The Two Hundred and Fiftieth Scientific Meeting of the Nutrition Society (Ninety-ninth of the Scottish Group) was held in the Teaching and Research Centre, Western General Hospital, Crewe Road South, Edinburgh, on Friday, 6 October 1972, at 11.15 hours, when the following papers were read:

The site of fat absorption in the hen. By C. C. WHITEHEAD, *Agricultural Research Council's Poultry Research Centre, West Mains Road, Edinburgh EH9 3JS*

In chicks, Renner (1965) has shown that in five successive segments of the small intestine little fat was absorbed until it had reached the third segment. Here absorption was maximum and most of the remaining absorbable fat was removed in the fourth segment. Although the absorbabilities of certain fats increase as chickens mature (Renner & Hill, 1960), there have been no studies on the site of fat absorption in the laying hen. Since the site may be influenced by the level, absorbability and chemical nature of the fat, diets containing triglycerides, monoglycerides and free fatty acids were given to laying hens. A fat-free diet was also given and cellulose was used as reference.

Table 1. *Absorption of different dietary fats in successive segments of the mesenteric small intestine of the hen and after complete passage through the gastro-intestinal tract*

Fat and dietary level	Absorption in mesenteric segment								Total	Total absorption*
	1	2	3	4	5	6	7	8		
Fat-free†	−8·9	7·7	0·6	−0·3	1·1	0·2	1·0	0·9	2·3	2·2
20% maize oil‡	5·9	33·3	26·3	17·3	5·3	4·3	−2·9	3·7	93·2	94·7
35% maize oil‡	2·2	24·9	25·1	16·7	13·2	6·9	−1·0	2·9	90·9	92·1
20% glycerol monostearate‡	2·4	24·6	16·0	4·9	−1·1	2·9	0·8	1·5	52·0	52·5
10% stearic acid‡	−83·1	69·2	25·2	6·3	1·2	0·4	1·0	0	20·2	21·2

*Total absorption of fat determined by analysis of faeces.

†Absorption expressed as % of diet.

‡Absorption expressed as % of dietary fat.

Table 1 shows the absorptions of the different fats in eight successive segments of the mesenteric small intestine. A considerable contribution of endogenous fat was noted with the fat-free diet, although no corrections were made for this in the other diets. With all diets maximum absorption occurred in the second and third segments. For poorly absorbed fats, such as glycerol monostearate or stearic acid, 90% of the absorption occurred in the first four segments. Maize oil could be

absorbed more distally, especially when dietary levels were high. These results show that in the hen, compared with the chick, fat can be absorbed in the small intestine nearer the gizzard.

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The effect of feeding on the concentration of glucose and insulin in portal and right atrial plasma in pigs. By D. M. ANDERSON, *Department of Biochemistry, Agricultural Research Council, Institute of Animal Physiology, Babraham*

Indwelling catheters were established in the right atrium and the portal veins of pigs weighing 20–50 kg. The portal catheters were inserted through the gastro-splenic vein. The animals were given 40 g/kg per d of normal pig pellets, either as one or two feeds.

In animals (5) fed twice each day, portal and atrial plasma glucose was between 700 and 1000 mg/l before the morning feed. The portal glucose differed from the atrial glucose by a range from −120 to +80 mg/ml. Portal glucose concentration increased within 10 min of feeding and reached a maximum (1500–2300 mg/l) 0·5–3·0 h after feeding. There was a tendency for portal glucose to show cyclical changes, with the peaks ascending or descending progressively. After feeding, atrial plasma glucose increased approximately in parallel with portal but the increase was only 200–600 mg/l. The second feed was given 6–7 h after the first, and the pattern and magnitude of portal and atrial glucose concentrations during the next 5–7 h were almost the same as after the first feed. During the remaining 10–12 h before the next feed, portal glucose concentration declined slowly, and the difference between portal and atrial glucose became progressively less and, in some pigs, portal glucose became lower than atrial.

