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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Eighty-eighth Meeting of The Nutrition Society was held at the Hannah Dairy Research Institute, Ayr, on Friday, 21 April 1967, at 11.30 am, when the following papers were read:

The effects of feeding a diet of low copper content to lambs. By N. F. SUTTLE and A. C. FIELD, *Moredun Institute, Edinburgh*, 9

Anaemia, achromotrichia, neonatal ataxia, loss of wool crimp and bone fractures have each been associated with copper deficiency in the sheep under field conditions (Underwood, 1962). There is, however, no information on the effect of Cu depletion *per se* on growth, development and reproduction in the sheep. We have developed a diet containing only 1·2 ppm dry matter Cu, using the following components (%): oat husks 37, maize starch 17, sucrose 16, dried skim milk 16, urea 2·1, arachis oil 2·5, $KHCO_3$ 2·1, NaCl 1·6, MgO 0·5, added water 4·2, + trace minerals and vitamins A, D and E. Two groups of ten lambs, weighing initially about 18 kg, have been given this diet for 64 weeks and one group also received a supplement of 10 ppm Cu. The lambs were kept in metal-free pens and given deionized water. Both groups increased in live weight at about 0·9 kg/week and consumed 12·1 kg food/kg live-weight gain up to the 48th week. Mean values for the Cu content of the erythrocytes and plasma in the two groups are given in Table 1. Plasma Cu

Table 1. *Mean concentration of copper in the erythrocytes and plasma (μg/100 ml) of copper-supplemented and unsupplemented lambs given a diet of low Cu content*

Blood fraction	Group	Sex	Weeks of treatment										
			0	4	8	12	16	20	24	28	32	48	64
Erythrocytes	Cu-supplemented	Both (10)	–	108	115	98	103	94	93	88	90	95	–
	Unsupplemented	♂ (4)	–	108	81	95	64	56	54	43	47	64	–
		♀ (6)		108	71	68	53	49	24	22	33	27	
Plasma	Cu-supplemented	Both (10)	114	130	122	111	109	103	107	88	78	100	94
	Unsupplemented	♂ (4)		118	110	81	70	47	30	31	31	36	37
		♀ (6)		122	114	50	38	23	14	8	8	12	13

concentrations fell most rapidly in unsupplemented female lambs reaching a minimum of about 10 μg/100 ml after 28 weeks, a value associated with deficiency symptoms in the field (Underwood, 1962) and some 20 μg/100 ml lower than that found in the male lambs. The decline in erythrocyte Cu was less rapid than that in plasma but was also greater in female lambs.

Loss of wool crimp was suspected after 24 weeks and by the 44th week a distinct band of poorly crimped wool was visible in three unsupplemented lambs with

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plasma Cu concentrations of 5, 11 and 50 μg/100 ml. Benzylamine oxidase activity in the plasma was measured at the 16th week and a significant correlation was obtained ($r=0{\cdot}77$; $P<0{\cdot}001$) between activity and plasma Cu content. There were, however, no differences between groups in haemoglobin levels and haematocrit values throughout the experiment and the radiographic appearance of radius bones at the 64th week. The studies are being continued to examine the effects of Cu depletion on reproduction and incidence of neonatal ataxia.

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Observations on human dietary deficiency of vitamin B_{12}. By F. R. ELLIS and F. WOKES, *Pathological Laboratory, Kingston Hospital and Vegetarian Nutritional Research Centre, Watford*

