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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Sixty-ninth Meeting of The Nutrition Society was held at the Royal Veterinary College, Royal College Street, London, NW1 on Saturday, 5 December 1964, *at* 10.30 *am, when the following papers were read:*

Barley supplementation and voluntary feed intake of fattening beef cattle under restricted and unrestricted grazing conditions. By R. S. MUSANGI, W. HOLMES and J. G. W. JONES, *Wye College* (*University of London*), *Ashford, Kent*

The feed intake and performance of Sussex × Ayrshire beef cattle grazing a perennial ryegrass–white clover ley from July to September under restricted and unrestricted conditions, with and without barley supplementation, were measured. Sixteen animals averaging 21 months of age were allocated at random to a 2 × 2 factorial experiment, each of the four treatments being replicated four times, and three observations were made at monthly intervals. Restricted and unrestricted grazing was achieved by stocking two paddocks at rates of 2.2 and 1.1 animals/acre, respectively; further, animals on restricted grazing were permitted access to the pasture for only 10 h daily from 8 am to 6 pm whilst for the remainder of the day they were housed. Barley was offered to the supplemented groups at the rate of 0·075 lb/lb live weight$^{0.73}$. Between 7 and 8 am daily and again between 2.30 and 3.30 pm each animal was dosed with 10 g chromic oxide and received half the barley ration when offered; rectal grab-samples were also taken at these times during estimation of feed intake which took place during two 4-day periods at the end of each month. Feed intakes were estimated by the faecal nitrogen–chromic oxide technique. The animals were weighed on 3 successive days before and after each feed intake estimate. Pasture availability was measured at the beginning of each feed intake estimate by cutting random sample areas of the paddocks. The results are shown in Table 1.

Table 1. *The effect of barley supplementation on the feed intake and performance of grazing beef cattle*

Pasture availability	Restricted		Unrestricted		Standard error of treatment means
Grazing pressure (animals/1000 kg herbage DM available)	4·2		1·0		—
Mean barley OM intake (kg/day)	—	3·3	—	3·4	—
Mean OM intake (kg/day)	7·3	9·0	8·2	11·0	±0·17
Mean OM digestibility of diet (%)	71·9	74·6	71·7	74·0	±0·47
Mean DOM intake (kg/day)	5·3	6·7	5·9	8·1	±0·13
Mean live weight (kg)	399	394	388	412	±7·6
Mean rate of live-weight gain (kg/day)	0·71	1·06	1·05	1·06	±0·109

Grazing pressure determinations showed that about one-quarter as much herbage dry matter was available to the restricted animals as to the unrestricted animals.

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Restriction had no effect on the digestibility of the pasture but supplementation improved the overall digestibility of the diet by 2–3 digestibility units; this together with their increased OM intake gave a greatly increased digestible organic matter (DOM) intake in the supplemented groups. The performances of the animals in all treatments did not differ except that the rate of live-weight gain of the unsupplemented–restricted group was significantly ($P = 0{\cdot}05$) lower than that of the other groups.

The results showed that grazing restriction with limited barley feeding achieved performances which equalled those of unrestricted grazing; excessive energy intake may have occurred with supplementation of unrestricted grazing.

Dietary carbohydrate and brain lipids in young mammals. By V. SCHWARZ, G. H. LYNN* and S. N. VARMA*, *Departments of Child Health and Pathology, University of Manchester, Manchester*, 13

Substitution of maltose for lactose in the synthetic diet of kittens has no untoward effect on their physical development. Considerable changes occur, however, in the composition of brain lipids: animals receiving maltose as the sole carbohydrate have double the sphingomyelin content but less cephalin (serine, ethanolamine and inositol phosphatides) compared with lactose-fed controls. The absence of dietary galactose is not reflected in the galactolipid content of the brain, either of experimental animals or human infants.

Radio–chromium sesquioxide as an intestinal marker in sheep and pigs. By T. K. EWER and H. F. SASSOON, *Department of Animal Husbandry, University of Bristol*

Body composition and the long–term nitrogen balance study of growing pigs. By D. W. ROBINSON, *Veterinary Field Station, University of Liverpool*

There appear to be several problems in relation to the pig which make it very difficult to establish the necessary basic information on the amino acid needs of the animal. Some of these are inherent problems common to all species and some refer to the methods of experimentation and the parameters used to measure efficiency in the pig. In the first category are factors such as the changing requirements during growth (Braude, 1958) the effect of genotype (Bowland & Berg, 1959) the endocrine status of the animal (Prescott, 1963) and the sex (Robinson & Lewis, 1964) as well as considerations such as the non-availability of amino acids. Problems associated with the method of experimentation have only recently been recognized. The use of growth rate and food conversion efficiency as the sole criterion of efficiency of utilization of dietary protein in meat producing animals is not entirely justified since the quantity of lean meat in the carcass is frequently of overriding importance.