In animals (4) fed once each day, feeding increased portal glucose concentration to 1500–2000 mg/l, 1·5–2·0 h after feeding. Portal glucose followed a cyclical pattern, with further rises 4–7 h and 14–18 h after feeding. The rises in atrial plasma glucose concentration were again much less marked than those in portal plasma, the cyclical pattern was less evident and there was some tendency for portal glucose to fall below the atrial concentration.

Animals were also given 300 g starch (soluble or potato) instead of their normal morning meal and this resulted in a rise in portal glucose concentration within 10–12 min. Ingestion of 75 g casein instead of a normal morning meal had no effect on either portal or atrial glucose concentration.

Portal and atrial insulin concentrations rose immediately after normal feeding, and followed a cyclical pattern similar to that of glucose. In animals fed twice a day, maximum insulin concentrations were reached 0·6–3·5 h after both feeds, and were 115–250 μU/ml in portal plasma and 50–160 μU/ml in atrial plasma. In animals fed once daily, maximum values were reached 0·7–5·5 h after feeding and

were in the range 120–317 μU/ml in portal and 60–80 μU/ml in atrial plasma. Feeding with 75 g casein (3) produced some short-lived increases in portal insulin concentrations but there was no effect on atrial plasma insulin.

The effect of feeding pattern on tissue alkaline phosphatase activity.

By F. W. HEATON and B. W. LOVELESS, *Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster*

The influence of feeding pattern on tissue alkaline phosphatase activity in the rat was investigated before studying the effect of magnesium deficiency on the enzyme. Two groups of male Wistar rats, weighing about 100 g, received 9 g/rat per d of a synthetic diet of normal composition for 16 d. Control animals were fed automatically (Loveless, Williams & Heaton, 1972) to ensure a normal frequency of feeding throughout the day, but experimental animals received the same amount of food all in one meal and consumed it within about 30 min. All animals were fasted for 18 h before being killed.

Table 1 shows that the meal-eating pattern of feeding lowered the alkaline phosphatase activity in serum, but raised the activity in both liver and small intestine.

Table 1. *Tissue alkaline phosphatase activity of rats with different feeding patterns*

(Mean values ± SEM, with no. of determinations in parentheses)

Tissue	Control	Meal-eating	Significance of P value
Serum (KA)	44·9 ±2·4 (12)	37·7 ±2·1 (12)	<0·05
Liver (i.u./g)	0·39±0·02 (9)	0·61±0·04 (10)	<0·01
Small intestine (i.u./mg nitrogen)	1·18±0·10 (12)	1·98±0·12 (12)	<0·001

KA, King-Armstrong units.

When fasting rats were allowed access to unlimited amounts of the same diet for 1 h, the serum alkaline phosphatase activity rose from 20·0±1·6 King-Armstrong (KA) units to a maximum of 39·7±4·5 KA units 7 h after the food was provided. Similar rats were killed 4 h after the provision of food, and the alkaline phosphatase activity in liver and small intestine was compared with that in animals which continued to fast. Identical activities were found in the liver of both groups, but the activity in the complete length of small intestine was higher in postabsorptive than in fasting rats (14·2±1·2 i.u. and 10·8±0·6 i.u. respectively; $P<0·05$).

It is concluded that ingestion of food stimulates both the production of alkaline phosphatase in the small intestine and its secretion into the blood, but that the effect of feeding pattern on the enzyme is not attributable to the short-term influence of food intake.

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Energy metabolism during growth in normal and congenitally obese rats.

By J. D. PULLAR and A. J. F. WEBSTER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Measurements of the efficiency with which increments of a particular foodstuff are used to promote energy retention usually involve measurements with mature animals which retain energy predominantly as fat. In these circumstances the efficiency of utilization of the food is assumed to be related entirely to the chemical composition of the food and not to the physiological state of the animal. During growth, the proportion of food energy that is used to promote protein synthesis varies considerably. The extent to which the partition of metabolizable energy between fat and protein synthesis influences its efficiency of utilization is not known.