Human dietary deficiency of vitamin B_{12} was first reported in some vegans consuming no animal food (Wokes, Badenoch & Sinclair, 1955). Since then various vegan foods fortified with vitamin B_{12} have been employed for its treatment (Ellis & Wokes, 1967). A few vegans with low intakes of these foods had serum vitamin B_{12} levels between 80 and 140 pg/ml. The average serum albumin of all the vegans studied did not fall significantly below the normal range, supporting the evidence (Hardinge & Crooks, 1964) that the vegan diet contains sufficient protein. A fall observed in serum globulin appears more significant. The biochemical and clinical effects observed, being removed by vitamin B_{12} therapy, were attributed to lack of the vitamin. The serum cholesterol remained normal except when vitamin B_{12} was provided in vegetable milk containing arachis oil, which lowered it whilst increasing the serum globulin. Low haemoglobin levels were also increased but, possibly due to high folic acid intakes, they were higher than would be expected from the clinical findings. There was no significant correlation between serum vitamin B_{12} levels and the EEG score (West & Ellis, 1966). Long-term vegans, on the diet for over 5 years tended to have more abnormal EEGs, confirming previous findings (Wokes & Smith, 1962). High serum folate levels associated with low serum vitamin B_{12} levels appear to be correlated with more abnormal EEG scores though not very significantly. The possible relationship between this and a recent hypothesis (Wilson & Langman, 1966) regarding serum folate and the endogenous cyanide metabolism (Wokes & Picard, 1955; Wokes & Barnard, 1959) were briefly discussed.

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Biogenesis of riboflavine metabolites. By D. W. West, E. C. Owen and Margaret M. Taylor, *Hannah Dairy Research Institute, Ayr*

Metabolites of riboflavine such as hydroxyethylflavin (RM1) are produced by ruminants and the rabbit but not by man, dog or newborn kids and calves. Subcutaneous injection of riboflavine into adult goats produces urine rich in riboflavine but devoid of RM1 or other metabolites. Owen, Proudfoot & West (1966) found that after taking solid food with riboflavine the kid excreted RM1 cyclically and they attributed this cyclical output to the caecum. Recent studies have, however, shown that production of RM1 in the adult rumen is also cyclic being maximal just before feeding. Just after feeding when RM1 is not always demonstrable in the rumen, the bacteria therein are diluted both by the incoming food and by copious oxygen-saturated saliva, and a rapid acidogenic fermentation, which inhibits RM1 production, sets in. Incubation in vitro produces RM1 in whole rumen contents of cow or goat after a day or two, and in caecal contents rather more readily. RM1 can be found in mixed bacterial cultures obtained from centrifugates of rumen contents incubated in various media for at least 2 days. Accompanying RM1 in such incubations was a new metabolite shown by various methods to be flavin-10-acetaldehyde, which is produced more readily than RM1.

The delay in the appearance of RM1 explains its cyclic excretion by the newly-weaned kid and also our failure at times to find RM 1 n the rumen. It also perhaps accounts for our difficulty in isolating bacteria responsible for the degradation of riboflavine to RM1, a difficulty which Stadtman (1958) likewise encountered.

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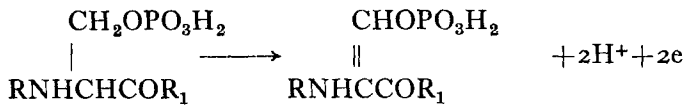
The reaction of casein with ferrous iron and oxygen. By W. Manson, *Biochemistry Department, Hannah Dairy Research Institute, Ayr*

There is evidence that cellular phosphoprotein takes part in the energy metabolism of tissues by functioning as a carrier of energy-rich phosphate (Friedkin & Lehninger, 1949; Davidson, Frazer & Hutchison, 1951; Johnson & Albert, 1953; Ahmed & Judah, 1963). The claims of Burnett & Kennedy (1954) and of Rabinowitz & Lipmann (1960) that the phosphoproteins of egg yolk and milk, phosvitin and casein, can take part in phosphate transfer reactions suggest that they, in addition to fulfilling their purely nutritional function, may behave similarly to cellular phosphoprotein. Grant & Taborsky (1966) after studying the interaction of Fe^{2+}, oxygen and phosvitin concluded that such behaviour involved conversion of serine phosphate residues in the protein to enol-phosphate groups. In this communication, the possibility of casein behaving analogously is discussed.

When a solution of $FeCl_2$ was added in the presence of air to a solution of either α_S- or β-casein at pH 6·5 a rapid uptake of oxygen occurred. This was greater than required to oxidize the iron, suggesting that oxidation of the casein had also taken

place. The caseins thus behaved as had phosvitin in the experiments of Grant & Taborsky (1966) who interpreted the reaction as being due to the production of enol-phosphate groups thus:



This view was upheld by their detection of inorganic phosphate and ammonia amongst the products of the reaction. However, in the present study neither α_s- nor β-casein yielded inorganic phosphate or ammonia. Furthermore contrary to the findings of Grant & Taborsky (1966) DL-*o*-phosphoserine when treated with Fe^{2+} and oxygen produced no ammonia, no inorganic phosphate and consumed no oxygen above the amount required for oxidation of the iron.