An experiment was carried out to investigate the influence of the protein : lysine ratio in the diet on the nitrogen metabolism and body composition of male castrate

* Former postgraduate student.

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pigs fed diets of low and moderate protein concentration. The body composition data were found to correlate well with the overall nitrogen balance results obtained on long-term continuous observation of up to 6 months. The comparison was made by calculating the area beneath the regression lines of nitrogen retention upon live weight over the whole of the growth period and comparing this with dissection data. The protein level had a more marked effect than the lysine level on the growth rate and food conversion efficiency although at each protein level growth was improved by the addition of supplementary lysine. Initially the nitrogen retention of pigs was highest on a diet containing 15% protein and 1·0% lysine, followed by 15/0·5, 10/1·0 and 10/0·5, respectively, but the pattern of nitrogen retention changed as the pigs grew and overall the pigs receiving the 10/1·0 (% protein/% lysine) diet had the highest retention, 10/0·5 the lowest and the other two groups were similar and intermediate.

REFERENCES

Bowland, J. P. & Berg, R. T. (1959). *Canad. J. Anim. Sci.* **39**, 102.
Braude, R. (1958). In *Scientific Principles of Feeding Farm Live Stock*, p. 168. London: Farmer and Stock Breeder Publications Ltd.
Prescott, J. H. D. (1963). The effects of sex and of certain steroids on the growth of the pig. PhD Thesis, University of Nottingham.
Robinson, D. W. & Lewis, D. (1964). *J. agric. Sci.* (In the Press.)

Endocrine factors and the response of pigs to amino acid supplementation.

By D. W. Robinson, *Veterinary Field Station, University of Liverpool*

Many factors may be involved in controlling the efficiency with which pigs utilize dietary protein and one of these is undoubtedly the endocrine status of the animal. It is well known that boars, castrate male pigs and gilts differ in their growth rate and food conversion efficiency under *ad lib.* feeding conditions (Charette, 1961) and also in their body composition (Wallace, 1944). Sex differences in pigs on different planes of nutrition have been observed by McMeekan (1940*a*, *b*) and Robinson (1964) and also on deficient dietary protein levels by Robinson & Lewis (1964) and Prescott (1963). The present study involving the use of forty individually fed pigs was undertaken to examine the response of gilts and castrate male pigs to diets supplemented in varying degrees with synthetic preparations of the first and second limiting amino acids.

The hypothesis that castrates have a lower requirement for protein is supported by the results of the present study in which castrates appear to have a lower requirement for the essential amino acids than gilts. In the grower phase between 50–100 lb live weight castrates failed to respond to the supplementation of methionine, an amino acid calculated to be deficient in the diet used, while gilts showed a significant improvement in the rate of gain and food conversion efficiency. In the finisher diets both castrates and gilts received suboptimal levels of lysine and other amino acids for maximum performance and both responded therefore to lysine supplementation equally well.

There was no response by castrate male pigs to amino acid supplementation in terms of increased lean meat production, but gilts showed a significant increase in the percentage of carcass lean.

REFERENCES

Charette, L. A. (1961). *Canad. J. Anim. Sci.* **41**, 30.
McMeekan, C. P. (1940*a*). *J. agric. Sci.* **30**, 276.
McMeekan, C. P. (1940*b*). *J. agric. Sci.* **30**, 387.
Prescott, J. H. D. (1963). The effects of sex and of certain steroids on the growth of the pig. PhD Thesis, University of Nottingham.
Robinson, D. W. (1964). *Anim. Prod.* **6**, 227.
Robinson, D. W. & Lewis, D. (1964). *J. agric. Sci.* (In the Press.)
Wallace, L. R. (1944). *Proc. N.Z. Soc. Anim. Prod.* p. 64.

Vascular changes in bone in calcium-deficient kittens. By F. KAYANJA and M. G. SCOTT, *Royal Veterinary College, London, NW*1, and P. P. SCOTT, *Royal Free Hospital School of Medicine, London, WC*1

Injection techniques using Berlin Blue and Micropaque at controlled pressures (Kayanja, 1963) were used to follow the changing pattern of blood vessels in growing bones. Control kittens received a stock diet, and a parallel series were given meat, low in calcium and high in phosphorus (Scott, Greaves & Scott, 1961); some deficient kittens were recovered by supplementing with 1–2 g $CaCO_3$ daily.