To investigate this problem we are using rats of the strain first described by Zucker & Zucker (1961). Individuals carrying two recessive genes (*fafa*), the 'fatties', synthesize an abnormally large amount of fat even if food intake is restricted and they are prevented from satisfying their voracious appetites. Litter-mates carrying one or more normal alleles have a normal body composition. It is possible therefore to select from one litter groups of fat and lean individuals which are conspicuously different in the way in which they partition retained energy between fat and protein synthesis during growth. Successive measurements of energy and nitrogen balance have been made on groups of four lean and four fat rats fed on a standard diet during their period of most rapid growth, from weaning to about 100 d of age.

The first results indicate that *ad lib.* intake of food was 36% greater, energy retention 94% greater, but N retention 17% less in the fat than in the lean rats. The proportion of energy retained as protein was more than twice as great in the lean rats as in the 'fatties'. Heat loss, at *ad lib.* intake, of both fat and lean rats kept during growth at an ambient temperature of 22° was related to body-weight $(g)^{0.4}$. Mean heat losses of fat and lean rats were 24·8 and 26·5 $kJ/g^{0.4}$. 24 h respectively. Preliminary measurements of spontaneous activity made with an Animex Activity Meter (on loan from LKB Instruments Ltd, South Croydon, Surrey) revealed no significant differences between fat and lean groups during the first 100 d of life. Mature fat rats over 800 g became, not surprisingly, very sluggish. There appeared to be no thermoneutral zone for either group of rats in which heat loss was independent of air temperature. In present experiments the influence of air temperature on energy exchanges is taken into account by replicating all experiments at 22° and at 28°.

The slopes of the lines relating energy retention to metabolizable-energy intake expressed in $kJ/g^{0.4}$. 24 h were 0·71 and 1·0 for lean and fat rats respectively. These preliminary results support the hypothesis that the efficiency of utilization of metabolizable energy during growth is inversely related to the proportion of metabolizable energy that is being used to promote protein synthesis.

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Estimation of body composition of sheep by isotopic dilution techniques. 1. Exchangeable potassium. By E. A. DOMINGO, T. E. TRIGG and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Very few attempts have been made to relate exchangeable potassium (K_e) to the body composition of farm animals which is subsequently directly determined. This experiment was conducted with the purpose of comparing the accuracy of this with that of other techniques as indirect measures of the body composition of sheep, using the direct method of slaughter and analysis as the standard for comparison.

Eighteen Shetland wethers, 10 months old, were divided at random into three groups of six animals. Each group was allocated to one of three dietary treatments. The high group was fed *ad lib.* with flaked maize, groundnut-cake and hay. The medium group was given the same diet, but to provide energy requirements for maintenance, and the low group was fed on hay in amounts below the maintenance requirements and the level of feeding was adjusted weekly. In this way the three groups were at a different physiological condition at slaughter and this was reflected in the live weight, though the percentage of body fat in the three groups was very close. Live weight at time of slaughter varied from 23·5 to 34·5 kg and body fat from 9·9 to 19·7%.

Each animal was injected with 250 μCi ^{42}KCl in 27·7 mg KCl. Specific activity in plasma K was estimated every 2 h from 12 to 24 h after injection. No changes were observed in specific activity with time and the seven results obtained were pooled for the final estimation of K_e. Activity in plasma was measured in a liquid scintillation counter, and that in urine, faeces and gut contents with a gamma counter. Total activity injected was corrected for the losses that occurred in urine and faeces in the first 24 h. Means and standard deviations were $4{\cdot}12 \pm 0{\cdot}92$, $2{\cdot}98 \pm 1{\cdot}06$ and $2{\cdot}92 \pm 0{\cdot}72$% of the injected activity in the low, medium and high groups respectively, and the corresponding activities in the gut contents were $5{\cdot}33 \pm 0{\cdot}66$, $2{\cdot}07 \pm 0{\cdot}32$ and $5{\cdot}1 \pm 0{\cdot}99$%.

Exchangeable K was $89{\cdot}4 \pm 4{\cdot}8$% of total body K.