The biological implication of these and other findings from related experiments was discussed.

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Studies on the protein requirements of beef cattle receiving high-energy diets. By M. KAY, G. MCKIDDIE and N. A. MACLEOD, *Rowett Research Institute, Bucksburn, Aberdeen*

Fifty-four Friesian steers were allocated at random to one of three isocaloric diets containing different levels of crude protein. The diets contained bruised barley and soya-bean meal, and provided either 11%, 14% or 17% crude protein in the dry matter. In the first experiment the steers started on the experiment at 75 kg live weight and in the second at 135 kg. The diets were given *ad lib.* and the slaughter weight was 400 kg live weight.

Up to 200 kg live weight, mean growth rate and feed conversion efficiency were significantly poorer for the low-protein diet than for the medium- or high-protein diets. At greater live weights there were no significant differences between treatments in either growth rate or feed conversion efficiency. Chemical analysis of the tenth rib joint did not reveal any significant treatment effects on the carcass although there was a tendency for the rib joints from the steers given the high- or medium-protein diets to have a higher proportion of fat and a lower proportion of water than those from steers given the low-protein diet.

The influence of dietary calcium concentration on epidermal lesions of zinc deficiency in lambs. By C. F. MILLS and A. C. DALGARNO, *Rowett Research Institute, Bucksburn, Aberdeen*

Determinations of the zinc and calcium contents of fodders used in areas in Finland where dairy cattle develop a Zn deficiency syndrome characterized by epidermal irritation have led to the suggestion that the Zn requirement of cattle may rise as the Ca intake increases (Haaranen, 1963). The object of the experiment reported here was to investigate the possible influence of Ca on Zn utilization by growing lambs.

Eighteen Border Leicester × Cheviot lambs were weaned when 2 months old on to a semi-synthetic basal diet (Mills, Dalgarno, Williams & Quarterman, 1967) supplemented with 18 ppm Zn. They were randomized by weight into three groups of six. The Ca contents of the diet given to these groups were adjusted to 0·6% (group A), 1·2% (group B) and 1·8% (group C) by adding calcium carbonate (BP). The concentration of supplementary Zn was progressively reduced in all diets as follows: 18 ppm (fed for 1 week), 13 ppm (for 2 weeks), 10 ppm (for 5 weeks), 5 ppm (for 10 weeks) and no supplement (2 weeks).

Differences in dietary Ca concentration had no significant effect on live weight. The rate of weight gain decreased in all groups shortly after the concentration of supplementary Zn was reduced to 5 ppm.

The mean plasma Zn concentration of all lambs fell progressively as the Zn content of the diet was reduced and in each of the above periods the mean plasma Zn of group C was lower than that of groups A and B.

Lambs were examined independently by three or four observers on several occasions and clinical lesions of Zn deficiency were scored on a 5-point scale according to severity. After 6 weeks on the diet containing 5 ppm supplementary Zn only two lambs of group A had developed mild lesions of hyperkeratosis around the eyes and mouth. Five lambs of group B had moderately severe lesions and six lambs of group C had severe lesions ($P<0{\cdot}01$).

This cross-breed develops keratinous horn buds when Zn-deficient (Mills *et al.* 1967); one lamb of group A, four of group B and six of group C had developed horn buds by the end of the experiment.

We conclude that high dietary concentrations of Ca adversely affect Zn utilization and, directly or indirectly, accelerate the development of epidermal lesions of Zn deficiency.

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Growth and voluntary food intake of steers fed on diets containing different levels of low-quality roughage. By R. C. ELLIOTT and W. D. C. REED, *Henderson Research Station, Salisbury, Rhodesia*, and J. H. TOPPS*, *Department of Agriculture, University College, Salisbury, Rhodesia*

*Present address: Department of Agriculture, University of Aberdeen.