In calcium deficiency a characteristic sequence of events occurred in regions undergoing resorption. First, fine blood vessels in bone became dilated; then the dilated vessels enlarged into sinusoids which virtually filled the resorption cavities; lastly, the calibre of the sinusoids became greatly reduced, the resulting space being filled mainly with fibrous connective tissue but also with haemopoietic tissue.

An increase in the arterial capacity of the bone vessels accompanied these latter changes.

When kittens showing the final stage of vascular change were supplied with calcium reorganization occurred very rapidly, initiated by a marked increase in the numbers of capillaries and followed by osteoblastic activity resulting in the formation of new bone.

Vascular changes resulting from calcium deficiency occurred in all bones. However, the order in which different parts of the skeleton were affected was as follows: (1) scapula and vertebras; (2) frontoparietals and metaphyses of long bones; (3) epiphyses and compact bone of diaphyses, pelvis, ramus of mandible; (4) patella; (5) body of mandible.

REFERENCES

Kayanja, F. (1963). *J. Physiol.* **170**, 44P.
Scott, P. P., Greaves, J. P. & Scott, J. G. (1961). *Brit. J. Nutr.* **15**, 35.

The effect of 5-day diets of various carbohydrates on the serum lipids in men. By I. MACDONALD, *Department of Physiology, Guy's Hospital Medical School, London, SE*1

When sucrose was the main carbohydrate in a diet with a low fat content the alterations in lipid metabolism which occurred were different from those with maize starch under the same conditions (Macdonald & Braithwaite, 1964). It seemed possible that these changes in fat metabolism which took 25 days might be accelerated

by giving fat-free diets so as to allow a greater variety of carbohydrates to be studied in a shorter period of time. Although the absence of dietary fat would in itself affect fat metabolism this influence would be a common feature in all experiments and the only variable would be that of the kind of carbohydrate employed. Accordingly, a practically fat-free diet was devised and administered with various carbohydrates for 5-day periods.

The volunteers were six adult men and the carbohydrates used were maize starch, liquid glucose BPC (a partial hydrolysate of starch), sucrose, maltose and glucose.

The serum lipid response to liquid glucose BPC, maltose and glucose was similar and consisted mainly of a reduction in the concentration of all fractions except the phospholipids. The serum lipid response to starch was a fall in cholesterol concentration whereas sucrose did not give rise to reduction in any fraction, but produced an increase in the serum glyceride fraction.

I am most grateful to the volunteers and also to Beecham Food and Drink Division Ltd for a research grant.

REFERENCE

Macdonald, I. & Braithwaite, D. M. (1964). *Clin. Sci.* **27**, 23.

The effect of mild cooking on the nutritive value of wheat. By C. K. Milner, *School of Agriculture, University of Cambridge*

In earlier work (Milner, 1963) strongly autoclaved wheat gave lower values than uncooked wheat in PER and NPR tests with weanling rats; mildly autoclaved wheat gave values slightly (non-significantly) higher than uncooked wheat. Shyamala & Kennedy (1962) reported that cooking wheat to Indian *chapatis* (dough of 3 parts whole-wheat flour + 1 part of water rolled into thin discs and cooked for 2 min each side on a hot-plate at 210°) increased its PER value considerably.

The effect of cooking to *chapatis* has now been compared with that of the stages in preparing *bulgur* by mild methods (i.e. hot steeping of whole wheat, then boiling and finally drying). The products were tested at levels providing 10% crude protein in 10-day rat experiments with the following results:

	Expts 1 and 2* (albino rats)		Expt 3 (hooded rats)				
Processing of wheat	PER		PER		NPR		TD
Uncooked control	0·88	(100)†	1·1	(100)†	2·3	(100)†	88
Steeped at 80° for 70 min freeze-dried	1·2	(136)	—	—	—	—	—
Steeped at 80° for 70 min air-dried at less than 40°	—	—	1·6	(145)	2·7	(118)	88
Steeped at 80° for 70 min, boiled 15 min, dried at less than 40°	1·3	(148)	1·4	(127)	2·5	(109)	88
Chapatis	1·2	(136)	1·4	(127)	2·5	(109)	87
SE of treatment mean			±0·1		±0·12		±0·5

* No standard error is given for Expts 1 and 2 since several animals lost weight.