Equations were obtained relating K_e to different components of the body and some of these relationships are shown in Table 1. The inclusion of live weight did not improve the regression to predict empty body nitrogen and it gave a slightly better prediction of fat-free empty body-weight.

Table 1. *Regressions relating exchangeable potassium (K_e) (g) alone or with live weight (W) (kg) to fat-free empty body-weight (FFEBW) (kg) and empty body nitrogen (EBN) (kg)*

Regression equation	R	RSD
$FFEBW = 0{\cdot}419K_e - 0{\cdot}551$	0·965	0·735
$FFEBW = 0{\cdot}276K_e + 0{\cdot}271W - 0{\cdot}015$	0·969	0·714
$EBN = 0{\cdot}013K_e + 0{\cdot}012$	0·927	0·034
$EBN = 0{\cdot}012K_e + 0{\cdot}001W + 0{\cdot}07$	0·927	0·035

Estimation of body composition of sheep by isotopic dilution techniques. 2. Deuterium oxide and tritiated water. By T. E. TRIGG, E. A. DOMINGO and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Total body water (TBW), total body fat (TBF), fat-free empty body-weight (FFEBW) and empty body nitrogen (EBN) were estimated using the eighteen sheep described in the preceding communication (Domingo, Trigg & Topps, 1973). Isotopes of hydrogen, as deuterium oxide and tritiated water, were used for the predictions of these body components. Both deuterium oxide and tritiated water were infused intravenously 8 h before slaughter, and serial blood samples were taken every 2 h. The dose-rates of deuterium oxide were similar to those recommended by Foot & Greenhalgh (1970), whereas ~ 250 μCi of tritiated water were given to each sheep. Water was obtained by vacuum sublimation from the plasma samples and the deuterium oxide concentration was estimated by infrared spectroscopy, and the activity of tritium was determined by liquid scintillation counting of deproteinized plasma.

Although contrary to most published results, tritiated water underestimated TBW by $4{\cdot}4 \pm 3{\cdot}1\%$ (mean $\pm$ SD), more accurate predictions of TBW, FFEBW and EBN were obtained with tritiated water than with deuterium oxide. Underestimation of the concentration of deuterium oxide in the water from sheep on the high plane of nutrition was an important factor responsible for the poorer results obtained with this isotope.

Simple and multiple regression analyses were used to obtain equations of body components (Table 1). More advantage was gained by using multiple regressions incorporating live weight (W) with deuterium oxide space (D) than with tritiated water space (T), for predicting either FFEBW or EBW. Of the multiple regressions using live weight and either of the two isotopic spaces, that including tritiated water space was the most accurate predictor.

Table 1. *Regressions relating deuterium oxide space (D) and tritiated water space (T) (l) either alone or with live weight to fat-free empty body-weight (FFEBW) (kg), empty body nitrogen (EBN) (kg) and total body fat (TBF) (kg)*

Regression equation	R	RSD
FFEBW = 1·09D − 0·197	0·858	1·43
FFEBW = 1·13T + 0·415	0·956	0·814
EBN = 0·036D − 0·018	0·889	0·041
EBN = 0·035T + 0·041	0·925	0·034
EBF = 0·406W + 0·552D + 0·211	0·725	0·659
EBF = 0·592W + 0·789T + 0·089	0·839	0·521

The results for exchangeable potassium (K_e) given in the preceding communication (Domingo *et al.* 1973), when combined with either deuterium oxide or tritiated water space for predicting FFEBW, gave the following regression equations:

FFEBW = $0{\cdot}372K_e + 0{\cdot}161D - 1{\cdot}21$ $R = 0{\cdot}967$ RSD = 0·735,

FFEBW = $0{\cdot}255K_e + 0{\cdot}468T - 0{\cdot}604$ $R = 0{\cdot}972$ RSD = 0·682.

The inclusion of K_e gave a more accurate prediction of FFEBW than that

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obtained with either of the hydrogen isotopes alone but the best prediction was given by measurement of tritiated water space combined with live weight.