Rates of body-weight gain and voluntary intakes of food and of digestible energy (DE) were measured using forty-eight steers given diets which contained either 5, 20, 35 or 50% roughage. Twelve steers were fed on each diet and measurements were made at the ages of 7–11 months, for 112 days, and of 19–23 months, for 102 days. In a concurrent trial on steers fitted with rumen cannulas, the digestibility of, and the pattern of ruminal volatile fatty acids (VFA) associated with the diets were measured.

Table 1. *Mean voluntary intake of digestible energy (kcal/kg $W^{0.73}$ 24 h) and mean gains of body-weight (kg/24 h) by groups of six steers given diets containing different levels of roughage*

Age of steers (months)	Roughage fed	Level of roughage in diet: 5% Intake	5% Gain	20% Intake	20% Gain	35% Intake	35% Gain	50% Intake	50% Gain
7–11	Hay	370	1·28	391	1·32	399	1·33	351	1·17
	Maize cob	348	1·16	367	1·13	406	1·36	333	1·09
	Mean	359	1·22	379	1·23	403	1·35	342	1·13
	SE of means			±9·4 (intake)	±0·046 (gain)				
19–23	Hay	385	1·68	429	1·74	402	1·59	355	1·33
	SE of means			±12·8 (intake)	±0·089 (gain)				

Maximal rates of body-weight gain and highest intakes of food and DE occurred with diets containing either 20 or 35% roughage. Molar proportions of VFA in the rumen of steers fed on the four diets differed markedly; acetate varied from 36·5% of total for the lowest roughage diet to 61·4% for the highest. However, when four groups of six steers were given the four diets in restricted quantities, which provided the same daily amount of DE (300 kcal/kg $W^{0.73}$) no differences occurred in rate of body-weight gain.

These data suggest that for growing steers, fed on either *ad lib.* or restricted amounts of food varying in roughage content from 5 to 50%, differences in body-weight gain can be largely accounted for by variations in intake of DE. Differences in the efficiency with which the energy from varying combinations of roughage and concentrate promoted gains in body-weight appear to be negligible.

Carcass dressing-out percentages were negatively correlated, however, with the roughage content of the diet eaten by the steers. This significant difference between groups may be due to either a smaller gut-fill of steers given low-roughage diets or a higher fat content of their carcasses.

Some observations on the changes occurring during the maturing of grass silage. By A. D. HUGHES, *Rowett Research Institute, Bucksburn, Aberdeen*

These observations were made on a series of twelve experimental tower silos, each holding about 8–10 cwt of silage. The silos were filled under as nearly as possible

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the same conditions with a ryegrass mixture cut at the ear emergence stage of growth. The grass used in all the silos was cut from the same field on the same day.

A silo was opened and sampled by boring after each of the following time intervals: 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18 and 20 months. Changes in the pH, dry matter, total nitrogen, water-soluble nitrogen, peptide nitrogen, amino nitrogen, and total volatile basic nitrogen, soluble carbohydrates and total volatile fatty acids were recorded.

Amino nitrogen values fell progressively with maturity from 30% down to 22% of the total nitrogen, whilst the total volatile basic nitrogen gradually increased from 9% to 15% of the total nitrogen. Soluble carbohydrates increased from 7% to 11% of the dry matter after 8 months and then decreased to 7%. The total volatile fatty acids increased steadily from 3·5% of the dry matter to 7%. This survey has shown that there was no major change in the overall nitrogen distribution after the first 2 months.

Some of the results obtained from these samples are shown in Table 1.