† Values relative to control wheat as 100.

NPR values are thought to be less influenced than PER values by palatability and other, non-protein, factors affecting food consumption, but an improvement even in NPR was seen on one treatment. Beaudoin, Mayer & Stare (1951) found that boiling wheat resulted in faster rat growth. Hutchinson, Moran & Pace (1964) showed that a short steaming process improved wheat quality for rats; our conclusion is that the same effect can be obtained with temperatures below 100°.

Our treatments were also evaluated in 14-day chick trials but no significant differences were found when the materials were fed as sole protein source in diets providing 13% crude protein. In contrast, other workers found that water-treated wheat or barley supported better growth in chicks than untreated materials (e.g. Willingham, Leong, McGinnis & Jensen, 1961). Their treatments increased the metabolizable energy of the grain for the chick and, in the case of barley probably acted by destroying a growth-depressant, β-glucan (Jensen, 1963). A similar explanation might hold for wheat, but it is also possible that the trypsin inhibitor in fresh wheat retards growth and that the sticky character of the gluten makes unprocessed wheat unpalatable. We are presently investigating these factors.

REFERENCES

Beaudoin, R., Mayer, J. & Stare, F. J. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 450.
Hutchinson, J. B., Moran, T. & Pace, J. (1964). *J. Sci. Fd Agric.* **15**, 413.
Jensen, L. S. (1963). *Proc. 16th annu. Calif. Anim. Ind. Conf.* p. 62.
Milner, C. K. (1963). *Proc. int. Congr. Nutr.* VI. *Edinburgh*, p. 491.
Shyamala, G. & Kennedy, B. M. (1962). *J. Amer. diet. Ass.* **41**, 115.
Willingham, H. E., Leong, K. C., McGinnis, J. & Jensen, L. S. (1961). *Poult. Sci.* **40**, 1470.

Lignin as an inert marker in studies of ruminant digestion. By P. PORTER, *University of Liverpool, Veterinary Field Station, Neston*

The use of lignin in ruminant digestion studies to assess digestibility of components by the ratio technique has frequently proved unsatisfactory because lignin itself has shown large apparent digestibilities. There is some evidence that lignin is metabolized, but the site of digestion and the validity of coefficients based on lignin remain the subject of controversy.

Although the inert qualities of lignin are suspect, it has advantages over artificial markers in being an integral part of the digesta, and its apparent digestibility may be a result of the crudeness of the 72% H_2SO_4 preparations. The technique of preparation was investigated and two methods of pretreatment designed to eliminate interference products of carbohydrates and proteins were compared: (a) acid and pepsin (Ellis, Matrone & Maynard, 1946), (b) acid detergent (Van Soest, 1963).

The second method produced fibre residues containing the least nitrogen but also gave a lower lignin yield. Investigations with soda lignin preparations revealed that the acid detergent reagent solubilized a proportion of the lignin which could not be recovered from solution. Lignins from the hay, duodenal contents and faeces of a sheep with exteriorized flow through a duodenal cannula were prepared and analysed (Table 1). The demethoxylation of lignin occurs mainly in the stomachs, suggesting that this is the main site for metabolism. The lignin prepared by the acid pepsin

Table 1. *Analysis of Klason sulphuric acid lignins after different preparative treatments*

Pretreatment	Sample	Percentage lignin	Percentage composition						Ash-free percentage compositon				
			C	H	N	O	Meth-oxyl	Ash	C	H	N	O	Meth-oxyl
EtOH/benzine 1·5 N-H_2SO_4	Hay	7·17	55·12	5·82	2·08	31·42	9·62	5·56	58·36	6·16	2·20	33·28	10·19
Pepsin (Ellis Matrone & Maynard, 1946)	Duodenal contents	13·69	52·47	5·03	2·33	34·31	8·73	5·86	55·73	5·34	2·48	36·45	9·21
	Faeces	20·86	50·43	4·28	1·87	32·46	8·03	10·96	56·63	4·81	2·10	36·46	9·02
Acid detergent (cetyltrimethyl ammonium bromide) (Van Soest, 1963)	Hay	4·63	52·60	5·99	1·53	27·49	9·03	12·39	60·03	6·84	1·75	31·38	10·31
	Duodenal contents	8·63	53·91	5·60	2·13	28·21	7·83	10·15	60·00	6·23	2·37	31·50	8·71
	Faeces	12·82	48·29	5·23	1·42	26·00	7·06	19·06	59·66	6·46	1·75	32·13	8·73

technique undergoes more extensive changes than the acid detergent lignin. It is therefore probable that the latter will act as a more valid marker.

