analyses of feed and corresponding faeces in balance trials with sheep, it was observed that whereas the potential of the detergent and probe techniques for extracting faecal nitrogen was virtually identical, their ability to extract nitrogen from the feeds varied considerably. Furthermore, with each method, more nitrogen resistant to extraction was consumed by the animal than reappeared in the faeces.

Estimates of the true digestibility of dietary nitrogen for roughage and concentrate rations agreed very well with those obtained using the extrapolation procedure.

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Single amino acids as non-protein nitrogen sources for adult sheep.

By MARGARET I. CHALMERS and A. D. HUGHES, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

The amino acids selected for investigation were (1) glycine, which on degradation gives equimolar ratios of ammonia and acetic acid (Van den Hende, Oyaert & Bouckaert, 1963), (2) alanine, which accounts for 10% of the total nitrogen in grass silages (Hughes, unpublished), and (3) lysine, one of the essential amino acids suggested as limiting for milk production (Bigwood, 1964).

Adult sheep fitted with rumen cannulas were fed a basal ration of 150 g cereals and 600 g hay. Equinitrogenous amounts of the individual amino acids (25 g glycine, 30 g alanine and 31 g lysine monochloride) were added to the cereal ration and fed for 14 days prior to experiment. Total ammonia, α-amino nitrogen and pH were estimated on rumen liquor samples taken at hourly intervals.

Glycine produced no increase in ruminal ammonia concentrations over 24 h. Glycine concentrations fell linearly with time over 17 h and could be accounted for as excess urea excreted in the urine. When polyethylene glycol (PEG) and glycine were administered into the rumen and mixed by the circulating pump, glycine disappeared from the rumen at the same rate as PEG and gave the same estimate of rumen volume.

Alanine disappeared from the rumen in 7 h. The ruminal ammonia concentrations increased over 6 h, indicating a slow rate of de-amination and a proportion of the alanine passing through the rumen.

Lysine feeding resulted in a slowly rising ruminal ammonia concentration, the lysine disappearing from the rumen in 7 h. No amines were detected in rumen

samples taken at 0, 1, 3 and 8 h after feeding lysine, and it was presumed that a proportion of the feed lysine travelled through the alimentary tract as such. Further evidence of this was obtained from analysing blood samples taken from the jugular vein 4 h after feeding lysine. The lysine concentration in blood plasma increased from 0·9 mg to 10·0 mg per 100 ml plasma, indicating that a substantial amount of lysine had been absorbed from the gastro-intestinal tract into the bloodstream.

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Estimation of urea in rumen contents and the rate of urea degradation in the rumen of adult sheep. By MARGARET I. CHALMERS, A. D. HUGHES and ALICE E. JAFFREY, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Of the methods described for urea determination only the enzymatic method using urease has the necessary specificity for estimating urea in a complex medium such as the rumen. The problem remains of estimating low concentrations of urea in the presence of large amounts of ammonia. The method developed involves the removal of ammonium ions by passing the sample through a column of Dowex-50 resin at pH 3·25, adjusting the pH of the eluate to 7, assaying the urea by incubation with urease and estimating the ammonia colorimetrically by the phenol-hypochlorite method. This method will accurately determine 4 mg urea per 100 ml rumen liquor.

Adult sheep fitted with rumen cannulas were fed a variety of urea-free diets and urea estimations were done on rumen samples taken at intervals throughout the day. No urea was detected. No urea was found in the rumen of sheep at 30 min after feeding 10 g urea in 150 g cereals.

Urea was administered into the rumen of sheep by a circulating pump fitted into the cannula, and the rumen contents were thoroughly mixed and sampled at 15 min intervals. Urea given 2 h after feeding 150 g cereals had disappeared from the rumen within 1 h, 75% of it within 15 min. This pattern was not influenced by (a) feeding urea for 5 months, (b) addition of starch to the cereal ration, or (c) administration of starch or acetic acid with the urea.

Rumen pH and the concentrations of total and free ruminal ammonia were determined over 6 h following the administration of urea by pump. The addition of starch lowered the rumen pH and decreased the percentage of free ammonia. The concentration of free ammonia present in the rumen was controlled by administering different molar proportions of acetic acid with the urea. The relationship between free ruminal-ammonia concentrations and urea toxicity will be discussed.