Table 1. *Changes in the composition of silage relative to time (June 1965 to February 1967)*

			Total nitrogen						
Age (months)	pH	Dry matter (%)	% fresh	% of DM	% CP	% peptide	% amino	% amide	% volat. basic
Initial grass	–	16·4	0·498	3·0	19·0	26·6*	3·9	5·3	0·5
2	4·05	16·8	0·500	3·0	18·6	7·3	30·0	2·5	8·8
3	3·96	16·8	0·505	3·0	18·8	4·5	26·3	2·2	10·0
5	3·85	17·6	0·518	2·9	18·4	4·6	28·0	1·7	10·6
8	3·94	17·2	0·508	3·0	18·5	4·5	26·4	2·3	10·2
14	4·06	17·5	0·504	2·9	18·0	3·1	24·1	2·1	11·3
18	4·16	17·2	0·500	2·9	18·2	3·2	21·5	1·9	14·7

	Soluble nitrogen					Soluble carbohydrates		Total VFA	
Age (months)	% of TN	% peptide	% amino	% amide	% volat. basic	% fresh	% of DM	% fresh	% of DM
Initial grass	53·1	50·1*	7·3	10·0	1·0	5·50	33·6	None	None
2	66·0	11·0	45·5	3·8	13·4	1·16	6·9	0·57	3·4
3	61·5	7·4	42·9	3·6	16·3	1·12	6·7	0·60	3·6
5	68·6	6·7	40·8	2·5	15·5	1·52	8·6	0·64	3·6
8	64·2	7·0	41·1	3·6	15·9	1·93	11·2	0·60	3·5
14	67·0	4·6	36·0	3·1	17·0	1·63	9·3	0·73	4·2
18	64·7	5·0	33·2	3·0	22·8	1·25	7·3	1·13	6·6

*Includes soluble protein.

This work is being followed up by a detailed study of the quantitative distribution of the amino acids and related nitrogenous compounds. Some findings concerning the quantitative distribution of the basic amino-acids will be discussed.

The effect upon the methane production of sheep of a short infusion of higher fatty acids into the rumen. By J. L. CLAPPERTON and J. W. CZERKAWSKI, *Hannah Dairy Research Institute, Ayr*

When long-chain fatty acids were continuously infused into the rumen of sheep, methane production fell (Czerkawski, Blaxter & Wainman, 1966). To investigate the effect of a single dose of fatty acids infused into the rumen, two wether sheep were confined in a respiration chamber for 4 consecutive days and were given 1000 g of hay once daily at 9 am. On the 2nd day, an emulsion of 40 g of a mixture of fatty acids resembling those of hay was infused into the rumen over a period of 2 h

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while the animal was feeding. The mixture used was 23 g technical linoleic acid (British Drug Houses Ltd, Poole, Dorset) and 17 g linseed oil fatty acids (British Drug Houses Ltd, Poole, Dorset) emulsified in water with sodium hydroxide. No infusion was given on the 1st, 3rd and 4th days. To measure the methane production, samples of the chamber air were removed every 2 h from 9 am to 11 pm, then at 6 am and again at 9 am when the chamber was opened so that the animal could be given food.

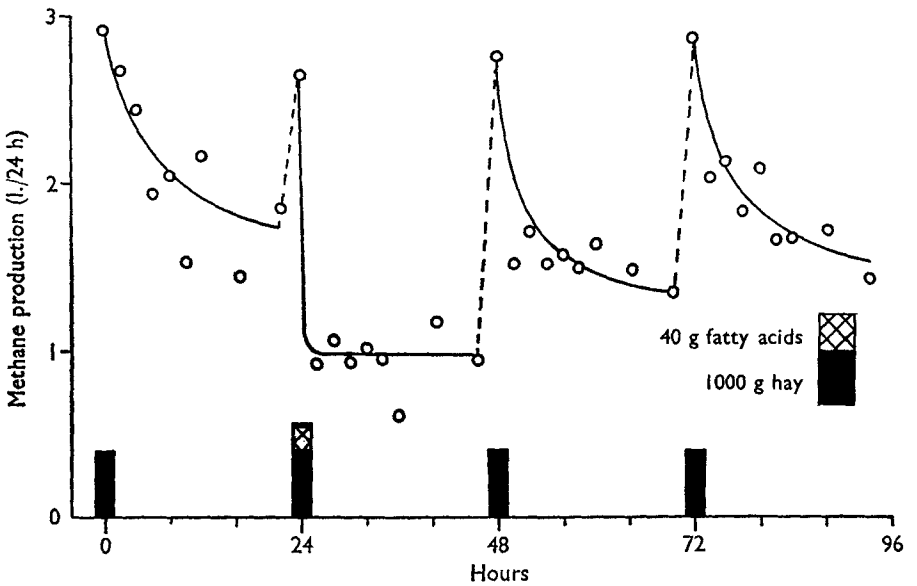

Fig. 1. Mean methane production of two sheep when fed 1000 g hay once daily before and after the infusion of 40 g fatty acids.