Acknowledgements are due to Mr A. G. Singleton for the surgical preparation of the sheep with duodenal cannula.

REFERENCES

Ellis, G. H., Matrone, G. & Maynard, L. A. (1946). *J. Anim. Sci.* **5**, 285.
Van Soest, P. J. (1963). *J. Ass. off. agric. Chem.* **46**, 829.

The performance of chicks on a diet containing autoxidized beef fat.

By J. L. L'Estrange and K. J. Carpenter, *School of Agriculture, University of Cambridge*, and C. H. Lea and L. J. Parr, *Low Temperature Research Station, Cambridge*

It is uncertain how far 'peroxide values' (PV) are useful indicators for the quality of animal feeding-stuffs. Lea, Parr, L'Estrange & Carpenter (1964) fed weanling rats one diet containing 5% beef fat oxidized to a PV of 93 μmoles/g and they thrived.

We have now fed similar, practical-type diets, including stabilized vitamins, to broiler cockerels. The tallows used were (1) fresh, (2) oxidized to a PV of 109 and (3) oxidized as in (2), then heated to destroy the 'peroxides'. Each was fed (A) as 5% of a diet with vitamin supplementation, including 4 mg/kg of stabilized α-tocopheryl acetate, to two groups of six chicks each and (B) in the same way without supplementary tocopherol. BHT (0·02%) was dissolved in each fat after its preparation. The diets were mixed at the start of the experiment, when the chicks were day-old and stored at 20°.

As before (Lea *et al.* 1964), PV (using cold, de-aerated chloroform–methanol (4 : 1) extracts) of the 'oxidized fat' diets decreased rapidly during storage; free fatty acid content of the extracts increased greatly for all diets. Analysis (Diplock, Edwin, Bunyan & Green, 1961) for α-tocopherol content of the 'B' diets showed a significant decrease over 8 weeks only with the 'oxidized fat' diet, from initially 8 to 3 mg/kg.

As with rats (Lea *et al.* 1964) the vitamin A stored in the livers of the birds was just significantly less on the 'oxidized fat' diets. Encephalomalacia was diagnosed in one

Diet (fat supplement)	Characteristics of dietary lipids before and after storage: Peroxide value (μmoles/g) Initial	Peroxide value (μmoles/g) Stored 8 weeks	Free fatty acid (as % oleic) Initial	Free fatty acid (as % oleic) Stored 8 weeks	Performance of chicks: No. of losses	Mean wt at 8 weeks (kg)	g food/g gain	Vitamin A at 8 weeks (i.u./liver)*
1A (fresh tallow)	3·0	1·8	8·3	57	2	1·53	2·05	550
1B (ditto, but without tocopheryl acetate)	3·0	1·2	8·3	55	2	1·55	2·08	620
2A (oxidized tallow)	71	6·1	8·6	46	—	1·62	2·11	350
2B (ditto, but without tocopheryl acetate)	71	6·1	8·6	47	1	1·63	2·13	440
3A (oxidized, heated tallow)	4·2	2·5	8·8	51	1	1·52	2·24	480
3B (ditto, but without tocopheryl acetate)	4·2	2·5	8·8	49	1	1·57	2·07	570
Standard error of treatment means	(—)	(—)	(—)	(—)	(—)	(0·05)	(0·04)	(49)

* All diets contained 3000 i.u. vitamin A/kg. Controls at day old had 73 (±18) i.u./liver.

chick receiving diet 2 B. Perosis occurred in five chicks on other diets; the strain used was apparently prone to this. The remaining cockerels grew extremely well without significant difference between treatments. Birds from each group were roasted but no flavour difference could be detected.

Thus a tallow oxidized to a PV higher than is likely to be encountered in practice, and used as 5% of a practical diet, has shown no adverse effect on the performance of broiler chicks.

REFERENCES

Diplock, A. T., Edwin, E. E., Bunyan, J. & Green J. (1961). *Brit. J. Nutr.* **15**, 425.
Lea, C. H., Parr, L. J., L'Estrange, J. L. & Carpenter, K. J. (1964). *Brit. J. Nutr.* **18**, 369.

Intakes and excretions of iron, copper and zinc by babies in the 1st week after birth. By ELSIE M. WIDDOWSON and PATRICIA A. CAVELL, *Medical Research Council Department of Experimental Medicine, University of Cambridge*

The intakes and urinary and faecal excretions of iron, copper and zinc by ten breast-fed babies were measured over a 3-day period at the end of the 1st week after birth.