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The effects of dietary supplements of wheat bran and cellulose on faeces. By M. A. EASTWOOD, T. HAMILTON, J. R. KIRKPATRICK and W. D. MITCHELL, *Gastrointestinal Unit, Western General Hospital, Department of Clinical Surgery, University of Edinburgh, Edinburgh*

In this study we have looked at recent suggestions that dietary wheat bran altered intestinal transit time, faecal weight and bile-acid excretion (Burkitt, 1971).

After a week's control period, eight subjects continued to eat their normal diet but this was supplemented with 16 g wheat bran/d taken for 3 weeks. This was followed by a further control period of 3 weeks. After this, cellulose was taken at 16 g/d for 3 weeks as a supplement to the normal diet. The diets remained constant during this period. During the 3rd week of each experimental period a dietary record was kept. Intestinal transit time (radio opaque markers), faecal weight (wet and dry), faecal bile acids and fats were measured. Serum cholesterol concentrations were also measured.

Bran and cellulose had no significant effect on the intestinal transit time, stool frequency or serum cholesterol. The stool wet weight increased significantly with both bran and cellulose. The total faecal bile-acid excretion was not increased by adding wheat bran to the diet, but it increased in the second control period in a highly significant manner. The striking difference, however, was in the faecal bile-acid output as mg/g dry weight in the treatment periods compared with the control periods. In the treatment periods the concentration of bile acids/g dry weight significantly decreased.

It would appear that bran and cellulose act as stool expanders, adsorbing and retaining water within the stool. In this way stool constituents, for example bile acids, are diluted. It does not appear that bran increases the excretion of bile acids. Bran and cellulose have no effect on the intestinal transit time.

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The use of the chromium complex of ethylenediaminetetra-acetic acid for studies of digestion in sheep. By E. D. GOODALL and R. N. B. KAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Polyethylene glycol (PEG) is commonly used as a reference substance for studies of digestion (Hydén, 1955) but in ruminant animals it may be precipitated when forages rich in tannins are consumed (Kay, 1969). The chromium complex of ethylenediaminetetra-acetic acid (Cr-EDTA) is not affected by tannins. It has

been used as a reference substance both in trace amounts labelled with ^{51}Cr (Downes & McDonald, 1964) and in the larger amounts permitting chemical estimation (Binnerts, Klooster & Frens, 1968). We have examined the latter approach.

A solution of Cr-EDTA was prepared by boiling 124 g Na_2-EDTA with excess $CrCl_3.6\ H_2O$ (100 g), precipitating the excess with NH_4OH (to pH 7·0), filtering and making up to 1 l with water. For sheep, 200 ml of this solution (about 4 g Cr) is a suitable daily dose. Samples for analysis are dried and ashed; the chromium is oxidized to dichromate and is estimated with automatic equipment from Technicon Instruments Co. Ltd, Basingstoke, Hants.

No ill-effect of repeated administration of Cr-EDTA to sheep and lambs has been observed, but a 200 ml dose contains 8·8 g NaCl and causes noticeable polydipsia and polyuria. However, Cr-EDTA does have certain drawbacks as a reference substance.

Cr-EDTA and PEG, given by rumen cannula, were used to estimate the rumen fluid volume in two sheep by the procedure of Hydén (1955). In thirty-two comparisons the volume estimated by Cr-EDTA (7·95 l, SE 0·13) was greater than that estimated by PEG (6·90 l, SE 0·16). The difference was observed whether the two substances were given simultaneously or on consecutive days.

It was evident that a little Cr-EDTA was absorbed, for the urine became tinged with purple after dosing. When 20 ml of the Cr-EDTA solution were injected intravenously into three sheep it was eliminated rapidly in the urine, 90–98% of the dose being excreted in 48 h. No Cr-EDTA could be detected in bile after dosing orally. Urinary excretion thus indicates quantitatively the absorption of Cr-EDTA from the gut.