The results are shown in Fig. 1. The daily curves were fitted to an equation of the type:

$$y = y_0 - \frac{\alpha t}{\beta + t},$$

where y is the volume of methane produced in any period of 2 h, y_0 is the value of y at the start of the day, t is the time from the beginning of the measurement of methane production and α and β are constants. Immediately after the infusion, the hourly methane production was reduced to less than half of the value obtained on the 1st day but recovered almost completely by the end of the 4th day.

If methane is produced by reduction of carbon dioxide and if the hydrogen or hydrogen donor is also utilized in the complete saturation of the infused fatty acids, then this saturation would account for only one-sixth of the observed reduction in methane production.

The results are consistent with the hypothesis that methane is produced from two types of substrate, one typified by cellulose and the other by soluble carbo-

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hydrates. According to this hypothesis the methane derived from cellulose is produced at a steady rate throughout the day and the methane derived from the soluble carbohydrate of the food rises rapidly soon after feeding and falls off to a steady value before the end of the day. It appears that the fatty acids reduce the production of methane from cellulose by only one-third but effect a much more marked reduction in methane synthesis from soluble carbohydrates.

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Effect of branched-chain fatty acids on methane production by sheep.

By J. W. CZERKAWSKI, J. L. CLAPPERTON and C. STRONG, *Hannah Dairy Research Institute, Ayr*

When straight-chain fatty acids were incorporated in the diets or infused into the rumens of sheep, there was a reduction in the rates of methane production (Czerkawski, Blaxter & Wainman, 1966). Methane production was reduced more effectively by the administration of unsaturated fatty acids than by the administration of saturated fatty acids. Since the fatty acids of rumen bacteria contain relatively large proportions of branched-chain fatty acids (Allison, Bryant, Katz & Keeney, 1962) it was of interest to determine the effect of these acids on methane production.

The preparation of mixed branched-chain fatty acids (Versatic, 1519) was kindly supplied by Mr. H. L. Bennister of Shell Chemical Co. It was a complex mixture of mainly tertiary monocarboxylic acids. The mean molecular weight of these acids was about 260 and the iodine value was negligible.

In some experiments, sheep provided with rumen cannulas were given a diet of dried grass and the fatty acids were infused continuously into the rumens until the daily methane production reached a steady value. Infusion was then stopped and the methane production was recorded until it returned to a normal value. In other experiments (change-over type) two sheep were given pelleted rations or the same rations supplemented with branched-chain acids (16 g/day), and the rates of methane production were determined.

When about 20 g palmitic acid/day were infused into the rumen, the methane production did not differ significantly from the control value, but when similar amounts of branched-chain acids were infused (16–25 g/day) the mean decrease in the production of methane was 32 ± 7 kcal/100 kcal of added fatty acids. The incorporation of the branched-chain acids in the rations resulted in a decrease of 80 ± 2 kcal of methane/100 kcal of added acids. The corresponding values obtained when linseed oil fatty acids were infused into the rumen or incorporated in the diets of sheep were 16 and 29 kcal/100 kcal of fatty acids given, respectively.

It appears that the branched-chain tertiary acids used here are more effective in depressing the production of methane than the straight-chain acids, but the mechanisms of these effects are at present obscure.

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Magnesium ammonium phosphate as a mineral supplement for growing lambs. By R. G. HEMINGWAY and NORA A. BROWN, *Department of Animal Husbandry and Veterinary Preventive Medicine, University of Glasgow*

Dicalcium phosphate (DCP) with 17·5% phosphorus and 23·5% calcium and magnesium ammonium phosphate (MAP) with 16% magnesium, 9% nitrogen and 20% P have been compared as P sources for growing lambs. Further comparison has been made with another compound (MIX) composed of equal parts of DCP and MAP and containing 8% Mg, 4·5% N, 19% P, 11·5% Ca.