All the babies were in negative iron balance, and the faeces contained on the average more than ten times as much iron as the food. Some of the babies were in negative and some in positive copper balance; all but one were in negative zinc balance. They were losing more than 1% of the body's iron and zinc each day.

It is not known how long these large negative balances continue; a possible explanation for them will be suggested.

The intakes and excretions of calorific constituents by babies. By D. A. T. SOUTHGATE and IRENE M. BARRETT, *Medical Research Council Department of Experimental Medicine, University of Cambridge*

Two groups of male babies, aged about 14 days, were studied for 3 days while they were receiving either human breast milk or a milk preparation based on dried cow's milk with added carbohydrate.

The intakes and excretions of nitrogen, fat and carbohydrates, and the heats of combustion of the milks, faeces and urines were determined.

The results show that the calorie conversion factors in conventional use are not suitable for calculating the calorific value of the milks consumed by babies and alternative factors are proposed.

The fate of oral riboflavine. By E. C. Owen and L. Dzialoszynski*, *Biochemistry Department, Hannah Dairy Research Institute, Ayr*

The authors each took 20 mg of riboflavine in the form of multivitamin tablets, and urine which was collected both before and after its ingestion, was examined by paper chromatography as in the following report. In both the control and experimental chromatograms of L.D., who had been taking the tablets for some time, riboflavine was visible in ultraviolet light together with a substance of R_f two-thirds of that of riboflavine. Ingestion of the tablets increased the amount of both these substances. Only a minute trace of riboflavine appeared in the control urine of E.C.O., whose chromatograms showed yellow-fluorescent spots of $R_f = 0{\cdot}01$, $0{\cdot}12$, $0{\cdot}14$. After the ingestion of the tablets new fluorescent spots appeared of $R_f = 0{\cdot}05$, $0{\cdot}1$ and $0{\cdot}24$, the last being riboflavine.

In a second experiment each of us took 200 mg of riboflavine (Laboratory Reagent; British Drug Houses Ltd). The results were similar except that the increases observed, especially that of riboflavine, were much more evident. Ethanolflavine was not found in the urine, though internal and external pilot spots of synthetic ethanolflavine (Cresswell & Wood, 1960) appeared in the expected position on the papers. Addition of 5 ppm of ethanolflavine before extraction also produced spots in the expected position, thus confirming the absence of the substance from the urines of E.C.O. and L.D.

In later experiments by us riboflavine was incubated in vitro with rumen contents, or beef liver or beef kidney. Only in the rumen incubations was ethanolflavine found. These results and the following result with the dog, support the hypothesis that ethanolflavine in ruminant urine and milk is a product of bacterial action in the rumen and recall the work of Stadtman (1958) and his colleagues who found ethanolflavine among the products of the action of a soil anaerobe on riboflavine.

We thank Dr H. C. S. Wood for a gift of ethanolflavine and Miss S. McLauchlan for assistance with the analysis.

REFERENCES

Cresswell, R. M. & Wood, H. C. S. (1960). *J. chem. Soc.* 4768.
Stadtman, E. R. (1958). *Proc. int. Congr. Biochem.* IV. *Vienna*, **11**, 19.

* On leave from the University of Poznan under the auspices of the British Council.

The fate of an oral dose of riboflavine in the dog. By E. C. Owen and L. Dzialoszynski*, *Biochemistry Department, Hannah Dairy Research Institute, Ayr*

Ethanolflavine was identified by Owen (1962) as the chief metabolite which appears in the urine and milk of the goat after ingestion of 2 g riboflavine. On two different occasions when a 2 g dose of riboflavine was given to a bitch, ethanolflavine could not be

* On leave from the University of Poznan under the auspices of the British Council.

demonstrated in the urine. Before its administration riboflavine was not present in the urine, but was plainly visible on paper chromatograms even in daylight thereafter (Crossland, Owen & Proudfoot, 1958).

When a concentrate of the bitch's urine was painted as a strip, 1 cm wide, at the origin of a chromatogram in the butanol : acetic acid : water (4 : 1 : 5) upper layer on Whatman no. 31, extra-thick, paper, a thin greenish-fluorescent band appeared in the ethanolflavine position. This band was cut from the dried chromatogram, washed with ether and eluted with water. The eluate was taken to dryness and acetylated (Owen, Montgomery & Proudfoot, 1961). No acetyl-ethanolflavine appeared when the acetylated product was chromatographed, thus confirming the absence of ethanolflavine.