Seven sheep, dosed with Cr-EDTA by rumen cannula, excreted 3·3% (SE 0·2) of the dose in the urine. In four other sheep dosed by oesophageal tube, an early peak in excretion was sometimes observed and up to 12% of the dose was excreted. In an attempt to locate the site of absorption one or other of two sheep having duodenal re-entrant cannulas was dosed with Cr-EDTA into the rumen and the duodenal digesta flows were exchanged for the subsequent 48 h. Urinary excretion of Cr by the dosed sheep, which could absorb Cr only proximal to the duodenal cannula, accounted for 1·7 and 1·9% of the dose, and that by the undosed sheep, absorbing Cr only distally to the cannula, for 2·9 and 2·0% respectively.

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Cannulation of the caecum in sheep. By J. C. MacRae*, C. S. W. Reid, D. W. Dellow, *Applied Biochemistry Division, DSIR, Palmerston North, New Zealand* and R. S. Wyburn, *Veterinary Clinical Sciences Department, Massey University, Palmerston North, New Zealand*

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As part of an examination of hind-gut function in sheep, six Romney Marsh wethers (1–3 years; 40–45 kg) were prepared with cannulas in their caecums. The cannulas were inserted either (a) 20–30 mm from the blind pole or (b) midway between the blind pole and the ileo-caecal valve (ICV). They were brought through the flank at distances of 100–260 mm from the lumber vertebrae. Each preparation was examined for adverse effects on caecal motility (radiological observations), digestibility of food and rate of passage of solid and liquid phase markers (^{103}Ru-phenanthroline and ^{51}Cr-EDTA respectively; Tan, Weston & Hogan, 1971).

Radiological observations indicated that mid-caecum cannulas (b) brought through the abdominal wall at the mid-flank caused least interference to caecal disposition and motility; these preparations also provided the most easily obtainable digesta samples. However, mid-caecum cannulas brought through the body wall above the mid-flank caused displacement of the caecum from its normal position on the ventral border of the abdominal cavity, and resulted in impairment of caecal motility and abnormal distribution of digesta, for a considerable time after the operation. Some adaptation to this interference appeared slowly to take place, and by 12 months after surgery caecal motility, and, consequently, digesta movement appeared to have partly regained many of its pre-operative characteristics despite the altered position of the caecum.

The point of exit of cannulas placed near the blind-pole (a) did not appear so critical, but an exit site high in the flank did tend to cause elongation of the caecum. These blind-pole cannulas were difficult to sample without the aid of some type of suction, with the attendant risk of mucosal damage.

Total digestibility of food was found to be a poor criterion for evaluation of post-operative normality after hind-gut surgery, even when the sheep were given high-cereal rations. Rate of passage of digesta, on the other hand, closely reflected the radiological observations. In sheep having postoperative disfunction of their caecums radio-opaque marker introduced at the ileum was not distributed throughout the caecum, but rather was quickly transferred from the region of the ICV to the colon. This was reflected in a significantly ($P<0{\cdot}001$) increased rate of faecal excretion of ^{103}Ru and ^{51}Cr introduced at the ileum.

The excretion patterns of both the solid and liquid phase markers were closely similar in any one sheep, suggesting that both phases of the digesta move through the hind-gut at the same rate.

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Effects of age and weaning on the apparent availability of dietary copper to young lambs. By N. F. SUTTLE, *Moredun Research Institute, Edinburgh EH17 7JH*

Mature sheep utilize less than 10% of the copper they ingest (Beck, 1963; Smith,

Field & Suttle, 1968) and the pre-ruminant lamb has been studied to see whether or not it can utilize dietary Cu more efficiently.