Blackface lambs aged 8 weeks were transferred to a low-P diet containing 0·06% P (Hemingway, 1963). Three groups, each of five lambs, were given appropriate amounts of one of these P supplements to add 0·15% P to the basal diet. Normal plasma P concentrations were not maintained and after a further 6 weeks (on 16 August) supplementation was increased to add 0·20% P. The lambs improved rapidly in condition. Comparable lambs fed the basal diet only during this period developed marked clinical signs of aphosphorosis.

Blood samples were obtained at regular intervals. Similar blood P concentrations were recorded for sheep fed all three sources of supplementary P. The increasing amounts of Mg in each diet (DCP, 0·04% Mg; MIX, 0·12% Mg; MAP, 0·20% Mg) resulted in elevated mean blood Mg concentrations.

Table 1. *Mean plasma phosphorus and magnesium concentrations and balance data of young lambs fed a low-phosphorus diet* (0·06% *P*) *supplemented with* 0·20% *P* (*five lambs/group*)

	mg P/100 ml			mg Mg/100 ml		
P source	DCP	MIX	MAP	DCP	MIX	MAP
16 Aug.*	3·37	3·96	4·73	1·71	1·89	1·92
30 Aug.	5·68	4·94	5·98	1·41	1·97	1·98
15 Sept.	5·71	5·72	4·88	1·87	2·15	2·35
30 Sept.	5·49	5·72	5·00	1·67	1·99	2·27
Mean	5·06	5·09	5·15	1·64	1·96	2·13

Balance data	g P/day			g Mg/day		
Intake	2·61	2·55	2·15	0·40	1·20	1·65
Urine	0·25	0·14	0·01	0·07	0·34	0·44
Faeces	1·03	1·07	0·98	0·30	0·54	0·79
Retained	1·33	1·34	1·16	0·03	0·32	0·42

Balance data (7-day collections) were obtained for the lambs (50–60 lb live weight) during September and October. Comparable proportions of dietary P (50–53% of intake) were retained by each of the three groups. The urine of lambs

* Supplementation increased from 0·15 to 0·20% P.

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fed DCP contained 9·6% of the total P intake but that of lambs fed MAP contained only 0·5% of the P intake. When Mg intakes were increased as MIX and MAP were included in the diets, elevated amounts of Mg were found in both urine and faeces and more Mg was apparently retained.

It is concluded that magnesium ammonium phosphate is an efficient source of P and Mg for ruminants.

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The One Hundred and Eighty-ninth Meeting of The Nutrition Society was held at the Sir Edward Lewis Hall, The Middlesex Hospital Medical School, Mortimer Street, London, W1, on Friday, 26 May 1967, at 10.30 am, when the following papers were read:

The distribution of polyethylene glycol in gut contents. By M. J. MANNERS and D. E. KIDDER, *School of Veterinary Science, University of Bristol*

To check the validity of experiments using polyethylene glycol (PEG) as a marker in studies of digestion in the piglet, we have examined the effect of adding solutions containing glucose and PEG to gut contents in vitro.

A solution containing, per 100 ml, 25 g glucose, 0·42 g PEG. (*M* 4000) and 3·4 g NaF as a preservative was prepared. Samples of contents were taken from the stomach and various sections of the small intestine of piglets and each was divided into two portions. To one portion, the glucose–PEG solution was added before centrifuging, the mixture was then centrifuged and the supernatant fluid taken. The second portion of the same gut contents was first centrifuged and the glucose–PEG solution then added to the supernatant. Glucose and PEG concentrations were determined on the supernatant fluids prepared in these two ways and dry-matter determinations were done on all the samples, making it possible to determine the ratio of water to glucose and PEG in each sample of supernatant fluid.

Where the glucose–PEG was added to the whole stomach contents, although the glucose:PEG ratio was much lowered (Table 1), the glucose was always distributed uniformly throughout the water of the sample. PEG distribution, however, was not uniform in the water in the whole stomach contents, where higher concentrations were found in the water of the supernatant than would have been present if it had dispersed evenly throughout all the water in the sample. A similar but