The absence of riboflavine from the control samples is paralleled by the effect in man (see preceding report) and it also resembles the observation of Owen & Hytten (1962) who found no riboflavine in the milk of one of two mothers until she ingested a dose of riboflavine.

We have to thank Dr T. A. Douglas of the Veterinary Biochemistry Laboratory, University of Glasgow, for the sample of canine urine.

REFERENCES

Crossland, A. C., Owen, E. C. & Proudfoot, R. (1958). *Brit. J. Nutr.* **12**, 312.
Owen, E. C. (1962). *Biochem. J.* **84**, 96P.
Owen, E. C. & Hytten, F. E. (1962). *Proc. Nutr. Soc.* **21**, xviii.
Owen. E. C., Montgomery, & Proudfoot, R. (1961).

The use of homocysteine in the estimation of ascorbic acid in urine.

By A. N. HOWARD* and B. J. CONSTABLE, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Techniques in general use for the estimation of ascorbic acid are unsatisfactory when applied to urine, because of the interfering materials present. A rapid and specific method is described for the estimation of ascorbic acid in urine based on the use of homocysteine, a reagent developed by Hughes (1956) for dehydroascorbic acid.

If urine is shaken with charcoal, both the ascorbic acid present and interfering substances are oxidized and the colourless filtrate does not decolorize dichlorophenolindophenol dye. On treatment with homocysteine at pH 7, the dehydroascorbic acid formed can be reconverted to ascorbic acid together with a small quantity of other dye reducing substances. These can be differentiated from ascorbic acid by carrying out the reaction in the presence of boric acid which prevents the reduction of ascorbic acid.

Human urine (20 ml) containing 4% (w/v) trichloroacetic acid as preservative and 2 ml acetone, is shaken with 0·25 g activated charcoal for 2 min and filtered. Two aliquots are treated as follows: (1) To 3 ml of filtrate is added 1 ml of water, 1 ml of homocysteine solution (1·5 mg/ml) and the quantity of 45% (w/v) anhydrous K_2HPO_4 solution required to bring the pH to 7·2 (determined by titration of an aliquot

* Present address: Department of Pathology, University of Cambridge.

using bromophenol blue indicator). (2) To 3 ml of filtrate is added 1 ml of 3% (w/v) boric acid solution, 1 ml homocysteine solution and the mixture adjusted to pH 7–7·2 with K_2HPO_4 solution as above. Mixtures (1) and (2) are left at room temperature for 20 min and a 3 ml aliquot titrated with 3 ml dichlorophenolindophenol dye (1 ml equivalent to 0·1 mg ascorbic acid) in the presence of 4 ml phosphate–citrate buffer of pH 2·2 (376 g citric acid, monohydrate, and 61·6 g anhydrous Na_2HPO_4 per l.) using a photoelectric colorimeter as described by Hughes (1956). Readings are taken exactly 20 sec after the addition of the dye. The amount of ascorbic acid is calculated from the difference in the titration values from (1) and (2) with reference to a standard curve constructed previously.

Recoveries of ascorbic acid added to urine were 96–103% even in the presence of added interfering materials. The new technique compared favourably with the formaldehyde method (Mapson, 1953) which is also specific but time-consuming.

REFERENCES

Hughes, R. E. (1956). *Biochem. J.* **27**, 303.
Mapson, L. W. (1953). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 280, p. 145.

Nitrogen metabolism in guinea-pigs receiving low daily intakes of ascorbic acid. By J. R. Evans, R. E. Hughes and R. Z. Thomas, *Welsh College of Advanced Technology, Cardiff*

Guinea-pigs with acute scurvy produced by completely depriving them of dietary ascorbic acid are of little value for metabolic studies. Such animals rarely survive for more than 3-4 weeks and during the final week it is difficult to distinguish between metabolic changes attributable to true avitaminosis C and those resulting from secondary effects such as inanition, tissue breakdown, etc.

In the experiments described in this note a condition of chronic hypovitaminosis C was produced. By adopting this procedure it was possible to follow changes in nitrogen metabolism over a period of weeks in guinea-pigs free from the secondary complications referred to above.

Groups of eight male guinea-pigs of mean initial weight 280 g were used. The diet and experimental conditions were as previously described (Evans & Hughes 1963). Group A was pair-fed with group B and received 8 mg ascorbic acid daily. Group B received 0·4 mg ascorbic acid daily.