Six male Finnish Landrace lambs, taken from their mothers at 1–2 d, were reared on a milk substitute (Nutrilamb; Scottish Agricultural Industries Ltd) supplemented with Cu, 5 μg/g, and iron, 50 μg/g dry matter, and weaned at 38–64 d on to a semi-purified diet (Suttle & Field, 1968) also supplemented with Cu, 4 μg/g. The daily dry-matter allowance was 5% of live-weight for both diets. On three occasions, 28 and 14 d before and 15d after weaning, each lamb was given single doses of ^{64}Cu (0·5–1·5 mCi as $^{64}CuCl_2$) and ruthenium 103 (2·5–5·0 μCi as a phenanthroline complex; Tan, Weston & Hogan, 1971). Doses were administered in 100 ml milk by suckling or in aqueous solution by intraruminal injection. The lambs were kept in metabolism crates and faeces were collected for 4 d after dosing. The faeces were freeze-dried for 24–48 h, pulverized, mixed and sampled for counting. Counts from ^{64}Cu and ^{103}Ru were separated by allowing the ^{64}Cu to decay completely and recounting each sample. The ratio of ^{64}Cu to the unabsorbed marker, ^{103}Ru, recovered in the faeces was used as the measure of Cu availability. The mean availabilities for Cu ($\pm$SE) on the three occasions were 71·0$\pm$3·7, 47·2$\pm$7·8 and 10·8$\pm$1·4% and the live-weight ranges 3·8–9·2, 7·0–15·2 and 8·0–24·0 kg respectively.

Cu availability was lower ($P<0·01$) and more variable when milk-fed lambs were dosed for the second time and, before weaning, decreased linearly with increase in age according to the following relationship: availability (%)$=97·1-1·18\pm0·35$ (age in d); $r=0·73$. After weaning, Cu availability decreased further and by a greater factor than that predicted from the above equation. Diminishing enterohepatic circulation of Cu, increased adsorption of Cu on to particulate matter and precipitation of CuS in the rumen are factors which may contribute to the observed changes.

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The effect of dietary molybdenum and sulphate on the binding of copper in sheep plasma. By B. S. W. SMITH and H. WRIGHT, *Moredun Research Institute, Edinburgh EH17 7JH*

The addition to a diet adequate in copper (10 parts/10^6) of molybdenum (25 parts/10^6) and SO_4 (5 g/kg) produces changes in the distribution of plasma Cu (Smith, Field & Suttle, 1968). Indirect evidence of the presence of a Cu fraction not found in normal plasma was obtained. We now have direct evidence of such a fraction.

A comparison between atomic absorption and an automated colorimetric method for determination of total plasma Cu concentration revealed that, though there was good agreement between the two methods for samples from unsupplemented

animals, the colorimetric method gave values 20–40% lower than atomic absorption with samples from supplemented animals. The latter method, which is a modification (A. C. Field, personal communication) of the procedure of Summers (1960), depends on dialysis of plasma Cu after release with 0·7 M-HCl. Since there was no interference from Mo or sulphide added to normal plasma it appears that the fraction of Cu which is not estimated by this method is not diffusible as a consequence either of insolubility or of binding to protein.

Further evidence was obtained by using ^{64}Cu. Plasma from normal and supplemented sheep (2 vol.) was acidified with 0·7 M-HCl (1 vol.) and the protein was precipitated with trichloroacetic acid (TCA) (4% final concentration). $^{64}Cu\ Cl_2$ (≡200 μg Cu/l plasma) was added (a) before HCl, (b) after HCl and before TCA, or (c) after TCA. In normal plasma all the ^{64}Cu was recovered in the supernatant fraction, regardless of treatment. In plasma from supplemented sheep, however, only 60–85% was recovered when ^{64}Cu was added as in (a) or (b), but when it was added after TCA all the radioactivity was present in the supernatant fraction.

There is therefore a fraction in plasma from supplemented sheep which can exchange with ^{64}Cu and either remains bound to protein or is insoluble in acid solution. Its chemical nature is unknown, but it cannot be the complex of Dowdy & Matrone (1968*a*,*b*), which is soluble under these conditions. Mo may contribute to the formation of a more stable Cu-protein bond, and further work is in progress to investigate this possibility. However, it should be noted that in these animals determination of total plasma Cu from a TCA filtrate will give an erroneous result.

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Variation in intake among group-fed pregnant Blackface ewes given restricted amounts of food. By JANET Z. FOOT, A. J. F. RUSSEL, T. J. MAXWELL and P. MORRIS, *Hill Farming Research Organisation, 29 Lauder Road, Edinburgh EH9 2JQ*

Previous work on group feeding of non-pregnant ewes indicated a two- or three-fold difference in intake of concentrate food by individuals of the same group. Sheep housed during late pregnancy frequently receive a large proportion of their energy supply in the form of concentrates. In this experiment the individual intakes of food by such animals were measured. Restricted amounts of hay and concentrates were given according to commercial practice; the hay was constant in amount and gave a theoretical allowance of 734 g dry matter (DM)/sheep per d, whereas the concentrates were increased from an amount which allowed 96 g DM/sheep per d to one giving 435 g as the ewes approached lambing.

Three groups of sixteen ewes were harnessed for faecal collection. Food intakes were estimated from 4 d faecal outputs on three occasions, every 3rd week during

the last 8 weeks of pregnancy. Chromic oxide was incorporated in the concentrates, and recoveries averaged 100%.

Intakes by individual sheep during the first two periods, when concentrate allowances were the same, were remarkably consistent. In some instances the concentrate intakes during the second period were almost identical to those measured 3 weeks earlier. The DM intakes from concentrates in the group consisting of eight primiparous and eight fully grown ewes ranged from trace amounts to 190 g, or 2–196% of the mean (g DM/kg $W^{0.75}$) during the first period. Half the primiparous ewes in this group had intakes of concentrates considerably below average, whereas only one of the older ewes had an intake as low as these. In the other two groups, ewes were uniform in maturity, and variation between individual concentrate intakes (g/kg $W^{0.75}$) was less marked; in the first period it was 69–142% of the mean for mature ewes and 50–182% for primiparous animals. As the amount of concentrate feed was increased, differences in intakes by individual sheep decreased until none were obtaining less than 70% of the mean. There was less competition between animals for the hay which made up the greater part of the diet.

The results demonstrate that younger ewes are at a disadvantage when competing with mature animals, for small amounts of concentrate feed, but that total digestible DM intakes (g/kg$W^{0.75}$) are similar between age groups.

The effect of level of nutrition at two stages of pregnancy on the performance of primiparous ewes. By A. J. F. Russel and Janet Z. Foot, *Hill Farming Research Organisation, 29 Lauder Road, Edinburgh EH9 2JQ*

The reproductive performance of hill ewes lambing for the first time at 2 years of age is generally not as good as that of ewes producing their second or subsequent lamb crops. The low birth weights and high incidences of mortality of lambs from primiparous ewes are commonly attributed to inadequate nutrition during the final weeks of pregnancy, but in commercial practice it is seldom possible to offer such animals preferential nutritional treatment. Russel and Foot (unpublished) observed poor reproduction performance in primiparous ewes in which the nutritional state, as assessed by concentrations of circulating metabolites, was satisfactory during late pregnancy and similar to that of mature ewes giving normal lambing performance.

In an experiment with sixty 2-year-old Scottish Blackface ewes, animals on a nutritional regimen similar to that encountered in practice, and which resulted in a body-weight loss of 4·5 kg between mating and 7 weeks pre-partum but provided adequate nutrition during the final 7 weeks of pregnancy, produced single lambs weighing 4·06 kg. A higher level of nutrition during the final 7 weeks of pregnancy failed to increase single-lamb birth weights (4·09 kg). Animals on a superior nutritional regimen from mating to 7 weeks pre-partum, giving a body-weight increase of 1·5 kg during this time, and which were adequately nourished during the final 7 weeks of pregnancy, produced single lambs weighing 4·25 kg. The birth weight of lambs from ewes treated similarly in early pregnancy, but receiving the higher

level of nutrition in the final 7 weeks was 4·30 kg. The generally satisfactory lambing performance of primiparous ewes in this experiment is attributed to the greater-than-average size and weight of the animals used.

None of the differences in mean lamb birth weight between treatments was statistically significant but, nevertheless, the results suggest that the considerable loss of weight commonly occurring in primiparous hill ewes during the earlier stages of pregnancy may adversely affect lamb birth weight. This effect is unlikely to be overcome by nutrition during late pregnancy at levels greater than those which prevent a measurable degree of undernourishment.