For the first 12 days of the experiment both groups showed a positive nitrogen balance. From day 13 onward the nitrogen balance in group B was negative, but in group A it remained positive. After 24 days on experiment the mean body-weight of group A was 321 g and that of group B 290 g.

Fractionation of the urinary nitrogen in subsequent experiments revealed a sharp rise in the ammonia and urea excreted by group B from day 5 onward. From day 5 to day 20 the mean daily excretion of ammonia nitrogen and urea nitrogen was 0·75 mg and 220 mg respectively for group A and 2·9 mg and 350 mg respectively for group B. No change occurred in the excretion of creatinine and uric acid. These results are

in general agreement with those reported by Banerjee for scorbutic monkeys (Banerjee, 1961). An increased excretion of inorganic sulphate occurred in group B, the increase paralleling that in the urea excreted.

Serum transaminase levels (SGOT and SGPT) were increased in group B. The mean SGOT value for group B was 33% higher than that for group A: the SGPT for group B was 60% above that for group A.

Subsequent work in vitro has shown that the activity of these enzymes is related to the presence of ascorbic acid in the reaction mixture. Both ascorbic acid and isoascorbic acid when present in 0·001 M concentration reduced the activity of guinea-pig tissue transaminases by 25%–40%. Reductic acid, cysteine, homocysteine and glutathione appeared to lack this activity.

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REFERENCES

Banerjee, S. (1961). *Proc. Symp. Proteins, Mysore*, 1960, p. 124.
Evans, J. R. & Hughes R. E. (1963). *Brit. J. Nutr.* **17**, 251.

Ruminal lipolysis and hydrogenation in the sheep. By I. H. BATH* and K. J. HILL, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

It is well established that ruminal micro-organisms are responsible for lipolysis and hydrogenation of dietary lipids (Shorland, Weenink, Johns & McDonald, 1957; Garton, Lough & Vioque, 1961) although there have been no studies of the composition of ruminal and intestinal digesta collected from conscious sheep maintained on diets containing added fat. In the present experiments sheep with ruminal and duodenal fistulas were maintained on diets of either hay plus 5% palm oil or hay plus 5% tallow and samples of digesta were taken at 4-hourly intervals for 32 h. Similar results were obtained for the two fats.

In sheep which received hay and tallow (85% glycerides and 13% free fatty acids and 1·3% phospholipids in dietary lipids) the glyceride content of the lipids in ruminal digesta 1, 5, 9 and 13 h after feeding was 12, 4, 2 and 1% respectively. The lipids in duodenal digesta contained 1·4–4·4% glycerides, the highest concentration appearing 5 h after feeding. Free fatty acids accounted for 71–90% and 84–91% of the lipids in ruminal and duodenal digesta respectively and phospholipids 8–15% and 7–12% respectively.

Source of lipid	Lipid fraction	Percentage in total (C_9–C_{20}) fatty acid												
		C_9–C_{13}	14:0	14:br	15:0	15:br	16:0	16:1	16:br	17:0	18:0	18:1	18:2	18:3
Diet	Total	1	3	T	1	T	28	4	1	1	15	35	6	3
Diet	PL	3	1	1	T	1	30	2	6	1	2	5	19	29
Rumen	FFA	T	1	T	1	T	27	1	1	2	55	10	T	0
Rumen	PL	8	8	11	3	4	28	3	3	2	12	12	4	2
Duodenum	FFA	0	2	T	1	2	25	0	2	2	52	13	1	0
Duodenum	PL	6	7	16	5	5	31	3	6	2	6	10	2	1

T, trace; PL, phospholipids; FFA, free fatty acids.

The fatty acid composition of the dietary lipids and of digesta samples taken 1 h after feeding (see table) confirms that hydrogenation proceeds rapidly and that little

* Present address: School of Veterinary Medicine, Trinity College, University of Dublin.

unsaturated lipid reaches the site of intestinal absorption. Whether the phospholipids found in the digesta represent, in part, hydrogenated dietary phospholipids or whether they are entirely of bacterial origin is not known, but the fact that lecithin is rapidly hydrolysed by rumen micro-organisms in vitro (Dawson, 1959) supports the latter possibility.

REFERENCES

Dawson, R. M. C. (1959). *Nature, Lond.*, **183**, 1822.
Garton, G. A., Lough, A. K. & Vioque, E. (1961). *J. gen. Microbiol.* **25**, 215.
Shorland, F. B., Weenink, R. O., Johns, A. T. & Mcdonald, I. R. C. (1957). *Biochem. J.* **67**, 328.